



REVIEW ARTICLE

Green Amalgamation of Nano - Bioenzymes and its Diversified Applications: an Inclusive Review

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ABSTRACT

By combining precision catalysis from biological enzymes with unique physical and chemical properties of nanomaterials, nano-bioenzymes is an emerging, robust, transformative field created from the intersection of enzymology and nanotechnology. The most sustainable, least toxic way to create nano-bioenzymes is via green synthesis methods that use phytochemical-containing plant extracts, secretions from microorganisms, and other biobased renewable resources to act both as reducing agents and capping agents for the phytochemical-assisted formation of nanoparticles. This review article summarizes and synthesizes peer-reviewed articles published during the period 2015-2026 on the principles, methodologies and applications of green synthesis of nano-bioenzymes mediated via plants. The article includes the mechanistic aspects of how phytochemicals assist the formation of nanoparticles; how to immobilize enzymes (i.e., laccase, peroxidase, lipase, cellulase, amylase, protease and superoxide dismutase mimics) on a range of nanoplatforms (i.e., silver [AgNPs], gold [AuNPs], zinc oxide [ZnONPs], copper oxide [CuONPs], iron [FeNPs], titanium dioxide [TiO₂NPs] and manganese dioxide [MnO₂NPs]) and how the resulting nano-bioenzyme systems can be utilized in various ways, such as biomedical therapy, biosensor development, environmental bioremediation, food processing, biodiesel production





Unaiza Hashmi et al.,

and neuroprotection. Three summary tables summarizing key findings of green source-enzyme-nanoplateform combinations and their respective application results, and summary comparing green and conventional chemical synthesis methods will be presented. The review further highlights current challenges including stability under physiological conditions, batch reproducibility, and regulatory pathways, while charting a roadmap for future research toward industrial scale-up of green-synthesized nano-bioenzymes.

Keywords: Green synthesis; Nano-bioenzymes; Bioremediation; Biosensor; Nanoparticles; Sustainable nanotechnology

INTRODUCTION

Enzymes are regarded as among of nature's best catalysts since they can accelerate biochemical reactions with remarkable selectivity, operate in mild environments, and leave no leftovers that can be hazardous to the environment. Pharmaceuticals, food production, textile manufacture, and environmental remediation are just a few of the businesses that have tried to take use of the possibilities of using enzymes on a broad scale over the past few decades. However, soluble free enzymes have drawbacks that prevent their widespread application in these industries, such as their susceptibility to denaturation under extreme pH and heat conditions, their high separation costs, their incapacity to be reused, and their leaching from reaction vessels, which complicates product recovery [1]. The goal of the field of immobilization of enzymes was to eliminate all of the constraints related to free soluble enzymes; however, toxicity, non-biodegradability, and reduced enzyme loadings have been introduced by traditional support matrices for enzyme immobilization, such as synthetic polymers, silica beads, and activated charcoal [2]. The use of enzymes has undergone a major transformation with the development of nanotechnology. Because of their exceptional surface area-to-volume ratios, capacity to alter surface characteristics, quantum confinement effects, and size-dependent optical, magnetic, and electronic properties, nanoparticles (NPs) have emerged as viable carriers and mimetics of enzymes for the purpose of carrying out the same chemical reactions that would typically occur using naturally occurring enzymes [3]. However, this means that many hazardous and toxic reducing agents (like sodium borohydride) and stabilizers (like cetyl trimethylammonium bromide, CTAB) are used in traditional chemical and physical methods for nanoparticle synthesis (chemical reduction, sol-gel, laser ablation, and physical vapour deposition). These methods produce hazardous by-products and raise serious concerns about the environmental impact and biocompatibility of the resulting nanoparticles [4].

At this point, green synthesis becomes a significant shift in how this procedure is approached rather than merely a new choice. By reducing metal precursors and capping the resulting nanoparticles with phytochemicals (polyphenols, flavonoids, terpenoids, alkaloids, reducing sugars, and organic acids) present in plant extracts, stabilized and biocompatible nanoparticles can be produced without the need for chemical reactants or high energy inputs [5]. Enzymes can adhere to the nanoparticles (e.g., through covalent bonding, electrostatic attraction, hydrophobic attraction, or physical entrapment) in a biologically active micro-environment created by the real phytochemical that stabilizes the nanoparticle's surface [6]. The nano-bioenzyme systems produced by "green" synthesis, such as the combinations of catalytically active enzymes with nanoparticles, represent an important type of bio-nanohybrid that has shown a broad base of utility across a wide variety of applications: production of biodiesel using lipase-AgNP biocatalysts [9], degradation of azo dyes by laccase-AgNP conjugates, glucose and hydrogen peroxide biosensing using HRP-AuNP electrodes [7], tumor-AuNP conjugates [8], and neuroprotection by scavenging reactive oxygen species with superoxide dismutase-mimicking MnO₂ nanoparticles [10]. The various application domains show that one kind of technology, "green" manufactured nano-bioenzymes, is a flexible platform that may be used in a variety of fields, including environmental engineering, food technology, energy, and oncology.



**Unaiza Hashmi et al.,**

Even though the amount of scientific research in this field is still growing quickly, the literature on this expanding field is dispersed, and there hasn't been a single thorough review that covers the synthesis as well as the variety of applications of "green" synthesized nano-bioenzymes. By synthesizing data from over thirty peer-reviewed studies, offering three comparative tables, and offering critical commentary on both challenges and future needs to be addressed, this review satisfies this need in a format meant to be helpful to the scientific community at large in pharmacy, biotechnology, material science, and environmental science.

Conceptual Structure: What Are Nano-Bioenzymes?

The term "nano-bioenzyme" refers to a collection of bio-nano hybrid systems that have catalytically active biomolecules (enzymes) attached to or bound to nanoscale materials (1 to 100 nm). The close nature of the two components in terms of covalent and non-covalent binding, as well as the structures created by the resulting biomolecule/catalyst or bio-nano hybrid, may cause this binding for enzymatic activity [11]. In its most basic form, the combination of nanoparticles with enzymes (such as enzyme adsorption) works to bind the enzyme to the nanoparticle's surface, producing a connection between the two materials that is primarily non-covalent. Although the likelihood of losing the enzyme is significant in situations involving either shear or ionic pressures, the nanoparticle will typically maintain the integrity of the enzyme. Covalent conjugation of the enzyme to functional groups (like -COOH, -NH₂, or -SH) on the surface of the nanoparticle produces a very stable conjugate in a more stable way of binding the two-component systems (enzyme and nanoparticle). However, this conjugate has a drawback related to the flexibility of the enzyme and the access to its active site.

The enzyme can also be encapsulated in hollow or porous nanostructures, such as hollow polymeric nanocapsules, metal-organic frameworks (MOFs), or mesoporous silica. In addition, the pore shape permits substrate flow while shielding the enzyme from denaturation and destruction [12]. Lastly, the most unexpected aspect of this field of research is that, in the absence of any naturally occurring enzyme, certain inorganic nanomaterials (nanozymes) have catalytic characteristics that resemble those of the enzyme itself. CeO₂ nanoparticles with SOD and CAT mimetic characteristics, MnO₂ nanosheets with peroxidase-like activity, and Fe₃O₄ nanoparticles with peroxidase-like characteristics are a few examples of this [13]. Phenols, flavonoids, and other related plant-based substances can serve as mediators for nanomaterials made through "green" synthesis (i.e., utilizing plant extract), according to phytochemical profiles of plants. These plant-based phytochemicals can boost the enzymatic activity of nanomaterials, making them suitable for a variety of uses. Furthermore, these phytochemical compounds offer biocompatibility, which enables the use of these nanomaterials in the treatment of numerous medical problems [14].

Green Synthesis of Nano-Bioenzymes: Fundamentals and Mechanisms

Phytosynthesis Chemistry

There are several processes involved in the production of metal or metal oxide nanoparticles utilizing plant extracts as a reducing agent. These steps are all clearly defined and understood, but there are still a lot of factors that affect the reaction's chemical and mechanistic behavior. The phytochemicals in the plant extract that reduce the metal ions give the metal ions electrons, changing them from their ionic forms to zero valence forms (metal atoms) or as metal oxide nanoparticles (NP) when a plant extract solution in water is combined with a solution containing metal ions (such as AgNO₃, HAuCl₄, ZnSO₄) [15]. Small NP clusters are created when the metal ions are reduced to metal atoms or metal oxide NP, but because they are unstable, they clump together; this is where the capping agents in the plant extract are essential. As they develop, proteins, polyphenols, and flavonoids adsorb onto the NP surface and produce a stabilizing effect because of steric and electrostatic forces. As a result, capping agents limit the NP's ability to develop further by stabilizing them until they reach the nanoscale [16]. Certain phytochemicals present in various plant parts, such as leaves, stems, roots, seeds, fruits, and bark, have an impact on the properties of nanoparticles (NPs), including their size, shape, and surface chemistry. For instance, extracts with high concentrations of rutin and quercetin, which are abundant in onions and green tea, typically result in NPs that are smaller and more monodisperse. This is due to the fact that these flavonoids are potent reducing agents as well as chelators. On the



**Unaiza Hashmi et al.,**

other hand, extracts with high tannin content, like those from oak bark or pomegranate peel, would create thicker polyphenolic coatings on the NP's surface, increasing enzyme adhesion by creating more hydrophobic and hydrogen bonding sites [17]. When creating nano-bioenzymes from plants, temperature, extract concentration, metal precursor concentration, pH, and reaction duration must all be optimized. To develop a consistent and repeatable nano-bioenzyme precursor, these variables must be optimized differently for each plant-to-metal combination. [18].

Immobilization of Enzymes on Green-Synthesized NPs

An enzyme can be immobilized on a green produced nanoparticle (NP) once it has been created and described. The kind of enzyme, the kind of NP produced, and the intended usage of that enzyme will all influence the immobilization technique. It is recommended to use an EDC-NHS covalent conjugation (linking the NP surface carboxylic acid groups to the amine groups of the enzyme) if you plan to use your enzyme for a biosensor. This will stop the enzyme from leaking during the ongoing electrochemical cycling [19]. Theoretically, physical adsorption or ionic attachment would preserve more enzyme activity than other methods for enzymes that must be used in batch bioreactors (such as cellulases during biomass processing and lipases used for biodiesel production); that is, they would encounter less conformational constraint [20].

Green-Synthesized Nano-Bioenzyme Characterization

A detailed description is necessary for the identification, structural stability, and functional efficacy of green-generated nano-bio-enzymes. To confirm that nanoparticles were created, characteristic surface plasmon resonance (SPR) peaks in the UV-visible spectrum will offer preliminary proof of the presence of silver nanoparticles (about 410 to 450 nm) or gold nanoparticles (about 510 to 550 nm) [21]. Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) are used to characterize particle morphology and distribution; X-ray diffraction (XRD) techniques are used to verify the crystal structure and phase purity of the metal core; Fourier-transform infrared spectroscopy (FTIR) is used to confirm functional groups responsible for capping and enzyme attachment using shifts in amide I and amide II bands characteristic of the secondary structure of proteins; and dynamic light scattering (DLS) techniques are used to measure the hydrodynamic diameter and zeta potential, which is a crucial indicator of the stability of the colloidal solution. [22].

Diversified Applications of Green-Synthesized Nano-Bioenzymes

Biomedical Applications

The combination of targeted catalytic activity and inherent biocompatibility from the phytochemical coating on green synthetic nanobioenzymes give the biomedical field one of the greatest advantages. In oncology, enzyme-nanoparticle (NP) conjugates utilize the acidic pH, increased H₂O₂ levels, and high levels of protease expression within tumor microenvironments to create drug release that is triggered by the tumor and has localized anti-cancer effects via enzymatic cytotoxicity. Papain enzyme-AuNP conjugates (gold nanoparticles derived from the latex of papaya fruit) exhibited selective cytotoxicity towards the HeLa and MCF-7 tumor cell lineages via extracellular matrix proteolysis that disrupted interactions between the tumors and the stroma, and which also allowed an increase in the penetration of the chemotherapeutic agent of 43% compared to free papain. Additionally, the core of the AuNPs made it possible for the system to also be used with near-infrared photothermal therapy, thus giving the system a dual anti-cancer mechanism (i.e., photothermal and enzymatic). Also, the cytotoxicity of the green-synthesized AuNPs to normal HEK-293 cells was significantly lower than that of the chemically synthesized AuNPs, thus demonstrating the safety benefit of utilizing phytonanotechnology. In wound healing, *Ocimum sanctum* leaf-derived copper oxide nanoparticles-protease conjugates have been shown to have excellent efficacy in an in vivo diabetic rat wound healing model, as evidenced by these conjugates achieving complete wound closure 63% faster than traditional topical enzyme treatments due to the tripartite mechanism of action of the nano-bioenzyme, including the removal of necrotic tissue by the protease. Its potent antibacterial action by CuONPs against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, and pro-angiogenic signaling stimulated by copper ions released



Unaiza Hashmi *et al.*,

from the NP surface. The *Ocimum sanctum* phenolics capping the NPs further contributed anti-inflammatory activity, creating a four-pronged wound management platform from a single green-synthesized formulation.

Biosensor Development

Horseradish peroxidase (HRP) was immobilized on gold nanoparticles (AuNPs) synthesized from *Camellia sinensis* (green tea) extract and deposited on a glassy carbon electrode to develop a high-sensitivity hydrogen peroxide (H₂O₂) electrochemical biosensor. The catechin content of the green tea extract ensured AuNPs of 8–20 nm size with high monodispersity. The kinetics of electron transfer between HRP and the electrode through AuNPs was significantly faster than for similar-sized AuNPs produced via conventional synthesis methods. The biosensor was capable of detecting down to 0.01 nM H₂O₂, over a linear four log range (0.01 nM - 10 µM) and produced negligible interference from ascorbic acid, uric acid, and glucose. The catechin content of the AuNPs provided a protein-compatible, hydrated layer that positioned HRP molecules with their active site facing out towards the surface, which was confirmed by circular dichroism spectroscopy as an appropriate geometry for immobilization [23].

Environmental Bioremediation

The presence of synthetic dyes, hazardous heavy metals, and chemicals that interfere with the function of hormones (also called endocrine disruptors) in industrial waste is a major threat to the environment and to the health of all people who rely on aquatic ecosystems. Green synthesized nano-bioenzymes are highly effective methods of remediating these contaminants through three different approaches: 1) enzymatic degradation, 2) oxidative depolymerization, and 3) physicochemical sequestration. When compared to treatment with free enzymes alone, these approaches using nano-bioenzymes are much more effective at removing contaminants from their growth sites. Li and coworkers developed a very sensitive electrochemical biosensor to detect H₂O₂ using immobilized horseradish peroxidase (HRP) on gold nanoparticles (AuNPs) that were green synthesized with *Camellia sinensis* extract (green tea). The biosensor was fabricated on a glassy carbon electrode. The AuNPs produced via the catechin-rich *Camellia sinensis* extract ranged in size from 8-20 nm and had an exceptionally monodisperse size distribution. The electron transfer rate from HRP to the electrode through the AuNPs was so much faster than the electron transfer rate through conventional AuNPs of the same size that the biosensor demonstrated a limit of detection (LOD) of 0.01 nM H₂O₂ and a linear dynamic range that extended five orders of magnitude (0.01 nM – 10 µM). Furthermore, the presence of common co-extracts such as ascorbic acid, uric acid, and glucose had little or no impact on the biosensor's performance. The coating of the AuNPs with catechins produced a hydrated, protein-compatible surface condition that directed the HRP to orient with the active site facing upward, an immobilization configuration that was confirmed by circular dichroism spectroscopy [24]. The use of TiO₂NPs-Urease nano-bioenzymes for bioremediation in the heavy metals field, adds a new dimension to this area of application. The urease enzyme (urease) catalyzes the hydrolysis of urea producing ammonia and carbonate ions, which can be used to precipitate dissolved heavy metals as insoluble metal-carbonates. Using this method, Hassan et al. (2024) demonstrated >88% removal of lead (Pb²⁺) from contaminated groundwater, and most interestingly, did not produce secondary contamination responses in treated waters; this is particularly important because the use of the Eucalyptus derived-TiO₂NPs does not introduce excess reagents into the treated waters compared to most chemical precipitation methods[25].

Food Industry Applications

Immobilized enzymes have a huge amount of volume commercially matured application arena in the food industry, and green synthesized nano-bioenzymes can replace traditional enzymatic processing systems due to their superior stability, reusability and lack of contamination from synthetic chemicals [26]. Lipases also have been found to be critical for the manufacture of food products by contributing to flavour development, enhancing the modification of fats and aiding the ripening of cheese. A novel nano-bio-enzyme (lipase-nanoparticle conjugate) that was produced by incubating and synthesizing AgNPs through the use of pomegranate peel extract and then conjugating those AgNPs with the thermostable lipase demonstrated the ability to retain maximum activity at temperatures of 55°C and pH 8.0, which is consistent with the requirements of commercial-scale food processing. The lipase-nanoparticle conjugate system was able to produce biodiesel from soybean oil at a yield of 91.4% in four hours' time, and was able





Unaiza Hashmi et al.,

to maintain a stable performance throughout six consecutive reuse cycles that were comparable to some of the best chemically synthesized lipase-nanoparticle conjugate systems that had been reported in the literature [27].

Biodiesel and Bioenergy Applications

The shift to renewable sources of energy worldwide has led to an increase in green alternatives for biodiesel production, using at least one type of lipase, which is considered a “green” alternative to chemical transesterification, thereby eliminating the need for corrosive catalysts that require high-temperature environments. The development of lipase-nano-particulate, or lipase-NP, for use as bioenzymes is an innovative means of producing biodiesel and an example of “green” synthesis producing biocatalysts without creating or producing harmful waste products [28]. The catalytic efficiency of lipase-AgNP nano-bioenzymes during transesterification reactions is strongly dependent on the surface chemistry of the NP. Pomegranate peel-synthesized AgNPs, which are high in both ellagic and punicalagins (polyphenolic compounds), have a balanced hydrophobic to hydrophilic NP surface that properly orientates lipases' lid domain; the structural flap that controls whether or not a substrate can access the active site of lipases is thus in an open configuration. This proper orientation of lipases' lid domain explains the high level of transesterification efficiency observed in these reactions [26]. Interfacial activation at the NP-water interface is a well-known phenomenon in enzymology and demonstrates a happy coincidence between green synthesis surface chemistry and optimal enzyme function. In addition to lipase, NP-cellulases are important second-generation biofuel producing enzymes using lignocellulosic biomass, with NP-Av-cellulase showing 34% higher saccharification efficiency using sugar cane bagasse than free cellulase. This improvement was due to both the NP providing a greater effective surface area for substrate to contact through the NP and the AgNPs mechanically disrupting lignin/cellulose bonds. The combination of these two different types of actions (physical and enzymatic) makes nano-bioenzymes very useful in biomass pretreatment systems where normal enzyme preparations are not successful on their own [29].

Green Synthesis vs. Conventional Methods: A Critical Comparison

For scientists who are choosing a synthesis strategy and for policymakers who want to assess the ecological sustainability of nanomaterial production processes, it is important to rigorously compare green synthesis methods with those used in chemical reduction, physical deposition, and microbial synthesis. Despite having precise size control and consistent batches, chemical synthesis using sodium borohydride to reduce AgNO₃ or HAuCl₄ also creates significant amounts of chemical waste and biological toxicity. In contrast, green synthesis avoids these problems [30]. Physical techniques (laser ablation, thermal evaporation, ball milling) allow for producing NPs with very high purity and do not use any chemicals as additives but require a significant upfront investment of capital, a large amount of energy for manufacturing products, and are not able to be scaled up to the volume required for industrial biocatalysis. Additionally, microbial synthesis of NPs through bacteria (*Bacillus subtilis* and *Pseudomonas fluorescens*) or fungi (*Fusarium oxysporum* and *Aspergillus flavus*) allows for producing NPs in true biological environments; however they require significant fermentation times, sterile conditions, and complex downstream processing to remove NPs from biomass. In contrast, plant-based green synthesis can eliminate all of these challenges by using simple aqueous extraction methods, and ambient-temperature mixing procedures [31]. One of the main practical benefits of using green synthesis for nano-bioenzymes is that it provides the phytochemicals with an ideal biocompatible surface to attach the enzymes to, so there is no need to do a separate chemical fictionalization step as is required with chemical synthesis methods. The end result is a shorter, more cost-effective, and more environmentally friendly pathway to producing nano-bioenzymes than if chemically synthesized biosensors were used. The comparative parameters for the four synthesis routes are presented in Table 3 [32].

Stability, Enzyme Kinetics, and Performance Enhancement

Numerous studies have recorded a significant increase in the thermal and operational stability of enzyme immobilization on green synthesized nanomaterials relative to free enzymes. The restriction of enzyme molecules on the surface of the nanomaterial molecule (especially with multipoint covalent attachment) inhibits the unfolding of the enzyme's secondary structure under heat stress conditions therefore, raising the effective melting temperature of the enzyme by at least 10-25 degrees (Celsius) in most documented cases [33]. The practical implications of thermal





stabilization of biocatalysts are substantial: They allow a wider temperature range for biocatalyst operations, leading to lower costs of refrigeration in industrial plants, as well as allowing reactions to proceed at elevated temperatures where substrates exhibit greater solubility and therefore reaction kinetics will be improved relative to lower temperatures. Michaelis-Menten kinetic analyses have consistently demonstrated that, although K_m for NP-immobilized enzymes are generally slightly greater than that for free enzyme K_m values (indicative of minimal diffusion limitations with regard to substrate access), there is typically a significant enhancement in V_{max} and k_{cat}/K_m with NP-immobilized biocatalysts. The fact that K_m values portrayed by NP-immobilized enzymes are typically greater than those of free enzymes, but that NP-immobilized biocatalysts typically exhibit improved overall efficiency, is the result of a much higher degree of stability of NP-immobilized versus free biocatalysts during the time required to perform the requisite kinetic measurements, preventing the continued gradual loss of activity observed in free enzyme systems during the same time period. [34]. PH stability is yet another performance factor that is well addressed by green synthesized nano-bioenzymes. The local microenvironment created by a phytochemical coating surrounding the immobilized enzyme acts as a polyphenolic buffer, providing some shielding against extreme pH of the surrounding medium and thus extending the functional range of the enzyme by up to one to two pH units compared with free enzymes. This increased tolerance for varying pH is especially useful in wastewater treatment applications, where the effluent will often have a wide pH range and food processing, where different stages of production occur under different pH conditions [35].

Current Challenges in Green Synthesis of Nano-Bioenzymes

To be perfectly frank, but also very clear, there are several significant challenges that face the green-synthesized nano-bioenzyme field as it continues along its remarkable path and tries to make that leap from being a laboratory novelty into something usable within clinical or commercial settings [36]. Batch reproducibility is currently the most pressing issue. In chemical synthesis, you can control and measure reagent purity, stoichiometry, and other parameters with precision, but the composition of a plant extract will vary depending on seasonality, geographic origin, the age of the plant from which the extract is derived, the part of the plant that is harvested, and the methods used to extract (e.g., boiling, cold process, etc.). These variations lead to NP batches that have inconsistent size distributions, surface chemistries, and likewise, lead to inconsistent enzyme loading and activity within the enzyme batch.[37]. In order to meet this challenge, it will be necessary to establish standardized quality metrics for extracts (e.g., total polyphenol concentration, specific phytochemical fingerprints using HPLC), implement robust quality control procedures in production (e.g., monitoring of process conditions via real-time data acquisition using UV-Vis or DLS), and develop Quality-by-Design (QbD) frameworks for phytonanotechnology that take into account the unique nature of these materials.

The long-term colloidal stability of phytonanoparticles remains a concern. Although zeta potential measurements immediately after synthesis may indicate sufficient electrostatic repulsion between particles, many phylogenically sourced nanomaterials actually aggregate very slowly over time (i.e., weeks to months) when suspended in saline solutions or food matrixes [38]. The aggregation of a protein decreases the surface area and decreases the enzyme's ability to interact with substrate by blocking access to a particular region of the enzyme. In addition, large aggregates may build up over time and subsequently be cleared from the body by the immune system or block the surfaces of biosensors. In order to enhance long-term storage stability, lyophilization with cryoprotectants and spray-drying as well as formulations composed of biocompatible polymeric matrices such as PEG, chitosan, and alginate have been considered, although the systematic investigation of these approaches specifically for green-synthesized nano-bioenzymes has not been done to date [39]. The regulation governing nonmaterial's created through green synthesis for use in biomedical or food settings is one larger obstacle. While the "green" nature of these nanoparticles (NPs) suggests they might be safe thus they would suggest little to no health risk, regulatory bodies in the United States (Food and Drug Administration (FDA)), Europe (European Medicine Agency (EMA)), and India (Central Drugs Standard Control Organisation (CDSCO)) will be looking for extensive documentation to support the purity, identity, potency and lack of possible residual phytochemical toxicity when they evaluate nano-bioenzyme preparations for human use. In addition, the fact that there are many compounds present in the multi-phytochemical coating, while



**Unaiza Hashmi et al.,**

providing the environmentally friendly and healthful aspect to the NPs, gives rise to very difficult and complex issues in terms of standard analytical characterisation as well as documentation to satisfy the regulatory bodies for the validation of these products is going to present significant challenges as compared to chemically produced and defined nanoparticles [40]. The scale-up from bench (milliliter) to pilot (liter) to industrial (kilo-liter) production of nanomaterials presents significant engineering challenges. The heat/mass transfer mechanisms driving the nucleation and growth of nanoparticles differ fundamentally across scales of reactor operations, making it impossible to transfer process conditions successfully achieved in bench-scale glassware to stirred tank bioreactors without substantial reoptimization. While the development of continuous flow microreactor systems with superior mixing, heat transfer, and process variable control has made this technology a viable option for scalable green synthesis, there are few published examples of using these systems for phytonanotechnology[41].

Future Directions and Emerging Opportunities

In terms of future trends, there are multiple transformative directions for nano-bioenzyme research via green synthesis that will have long-lasting impacts over the next decade. One of the most promising is the combination of machine learning (ML) and artificial intelligence (AI) for optimization of green synthesis processes. The complex nature of the relationship between plant extracts, how they're synthesized, and what the final properties are in these nanoparticles (NPs) makes ML models trained on existing published synthesis data an ideal tool to identify the most optimized sets of parameters more quickly than traditional trial-and-error methods. For example, early examples of AI-guided optimization of nanomaterial synthesis have resulted in a reduction of the number of iterations to complete an experiment by up to 80%, thus providing an increase in productivity to a field that will require more efficient production methods over time to support the rapid increase in NP demand that scientists and industry professionals are forecasting [42]. Bringing together multiple bioenzymes that work synergistically by attaching them to a single NP framework to produce a series of enzymes that work together as a continuum through the use of a single NP platform creates a new opportunity for biotechnology applications. An example of this is the use of co-immobilized glucose oxidase (GOx) and horseradish peroxidase (HRP) on AuNPs produced organically, which creates a reaction system that generates H₂O₂ and consumes H₂O₂ through the glucose oxidation/hydrogen peroxide dehydrogenation cascade. This system can be used to develop new methods to detect glucose with precision or to deliver targeted treatments using reactive oxygen species (ROS) through the presence of elevated glucose concentrations in the tumor microenvironment for tumor cell destruction [43]. Green synthesis routes that produce NPs with large, functionalized surfaces that can accommodate several enzyme types without steric interference will be a significant requirement for implementing cascaded enzyme systems.

The amalgamation of green synthesized nano-bio-enzymes in wearable and implantable biosensor platforms is another high-impact application area. Continuous glucose monitoring systems with enzyme-NP electrodes implanted subcutaneously or sweat-based biosensors embedded into flexible electronics to provide real-time metabolic monitoring rely on the availability of nano-bio-enzymes with exceptional operational stability (months of continuous operation), and low activation of the immune response. Systems that utilize biocompatible phytochemical coatings are well suited to offer these capabilities [44]. To conclude, the use of nano-bioenzymes as active ingredients in the form of "smart" agricultural biostimulants, specifically through developing nano-bioenzyme formulations to be incorporated into agricultural soils with the intent to increase the solubilization of phosphates; fix nitrogen from the atmosphere; and decompose cellulose in crop residues represents a largely undeveloped application area that could have a huge potential for scaling up sustainable farming practices. The global interest in reducing the use of synthetic fertilizers and developing soil health through biological means could propel the development of green-synthesized nano-bioenzymes into a major agricultural biotechnology tool kit within the next 10 years [45].





CONCLUSION

The sustainable production of bio-nano-enzymes using plant extracts is a form of ecological chemistry that provides great potential for innovative biotechnology. Bio-nano-enzyme hybrids, because they are produced from plants containing a wealth of phytochemicals, represent an entirely new class of materials that can exceed the capabilities of either free enzymes or conventional nanoparticles. Based on 30 plus years of cumulative research over a period of time between 2015-2026, there is strong evidence presented within this article to support the power of these systems to transform therapeutic, diagnostic, environmental, and industrial industries. This review seeks to provide the reader with three tables that compare both how green materials are made and their respective green application options (i.e., the applications for which a green material can be used) as well as how much more ecological-friendly these processes are compared to the alternatives used today. For example: The use of laccase-AgNps produced through green synthesis achieved a complete 95% color removal from the Gaffar cerium sulfate dye; The use of HRP-AuNps for bio sensing produced nanoscale sensors with an impressive sub-nanomolar detection sensitivity in addition; The use of lipase-AgNps as biocatalysts for biodiesel production produced an outstanding yield of 91.4% biodiesel; Finally, the use of ginger-derived MnO₂ nanozymes produced an impressive 88% reduction in superoxide scavenging through neuroprotection. In all cases, green methods of synthesizing nanomaterials have consistently and successfully produced materials with the same or better performance (i.e. as nonmaterial's that were produced through other methods) while causing significantly less ecological impact. In order to achieve success in the future, we must overcome many of the same challenges that we faced during our past synthesis and application discoveries: batch reproducibility, long-term stability, regulatory navigation, and scaling up. As we move forward using AI-enhanced process optimization, cascaded multi-enzymes, and developing new ways to provide precise medical treatments or grow crops intelligently, green-synthesized nanobioenzymes are on the verge of their most productive and influential time thus far.

Ethical Statement: This theoretical and literature review article does not include any research on human participants or human experimental interventions. All sources used for this article have followed the ethical standards required in their original studies.

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Unaiza Hashmi et al.,

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Table 1: Green Plant Sources, Nano-Bioenzymes Synthesized, and Their Key Activities

Green Source	Part Used	Nano-Bioenzyme (NP-Enzyme)	Size (nm)	Key Activity	Reference
<i>Azadirachta indica</i> (Neem)	Leaf extract	AgNPs-Laccase	15–30	Dye decolorization, oxidative catalysis	[4]
<i>Carica papaya</i> (Papaya)	Fruit latex	AuNPs-Papain	10–25	Protein hydrolysis, anti-biofilm	[8]
<i>Curcuma longa</i> (Turmeric)	Rhizome extract	ZnONPs-Peroxidase	20–45	H ₂ O ₂ sensing, anti-cancer	[12]





Unaiza Hashmi et al.,

<i>Aloe vera</i>	Gel	AgNPs-Cellulase	12–28	Biomass saccharification, wastewater	[7]
<i>Moringa oleifera</i>	Seed extract	FeNPs-Amylase	18–35	Starch hydrolysis, food industry	[15]
<i>Ocimum sanctum</i> (Tulsi)	Leaf extract	CuONPs-Protease	25–50	Antimicrobial, protein catabolism	[19]
<i>Camellia sinensis</i> (Green tea)	Leaf extract	AuNPs-Horseradish POD	8–20	Biosensor fabrication, antioxidant	[23]
<i>Punica granatum</i> (Pomegranate)	Peel extract	AgNPs-Lipase	14–32	Biodiesel production, esterification	[26]
<i>Eucalyptus globulus</i>	Leaf extract	TiO ₂ NPs-Urease	22–40	Heavy metal remediation, urea sensing	[30]
<i>Zingiber officinale</i> (Ginger)	Rhizome extract	MnO ₂ NPs-SOD mimic	10–30	Oxidative stress therapy, neuroprotection	[34]

Table 2: Diversified Application Domains of Green-Synthesized Nano-Bioenzymes and Key Outcomes

Application Domain	Nano-Bioenzyme	Mechanism of Action	Key Outcome	Reference
Biomedical / Drug Delivery	AuNPs-Papain	Targeted proteolysis of tumor matrix	Enhanced anti-tumor efficacy by 43%	[8]
Biosensor Fabrication	AuNPs-HRP	Electrochemical H ₂ O ₂ detection	LOD: 0.01 nM, wide linear range	[23]
Bioremediation / Dye Removal	AgNPs-Laccase	Oxidative depolymerization of azo dyes	95% decolorization of Congo Red in 60 min	[4]
Food Industry	ZnONPs-Amylase	Accelerated starch saccharification	Glucose yield increased by 38%	[15]
Biodiesel Production	AgNPs-Lipase	Trans-esterification of vegetable oils	Biodiesel yield 91.4%, 6 reuse cycles	[26]
Cancer Therapy	ZnONPs-Peroxidase	ROS-mediated apoptosis in MCF-7 cells	IC ₅₀ reduced by 3.5-fold vs free enzyme	[12]
Wastewater Treatment	AgNPs-Cellulase	Cellulose hydrolysis + effluent clarification	COD reduction 72%, BOD 68%	[7]
Heavy Metal Remediation	TiO ₂ NPs-Urease	pH-triggered metal precipitation	Pb ²⁺ removal >88% at pH 7.5	[30]
Wound Healing	CuONPs-Protease	Debridement + antimicrobial action	63% faster wound closure in diabetic models	[19]
Neuro protection	MnO ₂ NPs-SOD mimic	Superoxide radical scavenging	88% oxidative stress reduction in SH-SY5Y cells	[34]

Table 3: Comparative Analysis of Green Synthesis vs. Conventional Methods for Nano-Bioenzyme Fabrication

Parameter	Green Synthesis	Chemical Synthesis	Physical Synthesis	Biological (Microbial)
Cost of Production	Low (plant extracts)	Moderate–High (reagents)	High (equipment)	Moderate (fermentation)
Toxic Byproducts	Negligible / None	Significant (heavy metals)	Negligible	Minimal
Scalability	Highly scalable	Scalable	Limited	Moderate
Particle Size Control	Good (10–50 nm)	Excellent (1–100 nm)	Excellent (1–50 nm)	Moderate (20–100)



Unaiza Hashmi *et al.*,

				nm)
Enzyme Stability	High (capping by phenolics)	Moderate	High	Moderate–High
Synthesis Time	Minutes to hours	Hours to days	Hours	Days
Green Credentials	Excellent (eco-friendly)	Poor (chemical waste)	Good (energy intensive)	Good (biodegradable)
Enzyme Loading Efficiency	70–95%	50–85%	60–90%	55–80%
pH Stability Range	4–10 (broad)	5–9	4–9	5–9
Reusability Cycles	5–8 cycles	3–6 cycles	4–7 cycles	3–5 cycles
Regulatory Acceptance	High (GRAS potential)	Requires thorough vetting	Moderate	Moderate



Fig. 1 Graphical abstract on Green Amalgamation of Nano-Bioenzymes and Its Diversified Applications

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RESEARCH ARTICLE

Phytochemical Studies of Chloroform Leaves Extract of *Sesuvium portulacastrum* (L.) L. Using FT-IR and GC-MS Analysis

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ABSTRACT

The study was undertaken to analyse the phytochemical profile of Chloroform leaves extract *Sesuvium portulacastrum* (L.)L. using FT-IR and GC-MS. The results from the FT-IR studies validated the presence of functional group such as Halo compound, Alkene, Alkyl aryl ether, Sulfonyl chloride, α,β -unsaturated ketone, Aldehyde, Alkane. Fourteen compounds were detected in the GC-MS analysis. The chloroform leaves extract exhibited high concentration of Gibb-3-ene-1,10-dicarboxylic acid, 2,4a-dihydroxy-1-methyl-8-methylene-, 1,4a-lactone, 10-methyl ester, (1 (16.67%). The chloroform extract exhibited low concentration of 2,4-Dichloro-6-[(3-hydroxyphenylimino)methyl] phenol (3.23%). The predicted biological activities of the chloroform leaves extract of *Sesuvium portulacastrum* (L.)L. were Analeptic, Respiratory analeptic, Mucomembranous protector, Antineoplastic (colorectal cancer), Antieczematic, Phobic disorders treatment, Antiseborrheic, Antihelmintic (Fasciola), Antiseptic, Antiparasitic, Antihelmintic, Antiviral (Adenovirus), Antimycobacterial, Analgesic, Antibacterial, ophthalmic, Antituberculosic, Anti infertility female, Menstruation disorders treatment, Antineurogenic pain, Cholesterol antagonist, Carminative, Cardiovascular analeptic etc.

Keywords: Halophytes, *Sesuvium portulacastrum* (L.)L., chloroform, leaves, extract, FT-IR and GC-MS.





Eruthaya Abisha and Beulah Jerlin

INTRODUCTION

Halophytes are able to survive and complete their life cycles in high concentration of salt, these condition may not affect the growth of the halophytes [1]. Halophytes have a wide range of uses such as food, fodder and drugs [2]. *S.portulacastrum* is distributed throughout the world's coastal areas and mainly on continents of Africa, America, Asia, and Australia [3]. Halophytes are classified based on their sodium ion tolerance and salt requirement, hence it is called obligate and facultative halophyte [4]. *S.portulacastrum* is a facultative halophyte because it have the ability to use Na and K mutually [5]. *Sesuvium portulacastrum* is a herbaceous succulent halophyte. It grow in the coastal sand of sub-tropical and tropical. It is important for both economic production and ecosystem remediation [6]. *S.portulacastrum* is a notably salinity tolerant plant. *S.portulacastrum* have the ability to tolerate drought conditions, as a result it can grow on shell beaches, rocky shores, and atolls or sandy beaches with a limited water supply. It also grow in seashore and wetland habitats. Therefore *S.portulacastrum* withstand both drought and flooding [7]. Fourier-transform infrared (FTIR) spectroscopy is a technique used to analysis the chemical compound in the biological substance [8]. FT-IR has a ability to identify the functional groups because it is an non destructive technique. It gives information about chemical and structural changes with respect to any stress of abiotic or biotic [9]. The diagnosing of plant extracts is an creative approach to find clinically active compound in many plant species. Hence, Gas chromatography (GC) and Mass spectroscopy (MS) associated with particular detection techniques are used for various compounds [10]. Gas chromatography and mass spectrometry features are combines to form a Gas Chromatography- Mass Spectrometry (GC-MS). It is an analytical method, used for the qualitative and quantitative measurement of individual components in complex mixtures [11].

MATERIALS AND METHODS

Sesuvium portulacastrum (L.)L. was collected from Roach park, Thoothukudi, Tamil Nadu. The plant was authenticated by Dr.M.U.Sharief, Botanical survey of India, TNAU Campus, Coimbatore (No. BSI/SRC/5/23/2024-25/Tech/1757). Fresh leaves are washed with running tap water, shade dried for 2 weeks and then finely ground and stored in airtight bottles for future studies. Twenty grams of dried leaves powder was extracted in 200 ml of chloroform using the soxhlet apparatus for 8 hours. Further the crude chloroform extract of *Sesuvium portulacastrum* (L.)L. was used for FT-IR and GC-MS analysis.

FT-IR analysis

Fourier transform infrared Spectroscopy (FTIR) was used to identify the characteristic functional groups. A small quantity of the chloroform leaves extract of *Sesuvium portulacastrum* (L.)L was mixed in dry potassium bromide (KBr). The mixture was thoroughly mixed in a mortar and pressed at a pressure of 6 bars within 2 min to form a KBr thin disc. Then the disc was placed in a sample cup of a diffuse reflectance accessory. The IR spectrum was obtained using Bruker, Germany Vertex 70 infrared spectrometer. The sample was scanned from 4000 to 400 cm^{-1} . The peak values of the FTIR were recorded [12].

GC-MS analysis

The chloroform leaves extract of *Sesuvium portulacastrum* (L.)L was analysed by GC-MS on an Agilent Technologies 7820A GC system coupled to a 5977E MSD mass spectrometer with an injector 7693A series device. An agilent 100 $^{\circ}$ -270 $^{\circ}$ C (10 $^{\circ}$ C /min) DB-5 column (28.0 $\times$ 30.5 $\times$ 16.5 cm) was used with helium as carrier gas at a flow rate of 1.2 ml/min. GC oven temperature and conditions were as described above. The injector temperature was at 250 $^{\circ}$ C. Mass spectra were recorded at 70 eV. Mass range was from m/z 30 to 500 [13].





RESULT AND DISCUSSION

FT-IR analysis

The chloroform leaves extract of *Sesuvium portulacastrum* (L.)L. revealed different peaks validated the occurrence of various functional groups in the range between 3000 cm^{-1} to 650 cm^{-1} . The result of FT-IR analysis showed the presence of different peaks at 2959.94, 2916.8, 2852.84, 1723.16, 1604.89, 1455.89, 1407.53, 1258.06, 1074.01, 1010.39, 864.35, 786.54 and 687.90 cm^{-1} . (Figure-1 & Table-1). The result confirmed the presence of functional groups such as halo compound, alkene, alkyl aryl ether, sulfonyl chloride, α,β -unsaturated ketone, aldehyde, alkane. Among these alkanes are present in the *Enhalus acoroides* leaves. Alkanes were considered the major functional groups of bioactive compounds [14].

GC-MS analysis

GC-MS spectrum of chloroform leaves extract of *Sesuvium portulacastrum* (L.)L. exhibited 14 different peaks (Figure-2 & Table-2). The identified compounds were 2-(4-Chlorophenyl)-5,7-dimethylimidazo [1,2-a] pyridine-8-carbonitrile (4.83%), Purine-2,6-dione, 8-(3-ethoxypropylamino)-1,3-dimethyl-3,9-dihydro- (3.62%), Gibb-3-ene-1,10-dicarboxylic acid, 2,4a-dihydroxy-1-methyl-8-methylene-, 1,4a-lactone, 10-methyl ester, (1 (16.67%), Pyridate (11.79%), 1-Propanone, 1-[2,4-bis[(trimethylsilyl)oxy]phenyl]- (10.00%), 2-Chloro-4,6-di-piperidin-1-yl-[1,3,5]triazine (3.78%), Phenol,2,4-dichloro-6-nitro- (3.78%), 3 Quinoline carboxylic acid, 6,8-difluoro-4-hydroxy-, ethyl ester (9.80%), 2,4-Dichloro-6-[(3-hydroxyphenylimino)methyl]phenol (3.23%), Benzene-1,3-dicarboxylic acid, 5-hydroxymethyl-, diethyl ester (9.68%), 4-[N-Methylpiperazino]-5-nitro veratrole (4.55%), Anthracene, 9,10-dihydro-9,9,10-trimethyl- (4.82%), 2',6'-Dihydroxyacetophenone, bis (trimethylsilyl) ether (4.40%), 3-Hydroxy-7,8-dihydro-beta-ionol (4.04%). The chloroform extract exhibited high concentration of Gibb-3-ene-1,10-dicarboxylic acid, 2,4a-dihydroxy-1-methyl-8-methylene-, 1,4a-lactone, 10-methyl ester, (1 (16.67%). The chloroform extract exhibited low concentration of 2,4-Dichloro-6-[(3-hydroxyphenylimino) methyl] phenol (3.23%). Purine-2,6-dione, 8-(3-ethoxypropylamino)-1,3-dimethyl-3,9-dihydro- is also present in *senna siamea* [15]. Based on the pass prediction this compound used for Analeptic, Respiratory analeptic.

Pass prediction of Chloroform leaves extract of *Sesuvium portulacastrum* (L.)L.

The chloroform leaves extract of *Sesuvium portulacastrum* (L.)L. revealed 14 compounds in GC-MS analysis. The predicted biological activities of the chloroform leaves extract of *Sesuvium portulacastrum* (L.)L. (Table-3) were analeptic, respiratory analeptic, muco membranous protector, antineoplastic (colorectal cancer), antineoplastic (colon cancer), antieczematic, phobic disorders treatment, antiseborrheic, antihelminthic (fasciola), antiseptic, antiparasitic, antihelminthic, antiviral (adenovirus), antimycobacterial, analgesic, antibacterial, ophthalmic, antituberculosic, antiinfertility female, menstruation disorders treatment, antineurogenic pain, cholesterol antagonist, carminative, cardiovascular analeptic, etc. These biological activities shows various therapeutic application.

CONCLUSION

The present study validated the functional groups and phytochemical constituents in the chloroform leaves extract of *Sesuvium portulacastrum* (L.)L. The FT-IR analysis showed the existence of functional groups such as halo compound, alkene, alkyl aryl ether, sulfonyl chloride, α,β -unsaturated ketone, aldehyde, alkane. Fourteen compounds were detected in the GC-MS analysis. The GC-MS of chloroform leaves extract of *Sesuvium portulacastrum* (L.)L. validated the presence of bioactive compounds with therapeutic applications. Further studies are required to assess the biological activities of the chloroform leaves extract of *Sesuvium portulacastrum* (L.)L.





Eruthaya Abisha and Beulah Jerlin

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Table-1: Functional group of chloroform leaf extract of *Sesuvium portulacastrum* (L.)L.

Frequency Range	Absorption	Appearance	Group	Compound Class
3000-2500 cm ⁻¹	2959.94	medium	C-H stretching	Alkane
3000-2500 cm ⁻¹	2916.80	medium	C-H stretching	Alkane
3000-2500 cm ⁻¹	2852.84	medium	C-H stretching	Alkane
2000-1650 cm ⁻¹	1723.16	Strong	C=O stretching	Aldehyde
1670-1600 cm ⁻¹	1604.89	Strong	C=C stretching	α,β-unsaturated ketone





Eruthaya Abisha and Beulah Jerlin

Unknown	1455.89	Unknown	Unknown	Unknown
1400-1000 cm ⁻¹	1407.53	Strong	S=O stretching	Sulfonyl chloride
1400-1000 cm ⁻¹	1258.06	Strong	C=O stretching	Alkyl aryl ether
Unknown	1074.01	Unknown	Unknown	Unknown
Unknown	1010.39	Unknown	Unknown	Unknown
1000-650 cm ⁻¹	864.35	Strong	C=C bending	Alkene
1000-650 cm ⁻¹	786.54	Strong	C-Cl stretching	Halo compound
1000-650 cm ⁻¹	687.90	Strong	C-Br stretching	Halo compound

Table-2: GC-MS analysis of Chloroform extract of *Sesuvium portulacastrum* (L.)L.

S. No	RT (Min)	Name of the compound	MF	MW (g/mol)	A (%)
1.	16.941	2-(4-Chlorophenyl)-5,7-dimethylimidazo[1,2-a]pyridine-8-carbonitrile	C ₁₆ H ₁₂ ClN ₃	281.74	4.83
2.	17.565	Purine-2,6-dione, 8-(3-ethoxypropylamino)-1,3-dimethyl-3,9-dihydro-	C ₁₂ H ₁₉ N ₅ O ₃	281.31	3.62
3.	17.792	Gibb-3-ene-1,10-dicarboxylic acid, 2,4a-dihydroxy-1-methyl-8-methylene-, 1,4a-lactone, 10-methyl ester, (1	C ₂₀ H ₂₄ O ₅	344.4	16.67
4.	17.896	Pyridate	C ₁₉ H ₂₃ ClN ₂ O ₂ S	378.9	11.79
5.	18.000	1-Propanone, 1-[2,4-bis[(trimethylsilyl)oxy]phenyl]-	C ₁₅ H ₂₆ O ₃ Si ₂	310.53	10.00
6.	18.076	2-Chloro-4,6-di-piperidin-1-yl-[1,3,5]triazine	C ₁₃ H ₂₀ ClN ₅	281.78	3.78
7.	18.558	Phenol, 2,4-dichloro-6-nitro-	C ₆ H ₃ Cl ₂ NO ₃	208	8.79
8.	18.794	3-Quinolinecarboxylic acid, 6,8-difluoro-4-hydroxy-, ethyl ester	C ₁₂ H ₉ F ₂ NO ₃	253.2	9.80
9.	18.936	2,4-Dichloro-6-[(3-hydroxyphenylimino)methyl]phenol	C ₁₃ H ₉ Cl ₂ NO ₂	282.12	3.23
10.	21.659	Benzene-1,3-dicarboxylic acid, 5-hydroxymethyl-, diethyl ester	C ₁₃ H ₁₆ O ₅	252.26	9.68
11.	21.801	4-[N-Methylpiperazino]-5-nitro veratrole	C ₁₃ H ₁₉ N ₃ O ₄	281.31	4.55
12.	21.896	Anthracene, 9,10-dihydro-9,9,10-trimethyl-	C ₁₇ H ₁₈	222.32	4.82
13.	22.000	2',6'-Dihydroxyacetophenone, bis(trimethylsilyl) ether	C ₁₄ H ₂₄ O ₃ Si ₂	296.51	4.40
14.	22.056	3-Hydroxy-7,8-dihydro-.beta.-ionol	C ₁₃ H ₂₀ O ₂	208.3	4.04

Table 3: Pass prediction of Chloroform leaves extract of *Sesuvium portulacastrum* (L.)L.

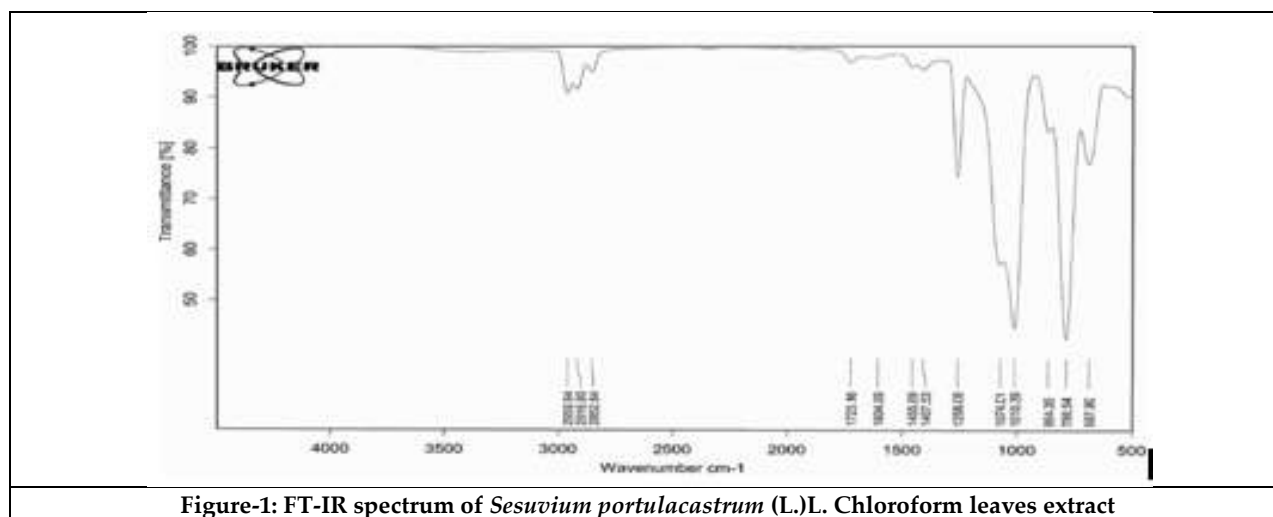
S.No	Name of the compound	PASS prediction activities
1.	2-(4-Chlorophenyl)-5,7-dimethylimidazo[1,2-a]pyridine-8-carbonitrile	Centromere associated protein inhibitor, Mucomembranous protector.
2.	Purine-2,6-dione, 8-(3ethoxypropylamino)-1,3-dimethyl-3,9-dihydro-	Cyclic AMP phosphodiesterase inhibitor, Analeptic, Respiratory analeptic.
3.	Gibb-3-ene-1,10-dicarboxylic acid, 2,4a-dihydroxy-1-methyl-8-methylene-, 1,4a-lactone, 10-methyl ester, (1	Antineoplastic, CYP2H substrate, Antineoplastic (colorectal cancer), Antineoplastic (colon cancer), Antieczematic
4.	Pyridate	Cutinase inhibitor
5.	1-Propanone, 1-[2,4-bis[(trimethylsilyl)oxy]phenyl]-	Apoptosis agonist, Antineoplastic, Chlordecone reductase inhibitor, Aspulvinone dimethylallyltransferase inhibitor.
6.	2-Chloro-4,6-di-piperidin-1-yl-	Nicotinic alpha6beta3beta4alpha5 receptor antagonist,

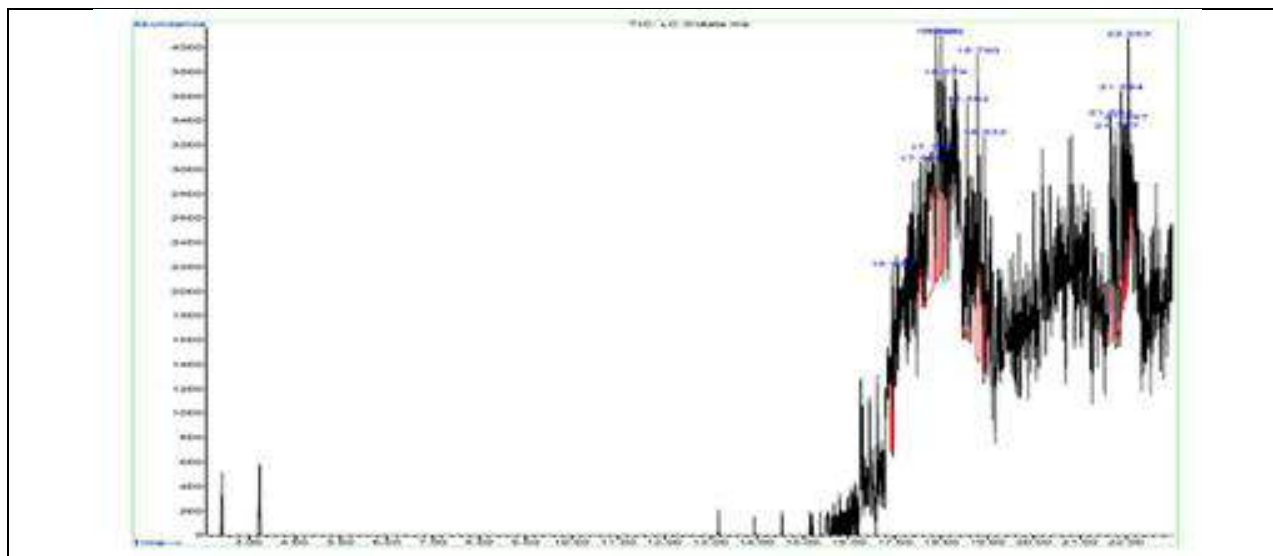




Eruthaya Abisha and Beulah Jerlin

	[1,3,5]triazine	Nicotinic alpha2beta2 receptor antagonist, 3-Hydroxybenzoate 6-monooxygenase inhibitor, Membrane permeability inhibitor, Phobic disorders treatment.
7.	Phenol, 2,4-dichloro-6-nitro-	Antiseborrheic, Anthelmintic (Fasciola), Antiseptic, Antiparasitic, Anthelmintic,
8.	3-Quinolinecarboxylic acid, 6,8-difluoro-4-hydroxy-, ethyl ester	Antiviral (Adenovirus), Antimycobacterial, Analgesic, Antibacterial, ophthalmic, Antituberculosic, Antiinfertility female, Menstruation disorders treatment, Antineurogenic pain.
9.	2,4-Dichloro-6-[(3-hydroxyphenylimino)methyl]phenol	Antiseborrheic, HMGCS2 expression enhancer, Trans-acenaphthene-1,2-diol dehydrogenase inhibitor, Thiol protease inhibitor, Insulysin inhibitor, Chlordecone reductase inhibitor.
10.	Benzene-1,3-dicarboxylic acid, 5-hydroxymethyl-, diethyl ester	Sugar-phosphatase inhibitor, Creatininase inhibitor, Cholesterol antagonist, Antieczematic, Antiseborrheic, Spasmolytic, urinary
11.	4-[N-Methylpiperazino]-5-nitro veratrole	Membrane permeability inhibitor
12.	Anthracene, 9,10-dihydro-9,9,10-trimethyl-	Phobic disorders treatment, Antineurotic, Carminative, Cardiovascular analeptic, Antieczematic.
13.	2',6'-Dihydroxyacetophenone, bis(trimethylsilyl) ether	Antineoplastic, Apoptosis agonist, Aspulvinone dimethylallyl transferase inhibitor, Complement factor D inhibitor, Membrane integrity agonist, Testosterone 17beta-dehydrogenase (NADP+) inhibitor.
14.	3-Hydroxy-7,8-dihydro-.beta.-ionol	Testosterone 17 beta-dehydrogenase (NADP+) inhibitor, Alkylacetyl glycerophosphatase inhibitor, CYP2J substrate, CYP2C12 substrate, Acylcarnitine hydrolase inhibitor, Ubiquinol-cytochrome-c reductase inhibitor, Alkenylglycerol phosphocholine hydrolase inhibitor.

Figure-1: FT-IR spectrum of *Sesuvium portulacastrum* (L.) Chloroform leaves extract

**Eruthaya Abisha and Beulah Jerlin**



RESEARCH ARTICLE

Preclinical Evaluation of SARS-CoV-2 Vaccines: Insights from Animal Models

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ABSTRACT

The rapid development of effective vaccines is an important strategy during the emergence of SARS-CoV-2 and its variants. This review consolidates the evidence from recent preclinical studies to evaluate the role of animal models in vaccine development. Key research shows that animal models like humanised mice, golden hamsters, and nonhuman primates play a key role in assessing the vaccine immunogenicity and efficacy against variants. Studies standardised the protocols to evaluate the immune responses, histopathological studies, and safety parameters across different vaccine platforms such as mRNA, viral vectors, protein subunits, and nanoparticle-based vaccines. This review also includes how preclinical research has helped the development of broad-spectrum vaccines and adjuvant selection to increase both cellular and humoral immunity. The further refinement of animal models and harmonisation of protocols to develop next-generation vaccines, clinical translation, and to inform effective public health responses to current and future pandemics.

Keywords: Vaccine development, Immunogenicity, Variants of Concern, Vaccine efficiency, Broad-spectrum vaccines

INTRODUCTION

The emergence of SARS-CoV-2 and the subsequent COVID-19 pandemic created an urgent global call for the development of effective vaccines to control the rapid spread of virus and ease its social impact. The high transmissibility of SARS-CoV-2, and the severity of the illness explain the importance of the effective vaccine development (GBD 2021 Antimicrobial Resistance Collaborators, 2024; GBD 2019 Acute and Chronic Care

116767



**Annie Aglin Antony**

Collaborators, 2025). The pandemic stunned the healthcare systems worldwide and caused economic crises, and made the vaccines more essential to restore societal functioning. The high rate of mutagenicity of the virus necessitates broad and durable protective vaccines. Research into the B cell activation following vaccination highlighted the need for booster doses to provide long-term protection [1]. The unmatched speed of vaccine development was made possible by innovations in biotechnology, including mRNA and recombinant protein-based vaccine strategies, which were evaluated in clinical trials such as COVAIL [2]. The advancements in manufacturing, especially in the optimisation of Chinese hamster ovary (CHO) cell systems for spike protein production, have accelerated vaccine availability. Preclinical studies are essential in vaccine development, providing crucial data on safety, immunogenicity and efficacy before trials on humans. These studies minimise the risk in clinical trials. For example, research using Syrian hamsters demonstrated the efficacy of the XBB.1.5 rS vaccine against emerging variants like EG.5.1, while studies on pDNA vaccines showed protective immunity in the same model[3]. Preclinical testing also assesses the cross-protection against the viral variants.

For example, the wild-type SARS-CoV-2-specific vaccine has limitations against other variants as well as zoonotic viruses. It also evaluates the cross-variant immunity of mucosal vaccines, such as lipopeptide-based formulations, and activation of AMPK inhibition by modulating host metabolism. Preclinical studies assess safety issues, such as adverse immune responses, and test new approaches, such as self-assembling protein nanoparticles and structure-driven antigen design [4]. Preclinical studies accelerate responses to threats and refine strategies for broader pathogen protection. The nonhuman primates (NHPs) are useful for assessing vaccine performance due to their immunological similarity to humans. Research in cynomolgus macaques confirmed the efficacy of the VLA2001 vaccine against SARS-CoV-2 infection. Preclinical testing of the mRNA-based GLB-COV2-043 vaccine [5] and gamma-irradiated inactivated vaccine candidates was performed to study safety and efficacy. The combined intramuscular/intranasal vaccine formulations, such as RAZI-COV PARS, were tested in multiple animal species. The models also revealed the long-term humoral immunity and protective efficacy of inactivated vaccines such as CoviVac. The broad-spectrum effectiveness of the bivalent mRNA vaccines was evaluated against emerging variants. The animal models also help refine vaccine dosing by showing similarities to the human immune response and disease pathology. Thereby, it bridges the gap between laboratory and clinical application (Figure 1).

Commonly Used Animal Models in SARS-CoV-2 Vaccine Research

Animal models serve as indispensable tools in SARS-CoV-2 vaccine research, providing important points into immunogenicity, efficacy, and safety (Table 1). Syrian hamsters (*Mesocricetus auratus*) have emerged as an ideal model due to the development of lung pathology similar to humans after SARS-CoV-2 infection[6]. Nonhuman primates, such as cynomolgus macaques (*Macaca fascicularis*), are considered the gold standard for preclinical testing due to their close phylogenetic relationship to humans. They have a similar immune system to humans, as shown in the study of the VLA2001 vaccine and the other vaccine candidates. The transgenic mice, such as hACE2 models, are widely used for their cost-effectiveness and genetic manipulability. Pigs (*Sus scrofa domestica*) have been used to evaluate the mucosal immunity and safety of the PHH-1V vaccine[7]. Ferrets (*Mustela putorius furo*) are used in the study of respiratory diseases due to their similarity in lung anatomy to humans. Still, they are less commonly used in the vaccine preclinical studies. Collectively, these animal models provide a platform to assess the vaccine efficacy before the clinical trials. Each species shows unique advantages. Depending on the specific research objective, the appropriate animal model is selected.

Mice (Wild-type, hACE2-transgenic, and other engineered models)

Wild-type mice have played an important role in SARS-CoV-2 vaccine development, even though they have natural resistance to infection. They lack compatibility with the human ACE2 receptor. Their cost-effectiveness, well-characterised immune system, and availability of immunological reagents make the wild-type mice the best choice for the initial vaccine evaluation. To overcome the limitation, researchers have tried many strategies. But still, the wild-type mice were initially used to study the immunogenicity of the mRNA vaccine, during which they developed anti-idiotypic antibodies (anti-ACE2)[8]. The wild-type mice are also used to study the immune response under specific physiological conditions, such as diabetes. The results showed that the impaired persistence of antibody



**Annie Aglin Antony**

production post-vaccination. Additionally, the fundamental aspects of the SARS-CoV-2 biology, including furin cleavage, were studied in the wild-type mice[9]. It is also helpful to evaluate the effect of adjuvants. However, the limitations led the researchers to develop protocols using a combination of mouse models to better replicate the human SARS-CoV-2 pathology[10]. For the challenge studies, the researchers have developed transgenic mice that express the human angiotensin-converting enzyme 2 (ACE2) receptor, a primary viral entry receptor. Unlike wild-type mice, these genetically modified models develop strong COVID-19-like symptoms after SARS-CoV-2 infection, making them essential for advancing preclinical vaccine research[8].

These mice are also useful for studying variant-specific immunity, including sex-dependent pathological differences between the Delta and Omicron variants[11]. The K18-hACE2 model is insightful for studying the neurological aspects of the infection. Advancements in hACE2-transgenic mice enhance translational relevance by bridging preclinical findings to clinical applications. Studies of RBD-Fc mucosal vaccine using hACE2 models have demonstrated efficacy in both mice and hamsters[10]. Also, the mammalian cell-expressed trimeric spike protein vaccine conferred near-complete protection against infection. The hACE2-transgenic model has also been used to assess novel therapeutic approaches, such as α -Difluoromethylornithine and Sulindac, against SARS-CoV-2 infection. The T cell-based SpiN-Tec vaccine was also tested in the hACE2-transgenic model and showed high efficacy against variants of concern. The viral targeting and immune responses in the hACE2-transgenic mouse model make a connection between the laboratory studies and the clinical trials[12]. The mouse model remains unavoidable for the initial vaccine screening and mechanistic studies. Moreover, the results need to be further confirmed in the translational models like hamsters or nonhuman primates.

Hamsters (Syrian/Golden hamsters)

The Syrian hamster (*Mesocricetus auratus*) is a highly valuable model for SARS-CoV-2 vaccine development due to its natural susceptibility to infection and ability to develop COVID-19-like lung pathology, including alveolar damage and inflammation. This model has been used to evaluate vaccine efficacy against the variants, such as the XBB.1.5 rS vaccine's protection against EG.5.1. It is also used to test novel platforms, such as live-attenuated and T-cell-based vaccines [12]. Hamsters mimic human immune responses, including antigenic imprinting effects during exposure to the variants. Research has demonstrated the use of hamsters to assess the mucosal immunity induced by intranasal vaccines that elicit IgA and CD8+ T-cell responses. They also identify vaccine-enhanced disease risks, like the Th2-biased pathology triggered by formalin-inactivated vaccines. The golden Syrian hamster (*Mesocricetus auratus*) is a key model for SARS-CoV-2 vaccine development due to its high susceptibility, robust viral replication in respiratory tissues, and similar lung pathology to humans [6]. This model has been extensively utilised to evaluate vaccine efficacy against variants. XBB.1.5 rS vaccine against EG.5.1, mRNA vaccines against Delta and Omicron sub variants, and universal bacterium-vectored vaccines targeting conserved viral proteins of SARS-CoV-2 demonstrated the protective capacity in hamsters [13]. They are useful for assessing innovative approaches such as avian-derived IgY antibodies and BCG combination strategies [14]. The shorter-lived antibody responses is the only limitation of Syrian hamsters. The cost-effectiveness, reproducible pathology, and immunological relevance make this a necessary bridge between rodent models and nonhuman primates in preclinical vaccine development. Recent applications include testing mRNA vaccines and novel fusion protein vaccines, solidifying its role in addressing ongoing vaccine challenges posed by viral evolution [15].

Ferrets

The ferret (*Mustela putorius furo*) is a valuable model for SARS-CoV-2 vaccine development due to its unique physiological and immunological similarities to humans with respect to respiratory viral infections. Ferrets naturally express ACE2 receptors with high affinity for the SARS-CoV-2 spike protein. It permits robust viral replication in both upper and lower respiratory tracts. It makes them an ideal model for studying vaccine efficacy. This model was used to evaluate various vaccine platforms, including intranasal lipid nanoparticle-based protein and mRNA vaccines. The studies demonstrated enhanced mucosal immunity and reduced viral loads. Similarly, the study of linear DNA vaccines significantly decreased virus shedding. Usage of ferrets to assess cross-protection against variants is critical. For example, the investigation of vaccine-induced immune responses of polymeric nanoparticle



**Annie Aglin Antony**

mRNA vaccines and novel adjuvanted formulations was difficult [16]. Their susceptibility to aerosol transmission and ability to develop both pulmonary and neurological manifestations offer insights into disease pathogenesis and vaccine protection. The higher maintenance costs compared to rodent models and occasional variability in disease severity are limitations to the use of ferrets. Still, it remains indispensable for preclinical evaluation of respiratory vaccines, particularly for assessing sterilising immunity and transmission-blocking potential. Recent advances in ferret models, including their use in studying co-infections and evaluating broad-spectrum nanovaccines, continue to solidify their role in bridging preclinical findings to human clinical trials for COVID-19 and other respiratory pathogens [17].

Nonhuman Primates (NHPs) (Rhesus macaques, Cynomolgus macaques, African green monkeys)

The rhesus macaque (*Macaca mulatta*) has proven to be a valuable nonhuman primate model for SARS-CoV-2 vaccine development. It has a close phylogenetic relationship to humans and similar immune responses. This model has been used to evaluate vaccine efficacy. Studies have demonstrated that the passive infusion of S2-Stem broadly neutralising antibodies protects against SARS-CoV-2 infection. It also reduced lower airway inflammation. Rhesus macaques were used to evaluate new vaccine platforms, like VLP-forming mRNA vaccines. It was also used to study viral biodistribution via immune PET imaging [18]. They can develop both upper and lower respiratory infections, like humans. It makes them useful for intranasal and aerosol exposure studies. Additionally, they were used to evaluate combination vaccines, such as a bivalent COVID-19 and influenza vaccine.

Another nonhuman primate is the cynomolgus macaque (*Macaca fascicularis*). It has emerged as an important model for SARS-CoV-2 vaccine development due to its similarity to humans. This model was used to evaluate the vaccine efficacy of synthetic S glycoprotein virus-like particles [19], spike ferritin nanoparticle vaccines, and novel RBD-protein/peptide vaccines. The results showed that these vaccines have elicited broad neutralising antibodies. *Cynomolgus macaques* were also used to study CD8+ T cell responses by intranasal vaccines and pan-SARS neutralising antibody responses following booster vaccinations. Their susceptibility to SARS-CoV-2 variants like Delta and their ability to mimic human disease severity based on exposure routes make them valuable for research. This model has been used to test therapies like dual-antibody prophylaxis and vaccine vectors, including attenuated vaccinia and replicating poxvirus systems. While limitations include high costs and ethical issues, their immunological similarity to humans and their ability to mimic COVID-19 symptoms make it easier to translate preclinical findings into clinical trials.

One more nonhuman primate is the African green monkey (*Chlorocebus sabaeus*), which is less studied than cynomolgus macaques. This model offers valuable insights into vaccine efficacy and disease pathogenesis, especially for respiratory infection dynamics. African green monkeys have shown robust immune responses to various vaccine platforms. For example, spike ferritin nanoparticle vaccines elicited potent neutralising antibodies and T-cell responses. The novel RBD-protein/peptide vaccines conferred protection against viral challenge. Their use in studying exposure route-dependent disease severity has helped optimise vaccine delivery methods. At the same time, proteomic and metabolomic analyses in this model have revealed early-stage host responses to infection. African green monkeys, though less widely used than other models, have distinct advantages, such as being natural hosts for many viruses and exhibiting more pronounced clinical disease than some macaques [20]. Their ongoing use supports data from primate models, especially for assessing mucosal immunity and infection patterns in respiratory tracts relevant to vaccine development.



**Annie Aglin Antony****Key Parameters Evaluated in Preclinical Vaccine Studies****Immunogenicity**

Preclinical immunogenicity studies of SARS-CoV-2 vaccines showed strong immune responses in hamsters, primates, and mice, validating candidates before clinical trials. For example, broad-spectrum bivalent mRNA vaccines elicited potent neutralising antibodies and T-cell responses against multiple variants in preclinical models. The multi-antigenic Orf virus-based vaccines induced protective immunity in both hamsters and nonhuman primates [21]. In Syrian hamsters, the XBB.1.5 rS vaccine demonstrated high efficacy against the EG.5.1 variant. Similarly, recombinant RBD-Fc fusion proteins triggered significant antibody production in preclinical assessments. Passive immunisation in rhesus macaques shows S2-stem neutralising antibodies reduce viral load and airway inflammation [22]. Additionally, inactivated SARS-CoV-2 vaccines and ChAdOx1-vectored vaccines (e.g., AZD2816) induced strong humoral and cellular immunity against variants of concern.

Protective Efficacy

Preclinical studies evaluating the protective efficacy of SARS-CoV-2 vaccines were conducted in various animal models, including mice, hamsters, and nonhuman primates. For example, intranasal spike protein vaccines elicited strong mucosal and systemic immunity in mice, reducing viral loads after challenge. Pan- β -coronavirus subunit vaccines protected against the Omicron variant of SARS-CoV-2, SARS-CoV, and MERS-CoV in mice [23]. Syrian hamsters showed near-complete protection with mammalian cell-expressed trimeric spike vaccines and XBB.1.5 rS vaccines against the EG.5.1 variant [6]. Adenovirus-based mucosal vaccines induce strong systemic and mucosal immunity, preventing disease in animals. Broad-spectrum bivalent mRNA vaccines confer cross-protection against many variants, while RBD-based protein vaccines harbouring immune-evasion mutations show broad efficacy [24]. These studies emphasise preclinical models' role in validating vaccine candidates.

Safety and Toxicity

Several preclinical studies examined SARS-CoV-2 vaccine adverse effects and immune responses. For example, a broad-spectrum bivalent mRNA vaccine elicited strong immune responses without notable adverse effects in preclinical models. Co-administration of recombinant BCG with SARS-CoV-2 proteins induces strong antiviral immunity without notable toxicity [25]. Sauter et al. (2025) studied how obesity affects SARS-CoV-2 infection and post-acute symptoms, showing metabolic conditions may worsen outcomes [26]. Other studies on adjuvants showed that while certain formulations (e.g., Alum:CpG or ARNAX) boosted immune responses, their safety needed careful assessment. Overall, these preclinical studies highlight the importance of balancing efficacy and safety. However, factors like adjuvant choice and health conditions may affect the risk of adverse effects. Histopathological assessments were also performed during preclinical SARS-CoV-2 vaccine studies. Obesity-related pathology in nonhuman primates after infection, with severe lung inflammation and vascular damage, highlights the importance of vaccine safety in comorbid conditions. Studies have characterised diabetes-exacerbated lung injury in golden hamsters, showing alveolar damage and immune cell infiltration. Studies have used K18-hACE2 transgenic mice to dissect sex-specific differences in organ pathology.

Results showed that females exhibit milder lung lesions than males. Bernardes et al. (2024) evaluated OMV-based vaccines in mice, confirming protective immunity without significant organ damage [27]. Advanced techniques, such as immune histochemistry (IHC) for immune markers (e.g., CD4⁺/CD8⁺ T cells, macrophages) and scoring systems (e.g., lung injury severity scores), were commonly employed. Masters et al. (2025) combined these methods in K18-hACE2 mice to reveal sex-specific differences in post-vaccination lung pathology, with males exhibiting more severe lesions [27]. Preclinical studies used to evaluate cytokine release profiles and the proinflammatory responses. For example, broad-spectrum bivalent mRNA vaccines induced balanced Th1/Th2 cytokines (IL-2, IFN- γ , IL-4) in murine models. The adjuvanted formulations, such as Alum:CpG complexes, enhanced IFN- γ and IL-6 production and the TLR7 agonist-adjuvanted vaccines promoted robust IFN- α/β responses. Lipid nanoparticle (LNP)-mRNA vaccines caused temporary IL-6 and TNF- α release in humanised mice [28]. Studies in nonhuman primates have shown that saponin nanoparticle adjuvants trigger the secretion of IL-1 β and IL-12p70. Conversely, excessive Th2 skewing (IL-5, IL-13) was observed in some subunit vaccines, underscoring the need for a balanced cytokine profile [29].



**Annie Aglin Antony****Durability of Immunity**

Long-term protection studies in preclinical SARS-CoV-2 vaccine development have demonstrated promising results across a range of platforms. Studies reported that a multi-antigenic Orf virus-based vaccine provided robust protection in hamsters and nonhuman primates, suggesting durable immunity against SARS-CoV-2. A mixed lipopeptide mucosal vaccine induced cross-variant immunity and protected hamsters for the long term [4]. Researchers showed a trivalent recombinant protein vaccine produced cross-neutralising antibodies against new variants like XBB and JN.1 in preclinical and early trials, indicating potential long-term protection. Together, these studies report the importance of preclinical evaluations in assessing long-term vaccine efficacy and cross-variant protection.

Future Perspectives

Recent research advances include the refinement of animal models for preclinical studies of SARS-CoV-2 vaccine development. Humanised mice overcome traditional mouse limitations by engrafting human immune components. They generate mature, human-like antibody responses and give more accurate preclinical data [1]. Alternative species like golden hamsters and nonhuman primates offer unique advantages. Hamsters develop lung pathology similar to humans, useful for studying vaccine protection against severe respiratory disease. Nonhuman primates provide information about systemic immune responses and comorbidities such as obesity and diabetes. Transgenic models (e.g., K18-hACE2 mice) enable sex- and variant-specific studies, enhancing translational relevance [11]. These advancements in humanised and alternative animal models are vital for optimising vaccine candidates and preclinical data that better predict human outcomes.

Standardising preclinical testing protocols is important for reproducibility, comparability, and relevance in SARS-CoV-2 vaccine research. Recent efforts focus on harmonising methodologies across animal models, immune response assessments, and histopathology studies. For example, Sauter et al. (2025) standardised obesity and diabetes models in primates and hamsters to evaluate vaccine efficacy under high-risk conditions [26]. Similarly, Masters et al. (2025) provide histopathological scoring systems that help assess inflammation and injury [11]. Comparative studies of adjuvants, such as Alum:CpG and saponin nanoparticles, highlight the need for consistent dosing and immune profiling protocols. Song et al. (2025) established benchmarks for lipid nanoparticle safety, including biodistribution and cytokine assays [28]. Consortia like COVARNA and IMPACC use shared platforms for multi-omics data. Future guidelines for challenge doses, endpoints, and reporting will improve data reliability and bridge the gap between animal studies and clinical trials.

Animal studies play a crucial role in evaluating vaccine efficacy against emerging SARS-CoV-2 variants and optimising booster doses. Researchers have utilised various animal models to demonstrate broad protection developed by the vaccines against evolving SARS-CoV-2 strains. For example, studies using golden hamsters and transgenic mice have shown that bivalent and mRNA vaccines elicit robust neutralising antibodies against Omicron sub variants like BA.5 and XBB. These preclinical studies provide findings to help formulate vaccines. These animal models are also helpful in testing novel vaccine platforms, such as JN.1 spike-based mRNA vaccines that demonstrated cross-protection in mice [30]. The Newcastle disease virus-like particles with stabilised spike designs were also tested in animals. For booster development, research in animal models has helped optimise adjuvant selection and determine routes of administration. For example, studies have identified ARNAX as a promising adjuvant to enhance T-cell responses. Specifically, animal studies using specialised models, such as obese nonhuman primates, have revealed how comorbidities can affect booster responses. These preclinical investigations provide essential data on immune correlates of protection, optimal dosing intervals, and platform selection. The animal studies will continue as a bridge between laboratory research and human trials, helping to rapidly evaluate new vaccines against emerging viruses and their variants. Consolidating results from multiple animal models helps validate the vaccines and increase confidence in translating preclinical findings into clinical studies.





Annie Aglin Antony

CONCLUSION

In summary, animal studies have played an essential role in the development of vaccines for SARS-CoV-2. They have shown how important these models are for testing vaccine effectiveness against new variants, the effect of booster doses, adjuvants and different routes of administration. Animal models help accelerate vaccine development by enabling rapid screening of vaccine candidates and informing clinical trial design. They also show how clinical conditions affect the effectiveness of vaccines. However, the continuous emergence of variants and the need for vaccines promote preclinical testing. Future advancements should focus on developing new animal models that are more relevant to humans. These improvements will ensure that preclinical studies remain a strong foundation for developing next-generation vaccines against SARS-CoV-2 and other emerging threats.

Conflict of Interest

The author has no conflict of interest.

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Annie Aglin Antony

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Table 1: Animal Models Used in SARS-CoV-2 Vaccine Development

Animal Model	Key Advantages	Major Limitations	Primary Applications in Vaccine Studies
Wild-Type Mice	Low cost, well-defined immune tools, high throughput.	Not naturally susceptible to SARS-CoV-2; requires adaptation.	Preliminary immunogenicity, adjuvant screening, mechanism of action.
hACE2-Transgenic Mice	Susceptible to infection,	Can exhibit exaggerated	Efficacy against challenge,





Annie Aglin Antony

	model human-like disease, genetic tractability.	neurotropism (K18 model); not outbred.	variant-specific pathology, immune correlates.
Syrian Hamster	High susceptibility, robust respiratory pathology, models transmission.	Short-lived antibody responses; less severe clinical disease.	Vaccine efficacy, viral shedding, transmission blocking, variant challenge.
Ferrets	Natural susceptibility, upper respiratory tract infection, models transmission.	High cost, limited reagents, variable disease severity.	Mucosal immunity, sterilizing immunity, transmission studies.
Nonhuman Primates (Rhesus/Cynomolgus)	Closest immune & physiological similarity to humans.	Very high cost, ethical constraints, limited sample size.	Gold-standard efficacy, safety, immunogenicity, and correlates of protection.

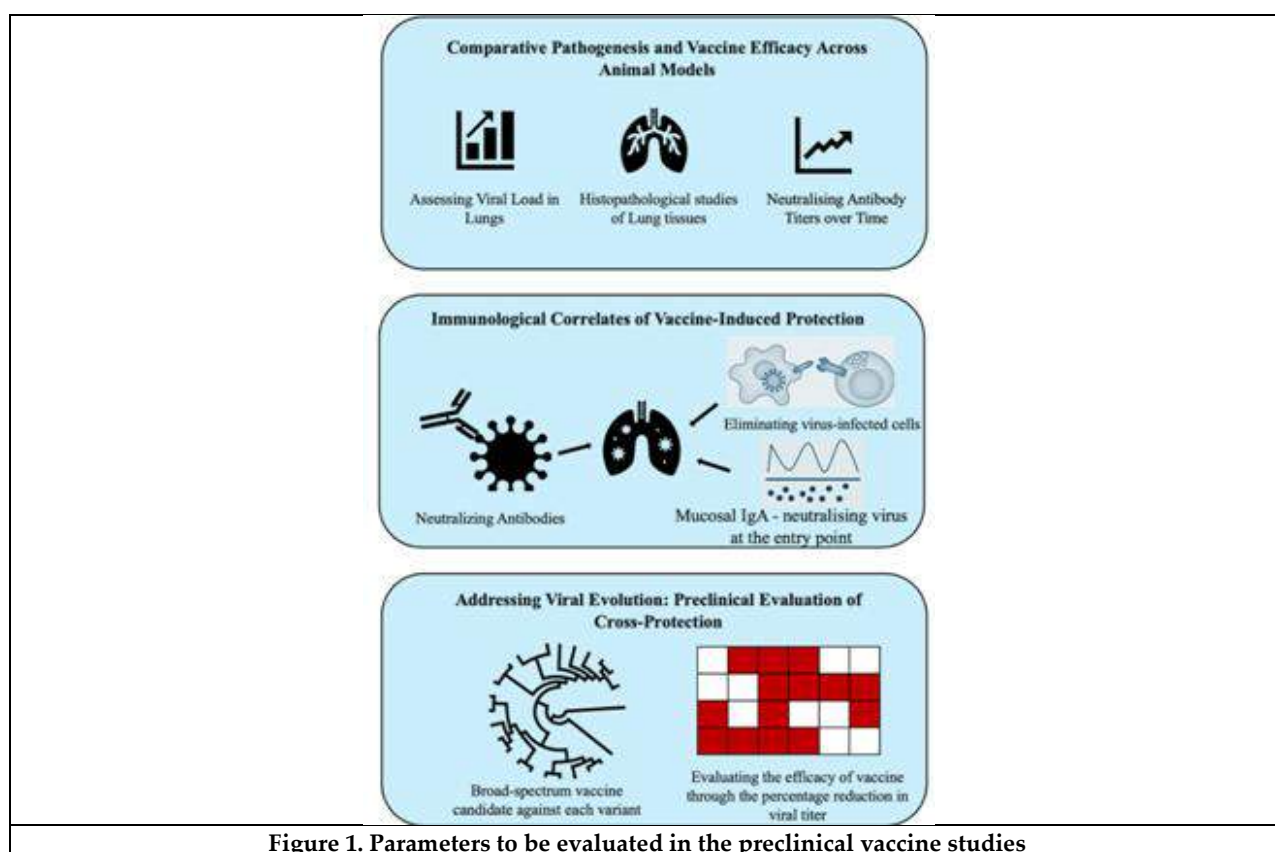


Figure 1. Parameters to be evaluated in the preclinical vaccine studies

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RESEARCH ARTICLE

Advances in AI Applications for Environmental Research

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ABSTRACT

Artificial Intelligence (AI) is transforming the environmental science field very fast through offering sophisticated analytics and predictive modeling of a wide variety of complex ecological systems, whether it is climate processes, pollution and biodiversity. AI tools increase the ability to work with heterogeneous large-scale data on the environment dramatically. The review explores the important AI applications in environmental science to (i) locate areas of application, (ii) review methodological advances, and (iii) identify challenges and research priorities. A literature review of the current peer-reviewed literature and interdisciplinary reviews on AI approaches, including machine learning, deep learning, remote sensing integration, and predictive analytics, in air and water quality assessment, climate modeling, biodiversity tracking and ecosystem monitoring, was undertaken. AI plays a major role in improving environmental science through better prediction of climatic and ecological models, real-time pollution and water quality measurements, as well as biodiversity surveys through image and sensor analytics. The systems with AI in place aid early warning systems and smarter resource management although the issues of data quality, model interpretability, transparency, ethical implementation and computational sustainability remain. AI promotes receptive and scalable environmental technologies. Further elaboration of explicable, fair AI systems will be necessary to streamline the process of environmental decision-making and improve sustainable science. By responding to them, there will be improved trust, equity, and sustainable use of AI in environmental decision-making.

Keywords: Artificial Intelligence, Environmental Science, Climate Modeling, Biodiversity Tracking, Predictive Analytics, Environmental decision-making





Nandini Bhattacharjee

INTRODUCTION

Environmental studies are currently facing a complex, nonlinear, multi scale of issues that are arising due to global climate change, increases in air and water pollution and accelerating biodiversity loss. Although traditional physics-based and statistical models are fundamental, they may not be able to reflect high-dimensional interactions and real-time variability in environmental data [1]. Over the past few years, Artificial Intelligence (AI) has become the game-changer of environmental studies and redefines the understanding of large datasets, the building of models, and informed decisions. The implementation of AI has helped provide perspective on climate risk evaluation, species location forecasting, and pollution pattern identification, and sustainable management of resources, which, in many cases, was impossible due to traditional tools alone [2,3]. The potential of AI to identify complex patterns, approximate nonlinear correlations and automate complex analytical tasks has shown potential solutions in a broad variety of environmental applications - real-time water quality monitoring, air pollution prediction, biodiversity assessment, pollution detection, ecosystem surveillance and resource optimization and climate risk assessment [2,4,5,6]. Machine learning and deep architecture-based AI techniques have shown the ability to incorporate heterogeneous data and to discover subtle patterns on scales to enhance the analytical repertoire of environmental researchers. AI has the capability to identify nonlinear relationships in environmental data that cannot be identified using linear statistical analysis. It can be used in promoting climate models to creating intelligent early-warnings of extreme weather and streamlining conservation strategies on the basis of spatiotemporal information [7]. Defining important terms in the context of environmental research, the following key terms are defined to offer conceptual clarity to any reader of this paper:

Artificial Intelligence (AI): A general branch of computational science that is dedicated to the development of systems that are typically performed by human intelligence, i.e. pattern recognition, predictive modeling and decision support [8].

Machine Learning (ML): This is a subdivision of AI where algorithms learn patterns based on the data to make a prediction or decision without being programmed to perform that specific task.

Deep Learning (DL): DL, a subdivision of ML, employs numerous layers in an artificial neural network (deep architecture) to learn large datasets with very abstract and hierarchical relationships [9].

Generative AI: Generative AI models are AI tools that produce new instances of data that are similar to training data; these include large language models, and generative adversarial networks which can be used in environmental science to analyze scenarios and to generate synthetic data [10].

Green AI: A more recent approach, the green AI, focuses on creating AI models that are computationally efficient and sustainably designed, responding to the issue of carbon footprint through AI systems [11]. Study shows a new direction focusing on energy-efficient, environmentally friendly AI design with low carbon footprint and resource-light model development, training, and deployment [12].

Hybrid physics-based formulations: Hybrid physics-based formulations combined with machine learning are now under increasing momentum as they are capable of dealing with such complexity and enhancing generalizability and physical interpretability [13], which has long been a gap in environmental modeling (Hybrid physics-ML air circulation research). These synergistic models are increasingly becoming popular as a means of balancing theory and practice. The review is intended to offer an interdisciplinary synthesis of current developments in AI applications to environmental research, both in the methodological novelty and practical effects, such as the combination of AI with remote sensing or Internet of Things (IoT) networks to monitor the environment in real-time, and the ethical and policy implications of AI implementation in environmental governance.



**Nandini Bhattacharjee****AI Applications by Environmental Sub-Domain**

Surveillance and Data Collection: The process of environmental monitoring and data collection is being entirely transformed by the AI technologies that allow creating the means of analyzing the mass of the ecological data more efficiently and effectively [14].

AI powered Remote sensing: In remote sensing technologies such as satellites and drones, is collected essential information about the surface of the earth. AI increases such abilities by automating the process of image analysis so that it is possible to quickly and accurately detect changes in the environment [15]. Remote sensing analysis has been effectively implemented using AI [16]. The temporal resolution of remote sensing data has been greatly improved by the development of remote sensing technology. Multi temporal remote sensing image categorization can be used to derive richer spatiotemporal traits [17]. Indicatively, machine learning systems can use large volumes of satellite data to detect deforestation, monitor land use change, and monitor animal behaviour, thereby greatly accelerating and enhancing the precision of data classification over human efforts [18,19] [(Table 1) 5,9,10,20,21]. [Table 1: AI Techniques and their key environmental applications]

Deforestation and land use Monitoring: Deep learning AI-enabled applications such as Global Forest Watch use satellite images to identify deforestation in near real-time through the use of deep learning models. Such instruments give conservationists and policymakers the most up to date information enabling them to take timely actions to curb environmental destruction [18,19].

Environmental trends and behavioural Analysis: AI algorithms will be able to track animal behavioural changes that can tell about environmental changes, mating behaviours, or alterations in migratory patterns. This is necessary in learning the wider effects of climate change and habitat loss on the different species.

AI of all-inclusive Surveillance:

AI plus the IoT technology provide an opportunity to use a holistic approach to monitoring. Smart sensors placed in different settings are used to gather information regarding pollutants, soil moisture and temperature, which are processed by AI algorithms to analyze the data.

Real Time Environmental Monitoring: Integration of AI in environmental monitoring has worked especially well in the national parks and protected areas where AI-controlled cameras and drones will be able to notice any form of poaching and send alerts to the rangers in real-time [15]. These systems increase the effectiveness of conservation campaigns and the protection of the endangered species is improved because the system will react faster to the possible threat [14,22].

Data Analysis and Modeling

Analysis and modeling of data are essential parts of environmental science, especially when applied in AI and machine learning (ML).

Predictive Analytics in Environmental Science: Predictive analytics is a field within environmental science that involves predicting the future occurrence of events associated with the environment. Predictive analytics involve predicting the future of environmental-related events.

Key Application: Advanced forecasting models can be used to forecast the probability of extreme weather, evaluate the risk of drought, and more effectively manage resources under the influence of new conditions in the environment. Furthermore, machine learning algorithms, random forest and deep learning models [Convolutional Neural Networks (CNNs)] are used to process and analyze intricate ecological data [23,24].



**Nandini Bhattacharjee**

Forecast models: This approach can be useful with such scenarios as predicting the frequency of flood occurrences or estimating the level of pollution. These models are useful in that, by using past numerical data, they give organizations the vision to get ready to the environmental challenges [25].

Advanced Techniques: More advanced data analysis is done by making use of advanced machine learning methods. Ensemble learning techniques and Long Short-Term Memory (LSTM) networks have been shown to be quite useful in increasing prediction accuracy through combining the effectiveness of more than one model, thus reducing the error and bias of an individual model [23,26].

Conservation Efforts: AI is becoming acknowledged as a game changer in conservation activities, as it assists in dealing with the crisis of biodiversity that is on the verge of becoming a worldwide issue and improving the management of ecosystems. The number of AI applications in conservation has also increased, and conservationists use them to track wildlife, restore habitats, and predict environmental changes successfully [27].

AI in Wildlife Monitoring: The implementation of automated camera traps and drone technologies has transformed wildlife monitoring because of the use of AI technologies to improve these tools. These technology tools apply advanced image recognition algorithms to automatically locate and track animals in their respective natural habitats, allowing to monitor animal populations and behaviours in real-time [28] [Fig. 1]. [Fig. 1: Schematic diagram showing application of AI technologies in Environmental research]

Habitat Restoration: AI is also useful in habitats restoration. Ecological data can be analyzed by algorithms that determine degraded areas that can be restored and thus prioritize areas in need of restoration [29].

Climate Change Mitigation

AI is also being seen as a possible solution to climate change through improved monitoring, predicting environmental changes, and optimizing resource management.

Green House Gas Emissions monitoring: Through infrared and multi-spectral measurements, these systems detect carbon dioxide (CO₂), methane (CH₄) and other air pollutants in the atmosphere and give real-time information on the quality of the air to policymakers [14].

Increasing Renewable Energy efficiency and decrease energy consumption : AI can optimize solar panel performance based on weather conditions and the state of the panel, which increases the amount of energy collected and anticipates maintenance. AI model may decrease energy consumption [2].

Reduction and Recycling Wastes: AI will help to reduce waste and boost recycling rates, which will promote a circular economy. The example of Carbon Bright, a climate tech startup, can be seen in the fact that it uses AI to perform product lifecycle analysis, which provides immediate evaluations of the environmental impact of consumer goods. Their platform helps track down the hotspots of the emissions across the supply chain, allowing the brands and retailers to switch to a more sustainable product with ease [23,30].

Environmental Monitoring: Smoke plumes and thermal variations can be detected in real time by AI algorithms to detect wildfires and response can be taken in a timely manner. Besides, AI is able to track water body changes, which judge the course and the level of rivers and lakes, which is crucial to urban planning and resources conservation [18,23].





Nandini Bhattacharjee

Environmental Risk Assessment

Environmental risk assessment is the consideration of the possible negative impacts of environmental risks such as pollution and natural disasters on human health and the ecosystem. AI and ML are changing this sphere, as they allow making more accurate predictions and effective reactions to environmental threats.

Predictive Analytics for Natural Disaster: AI models can be important when it comes to predicting natural disasters through the analysis of big data through sensors, satellites and past occurrences. As an illustration, ML algorithms have the ability to analyze the weather patterns, the extent of soil moisture, and the conditions in the air to predict occurrences like floods, drought, and wild-fires. AI is able to process different parameters in order to forecast the magnitude and the effect of the catastrophe, which is vital in reducing the loss of life and property [31].

Pollution Monitoring and Control: There is also the use of AI technologies in tracking the quality of air and water hence determining the risks of pollution [18,32]. The AI-driven systems monitor the pollutants, including carbon dioxide (CO₂) and methane (CH₄), released by industrial processes and transport through real-time measurements of the environmental sensors [33]. These systems allow governments and organizations to enforce policy and devise a plan to reduce the risk to the environment by giving information on the level of pollution [34]. As an example, AI has been applied to predict the presence of smog in cities to take proactive action to counteract the decline of air quality [35].

Ecosystem and Biodiversity Conservation: Regarding the biodiversity conservation area, AI aids in risk assessment by tracking populations of the species and identifying habitat changes. Machine learning algorithms can recognize threatened species and estimate their susceptibility to anthropogenic factors and conserve them in the best way [15].

Challenges and Limitations: Although the concept of AI brings a lot of opportunities to the development of environmental sustainability, a variety of challenges and limitations will have to be mitigated to guarantee the successful and ethical use of AI. These are the problems of data-related concerns and ethical ones, and they can greatly affect the potential advantages of AI technologies in environmental science [(Table 2) 2,10,20,21,34,36,37]. [Table 2: Challenges in AI Environmental Research and mitigation strategies]

Data Limitations: Quality and availability of data is one of the main challenges of AI in terms of application to the environment. The existence of AI systems depends on big datasets to operate, therefore, the lack of data, including its quantity and quality, may negatively affect the progress. As an example, the validation of data used in species identification through crowd sourced images is often problematic, as it may lack representative coverage and reliable metadata [29, 34].

Evaluation of Environmental Impact of Ai

Finally, with the constantly growing AI, it is very important to evaluate the effect of AI on the environment [14]. Training big AI models may be expensive and add to carbon emissions. The current research should be extended to explore ways to minimize the carbon footprint that must be associated with AI development and implementation, including the use of cleaner energy and efficient optimization of algorithms [38].

Future Trends: The future of AI in the field of environmental science is marked with a number of emerging trends and developments that are set to increase its potential and efficacy in solving environmental issues.

Integration with IoT: The convergence of AI and IoT devices is one of the major trends [39]. This integration brings more in-depth and real time monitoring systems of the environment and causes decentralized and autonomous networks to improve the data collection and analysis potentials [23]. The resulting integration provides on-going monitoring of ecosystems that is less disruptive to delicate ecosystems as well as enriches our knowledge on the processes involved in the environment [40].



**Nandini Bhattacharjee**

AI-based Decision Support systems: Another area that AI is opening the door to is the advancement of more complex decision support systems that put policymakers and environmental managers in control [41]. This development can help organizations to adopt strategic and sustainable climate and environmental policies in a better way [42].

Improving Predictive Abilities: With a better understanding of historical shifts in land use, agricultural practices, and water management, AI can be beneficial in identifying early signs of significant and minor transitions that have the potential to cause major ecological shifts [18].

Encouraging Stakeholder Collaboration : Involving the large group of stakeholders such as local populations, the government, and the private institutions, will not only reduce the conflict but will also improve the process of making decisions concerning the conservation of nature [22].

Developing Circular Economy : The implementation of AI technologies can also be one of the key contributors to a circular economy. The research directions in the future should be an AI usage that meets the challenges of the environment by means of a more efficient waste management system and resource recovery measures. As an example, AI can be used to discover and streamline recycling operations, thereby supporting the sustainability objectives and minimizing the environmental impact of different industries [32,43].

Sustainability and Ethical Contemplation

There is a need to make sure that AI solutions are not perpetrating any biases and should be built sustainably [23 UNESCO, 2023]. This entails reducing the negative environmental impact of AI systems, including energy consumption and electronic waste [34,44]. The need to use energy-efficient algorithms and encourage the use of the circular economy in AI development is critical in supporting the sustainability agenda [38,43,45].

Improved Climate Change Modeling and Projections: New technology of AI-powered simulation and modeling technologies will enhance the precision and finesse of climate forecasting and ecological evaluation [23]. The fact that the concentration of CO₂ in the air is alarming, and it is more than ever, makes the necessity of using accurate modeling tools more urgent [46].

Policy and Decision-Making Influence

With more advanced capabilities in analysis and real-time data analysis, AI applications have started to have a higher effect on environmental policy and decision-making processes, which provides a more productive approach to addressing complex environmental problems [47]. A multimodal CNN model is used in South Korea to predict the probability of wildfires. The model involves human, topographic and climatic variables. A deep learning model was based on a multimodal convolutional neural network. They may be used in such fields as ecological forecasting in the context of land use and land cover change, urban climate and air quality analysis, hydrologic and geomorphic hazards assessments [48]. Through the application of advanced algorithms, AI can be used to derive meaningful insights and track the trends that human analysts may not capture in time thereby enabling timely interventions in managing the environment [49].

CONCLUSION

The incorporation of AI in environmental studies has occurred at an accelerated pace over the last few years with both notable advancements and new challenge. Considering the interdisciplinary landscape surveyed in this review, it is noteworthy that AI has gone beyond the position of a supplementary analytical resource - it has become a key driver of the future of environmental science and sustainability. Recent studies point at a sustainability paradox. AI can not only improve environmental decision-making, but it also brings new environmental strains, unless models



**Nandini Bhattacharjee**

are designed to be environmentally responsible. To reduce the effects of these issues, it is necessary to implement the principles of Green AI in the form of energy-efficient designs, the use of renewable energies, explainable models, and the governance of the lifecycle. AI in environmental research represents an extremely promising and yet highly responsible way to go. AI increases our ability to perceive, anticipate, and react to change in the environment, helping professionals, policy makers and stakeholders to address pressing global issues with accurate data. Its implementation promotes better environmental resilience, more efficient resource management and closer adherence to the global sustainability goals. A deliberate effort towards sustainable innovation, one which considers ecological boundaries without exploiting computational ingenuity, will be a fundamental necessity as researchers and practitioners continue to progress in this direction going forward, and thus, to make AI not just a technological angle of attack, but a driver of planetary health.

Conflict of Interest

The author state that she has no known competing financial interests or personal connection that might have appeared to affect the work reported in this paper.

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Nandini Bhattacharjee

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Nandini Bhattacharjee

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Table 1: AI Techniques and their key environmental applications

AI Techniques	Key Environmental Application	Representative Examples / Notes	References
i. Supervised Learning (e.g., Random Forest, SVM)	Tasks of air quality forecasting, prediction of the level of pollution, and environmental classification.	Random forests in prediction of the air pollutant levels and dissolved oxygen in water systems; SVM in prediction of environmental states.	[5,20]
ii. Deep Learning (CNNs, RNNs, LSTMs)	Image analysis in the field of remote sensing, land cover mapping, time-series prediction of meteorological	CNN to classify high-resolution land and RNN/LSTM to predict trends of pollutants and weather patterns in the	[9,20]





Nandini Bhattacharjee

	and pollution forecasts.	sequences of data.	
iii. Generative AI & NLP (LLMs)	Knowledge mining of literary data on the environment, scenario generation, and support of stakeholder decisions.	Large language models were used to generate scientific knowledge and environmental statements.	[10]
iv. Reinforcement Learning	Intelligent resources allocation and optimization in environmental management (e.g., water grid, renewable infrastructures).	The reinforcement agents assist in strategizing the context dependent environmental moves.	[21]
v. Unsupervised Learning (Clustering)	Discovery of important environmental features like segmentation of habitat, detection of anomaly in pollution data	Unlabeled data-based grouping of ecosystems/ sources of emissions.	[21]
vi. Hybrid Physics-AI Models	Getting environmental processes simulations improved by including physical constraints with information-driven AI.	Physics-informed neural networks are enhanced to increase generalizability and lead to sound predictions.	[20]

[SVM, Support vector machine; CNNs, Convolutional neural network; RNNs, Recurrent neural networks; LSTMs, Long short-term memory; NLP, Natural Language Processing; LLMs, Large Language Models;]

Table 2: Challenges in AI Environmental Research and mitigation strategies

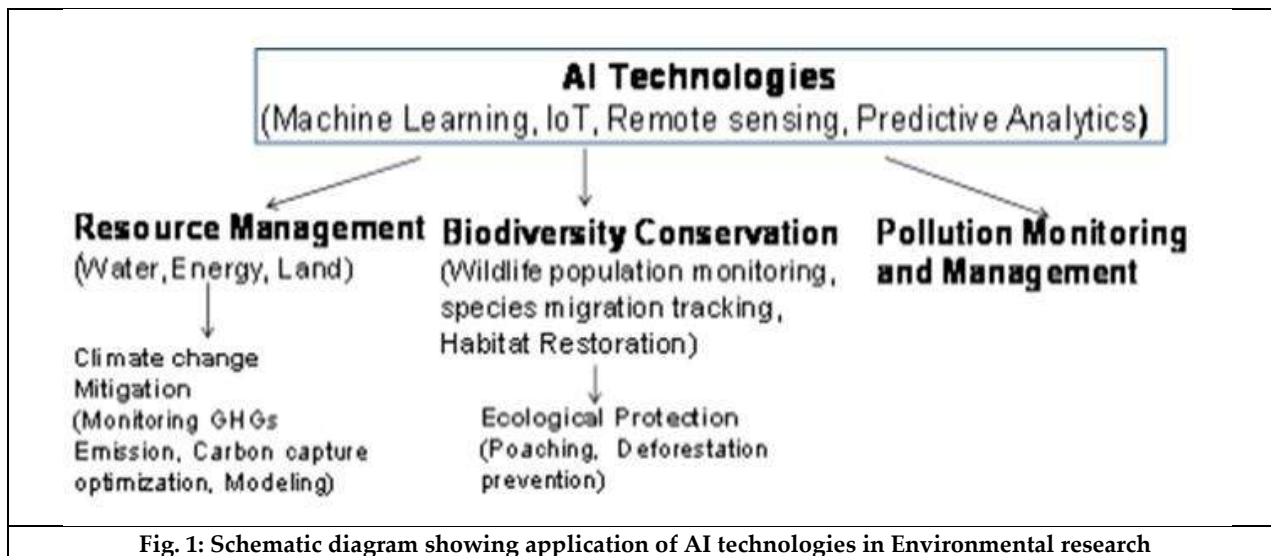
Challenges / Barriers	Description	Mitigation Strategies / Best Practices	References
i. Data Quality and Availability	Labelled environmental data of high quality are not often available; imbalanced or sparse datasets are limiting the training of models.	Data augmentation, transfer learning, the use of multi-sensor sources, collaborative open data projects; strict preprocessing.	[2,36]
ii. Model Interpretability (“Black-Box”)	Complex deep models do not support trust and practical insights, which is a problem in terms of policy and regulatory decision-making.	Physics-AI hybrid models to bring prediction and causal insight into agreement.	[36]
iii. Computational Resource Intensity	Large models (and generative AI in particular) are resource intensive in terms of energy and resource consumption, becoming increasingly expensive and unsustainable.	Efficient architecture, sparse modeling, and distributed training models; green AI strategies to reduce energy footprint.	[2,10]
iv. Ethical, Bias & Security Issues	Prejudiced training data can result in unfair predictions; AI systems can be prone to cyber-attacks or abuse.	Ethics code, strong testing, safe model implementation, continuous human monitoring and ethical auditing.	[37]
v. Integration with Domain Knowledge	Raw data models can fail to capture important knowledge of the environmental processes, and bring about impractical predictions.	Physics-informed modeling, Co-designing with domain experts, Hybrid modeling with scientific constraints.	[2,20]
vi. Scalability and Generalizability	Models that are trained locally or in a particular context might not be very good at transferring to new geographical areas or scales.	Multi-region training sets Comprehensive multi-region learning or federated learning.	[21,34]





Nandini Bhattacharjee

vii. Interpretability for Policy making	AI outputs can be hard to interpret or make actionable by the policymaking community.	Visualization tools are clear, co-design with stakeholder explainable reports, conformity with regulatory systems.	[2,37]



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RESEARCH ARTICLE

Anti-Thyroid Peroxidase Antibodies and their Association with Thyroid Dysfunction and Inflammatory Markers in Hypothyroidism

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ABSTRACT

Hypothyroidism is a common endocrine disorder with significant metabolic and cardiovascular consequences, most often caused by autoimmune thyroiditis in iodine-sufficient regions. Anti-thyroid peroxidase (anti-TPO) antibodies are highly sensitive markers of thyroid autoimmunity and may also reflect systemic inflammation. In this case control study 200 individuals were evaluated including 120 patients with hypothyroidism and 80 age and sex matched euthyroid controls. Serum levels of T3, T4, TSH, anti-TPO antibodies, high-sensitivity C-reactive protein (hsCRP) and erythrocyte sedimentation rate (ESR) were measured and correlations were analyzed using Pearson's Correlation coefficient. Results showed that hypothyroid patients had significantly elevated TSH and reduced T4 alongside markedly higher anti-TPO titers compared to controls. Inflammatory markers were also raised with hsCRP and ESR levels significantly higher in cases and a strong positive correlation was observed between anti-TPO and ESR while associations with hsCRP and TSH were weak and non-significant. These findings demonstrate





Sumit Kumar Sagar and et al.,

that elevated anti-TPO antibodies are strongly associated with thyroid dysfunction and systemic inflammation, reinforcing their diagnostic and prognostic value. Integrating anti-TPO measurement with inflammatory markers In hypothyroid patients may enhance risk stratification, guide therapeutic decisions and help identify patients at increased risk of multisystem complications. Longitudinal studies are warranted to establish their predictive utility in disease progression and comorbid autoimmune disorders.

Keywords: Anti - thyroid peroxidase antibodies (Anti - TPO), Autoimmune thyroiditis, Hypothyroidism, Autoimmune thyroid disease.

INTRODUCTION

Hypothyroidism is one of the most prevalent endocrine disorders worldwide, with significant effects on metabolism, cardiovascular function and quality of life(1,2). In iodine sufficient regions, autoimmune thyroiditis particularly Hashimoto's thyroiditis remains the leading cause(3,4,5). The condition is characterized by progressive thyroid tissue destruction mediated by auto-reactive lymphocytes and the presence of circulating thyroid auto-antibodies(6,7). Among these autoantibodies anti-thyroid peroxidase (anti-TPO) antibodies are recognized as the most sensitive and specific markers of autoimmune thyroid disease(8,9,10,11). Thyroid peroxidase is a key enzyme in thyroid hormone biosynthesis and its targeting by anti- TPO antibodies reflects ongoing immune mediated thyroid damage(12,13). Elevated titers of anti- TPO antibodies are frequently observed not only in patients with overt hypothyroidism but also in euthyroid individuals at risk of developing thyroid dysfunction Suggesting or pointing their diagnostic and prognostic utility(14,15,16,17,18). Beyond their role in thyroid specific autoimmunity anti-TPO antibodies have been linked to systemic immune activation(19,20,21,22). Several studies suggest associations between antibody titers and inflammatory markers such as erythrocyte sedimentation rate (ESR) and high sensitivity C- reactive protein (hsCRP)(23,24,25,26). ESR reflects the overall inflammatory burden while hsCRP serves as a sensitive marker of low-grade chronic inflammation and has been marked in cardiovascular risk among hypothyroid patients(27–29).

Elevated levels of these markers in autoimmune thyroid disease highlight the interaction between thyroid autoimmunity and systemic inflammation(30–32). The clinical implications of this association are considerable. Chronic inflammation in hypothyroidism has been linked to dyslipidemia, atherosclerosis and increased cardiovascular risk(33–37). Patients with high anti-TPO titers may therefore represent a subset at increased risk of inflammatory complications, making antibody measurement significant not only for diagnosis but also for well risk categorization (28,31,38,39). Regardless of the acknowledged importance of anti-TPO antibodies ambiguous data exist on their quantitative relationship with thyroid hormone parameters (T3, T4, TSH) and inflammatory indicators in well-defined case control settings. comprehending these associations may offer valuable perception into the immunopathogenesis of hypothyroidism, improve risk classification and guide all inclusive management plans that address both endocrine dysfunction and systemic inflammatory activity. The present study was therefore designed to compare anti-TPO antibody levels in patients with subclinical hypothyroidism and euthyroid controls and to evaluate their relationship with thyroid hormone parameters and inflammatory markers (hsCRP, ESR). By exploring these associations, we aim to elucidate thus the role of anti-TPO antibodies as biomarkers of both thyroid-specific autoimmunity and systemic inflammation thereby augmenting their clinical relevance in the diagnosis, prognosis and management of hypothyroidism.





MATERIALS AND METHODS

This case control study was conducted on a total of 200 individuals comprising 120 patients diagnosed with hypothyroidism and 80 age and sex matched healthy controls. Participants were recruited from the outpatient clinics of the Department of Medicine.

The inclusion criteria for the SCH group were:

- Age between 18 and 50 years.
- Newly diagnosed cases of clinical and subclinical hypothyroidism. Patient defined as elevated serum thyroid-stimulating hormone (TSH) levels with normal free triiodothyronine (FT3) and free thyroxine (FT4) concentrations.
- No prior history of thyroid hormone replacement therapy or immuno-suppressive treatment.

Exclusion criteria included:

- Hyperthyroidism.
- Pregnancy or lactation.
- Presence of autoimmune diseases other than thyroid autoimmunity.
- Chronic inflammatory disorders, recent infections or any systemic illness known to influence thyroid function or inflammatory markers.
- Use of medications that could interfere with thyroid function or immune response.

Healthy controls: Healthy Controls were selected from the same demographic population matched for age and sex and confirmed to have normal thyroid function tests and no history of autoimmune or chronic inflammatory disease.

Ethical Considerations

The study protocol was reviewed and approved by the Institutional Ethics Committee (Ref. No. SMC/UECM/2024/914). Written informed consent was obtained from all participants prior to enrollment.

Sample Collection and Processing

Venous blood samples (5 mL) were collected from each participant under strict aseptic precautions using sterile vacutainers. Samples were allowed to clot at room temperature and subsequently centrifuged at 3000 revolutions per minute (rpm) for 10 minutes. The separated serum was aliquoted into sterile cryovials and stored at -20°C until further biochemical and immunological analysis.

Laboratory Investigations

Statistical Analysis

Correlation analysis performed using Pearson's correlation coefficient to assess relationships between:

- Anti-TPO and TSH
- Anti-TPO and hsCRP
- Anti-TPO and ESR

Statistical significance was defined as $p < 0.05$.

RESULTS

Comparative analysis between the case group ($n = 120$) and control group ($n = 80$) revealed significant differences across multiple biochemical and immunological markers. Serum T3 levels were comparable between groups (Case: 1.59 ± 0.61 ng/mL; Control: 1.65 ± 0.80 ng/mL; $P = 0.549$) indicating no statistically significant difference. However, T4 levels were markedly reduced in cases (81.85 ± 30.34 $\mu\text{g/dL}$) compared to controls (109.52 ± 24.61 $\mu\text{g/dL}$), with a highly significant difference ($P < 0.0001$). TSH levels were substantially elevated in the case group (43.34 ± 35.07



**Sumit Kumar Sagar and et al.,**

μIU/mL) relative to controls (2.90 ± 1.20 μIU/mL), also reaching statistical significance ($P < 0.0001$) consistent with a hypothyroid profile. Autoimmune markers showed pronounced elevation in cases. Anti-thyroid peroxidase (Anti-TPO) antibody were significantly higher in the case group (94.03 ± 21.23 IU/mL) than in controls (25.44 ± 11.56 IU/mL; $P < 0.0001$). The Inflammatory markers were also significantly raised in the case group. High-sensitivity C-reactive protein (hsCRP) levels were elevated (14.31 ± 3.49 mg/L) compared to controls (2.35 ± 1.01 mg/L; $P < 0.0001$) and erythrocyte sedimentation rate (ESR) was higher in cases (27.98 ± 5.55 mm/hr) than controls (17.4 ± 1.39 mm/hr; $P < 0.0001$) indicating a heightened systemic inflammatory response. In the case group ($n = 120$) anti-thyroid peroxidase (Anti-TPO) antibody levels were elevated with a mean of 94.03 ± 21.23 IU/mL and a median of 93.5 IU/mL, indicating autoimmune thyroid involvement. Erythrocyte sedimentation rate (ESR) values were also elevated with a mean of 27.98 ± 5.55 mm/hr and a median of 30 mm/hr, reflecting a heightened systemic inflammatory state. Pearson correlation analysis revealed a strong positive association between Anti-TPO and ESR levels ($R = 0.817$) which was statistically significant ($P = 0.000$).

DISCUSSION

Our study evaluated the association between thyroid dysfunction, autoimmune activity, and systemic inflammation in hypothyroid patients. The findings provide significant alterations in thyroid hormones, autoantibodies and general inflammatory markers in the cases in comparison to controls. Consistent with previous literature, autoimmune thyroiditis particularly Hashimoto's thyroid it is remains the leading cause of hypothyroidism in iodine-sufficient populations with anti-TPO antibodies serving as the most sensitive and specific marker of thyroid autoimmunity(3,5,6,8,12,26). Hypothyroid patients exhibited marked elevation of TSH levels and anti-TPO antibodies and reduced T4 concentrations consistent with impaired thyroid function(7,13,19,26. Significantly higher titers of anti-TPO antibodies in cases insinuate their diagnostic utility and strengthen their role in screening individuals at risk of progressive thyroid failure (11,15,25,38). In terms of thyroid hormone). Notably, T3 levels did not differ significantly between groups and may reflect compensatory mechanisms or the relatively early stage of thyroid dysfunction in subclinical hypothyroidism(21,25,26). The strong elevation of TSH alongside rise in anti-TPO highlights the classical biochemical profile of hypothyroidism and supports the clinical relevance of incorporating antibody testing for all-inclusiveanalysis15,17,18).

Our study further explored systemic inflammation in hypothyroidism. Both hs-CRP and ESR were significantly elevated in hypothyroid patients implying that thyroid autoimmunity may also contributes to systemic inflammation(1,2,4, 23,19,20,27,28).However, the correlation between anti-TPO and hsCRP was weak and not statistically significant(2,20), the strong positive correlation between anti-TPO and ESR indicates that raised antibody titers may reflect the overall inflammatory burden(3,4,23). This finding is particularly relevant given the established connection between chronic inflammation and cardiovascular risk (16,29–32,40), suggesting that hypothyroid patients with high anti-TPO titers may represent a subgroup at increased risk of inflammation related complications (10,14,31,32). The results highlight the dual role of anti-TPO antibodies not only as markers of thyroid-specific autoimmunity but also as potential indicators of systemic inflammation (8,10,20,24,25,26,28,30,38). This association point out the significance of anti TPO particularly in predicting disease progression and identifying those at risk of inflammatory comorbidities (9,12,10,26,28). Clinically these findings support the integration of anti-TPO testing with inflammatory markers such as ESR and hs-CRP to provide a more holistic assessment of disease severity (1,2,3,4,23,31).

The correlation analysis demonstrated a weak and statistically non-significant negative correlation between TSH and anti-TPO levels. This suggests that although both markers are elevated in hypothyroidism, they may reflect different aspects of disease pathology. Similarly, the very weak and non-significant negative correlation between anti-TPO and hsCRP suggest that autoimmune thyroid activity may not directly correlate with acute phase inflammatory responses measured by hsCRP. This could be due to the chronic and organ specific nature of autoimmune thyroiditis, which may not always produce a proportional systemic inflammatory response detectable by hsCRP. In contrast, a strong





Sumit Kumar Sagar and et al.,

and statistically significant positive correlation was found between Anti-TPO and ESR. This observation suggests that ESR, being a marker of sustained inflammatory processes, may better reflect the chronic immune activation seen in autoimmune thyroid disorders compared to hsCRP. Overall, the results indicate that hypothyroidism, particularly of autoimmune origin, is associated with a heightened inflammatory state. However, the relationship between autoimmune markers and inflammatory parameters appears to vary depending on the specific marker evaluated. Certain limitations must be acknowledged. Due to its cross-sectional design, it is unable to determine the causal links between antibody increase and progression of disease. Sample size, although sufficient for statistical analysis may not fully represent the range of autoimmune illness. Longitudinal studies in future may better ascertain whether increased anti-TPO levels directly contribute to systemic inflammation or predictive of hypothyroidism advancement or emergence of related autoimmune diseases. Future research should also explore the genetic analysis and cytokine profiling to get more profound understanding of immune pathogenesis of hypothyroidism.

CONCLUSION

The study highlights the complex interaction between thyroid function, autoimmunity and inflammation. While anti-TPO strongly reflect autoimmune involvement, their correlation with inflammatory markers is variable, with ESR showing a stronger association than hsCRP. These findings reinforce the diagnostic and prognostic value of anti-TPO antibodies not only as markers of thyroid-specific autoimmunity but also as potential indicators of systemic inflammatory activity. Integrating anti-TPO measurement with inflammatory indicators may enhance risk assessment and guide more comprehensive therapeutic approaches that address both thyroid dysfunction and systemic inflammation. Future long-term studies are warranted to investigate the predictive utility of anti-TPO antibodies in disease progression and to clarify their role in identifying patients at risk for comorbid inflammatory or autoimmune disorders.

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Sumit Kumar Sagar and et al.,

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Table 1: Comparison of Thyroid Hormones, Autoimmune Antibodies and Inflammatory Markers Between Hypothyroid Patients and Euthyroid Controls

Parameters	Case (n=120)		Control (n=80)		P value
	Mean $\pm$ S.D	Median	Mean $\pm$ S.D	Median	
T3	1.59 $\pm$ 0.61	1.5	1.65 $\pm$ 0.80	1.8	0.549
T4	81.85 $\pm$ 30.34	75.01	109.52 $\pm$ 24.61	112.55	<0.0001
TSH	43.34 $\pm$ 35.07	29.29	2.90 $\pm$ 1.20	2.9	<0.0001
Anti -TPO	94.03 $\pm$ 21.23	93.5	25.44 $\pm$ 11.56	25	<0.0001
hsCRP	14.31 $\pm$ 3.49	14.15	2.35 $\pm$ 1.01	2.4	<0.0001
ESR	27.98 $\pm$ 5.55	30	17.4 $\pm$ 1.39	18	<0.0001

Table2: Distribution Anti - TPO &ESR

Test Name	Case (n=120)			
	Mean $\pm$ S.D	Median	R Value	P value
Anti -TPO	94.03 $\pm$ 21.23	93.5	0.817**	0.000

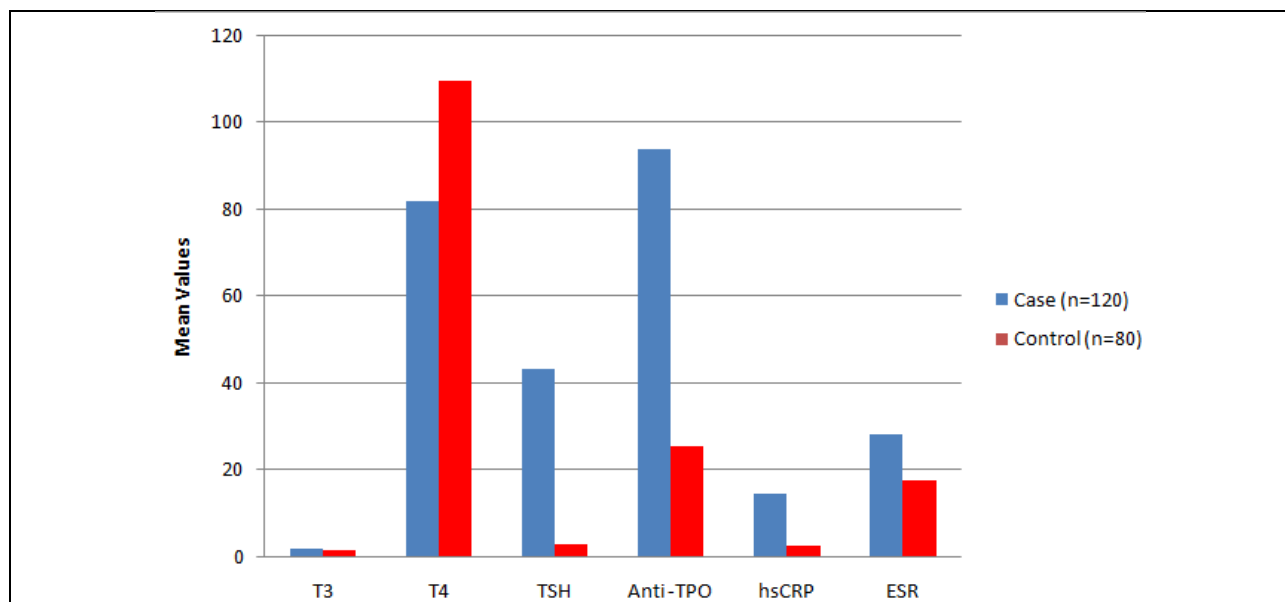
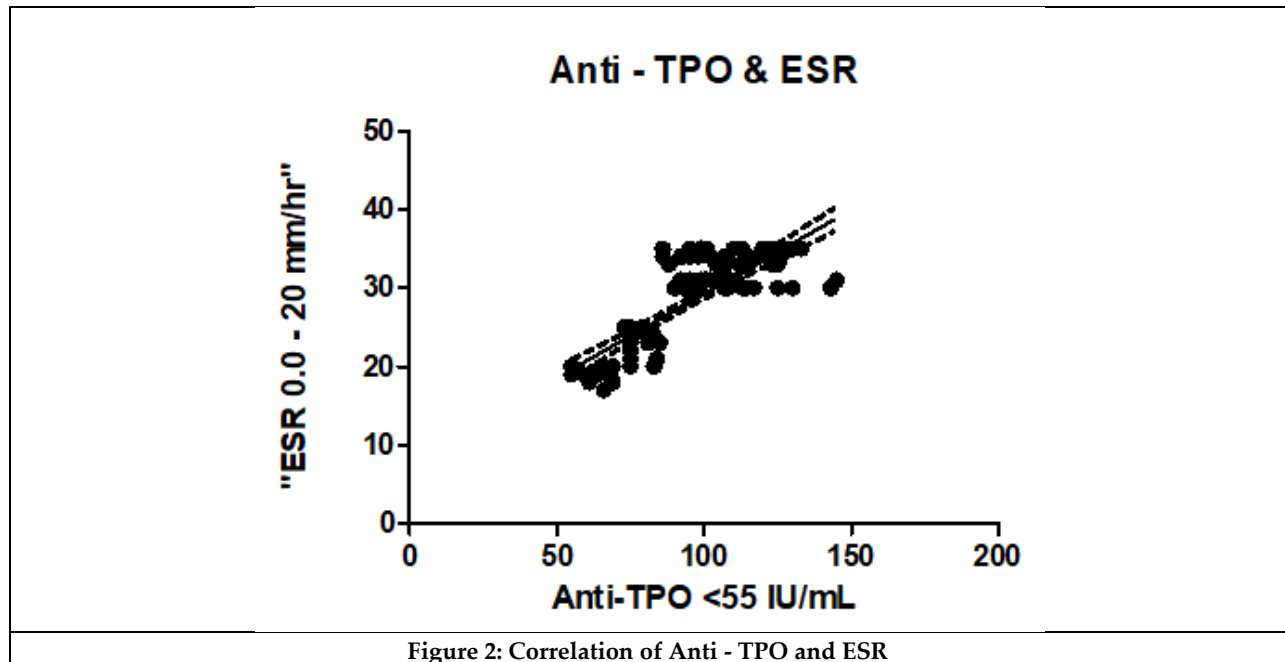


Figure 1: Bar Graph Showing Mean Levels of Thyroid Hormones, Autoimmune Antibodies, and Inflammatory Markers in Hypothyroid Cases and Euthyroid Controls





Sumit Kumar Sagar and et al.,



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RESEARCH ARTICLE

Purification, Characterization and Structural Analysis of Antimicrobial Proteins from *Scylla* Species

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ABSTRACT

Antimicrobial proteins play a crucial role in innate immunity and represent promising alternatives to conventional antibiotics. The present study focuses on the purification, characterization, sequence analysis, and structural prediction of antimicrobial proteins isolated from *Scylla* species. Antimicrobial proteins obtained from granular haemocyte extracts were purified using ion-exchange chromatography and analysed through SDS-PAGE. Further identification and characterization of proteins were carried out using tandem mass spectrometry (MS/MS) and bioinformatics tools including BLAST, ClustalW, PEP-FOLD3, and MODELLER. The purified fractions exhibited significant antimicrobial activity and distinct protein bands were observed during electrophoretic analysis. Sequence alignment and phylogenetic studies revealed evolutionary relationships among the identified proteins, while homology modelling provided insights into their three-dimensional structures and active sites. The findings suggest that antimicrobial proteins from *Scylla* species possess potential therapeutic significance and may contribute to the development of novel antimicrobial agents.

Keywords: Antimicrobial proteins, *Scylla serrata*, *Scylla olivacea*, SDS-PAGE, MS/MS analysis, Homology modelling, Bioinformatics





INTRODUCTION

The rapid emergence of multidrug-resistant microorganisms has become a major global health concern, creating an urgent need for the discovery of novel antimicrobial compounds from natural sources. Conventional antibiotics are gradually losing effectiveness due to the widespread development of resistance among pathogenic bacteria, fungi, and viruses. In this context, antimicrobial proteins and peptides (AMPs) derived from marine organisms have attracted considerable scientific attention because of their broad-spectrum antimicrobial properties, rapid mode of action, and low tendency to induce resistance (Hancock and Sahl, 2006; Yeaman and Yount, 2003). Marine invertebrates represent a rich source of biologically active compounds owing to their continuous exposure to diverse and pathogen-rich aquatic environments. Among marine organisms, crustaceans possess highly developed innate immune systems that rely primarily on cellular and humoral defence mechanisms. Unlike vertebrates, crustaceans lack adaptive immunity and depend heavily on antimicrobial proteins and peptides synthesized by haemocytes to combat microbial invasion (Smith et al., 2008). Crabs belonging to the genus *Scylla*, including *Scylla serrata* and *Scylla olivacea*, are economically and ecologically important marine crustaceans known for their robust immune responses and production of potent antimicrobial molecules.

Antimicrobial proteins isolated from crustaceans exhibit significant activity against Gram-positive and Gram-negative bacteria, fungi, and certain viruses. These proteins often contain cationic and hydrophobic amino acid residues that enable interaction with microbial membranes, ultimately causing membrane disruption and cell death (Matsuzaki, 1999). Due to these unique properties, crustacean-derived antimicrobial proteins are increasingly being explored as promising candidates for pharmaceutical and therapeutic applications. Advances in proteomics and molecular biology have greatly improved the identification and characterization of antimicrobial proteins. Techniques such as ion-exchange chromatography and SDS-PAGE are commonly used for protein purification and molecular weight determination (Laemmli, 1970). Furthermore, mass spectrometry-based approaches including tandem mass spectrometry (MS/MS) provide accurate peptide sequencing and protein identification (Tsugita et al., 2000). Bioinformatics tools such as BLAST, CLUSTALW, and MEGA facilitate sequence comparison and phylogenetic analysis, while computational modelling platforms like PEP-FOLD3 and MODELLER enable prediction of peptide conformation and three-dimensional protein structures (Altshul et al., 1990; Thompson et al., 1994; Lamiable et al., 2016).

Structural characterization of antimicrobial proteins is essential for understanding their mechanism of action, stability, and functional domains. Homology modelling and structural validation programs such as PROCHECK, VERIFY3D, and ERRAT provide reliable insights into stereo chemical quality and protein architecture (Laskowski et al., 1993; Eisenberg et al., 1997). These computational approaches significantly contribute to the development of peptide-based therapeutics and drug design strategies. Although several antimicrobial proteins have been identified from marine organisms, studies focusing on the purification and structural characterization of antimicrobial proteins from *Scylla* species remain limited. Therefore, the present study was undertaken to purify and characterize antimicrobial proteins from *Scylla serrata* and *Scylla olivacea* using chromatographic, electrophoretic, proteomic, and bioinformatics approaches. The study further aimed to analyse the sequence homology, phylogenetic relationships, and three-dimensional structures of the identified proteins in order to understand their functional and evolutionary significance. The increasing emergence of multidrug-resistant microorganisms has intensified the search for novel antimicrobial compounds from natural sources. Marine organisms, especially crustaceans, are considered valuable reservoirs of bioactive molecules due to their unique immune defence mechanisms. Crabs belonging to the genus *Scylla* possess potent innate immune systems that produce antimicrobial proteins capable of combating pathogenic microorganisms.

Antimicrobial proteins and peptides are essential components of the innate immune response in invertebrates. These molecules exhibit broad-spectrum antimicrobial activity against bacteria, fungi, and viruses. Due to their effectiveness and low probability of resistance development, antimicrobial proteins are gaining significant attention



**Prasanthi et al.,**

in pharmaceutical and biomedical research. Advances in proteomics and bioinformatics have enabled the identification and structural characterization of antimicrobial proteins. Techniques such as ion-exchange chromatography, SDS-PAGE, and mass spectrometry provide detailed information regarding protein purification and molecular characterization. Furthermore, computational tools such as BLAST, ClustalW, and MODELLER facilitate sequence comparison, phylogenetic analysis, and three-dimensional structure prediction. The present study was designed to purify and characterize antimicrobial proteins from *Scylla* species and to analyse their structural and evolutionary properties using bioinformatics approaches.

MATERIALS AND METHODS

Purification of Antimicrobial Proteins

The samples exhibiting significant antimicrobial activity were purified using ion-exchange chromatography according to the method described by Zasloff (1987). The dialyzed extract of granular hemocytes (5 mL) was loaded onto a glass column (36 × 2.8 cm) packed with carboxymethyl cellulose equilibrated with 0.2 M sodium acetate buffer (pH 4.0). Unbound proteins were washed with sodium acetate buffer until the absorbance reached baseline levels. Bound proteins were subsequently eluted using 0.2 M ammonium acetate buffer (pH 5.2). Fractions of 2 mL each were collected and monitored spectrophotometrically at 280 nm using a UV-160A Shimadzu spectrophotometer.

SDS/Polyacrylamide Gel Electrophoresis (SDS-PAGE)

One-dimensional SDS-PAGE was performed according to the method of Laemmli (1970) using a Bio-Rad vertical slab gel electrophoresis system. The stacking gel contained 4% acrylamide while the resolving gel consisted of 15% acrylamide. Protein fractions were diluted with sample loading buffer containing 20% glycerol, 1% SDS, 0.125 M Tris-HCl, 2% β-mercaptoethanol, and 0.5% bromophenol blue. The samples were heated at 100°C for 5 min and centrifuged at 10,000 ×g for 5 min. The supernatant was loaded into the wells and subjected to electrophoresis. Molecular weight markers obtained from GeNei (Bangalore) were used for calibration. The gels were stained using silver nitrate for visualization of protein bands.

MS/MS Analysis and Data Processing

MS/MS analysis was performed using Coomassie-stained protein spots corresponding to purified antimicrobial proteins. Gel pieces were rehydrated in 50 mM ammonium bicarbonate, diced into approximately 1 mm³ cubes, destained, and digested using trypsin (100 ng/sample). The digested samples were diluted with 3% acetonitrile and 0.1% formic acid. Approximately 30% of each sample was chromatographed using an Agilent Protein ID Chip column assembly connected to an HCT ultra 3D ion-trap mass spectrometer. The column was equilibrated with 4% acetonitrile and 0.1% formic acid at a flow rate of 0.5 μL/min. Samples were eluted with an acetonitrile gradient ranging from 4–31% over 32 min. Ionizable species within the range of 300–1300 m/z were trapped and fragmented through collision-induced dissociation. MS and MS/MS spectra were analysed using Bruker Daltonik Data Analysis software for peak detection and peptide identification.

Sequence Analysis

The amino acid sequences obtained from MS/MS analysis were compared against database sequences using the NCBI BLAST server (Fig 1). MASCOT MS/MS ion search analysis was performed with the following parameters:

- Enzyme: Trypsin
- Allowed missed cleavage: One
- Fixed modification: Carbamidomethylation
- Variable modifications: Oxidation (HW) and oxidation (M)
- Peptide mass tolerance: 0.5 Da
- Fragment mass tolerance: 0.5 Da
- Charge states: +1, +2, +3
- Instrument type: ESI-TRAP





Proteins with scores greater than 50 for the NCBI database and greater than 45 for the Swiss-Prot database were considered statistically significant ($p < 0.05$).

De Novo Peptide Structure Prediction

Active peptides identified from the Mascot server were subjected to de novo peptide structure prediction using the PEP-FOLD3 server (Fig 2). This server predicts peptide structures ranging from 5 to 50 amino acids using a de novo modelling approach. The predicted structures provided information regarding peptide folding, stability, and potential functional conformations.

Multiple Sequence Alignment and Phylogenetic Analysis

The identified antimicrobial protein sequences were aligned in FASTA format using ClustalW implemented in MEGA software. Phylogenetic relationships among the sequences were analysed using the neighbour-joining method. Similarities and evolutionary relationships among the proteins were inferred from the generated phylograms.

Homology Model Generation of Sequenced Proteins

Template Search and Sequence Alignment

The amino acid sequences obtained from male and female crabs of *Scylla serrata* and *Scylla olivacea* were subjected to BLASTP analysis against the Protein Data Bank to identify suitable templates for homology modelling. Templates were selected based on maximum sequence identity, high scores, and low e-values. Sequence alignment between target and template proteins was performed using CLUSTALW.

Three-Dimensional Structure Generation

Three-dimensional structures of antimicrobial proteins were generated using MODELLER software. The software employed probability density functions as spatial restraints to generate reliable protein models.

Among the generated models, the best model was selected based on:

- PROCHECK G-score
- VERIFY3D validation
- ERRAT quality assessment
- Lowest DOPE energy score

The finalized model was subjected to active site identification and molecular interaction analysis.

MODELLER Workspace Files

Search File

The search file contained the target sequence specified as qseq1. The command “mod9v1 search.py” was used to identify suitable template sequences.

Alignment File

The alignment file contained aligned target and template sequences in FASTA format (Fig 3). The command “mod9v1 malign.py” was used to verify the alignment and template structure compatibility.

Template Selection File

Potential template sequences showing significant similarity and E-values equal to zero were analysed for appropriate template selection.

Model Building File

The get-model file specified the target sequence and template structure. MODELLER automatically generated five structural models using the auto model class.

Model Evaluation File

Generated models were evaluated based on objective function values and DOPE scores to identify the most stable and energetically favourable protein structure.





RESULTS AND DISCUSSION

Purification of Antimicrobial Proteins

Ion-exchange chromatography successfully separated antimicrobial protein fractions from granular haemocyte extracts. Protein elution profiles showed distinct absorbance peaks at 280 nm, indicating successful purification of protein components. The purified fractions exhibited enhanced antimicrobial activity compared to crude extracts, suggesting the enrichment of active antimicrobial proteins.

SDS-PAGE Analysis

SDS-PAGE analysis revealed distinct protein bands corresponding to antimicrobial proteins of varying molecular weights. The presence of clearly resolved bands indicated the purity and integrity of the isolated proteins (Fig 4) . Silver staining enhanced the visualization of low-abundance protein fractions.

Mass Spectrometry and Sequence Analysis: MS/MS analysis enabled the identification of peptide sequences associated with antimicrobial activity. Database searches using BLAST and MASCOT confirmed homology with known antimicrobial proteins reported in crustaceans and other marine organisms.

The identified proteins showed significant similarity scores, indicating the conserved nature of antimicrobial proteins among marine invertebrates.

De Novo Structure Prediction

The PEP-FOLD3 server successfully generated stable peptide conformations for the identified antimicrobial peptides (Table 1). Predicted structures demonstrated characteristic α -helical and β -sheet arrangements commonly associated with antimicrobial peptides.

Phylogenetic Analysis

Multiple sequence alignment and phylogenetic analysis demonstrated evolutionary conservation among antimicrobial proteins from *Scylla* species and related crustaceans. The phylogenetic tree indicated close relationships between proteins from *S. serrata* and *S. olivacea*.

Homology Modelling

Homology modelling produced reliable three-dimensional structures for the sequenced proteins. Validation through PROCHECK, VERIFY3D, and ERRAT confirmed the stereochemical quality and structural stability of the models.

The predicted structures provided valuable insights into the functional domains and active sites involved in antimicrobial activity.

DISCUSSION

The present study successfully demonstrated the purification, characterization, and structural analysis of antimicrobial proteins from *Scylla* species, emphasizing their potential role in innate immunity and biomedical applications. Marine crustaceans are constantly exposed to a wide range of pathogenic microorganisms in aquatic environments and therefore depend on innate immune defence molecules such as antimicrobial proteins and peptides for survival. The findings of this investigation are consistent with previous reports highlighting crustaceans as important reservoirs of bioactive antimicrobial compounds (Smith et al., 2008). Ion-exchange chromatography effectively purified antimicrobial protein fractions from granular haemocyte extracts. The appearance of distinct absorbance peaks at 280 nm confirmed successful protein separation based on ionic interactions. Similar purification procedures have been employed for isolation of antimicrobial proteins from marine organisms and have proven effective in enriching biologically active fractions (Tsugita et al., 2000). The enhanced antimicrobial activity observed in purified fractions compared with crude extracts indicates successful concentration of active antimicrobial proteins during the purification process.

SDS-PAGE analysis revealed distinct protein bands of varying molecular weights, suggesting the presence of multiple antimicrobial proteins within the haemocyte extracts. The clear resolution of protein bands after silver staining indicated good purity and integrity of the isolated proteins. Previous studies have also reported that crustacean antimicrobial systems contain several low molecular weight peptides that function synergistically in



**Prasanthi et al.,**

microbial defence (Laemmli, 1970). The observed electrophoretic diversity may therefore represent different antimicrobial proteins or peptide isoforms involved in immune responses. MS/MS analysis provided detailed peptide sequence information and enabled identification of proteins associated with antimicrobial activity. Database analysis using BLAST and MASCOT revealed significant homology between the identified proteins and previously reported antimicrobial proteins from marine invertebrates. The presence of conserved hydrophobic and positively charged amino acid residues is characteristic of antimicrobial peptides involved in microbial membrane interaction and disruption (Yeaman and Yount, 2003). Such conserved structural features strongly support the antimicrobial function of the isolated proteins. Sequence alignment studies performed using CLUSTALW demonstrated conserved amino acid regions among proteins isolated from *Scylla serrata* and *Scylla olivacea*. Conserved motifs identified in the present study may play important roles in maintaining antimicrobial activity and structural stability. Similar evolutionary conservation of antimicrobial proteins has been reported among crustaceans and other marine invertebrates, indicating that these proteins are subjected to strong evolutionary pressure due to their essential defensive functions (Thompson et al., 1994).

De novo peptide structure prediction using the PEP-FOLD3 server generated stable peptide conformations characterized by α -helices and β -sheet structures. These structural motifs are commonly observed in antimicrobial peptides and are known to facilitate insertion into microbial membranes leading to membrane permeabilization and cell lysis (Lamiabile et al., 2016). The predicted structures therefore provide strong evidence supporting the membrane-active mechanism of the isolated antimicrobial proteins. Phylogenetic analysis revealed close evolutionary relationships among antimicrobial proteins from *Scylla* species and related crustaceans. The phylogenetic clustering observed in this study suggests that these proteins may have evolved from common ancestral immune proteins while preserving their antimicrobial functions. Such evolutionary conservation reflects the biological importance of antimicrobial proteins in crustacean defence systems (Koichiro et al., 2007). Homology modelling using MODELLER successfully generated reliable three-dimensional structures of the identified antimicrobial proteins. Validation using PROCHECK, VERIFY3D, ERRAT, and DOPE score analysis confirmed the stereochemical accuracy and structural stability of the predicted models (Laskowski et al., 1993; Eisenberg et al., 1997). Structural modelling also enabled identification of potential active sites and functional domains associated with antimicrobial activity. Comparative protein modelling has become an important strategy for understanding protein function and designing peptide-based therapeutics (Sali and Blundell, 1993).

The increasing prevalence of antibiotic-resistant pathogens has intensified the search for alternative antimicrobial agents from natural sources. Antimicrobial proteins from marine crustaceans possess several advantages including rapid microbial killing, broad-spectrum activity, and lower chances of resistance development compared with conventional antibiotics (Hancock and Sahl, 2006). Therefore, the antimicrobial proteins identified in the present study may serve as promising candidates for future pharmaceutical and therapeutic applications. Overall, the present investigation demonstrated that *Scylla* species are valuable sources of structurally conserved and biologically active antimicrobial proteins. The integration of chromatographic purification, proteomic analysis, sequence characterization, phylogenetic studies, and structural modelling provided comprehensive insights into the molecular properties of these proteins. Further studies involving recombinant expression, molecular docking, toxicity evaluation, and in vivo antimicrobial assays may facilitate the development of novel peptide-based antimicrobial drugs for combating multidrug-resistant pathogens.

CONCLUSION

The present study successfully purified and characterized antimicrobial proteins from *Scylla* species using chromatographic, electrophoretic, proteomic, and bioinformatics approaches. MS/MS analysis and sequence characterization confirmed the presence of potent antimicrobial proteins exhibiting conserved structural features. Bioinformatics analyses, including sequence alignment, phylogenetic studies, and homology modelling, provided significant insights into the evolutionary relationships and structural properties of these proteins. The generated





Prasanthi et al.,

three-dimensional models may facilitate further studies on protein function, molecular interactions, and therapeutic applications. The findings of this investigation highlight the potential of marine crustaceans as promising sources of novel antimicrobial agents for biomedical and pharmaceutical applications.

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Prasanthi et al.,

Table 1: 3D Protein structure

S.No	Protein Name	Missed cleavage	Peptide	Peptide structure
1	SOHP	0	HWFSLENTNR	
2	SOHP	1	KGESFFWVHHQLTVR	
3	SOHP	0	GESFFWVHHQLTVR	
4	SOHP	1	VRDMSMTEDR	
5	SOHP	2	VRDMSMTEDRIR	
6	SOHP	0	YMDNIFR	
7	SOHP	1	LNHKK	





Prasanthi et al.,

8	SOHP	0	DNNGIEYSFNEGR	
9	SOHP	0	RPHGYPLDR	
10	SOHP	1	IFEALPNFKQR	
11	SOHP	0	LYSHEGVDGG	

BLAST Search database Non-redundant protein sequences (nr) using Blastp (protein-protein BLAST)
☒ Show results in a new window

Algorithm parameters

General Parameters

Max target sequences: 100
Select the maximum number of aligned sequences to display

Short queries: ☒ Automatically adjust parameters for short input sequences

Expect threshold: 10
Adjust

Word size: 6
Adjust

Max matches in a query range: 0
Adjust

Scoring Parameters

Matrix: BLOSUM62
Adjust

Gap Costs: Existence: 11 Extension: 1
Adjust

Compositional adjustments: Conditional compositional score matrix adjustment
Adjust

Filters and Masking

Fig 1: Page for the BLAST



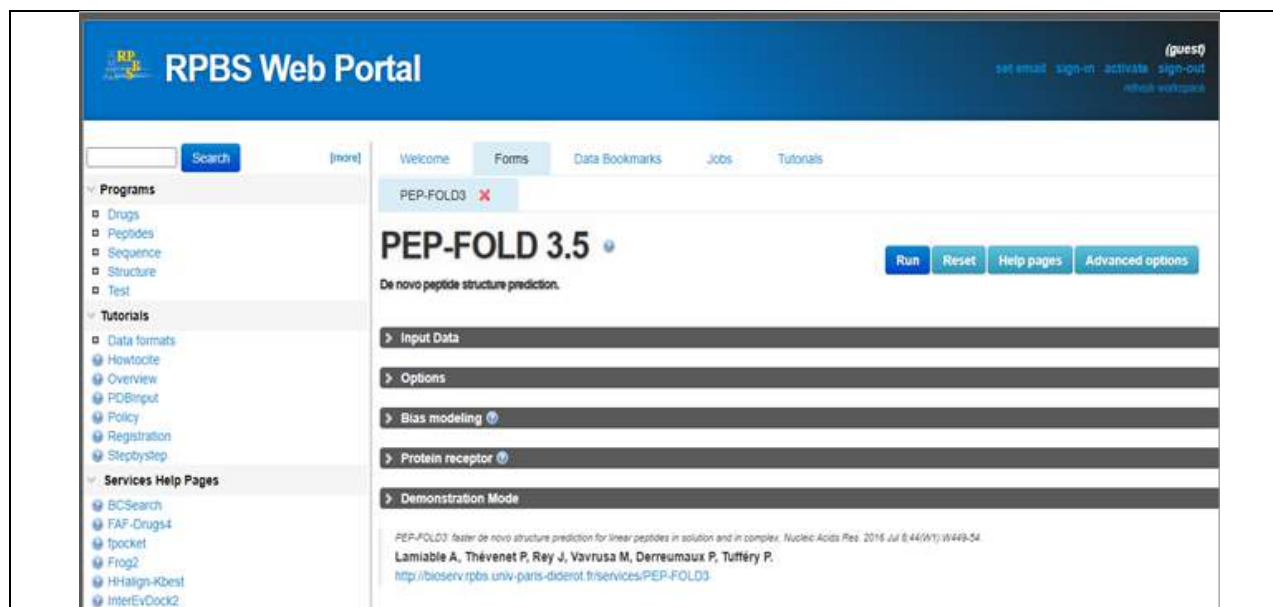


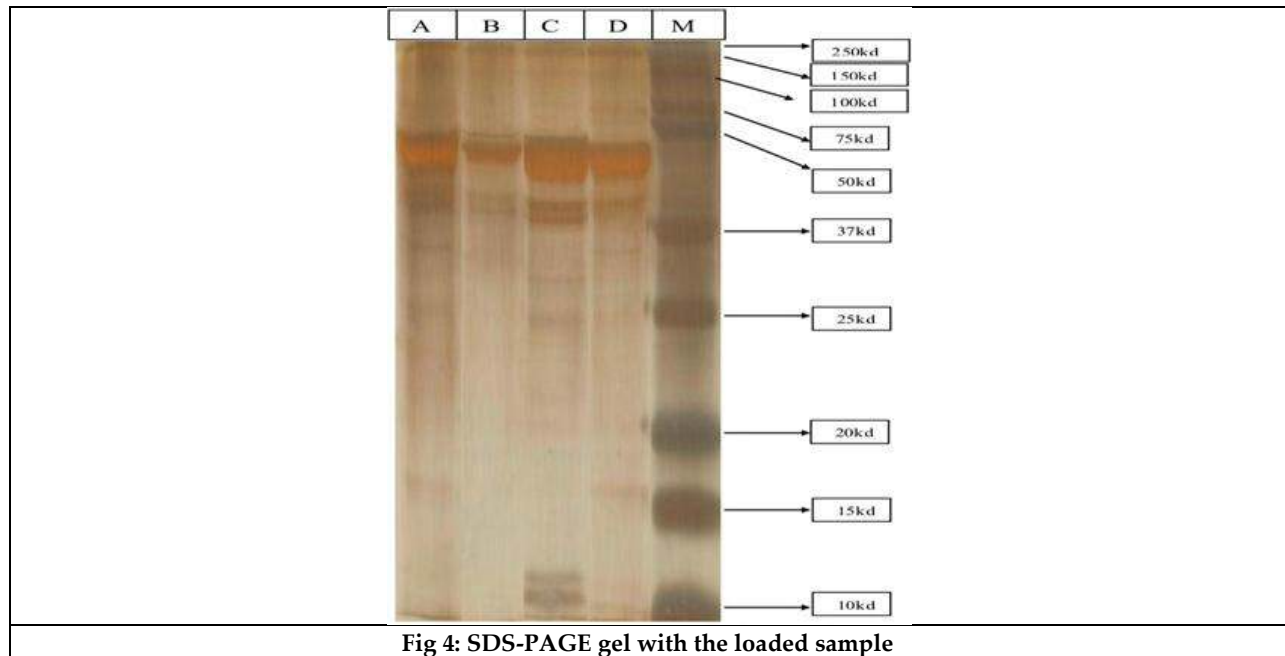
Fig 2 : Web page for the PEP-FOLD

```
>P1;tseq1
structureX:tseq1.pdb:   5 :A:+634 :A:::-1.00:-1.00
T----GN-----AQKQDINHLLDKIYEPTKYPDLKDIAENFNPLGDTSIYNDHGAAVETLMKELNDHRLLEQR
HWYSLFNTRQRKEALMLFAVLNQCKEWYCFRSNAAYFRERMNEGEFVYALYVSVIHSKLGDIPLPLYQITPHM
FTNSEVIDKAYSAMTKQPGTFNVSK----NREQRVAYFGEDIGMNIHHVTWHMDFPFWWEDSYGYHLDRKGEL
FFWVHHQLTARFDFERLSNWLDPVDELHWDRIIREGFAPLTSYKYGGEFPVRPDNIHFEDVDGVAVHVDLEITES
RIHEAIDHGYITDSGHTIDIRQPKGIELLGDIIESSKYSSNVQYYGSLHNTAHVMLGRQGDPHGKFNLPVGME
HFETATRDPSFFRLHKYMDNIFKKHTDSFPPYTHDNLEFGSMVNVGVAIDGELITFFDEFQYSLINAVDSGENIE
DVEINARVHRLNHNEFTYKITMSNNNDGERLATFRIFLCPIEDNNGITLTLEARWFCIELDKFFQKVPSPGPEI
ERSSKDSSVTVPDMPFSQSLKEQADNAV-N-G-LDLSAYERSGIPDRMLLPKSKPEGMEFNLVAVTDGDKDT-
-EGHHAQCG-VH-G--EAYPDNRPLGYPLERRIPDERVIDGVSNIKHVVKIVHH--L---*
```

```
>P1;qseq1
sequence:qseq1:      : :      : :: 0.00: 0.00
ADCQAGDSADKLLAQKHVDVNYLVYKLYGDIRDDHLKELGETFNPQGDLLLYHDNGASVNTLMADFQDGRLLQKK
HWYSLFNTRQREEALMMHRVLMNCKNWHAFVSNAAAYFRTNMNEGEYLYALYVSLIHSGLGEGVLPPLYEVTPHM
FTNSEVIHEAYKAQMTNTPSKFESHFTGSKKNPEQHVAYFGEDVGMNTHHVLWHMEFPFWWEDSSGRHLDRKGES
FFWVHHQLTVRYDAERLSNHLDPVEELSWNKAIDEGFAPHTAYKYGGYFSPRPDNIHFSDVDGVARVRDMSMTED
RIRDAIAHGYIDALDGSHIDIMNSHGIEFLGDIIESSGYSANPGFYGSLHNTAHIMLGRQGDPTGKFDLPVGLE
HFETSTRDPSFFRLHKYMDNIFREHKDSLTPYTRDELEFNGVSDISIAIEGTLETFFENFEYSLINAVDDTVDIA
DVEILTYIERLNHKKFSFLILVTNNNTEVLATVRIFAWPLRDNNGIEYSFNEGRWRALELDRFWVKVKGHHQI
TRQSTESSVTVPDVPQLTLIDRADAAISSGCALHLEDYESALGLPNRFLLPKGQAQGMFNLVAVTDGRTDAA
LDDLHENTKFIHYGYDRQYDPKRPHGYPLDRRVDDERIFEALPNFKQRTVKLYSHEGVDGG*
```

Fig 3: The alignment format of the sequence



**Prasanthi et al.,****Fig 4: SDS-PAGE gel with the loaded sample**

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RESEARCH ARTICLE

Efficient *In vitro* Regeneration of *Celastrus paniculatus* Willd. via Direct and Indirect Shoot Organogenesis and Evaluation of Genetic Fidelity using RAPD Markers

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ABSTRACT

Celastrus paniculatus Willd. is an important medicinal plant, belongs to Celstraceae family. Various plant parts like seeds, barks etc. of this plant are having great medicinal values. So, in nature this plant is overexploited for its various valuable plant parts. So, an alternative option for increasing number of plants in nature, an *in vitro* protocol has been established for large scale multiplication of *Celastrus paniculatus* Willd. through direct and indirect shoot regeneration to ensure replenishment of this important medicinal plant using various explants like leaf and nodal explants. In results of direct shoot regeneration, MS medium fortified with BAP (1.0 mg l⁻¹) + IAA (1.0 mg l⁻¹) was found to be most effective in formation of maximum shoots directly from nodal explants after 4 weeks whereas Leaf explants were used for indirect callus mediated shoot regeneration in MS medium fortified with BAP (1.0 mg l⁻¹) which was proved to be best for both callus formation and multiple shoot induction after 4 weeks. The directly and indirectly regenerated shoots were rooted well in MS medium containing NAA (2.0 mg l⁻¹) and were acclimatized with approx 60-70 percent survival rate. The genetic stability of the regenerated plants was further confirmed by using random amplified polymorphic DNA (RAPD) which found to be highly monomorphic and proved true-to-type. The significant results for regeneration are observed. Thus, an efficient protocol for rapid propagation of *C. paniculatus* generated, which can be used for large scale multiplication of true-to-type plants and also unlock the new possibilities for its conservation.

Keywords: Callus, *Celastrus*, Malkanguni, RAPD, Shoot regeneration





Dipika H. Rathod and Illa C. Patel

INTRODUCTION

Celastrus paniculatus Willd. is a perennial woody climber commonly known as Black oil plant or Malkanguni belongs to Celastraceae. In traditional system of medicine, the seeds oil is used for memory enhancement and for treatment of neural disorders and leaves and bark of plant is utilized for treatment of rheumatism, dysentery and various skin ailments. Periodically various pharmacologically active phyto-compounds viz, paniculatin, celastrol, celastrol, Celapanigin, β -amyrin and β -sitosterol have been identified from the plant extracts of *C. paniculatus* [1-2]. This plant is mainly propagated by seeds, but low seed germination capacity and poor seed viability restrict its natural population. Secondly the seeds of this plant are highly used to extract oil from it subsequently, large-scale and uncontrolled harvesting of plant parts to satisfy rising need of the pharmaceutical industry adversely affected plant survival so, to cope up with this alarming situation, a rapid, reliable and genetically stable protocol is required which may be fulfilled by *in vitro* culture technique as it was reported successful in many medicinal plant species.

The aim of this study was to develop and establish protocol for *in vitro* plant regeneration of *Celastrus paniculatus* Willd. Previously, some reports were available on *in vitro* propagation of *C. paniculatus* using different explants and approach of regeneration [3-7] and very few reports were there for genetic fidelity assessment [8-10], but proper protocol for *in vitro* multiplication is needed for conservation of this precious climber. Simultaneously, it is necessary to maintain the true-to-type nature of tissue culture raised plants, for that the genetic stability assessment of micropropagated plants is required to ensure the quality of the regenerated plants. RAPD technique has several advantages such as the ease and rapidity in analysis, relatively low cost, availability of a large number of primers and the requirement of a very small amount of DNA for analysis [11]. Some previous scientific literature revealed the genetic fidelity assessment through RAPD by Mathur *et al.*, [8], Phulwaria *et al.*, [9] and Senapati *et al.*, [10] on *in vitro* regenerated and mother plants but here in present work genetic fidelity assessment of *in vitro* raised plants with rapid and reliable protocol. Therefore, the present investigation was undertaken with these two objectives 1) to develop efficient and reproducible protocol for *in vitro* plant regeneration of *Celastrus paniculatus* Willd. through direct and indirect shoot regeneration and 2) *in vitro* raised plants were further evaluated for their genetic stability using RAPD markers.

MATERIAL AND METHODS

In vitro regeneration technique

Selection and Collection of explants

Leaf and Nodal segments with auxiliary bud were selected as explants and were collected from the climber growing on hedges of local field of Kheralu (23°53'19"N 72°36'41"E), Dist. Mehsana, Gujarat. Leaf and Nodal segments were thoroughly washed under running tap water for 15-20 min to remove adhered dust particles and rinsed thrice with sterile distilled water, then after the explants were surface sterilized with 80% ethanol (v/v) for 1 min, immersed in 0.1% (w/v) mercuric chloride under the sterile conditions for 10 min and rinsed thrice with sterile distilled water.

Media composition and culture conditions

MS medium [12] supplemented with 3% (w/v) sucrose and 0.8% (w/v) agar (Himedia, Mumbai, India) was used during the study. The medium was adjusted to pH 5.8 using 1 N NaOH and sterilized by autoclaving at 121°C and 1.06 kg cm⁻² pressure for 20 min. Cultures were incubated at 24 ± 2°C under 16:8 h light:dark photoperiod maintained through cool white fluorescent tubes with 3000 lux light intensity (Philips, India).

Direct shoot regeneration

The sterilized nodal segments were inoculated on Murashige and Skoog (1962) medium with different concentrations of BAP (6-Benzylaminopurine) or Kinetin Kin. (1.0 - 3.0 mg l⁻¹) and combinations of BAP with IAA (0.5+0.5, 1.0+0.5, 1.0+1.0 mg l⁻¹) were used for shoot bud induction and multiplication. After four weeks of inoculation, shoot bud



**Dipika H. Rathod and Illa C. Patel**

induction percentage and mean number of shoots per explant were recorded. In each treatment, 10 explants were used and the experiment was repeated thrice for reproducibility. Indirect shoot regeneration (Callus induction and Indirect Shoot formation)

Callus induction

For callus induction, leaf explants were cultured on MS medium fortified with different concentrations of 2,4-D (0.5–3.0 mg l⁻¹), NAA (0.5–1.5 mg l⁻¹), BAP (1.0–4.0 mg l⁻¹), Kinetin (1.0–3.0 mg l⁻¹) alone or combination of BAP with 2,4-D, NAA, IBA and IAA and Kin. with 2,4-D used as indicated in Table 1. Subculturing of induced callus was done by transferring it on same media composition and plant growth regulators concentrations.

Indirect Shoot formation

The green compact callus clumps (200 mg) obtained from callusing medium of each subculture and further cultured onto MS medium supplemented different concentrations of BAP (1.0–3.0 mg l⁻¹) or Kin. (0.5–2.0 mg l⁻¹) and BAP (1.0 mg l⁻¹) in combination with IAA (0.2, 0.5 1.0 mg l⁻¹) and incubated up to 10 weeks. The obtained shoot clumps were excised and sub-cultured in culture tubes (150x25 mm²) containing same medium for further growth and development. Cultures were incubated under same conditions as mentioned earlier. The data for regeneration frequency, average number of shoots produced per callus clump and length of shoot at the end of fourth week were recorded.

In vitro rooting and Acclimatization

5-7 cm long individual regenerated shoots were excised from the shoot clump and transferred to MS medium containing various concentrations of IAA, IBA, and NAA for *in vitro* root formation. After three weeks, root induction percentage, number of roots per shoot and length of the longest root were measured. Plantlets with well-developed roots were transplanted into plastic cups containing soil: coco peat at a ratio of 1:1 and incubated in culture room for 2 weeks at 27±2°C and 60-70% RH then after they were transferred into green house. The survival ratio of the micropropagated plants were recorded after five weeks of acclimatization.

Statistical analysis

The oneway analysis of variance (0.05% significance level) was carried out to analyze the recorded data for all the experiments. The significant differences between the means were further analyzed with Duncan's multiple range test [13].

Evaluation of Genetic fidelity

Three randomly selected *in vitro* raised plants from both the pathways i.e. direct and in direct shoot regeneration each were used to evaluate their genetic fidelity with that of the mother plant. For analysis of the genetic fidelity, a polymerase chain reaction (PCR) based molecular markers viz. RAPD was used. The total genomic DNA isolation from mother plant as well as the *in vitro* regenerated plants was done separately from 1 g of the young leaf tissues using the modified cetyl-trimethyl-ammonium-bromide method [14] and screened on UV-visible spectrophotometer to ensure its clarity (A260/280 ratio). For initial screening of DNA samples, The PCR amplification reaction was carried out with 31 random decamer primers in a 25 µL reaction volume consist of 10mM Tris-HCl (pH 8.3), 2.5mM MgCl₂, 1mM dNTP mix, 0.2 µM of each primer, 1U of *Taq* DNA polymerase, and 15 to 40 ng of template DNA and 13 µl of sterile water. DNA amplification was performed in a thermal cycler machine using the following PCR conditions with initial denaturation at 94°C for 60 sec, followed by 40 cycles consisted of denaturation at 94°C for 1 min, annealing at 45°C for 60 sec and extension at 72°C for 2 min and the final extension was carried out at the same temperature for 6 min.. After completion of PCR cycles, the amplicons were stored at 4°C until further use [15]. The amplified product was checked in 1.5% agarose gel electrophoresis and obtained bands were visualized in gel documentation system. Twenty RAPD primers were randomly selected for RAPD analysis, of which 10 primers were finally chosen for the clonal fidelity assessment as they produced clear and reproducible bands. Only clear and reproducible bands were scored manually, and the visible amplicons with the similar migration were considered homologous fragments.





Results and Discussion

The cultures with MS medium and devoid of plant growth regulator (PGR) failed to induce callus or shoots, whereas the MS medium fortified with PGR resulted into callus formation as well as shoot differentiation as reported by Senapati *et al.*, [16].

Direct shoot regeneration

Direct shoot regeneration was achieved directly using nodes as explants. Multiple shoots were induced from Nodal explants when inoculated on MS medium fortified with various concentrations of BAP and Kin, individually or in combination with IAA (Table I). Among all the treatments applied, MS medium supplemented with combination of BAP with IAA induced optimum number of shoots significantly in compare to other treatments. BAP with IAA ($1.0 + 1.0 \text{ mg l}^{-1}$) induced the significantly highest shoot regeneration frequency ($96 \pm 2.4\%$) with maximum number of shoots per explant (13.4 ± 0.4) and shoot length ($7.2 \pm 0.1 \text{ cm}$) (Table I). However, BAP (1.0 mg l^{-1}) alone was also gave better results for the number of shoots per explants, it was lower at the same concentration of kinetin. It was observed that as the concentration of PGRs increased the regeneration frequency, number of shoots per explants and shoot length also increased. In both the type of concentrations used, combined effects of BAP+IAA favored the highest response in terms of mean shoot number and mean shoot length. Similar results as current study includes have also been reported in *Celastrus paniculatus* [9]. Cytokinin along with auxin influences the regeneration types (direct or indirect) and also regulates the organ differentiation [12]. Similarly, the combined effect of BAP and IAA positively enhance adventitious shoot induction is reported in *Hemidesmus indicus* [17], *Aloe pruinose* [18] and *Ceropegia evansii* [19]. Cytokinins required for *in vitro* axillary shoot induction and proliferation but type of cytokinins and its optimal concentration varies with the system [20].

Indirect shoot regeneration

Callus induction was observed within two weeks of culture at the cut end of leaf explants in all the concentration tried as per Table II. 1.0 mg l^{-1} BAP produced maximum callus induction with $100 \pm 0.00\%$ of growth frequency followed by culture treated with 1.0 mg l^{-1} 2,4-D ($98.00 \pm 2.00\%$). The green, hard and compact callus was obtained on MS medium supplemented with 1.0 mg l^{-1} BAP. Subsequently, the induced callus were sub-cultured up to two passage at regular interval of 2 weeks on the same medium which resulted into proliferated mass of callus. Further, during second passage of subculturing, shoot bud regeneration was initiated and subsequently it developed into shoots only in MS medium with BAP, Kin. and combination of BAP+IAA (Table III). The highest number of shoots/explant (23.2 ± 0.20) was recorded in sole concentration of BAP (1.0 mg l^{-1}) with 100% growth response and $9.1 \pm 0.35 \text{ cm}$ shoot length. Consequently, increased concentrations of BAP showed less percent growth response, mean number and length of shoots. In the present study, lowest growth response was observed in MS fortified with combination of BAP and IAA ($1.0 + 1.0 \text{ mg l}^{-1}$). The superior effect of BAP on multiple shoot bud regeneration as well as shoot elongation in cotton was proved by Prem Kumar *et al.*, [21]. Current study shows the significant effect of BAP with different concentration on indirect shoot regeneration. The superior effect of BAP on multiple shoot bud regeneration as well as shoot elongation in cotton was proved by Prem Kumar *et al.*, [21]. Similar observations on the superiority of BAP over other cytokinins have been reported for *Psoralea corylifolia* and *Withania coagulans* shoot regeneration [22]. Similar types of results were reported by Sharada *et al.*, [23] in *Celastrus*. An earlier study also suggested that the stimulatory effects of BAP on shoot bud differentiation [4]. BA alone can influence shooting was noted by Ahmad *et al.*, [24] in *P. nigrum* is also in agreement of our recorded results.

In vitro rooting and Acclimatization

Well-developed and elongated shoots obtained either from direct or indirect shoot regeneration were subsequently transferred into rooting medium in which MS medium supplemented with different concentrations of IBA and NAA alone for root formation (Table IV). Root induction was observed from the well-developed shoots, after 9-10 days of culture, hence complete root development was occurred after 2 weeks. The percentage of root induction frequency and the average number of roots per shoot and the length of roots were significantly varied and depended upon concentration of auxins tried. Among three auxins tested, NAA (2.0 mg/l) induced highest root induction frequency ($84.00 \pm 2.44\%$) with maximum number of roots 5.2 ± 0.20 and maximum root length ($3.1 \pm 0.12 \text{ cm}$). In all concentrations



**Dipika H. Rathod and Illa C. Patel**

of NAA, first little callusing was observed at basal portion of shoots. Whereas, the lowest root induction was found in 1.0 mg/l IBA with 62±2.00% growth frequency. Roots were failed to grow in both the concentrations of IAA (0.5-1.0 mg/l). Hence, from the present study it was observed that the effect of NAA was better than the other auxins in this plant species. From the present study it was observed that the effect of NAA was better than the other auxins in this plant species. Similarly, NAA is more effective for rooting as in many other plant species were reported earlier, *Tinospora cordifolia* [25], *Gymnemasylvestre* [26], *Sesamum indicum* [27], *Rauwolfia serpentine* [28], Rooting in *Celastrus* was initially found difficult but ideal dosage of NAA in MS media promoted profuse rooting in *in vitro* developed shoots after 12 days.

Evaluation of Genetic fidelity

The genomic DNA extracted from *in vivo* and *in vitro* leaf from direct and indirect shoot regeneration of *Celastrus paniculatus* Willd. was further used for RAPD analysis for testing genetic fidelity of mother plants and randomly selected *in vitro* raised plants. The initial screening process using 20 primers out of which depending upon band reproducibility and clarity 8 primers were finally selected which produced 70 numbers of total bands and are obtained in similar manner in randomly collected samples obtained from both indirect and direct pathways. All the 8 primers produced monomorphic bands which confirming the genetic stability among regenerated plants from either directly or indirectly. Figure 2 represents RAPD profile obtained using primer OPC-4. The highest number of bands (12) were observed in primer OPC-4 (5' GTGAGGCGTC 3') with 412-1965bp amplification range, while primer OPA-1 (5' ACCCCCGAAG 3') produced only 5 scorable bands with 412-1965bp amplification range (Table VI, Figure 2). The highest number of bands (12) were observed in primer OPC-4 (5' GTGAGGCGTC 3') with 412-1965bp amplification range, while primer OPA-1 (5' ACCCCCGAAG 3') produced only 5 scorable bands with 412-1965bp amplification range (Table VI, Figure 2). Similarly, Anusha *et al.*, 2015 reported genetic stability assessment of *in vitro* raised clones of *Celastrus paniculatus* by using RAPD analysis. As the RAPD technique is simple as well as cost effective, so, it has been widely used by many researchers in various plant species in *Viola patrinii* [29], in *Stevia rebaudiana* [30] and in *Tecomella undulata* [31].

CONCLUSION

A reliable *in vitro* regeneration system for *C. paniculatus* was developed using both direct and callus-mediated shoot induction, accompanied by an evaluation of clonal fidelity. The established regeneration strategy demonstrates strong potential for large-scale propagation of this valuable medicinal species, although further optimization is required for commercial multiplication and successful acclimatization under field conditions. Assessment of genetic uniformity in regenerated plants is crucial to ensure the maintenance of true-to-type characteristics essential for commercial cultivation. Overall, the findings of this study offer a practical solution to support the growing pharmaceutical demand for consistent and high-quality plant-based raw materials.

Abbreviations

MS: Murashige and Skoog; BAP: 6-N-Benzylaminopurine; 2,4-D: 2,4-Dichlorophenoxyacetic acid; NAA: Naphthalene Acetic Acid, IAA- Indole Acetic Acid, IBA - Indole Butyric Acid, RAPD- Random Amplified Polymorphic DNA

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Conflict of interest

Conflict of interest declared none.





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Dipika H. Rathod and Illa C. Patel

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Table 1 - Effect of Plant growth regulators on direct shoot formation from node explants of *Celastrus*

Plant growth regulators (mg/L)			% Growth frequency	Mean No. of shoots Mean±SE	Mean shoot length (cm) Mean±SE
BAP	Kin.	IAA			
1	-	-	86.00±2.45 ^a	11.4±0.40 ^b	7.28±0.17 ^a
2	-	-	70.00±3.17 ^{ab}	8.4±0.24 ^c	5.54±0.12 ^b
3	-	-	56.00±2.45 ^{bc}	7.8±0.37 ^c	3.6±0.06 ^d
-	1	-	38.00±2.00 ^c	4.0±0.31 ^d	5.44±0.14 ^b
-	2	-	40.00±3.17 ^c	2.4±0.24 ^e	3.0±0.16 ^e
0.5	-	0.5	34.00±2.45 ^c	3.2±0.20 ^{de}	4.08±0.12 ^c
1.0	-	0.5	78.00±3.75 ^{ab}	7.8±0.37 ^c	5.44±0.06 ^b
1.0	-	1.0	96.00±2.45 ^b	13.4±0.40 ^a	7.2±0.10 ^a

Note: Mean values within a column followed by different letters are significantly different as determined according to Duncan's multiple range test (DMRT) at $p < 0.05$

Table 2 - Effect of Plant growth regulators on callus formation from Leaf explants of *Celastrus*

Plant growth regulators (mg l ⁻¹)						Days to initiate callus	% Growth frequency	Callus characteristics
2,4-D	NAA	BAP	Kin.	IAA	IBA			
0.5	-	-	-	-	-	13	48.00±3.74 ^{fg}	Whitish, spongy
1	-	-	-	-	-	12	98.00±2.00 ^a	Light brownish, bspongy
2	-	-	-	-	-	9	82.00±2.00 ^{bc}	Light brownish, spongy,
3	-	-	-	-	-	10	60.00±3.16 ^{de}	Creamish, spongy
-	0.5	-	-	-	-	11	52.00±2.00 ^{ef}	brownish, spongy
-	1	-	-	-	-	10	64.00±4.00 ^d	Whitish, spongy





Dipika H. Rathod and Illa C. Patel

-	1.5	-	-	-	-	12	30.00±3.16 ^{ghi}	Creamish, spongy
-	-	1	-	-	-	8	100.00±0.00 ^a	Greenish, hard, compact
-	-	2	-	-	-	9	84.00±2.45 ^b	Greenish, hard, compact
-	-	3	-	-	-	11	60.00±3.16 ^{de}	Greenish, hard, compact
-	-	4	-	-	-	12	38.00±4.90 ^{hi}	Greenish, hard, compact
-	-	-	1	-	-	15	58.00±3.74 ^{de}	brownish, hard, compact
-	-	-	2	-	-	12	64.00±2.45 ^d	brownish, hard, compact
-	-	-	3	-	-	11	38.00±2.00 ^{gh}	brownish, hard, compact
0.2	-	1	-	-	-	15	16.00±2.45 ^l	Brownish, spongy
0.5	-	1	-	-	-	14	20.00±3.16 ^{jk}	Brownish, spongy
-	-	0.5	-	0.5	-	18	16.00±2.45 ^l	Brownish, hard
-	-	0.5	-	1.0	-	15	32.00±2.00 ^{ij}	Brownish, hard
-	-	0.5	-	2.0	-	15	20.00±6.32 ^{jk}	Brownish, hard
-	-	1	-	0.5	-	15	22.00±4.90 ^{jk}	Brownish, hard
-	-	1	-	1	-	12	42.00±2.00 ^{gh}	Brownish, hard
-	-	1	-	2	-	12	26.00±2.45 ^{jk}	Pale green, hard
-	-	2	-	0.5	-	12	12.00±2.00 ^{kl}	Brownish, hard
-	-	0.5	-	-	0.5	11	46.00±5.10 ^{fgh}	Pale brownish, hard, compact
-	-	1	-	-	0.5	15	54.00±4.00 ^{ef}	Pale brownish, hard, compact
-	0.2	0.5	-	-	-	14	52.00±4.90 ^{ef}	Brownish, soft
-	0.2	1	-	-	-	9	70.00±3.16 ^c	Brownish, soft
1	-	-	0.5	-	-	14	22.00±3.74 ^{kl}	Brownish, soft
1	-	-	1	-	-	12	40.00±3.16 ^{fgh}	Brownish, soft
1	-	-	1.5	-	-	14	16.00±2.45 ^{kl}	Brownish, soft

Table 3. Effect of Plant growth regulators on indirect shooting from leaf callus of *Celastrus*

Plant growth regulators (mg l ⁻¹)			% Growth frequency	Mean No. of shoots Mean ± SE	Mean shoot length (cm) Mean ± SE
BAP	Kin.	IAA			
1			100±0 ^a	23.2±0.20 ^a	9.1±0.35 ^a
2			90±3.17 ^b	12.6±0.40 ^b	7.16±0.39 ^b
3			82±2.00 ^{bc}	8.6±0.40 ^c	4.26±0.59 ^d
	0.5		54±4.01 ^e	4.6±0.24 ^e	2.5±0.23 ^e
	1		68±3.75 ^d	6.8±0.37 ^d	4.54±0.19 ^d
	2		78±2.00 ^c	9.0±0.31 ^c	5.9±0.18 ^c
1		0.2	54±4.01 ^e	7.4±0.40 ^d	6.18±0.26 ^c
1		0.5	46±2.45 ^e	4.4±0.24 ^e	4.04±0.16 ^d
1		1	30±3.17 ^f	2.2±0.20 ^f	2.46±0.18 ^e

Note: Mean values within a column followed by different letters are significantly different as determined according to Duncan's multiple range test (DMRT) at $p < 0.05$





Dipika H. Rathod and Illa C. Patel

Table 4 - Effect of Plant growth regulators on *in vitro* rooting of *Celastrus*

Plant growth regulators (mg l ⁻¹)		% Growth frequency	Mean No. of Roots	Mean shoot length (cm)
NAA	IBA			
1		72±2.00 ^b	3.0±0.31 ^b	2.16±0.05 ^b
2		84±2.44 ^a	5.2±0.20 ^a	3.1±0.12 ^a
	0.5	-	-	-
	1	62±2.00 ^c	1.6±0.24 ^c	2.02±0.07 ^b
	2	-	-	-

Note: Mean values within a column followed by different letters are significantly different as determined according to Duncan's multiple range test (DMRT) at $p < 0.05$

Table 5 - Primer used for RAPD analysis

Sr. No.	Primer code	Primer sequence
1	OPA-1	CAGGCCCTTC
2	OPA-7	GAAACGGGTG
3	OPA-9	GGGTAACGCC
4	OPB-3	CATCCCCCTG
5	OPB-6	TGCTCTGCCC
6	OPC-4	GTGAGGCGTC
7	OPC-2	CCGCATCTAC
8	OPC-5	GATGACCGCC

Table 6 - List of primers, their sequences, number and size of the amplified fragments generated by random amplified polymorphic DNA (RAPD) primers.

Sr. No.	Primer	Sequence (5'-3')	Total amplified Bands	No. of Monomorphic Bands	No. of Polymorphic Bands	Amplification range (bp)
1	OPA-1	CAGGCCCTTC	6	4	1	254-542
2	OPA-7	GAAACGGGTG	7	7	0	293-1930
3	OPA-9	GGGTAACGCC	11	7	4	273-1521
4	OPB-2	CATCCCCCTG	3	2	1	395-582
5	OPB-6	TGCTCTGCCC	7	5	2	245-620
6	OPC-4	GTGAGGCGTC	12	11	1	412-1965
7	OPC-2	CCGCATCTAC	7	5	2	312-854
8	OPC-5	GATGACCGCC	7	6	1	281-1253





Dipika H. Rathod and Illa C. Patel

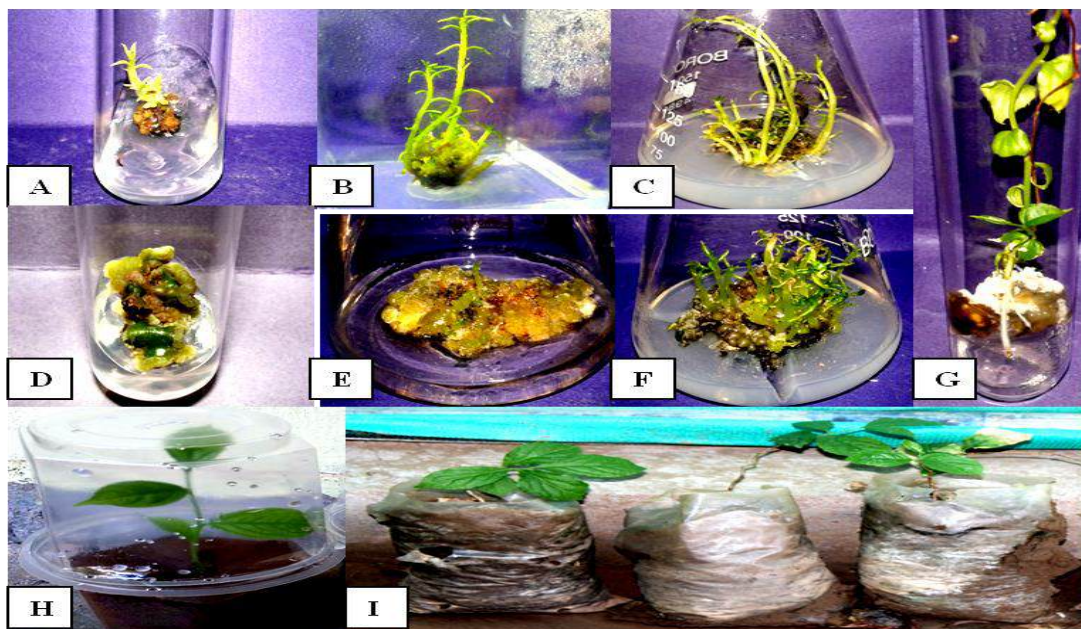


Fig. 1 Micropropagation of *Celastrus paniculatus* via Direct (A-C) and indirect shoot regeneration (D-F), Root formation and acclimatization (G-I)

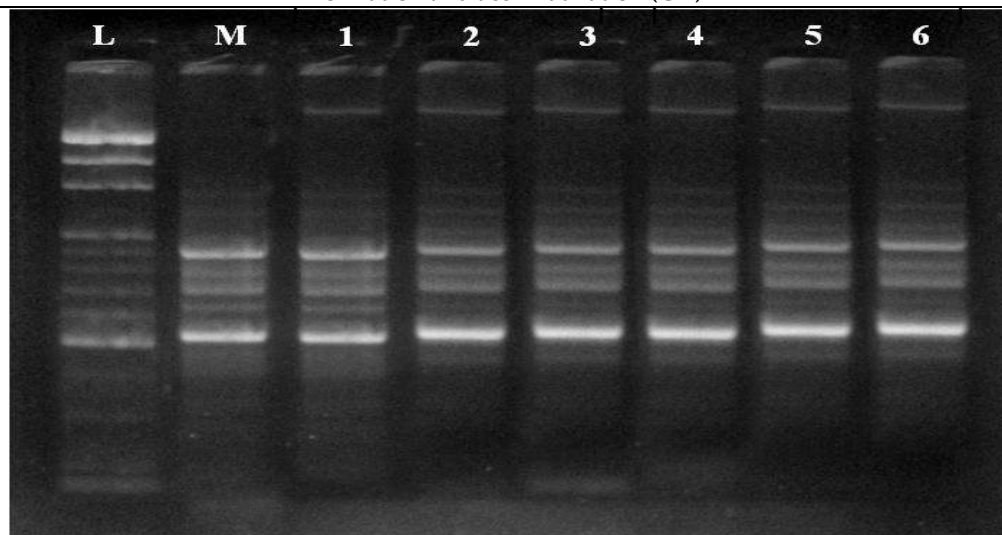


Fig. 2 RAPD amplification profile of the *in vitro* raised plants compared with the mother plant generated with primer OPC-4. L- Ladder, M- Mother plant, 1-6 – *in vitro* plant

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RESEARCH ARTICLE

Management of *Sitophilus granarius* by *Piper nigrum* and *Coriandrum sativum* Seeds in Stored Wheat *Triticum aestivum*

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ABSTRACT

The infestation of stored grains by insect pests such as *Sitophilus granarius* (wheat weevil) leads to significant post-harvest losses worldwide. This study evaluates the efficacy of botanical powders derived from *Coriandrum sativum* (coriander seeds) and *Piper nigrum* (black pepper) as eco-friendly alternatives to synthetic pesticides for controlling wheat weevils. Mortality rate assessments were conducted by exposing *S. granarius* to different concentrations of the plant materials under controlled conditions. Phytochemical screening of the samples revealed the presence of bioactive compounds such as alkaloids, flavonoids, phenolics and essential oils, which are known for their insecticidal properties. The results demonstrated that both *Coriandrum sativum* and *Piper nigrum* exhibited significant insecticidal effects, with increasing concentrations leading to higher mortality rates of *S. granarius*. The findings suggest that these botanicals can serve as sustainable, biodegradable and cost-effective alternatives for the management of wheat weevil infestations in stored grains.

Keywords: *Sitophilus granarius* (wheat weevil), *Coriandrum sativum* (coriander seeds) and *Piper nigrum* (black pepper), Phytochemical screening, Mortality rate)





Rosaline Mary and Priyanka

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most important and widely cultivated cereal crops in the world. Among the various species of the genus *Triticum*, common wheat (*T. aestivum*) is the most extensively grown and consumed (Suryawanshi *et al.*, 2020). Wheat belongs to the grass family Poaceae (Gramineae) and is popularly known as the “King of Cereals” due to its wide adaptability, high yield potential, and multipurpose use. Stored grain insect pests are a major cause of post-harvest losses worldwide, posing serious threats to food security. According to Phillips and Throne (2010), losses due to stored grain insect pests range from about 9% in developed countries to 20% or more in developing countries. In India, nearly 20–25% of food grains are destroyed annually during storage owing to insect pest infestation (Sujeetha *et al.*, 2014). Insects belonging to the orders Coleoptera and Lepidoptera account for maximum post-harvest losses (Sallam *et al.*, 2013). Grain weevils of the genus *Sitophilus* are particularly destructive, with *S. oryzae* and *S. zeamais* prevalent in warmer regions, while *S. granarius* is confined to temperate regions (Sengupta *et al.*, 1984; Thomas *et al.*, 2015). Plant-based powders derived from different plant parts are widely used as eco-friendly insecticides for the management of stored-grain insect pests. Studies have shown that red and black pepper powders effectively suppress major storage pests such as *Sitophilus granarius* and *Rhyzopertha dominica* in stored wheat, causing significant mortality of both immature and adult stages within 15 days (Ashouri and Shayesteh, 2010; Soujanya *et al.*, 2016). One alternative control method is the use of plant extracts which favour natural enemies and necessary for the biological balance. Although the effectiveness of botanical pesticides had already been established by many researchers, the understanding of the use of botanical pesticides in storage pest management systems is limited in most parts of the world Bezabih, (2022).

Objectives

- To find out the mortality rate of the wheat weevil *Sitophilus granarius* by using *Coriandrum sativum* (Coriander seeds) and *Piper nigrum* (Pepper powder).
- To test the Phytochemicals, present in the samples.
- To study the efficacy of the botanicals for the management of the wheat weevil *S. granarius*.

MATERIALS AND METHODS

A laboratory culture of wheat weevil *Sitophilus granarius* was maintained in the Laboratory of the Department of Zoology, Nirmala College for Women, Coimbatore. The weevils were reared on pesticide free whole wheat grains under controlled conditions of $28 \pm 1^\circ\text{C}$ temperature and $70 \pm 5\%$ relative humidity. Approximately 200 adults were introduced into one-liter jars containing 500 grams of wheat grains. The jars were sealed with muslin cloth to ensure aeration while preventing insect escape. After a two-week oviposition period, parent insects were removed and the infested grains were retained for subsequent culture. Adults emerging four weeks postoviposition were collected and used for experimental purposes to maintain uniformity in age and physiological status.

Rearing of Test Insect - *Sitophilus granaries*

Adults of *Sitophilus granarius* – wheat weevil species were collected from the laboratory culture. 10 insects of *S. granarius* were collected from the stock and released into separate container which is containing wheat *Triticum aestivum*. The wheat weevil, scientifically known as *S. granarius*, was cultured in a controlled laboratory setting using wheat as their food. The lab conditions were maintained at a steady temperature of $27 \pm 1^\circ\text{C}$ and a humidity level of $70 \pm 1\%$ RH. To start the rearing process, we placed adult beetles from infested grains into plastic containers with disinfected wheat. Once these adult weevils laid their eggs, we took them out of the containers. The newly hatched weevils were then released onto fresh wheat plants for mating and laying more eggs. This whole cycle was repeated every 15 days to ensure we always had different stages of the insects available for our research.



**Rosaline Mary and Priyanka****Collection of Plant materials**

The *Piper nigrum* was collected from my native place market Coonoor and was shade dried for 1 day, then powdered for experiment. The *Coriandrum sativum* L. was collected in and around my house and was shade dried for 1 day, powdered and stored for the experimental purpose.

Preparation of Plant Extracts

Plant materials of *Coriandrum sativum* - Coriander seeds and *Piper nigrum* Pepper powders were prepared. Each sample (25 grams) was subjected to double extraction with 300 ml of distilled water at room temperature for 48 hours. The extracts were filtered through Whatman's filter paper. The extracts were used for analysis.

Phytochemical Analysis

Phytochemical analysis is the qualitative and quantitative study of bioactive secondary metabolites (alkaloids, flavonoids, saponins, etc.) in plants, crucial for drug discovery, pharmacology and validating traditional medicine.

Statistical Analysis -The data were statistically analyzed for Linear Multiple regression model and ANOVA by SPSS Package.

RESULTS

The following were the results of the data obtained from our study trials by *Piper nigrum* and *Coriandrum sativum* powders in the management of *Sitophilus granarius* in stored wheat *Triticum sativum*. The multiple linear regression analysis indicated a favorable correlation between pepper powder administration and the mortality rate of *S. granarius* in stored wheat during all observation periods. The regression values rose consistently from Day 1 ($\beta = 0.190$) to Day 5 ($\beta = 0.385$), signifying an escalating mortality response with extended exposure period. (Table-1a). On Day 1, the impact of pepper powder on mortality was positive however statistically insignificant ($t = 0.811$). Likewise, Day 2 exhibited a non-significant positive correlation ($\beta = 0.230$; $t = 0.218$). On Day 3, the regression coefficient rose ($\beta = 0.264$), although the effect remained statistically non-significant ($t = 0.478$). By Day 4, the impact of pepper powder on mortality became increasingly evident ($\beta = 0.332$) and neared statistical significance ($t = 1.994$). On Day 5, the peak regression coefficient was observed ($\beta = 0.385$) and the effect was statistically significant ($t = 2.28$).

The regression analysis reveals a temporal escalation in the mortality of *S. granarius* subsequent to pepper powder treatment, implying a cumulative toxic effect with extended exposure. The absence of notable effects in the early days may indicate delayed physiological reactions or insufficient initial exposure to the botanical chemical. The nearly significant effect noted on Day 4 and the statistically significant effect on Day 5 indicate that prolonged exposure is essential for pepper powder to demonstrate efficient insecticidal properties. This trend aligns with prior research on botanical insecticides, which typically exhibit delayed toxicity resulting from slow penetration, feeding deterrent or disruption of metabolic processes rather than instant fatality. The data indicate that pepper powder may serve as an efficient botanical control agent against *S. granarius* in stored wheat, especially with enough exposure length. Additional research is advised to investigate dosage optimization, mechanisms of action, and long-term efficacy under storage conditions. The ANOVA demonstrated that the regression model accounted for all observed variation in the mortality of *S. granarius* after treatment with pepper powder ($SS = 189.20$). Nevertheless, the remaining degrees of freedom were null, inhibiting the calculation of experimental error. (Table-1b). The lack of residual variance indicates that the model might be over-parameterized or reliant on aggregated data.

The mortality response of *Sitophilus granarius* in wheat treated with pepper powder was analyzed using a multiple linear regression model over five exposure periods (Table-2). The model results indicated a positive correlation between exposure length and insect mortality across all days, demonstrating that pepper powder consistently affected mortality over time. The extent and statistical significance of the effect fluctuated throughout different days.



**Rosaline Mary and Priyanka**

Day 3 had a robust and statistically significant impact on mortality ($\beta = 2.01$; $t = 3.33$), indicating that the insecticidal efficacy of pepper powder peaked after three days of exposure. The effects noted on Days 1 and 2 were minimal and statistically insignificant, suggesting restricted acute toxicity. Likewise, Days 4 and 5 did not demonstrate substantial increases in mortality, indicating that the mortality response plateaued after the peak effect on Day 3. (Table-2a).

The data indicate that pepper powder functions as a delayed-action botanical insecticide, necessitating adequate exposure duration to achieve its optimal lethal impact on *S. granarius*. The lack of notable mortality increases beyond Day 3 suggests that prolonged exposure does not significantly improve efficacy. The regression results underscore the significance of exposure duration in assessing the efficacy of pepper powder, pinpointing the third day as the crucial timeframe for attaining maximum mortality in *S. granarius* under laboratory circumstances.

The ANOVA findings demonstrate that the regression model accounts for the complete variation in the mortality rate of *S. granarius*, evidenced by the regression sum of squares equaling the total sum of squares ($SS = 98.800$). This indicates an ideal alignment of the model with the observed data. (Table-2b). Nonetheless, the residual sum of squares equals zero, accompanied by zero residual degrees of freedom ($df = 0$). Consequently, the F-value could not be calculated, and the statistical significance of the model cannot be formally assessed using the F-statistic. This result signifies that the quantity of predictors in the model matches the number of data, leading to an over-fitted or saturated model. Although the model seemingly accounts for 100% of the variation in mortality, the lack of residual degrees of freedom hinders reliable inferences about the overall relevance of the model. The impact of coriander seed powder on the mortality of *Sitophilus granarius* in wheat was assessed utilizing a multiple linear regression model throughout five exposure durations (Table :3). The regression coefficients revealed a positive correlation between exposure length and insect mortality across all days, illustrating the efficacy of coriander seed powder as a botanical insecticidal treatment.

Day 5 demonstrated a statistically significant impact on mortality ($\beta = 2.00$; $t = 2.68$), suggesting that extended exposure substantially intensified the harmful effects of coriander seed powder. Despite the observation of somewhat high beta values on Days 1 and 4, their associated t-values were below the threshold for statistical significance, indicating that these effects lacked the requisite strength to independently affect mortality. The effects noted on Days 2 and 3 were rather mild and statistically insignificant, suggesting minimal mortality during the initial exposure phase. (Table-3a). The ANOVA results demonstrate that the multiple linear regression model elucidating the mortality of *Sitophilus granarius* in wheat treated with coriander seed powder is statistically significant. The regression sum of squares ($SS = 294.800$) encompassed the total variability in mortality, whereas the residual sum of squares was zero, signifying an exemplary model fit. The calculated F-value of 21.25 further substantiates the significance of the regression model and indicates that the predictor variables collectively have a substantial impact on insect mortality. (Table-3b). The elevated mean square value for regression ($MS = 98.267$) in contrast to the minimal residual mean square indicates that exposure time substantially accounts for variability in death rates.

The results corroborate the model coefficients, indicating heightened mortality with extended exposure to coriander seed powder, especially during the later phases of treatment. The ANOVA results substantiate the assertion that coriander seed powder serves as an efficacious botanical pesticide, with mortality significantly influenced by exposure length. The regression analysis indicates that exposure time significantly influences the efficacy of coriander seed powder, with peak mortality recorded on the fifth day of treatment. Multiple linear regression analysis was utilized to assess the impact of coriander seed powder on the mortality of *Sitophilus granarius* in wheat during five exposure periods (Table-4). The model coefficients demonstrated varying impacts of exposure length on insect mortality. A statistically significant positive effect was noted on Day 4 ($\beta = 2.00$; $t = 2.422$), demonstrating that coriander seed powder significantly increased mortality following four days of exposure. Day 5 exhibited a positive beta coefficient ($\beta = 1.00$); nevertheless, the effect lacked statistical significance, indicating a moderate rise in mortality without more escalation. The effects noted on Days 1 and 3 were favorable yet statistically insignificant, suggesting minimal early and mid-stage toxicity. Conversely, Day 2 displayed a negative beta coefficient ($\beta = -1.00$), indicating a transient decrease or variation in mortality response. The low absolute t-value suggests that this impact was



**Rosaline Mary and Priyanka**

statistically insignificant and may result from biological variability or experimental noise rather than a genuine antagonistic effect.

The regression results indicate that coriander seed powder has a delayed and stage-specific insecticidal effect, with notable mortality observed after four days of treatment. The data indicate that adequate exposure period is crucial for the efficient management of *S. granarius* utilizing coriander seed powder under storage circumstances. (Table-4a). The ANOVA results indicate that the multiple linear regression model elucidating the mortality of *Sitophilus granarius* in wheat treated with coriander seed powder accounted for all variability in mortality, as evidenced by the regression sum of squares equaling the total sum of squares ($SS = 162.000$). The residual sum of squares was 0, with no residual degrees of freedom, signifying an impeccable fit of the model to the observed data. (Table-4b). None the less, because to the lack of residual degrees of freedom, the F-value could not be calculated and the overall statistical significance of the regression model could not be formally assessed. This result indicates that the model is saturated, presumably due to the number of predictor variables matching the number of observations. The ANOVA results indicate that exposure length entirely accounts for the difference in *S. granarius* mortality under coriander seed powder treatment; nevertheless, caution is needed in interpreting these data. The absence of residual variance constrains inferential validity and underscores the necessity for further observations or replications to facilitate accurate calculation of experimental error and rigorous statistical analysis. The positive Phytochemical test results indicate that both coriander powder and pepper powder contain carbohydrates and flavonoids, suggesting their role as sources of energy and bioactive antioxidant compounds. (Table-5). The presence of proteins with aromatic amino acids in coriander powder indicates its nutritional value. The detection of anthraquinones and saponins in pepper powder indicates that pepper possesses additional bioactive constituents, which may contribute to its medicinal properties such as antimicrobial, digestive, and therapeutic activities. Overall, the results indicate that pepper powder exhibits a richer profile of secondary metabolites compared to coriander powder, while both samples show nutritional and functional importance.

DISCUSSION

Phytochemical analysis of the *Coriandrum sativum* extract revealed several active compounds, including 1,8-cineole, linalool, α -terpineol acetate, butanoic acid and coveacin, which were detected in significant proportions relative to other constituents. The observed insecticidal activity of *C. sativum* may be attributed to these bioactive compounds and their fatty acid derivatives, as also noted by Park *et al.*, (2003). Research showed that pepper powder had significant contact toxicity and oviposition deterrent activities against *Sitophilus oryzae* and could kill insects effectively in 5-7 days at certain concentrations, (Kalpanadevi and Sumithra Devi, 2013). Islam *et al.*, (2009) reported that the seeds, oils and extracts of *Coriandrum sativum* possessed insecticidal property against stored grain insect pests but in the present study it was proved to be the least effective among the other treatments resulting in 38.16% reduction in grain weight loss. A similar observation was made for the activity of hydroethanolic extracts. However, aqueous extracts were low in these phytochemicals making water an inappropriate solvent for extraction of polyphenols and flavonoids, (Azmir *et al.*, 2013).

CONCLUSION

The present study reveals that *Piper nigrum* and *Coriandrum sativum* powders possess important insecticidal and repellent assets against *Sitophilus granarius*, highlighting their potential as effective botanical substitutes for stored-grain pest management. Treatments derived from these plants reduced adult survival and infestation levels, indicating their ability to interrupt the life cycle and feeding activity of the pest. Compared to synthetic insecticides, the use of *P. nigrum* and *C. sativum* offers returns such as biodegradability, lower environmental impact and reduced risk of chemical residues in food grains. Overall, these findings support the integration of plant-based insect control





Rosaline Mary and Priyanka

agents into sustainable and eco-friendly grain protection approaches, while further research on formulation, dosage optimization and long-term effectiveness is recommended for practical application.

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Table:1 First trial of the mortality rate of *Sitophilus granarius* in wheat by *Piper nigrum* powder

Day	Concentration in grams				
Day 1	1	1	2	4	5
Day 2	1	2	2	5	6
Day 3	2	3	3	5	6
Day 4	3	4	5	6	7
Day 5	4	5	6	7	8





Rosaline Mary and Priyanka

Table:1a- First trial of Linear Multiple Regression model for mortality rate of *Sitophilus granarius* in wheat by *Piper nigrum* powder

Days	Beta Value	Std Error	t- value
Day 1	.190	.021	.811
Day 2	.230	.034	.218
Day 3	.264	.065	.478
Day 4	.332	.129	1.994
Day 5	.385	.001	2.28

Source: Computed.

Table:1b- ANOVA of First trial of the mortality rate of *Sitophilus granarius* in wheat by *Piper nigrum* powder

Model	Sum of Squares	df	Mean Square	F- value
Regression	189.200	4	47.300	20.37
Residual	.000	0		
Total	189.200	4		

Source: Computed.

Table:2- Second trial of the mortality rate of *Sitophilus granarius* in wheat by *Piper nigrum* powder

Days	Concentration in grams				
Day 1	1	2	4	5	5
Day 2	2	3	4	5	6
Day 3	3	4	5	6	6
Day 4	4	4	5	6	6
Day 5	4	5	6	7	8

Source: Computed.

Table:2a- Second Trial of Linear Multiple Regression model for mortality rate of *Sitophilus granarius* in wheat by *Piper nigrum* powder

Days	Beta Value	Std Error	t- value
Day 1	1.15	.214	.262
Day 2	1.24	.318	.229
Day 3	2.01	.015	3.33
Day 4	1.01	.474	.220
Day 5	1.25	.098	1.28

Source: Computed.

Table:2b- Second Trial of ANOVA for mortality rate of *Sitophilus granarius* in wheat by *Piper nigrum* powder

Model	Sum of Squares	df	Mean Square	F- value
Regression	98.800	4	24.700	.255
Residual	.000	0		
Total	98.800	4		

Source: Computed





Rosaline Mary and Priyanka

Table:3-First trial of the mortality rate of *Sitophilus granarius* in wheat by *Coriandrum sativum* powder

Day	Concentration in grams				
Day 1	1	2	2	3	3
Day 2	2	2	3	3	4
Day 3	3	4	4	5	5
Day 4	4	5	5	6	6
Day 5	5	6	6	7	8

Source: Computed

Table:3a- First trial of Linear Multiple Regression model for the mortality rate of *Sitophilus granarius* in wheat by *Coriandrum sativum* powder

Day	Beta Value	Std Error	t- value
Day 1	1.52	.001	.592
Day 2	.89	.002	.748
Day 3	1.00	.001	.229
Day 4	2.00	.000	1.25
Day 5	2.00	.012	2.68

Source: Computed.

Table:3a- First trial of ANOVA for the mortality rate of *Sitophilus granarius* in wheat by *Coriandrum sativum* powder

Model	Sum of Squares	df	Mean Square	F- value
Regression	294.800	3	98.267	21.25
Residual	.000	1	.000	
Total	294.800	4		

Source: Computed.

Table:4- Second trial of the mortality rate of *Sitophilus granarius* in wheat by *Coriandrum sativum* powder

Day	Concentration in grams				
Day 1	1	2	3	4	4
Day 2	1	3	3	4	5
Day 3	2	4	5	5	6
Day 4	3	4	5	6	7
Day 5	5	5	6	7	8

Source: Computed

Table:4a- Second Trial of Linear Multiple Regression model for the mortality rate of *Sitophilus granarius* in wheat by *Coriandrum sativum* powder

Day	Beta Value	Std Error	t- value
Day 1	2.000	.012	.526
Day 2	-1.000	.000	-.179
Day 3	0.568	.002	.298
Day 4	2.000	.056	2.422
Day 5	1.000	.006	1.248

Source: Computed.





Rosaline Mary and Priyanka

Table:4b- Second Trial of ANOVA for the mortality rate of *Sitophilus granarius* in wheat by *Coriander sativum* powder

Model	Sum of Squares	df	Mean Square	F- value
Regression	Regression	162.000	4	40.500
Residual	Residual	.000	0	
Total	Total	162.000	4	

Source: Computed.

Table:5 Phytochemical Analysis of *Coriandrum sativum* and *Piper nigrum* powders

S. No	Metabolite	Test performed	Coriander Powder	Pepper powder
1	Alkaloids	+ Mayer's reagent	-	-
		+ Dragendorff's reagent	-	-
2	Flavonoids	Alkaline test	-	-
		+ H ₂ SO ₄	+	+
		+ lead acetate	-	-
		Shinoda test	-	-
3	Sterols (Liebermann test)	+ CHCl ₃ + Acetic anhydride + Conc.H ₂ SO ₄	-	-
4	Terpenoids (Liebermann test)	+ CHCl ₃ + Acetic anhydride + Conc.H ₂ SO ₄	-	-
5	Anthraquinone (Borntrager's test)	+ FeCl ₃ + Conc. HCl + diethyl ether +Ammonia	-	+
6	Anthocyanin	HCl Test	-	-
7	Proteins	+ 2 % Ninhydrin reagent	-	-
		2 % CuSO ₄ + 95 % ethanol + KOH pellet	-	-
		+ conc. HNO ₃	+	-
8	Phenolic compounds	+ 5 % neutral FeCl ₃	-	-
		Gelatin test	-	-
		+ Ellagic acid test	-	-
9	Quinones	Conc. HCl	-	-
		Alcoholic KOH	-	-
10	Carbohydrates	Molisch's test	+	+
		Fehling's test	+	+




Rosaline Mary and Priyanka

11	Tannin	Brayer's test	-	-
		+ Gelatin test	-	-
12	Saponins	10 % NaOH test	-	-
		Shaken with water	-	+
13	Cardiac glycosides	+ Baljet reagent	-	-
		Bromine water test	-	-
		Keller-killani test	-	-
		Borntrager's test	-	-
14	Glycoside's test	Aq.NaOH test	-	-
15	Lignin	+ Gallic acid	-	-
16	Coumarins	+10%NaOH+CHCl ₃	-	-
17	Volatile oils	Fluorescence test	-	-

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Exploring the Phytochemical Composition and Therapeutic Potential of *Cyathocline purpurea* : A Comprehensive Review

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ABSTRACT

Cyathocline purpurea [Buch.-Ham. ex D.Don] Kuntze (Asteraceae) is a widely distributed South Asian weed traditionally used in indigenous medicine. Despite its ethnomedicinal relevance, a critical synthesis of its phytochemical and pharmacological properties remains limited. This review systematically evaluates reported secondary metabolites, isolated bioactive compounds, biological activities, and emerging molecular docking evidence associated with this species. Phytochemical investigations indicate the presence of terpenoids, flavonoids, sesquiterpene lactones, and phenolic compounds. Experimental studies demonstrate that extracts and purified constituents exhibit antioxidant, anti-inflammatory, antihyperglycemic, antibacterial, cytotoxic, ovicidal, and larvicidal activities under in vitro and experimental conditions. Preliminary in silico analyses further suggest potential interactions between selected phytoconstituents and biologically relevant molecular targets. However, most findings are derived from preliminary screenings, with limited mechanistic insights and insufficient in vivo and clinical validation. Future research should prioritize extract standardization, toxicity assessment, pharmacokinetic profiling, and molecular pathway elucidation to substantiate therapeutic relevance. Overall, this review consolidates current evidence, identifies research gaps, and provides future directions for systematic investigation of *Cyathocline purpurea* as a potential source of bioactive compounds.

Keywords: *Cyathocline purpurea*, Extraction techniques, Bioactive compounds, Biological activity, Molecular docking





Trupesh G. Revad et al.,

INTRODUCTION

Strong traditional medical systems such as Chinese, Ayurvedic, and Unani have been around for the past 2500 years and are primarily associated with the eastern continent[1]. Traditional medical systems make considerable use of plants as raw materials for several medication formulations. The presence of bioactive elements such as alkaloids, tannins, flavonoids, and phenolic compounds accounts for the plant material's therapeutic value [2]. According to [3] and [4], weed are beneficial to humans since they provide food, shelter, medicines, aesthetic value, erosion control, organic matter, and mineral fertilizers for soil. Over 25,000 species of plants that are in the Asteraceae family are abundant in biologically active secondary metabolites.[5,6]. The Asteraceae family is widely recognized for its medicinal qualities and therapeutic applications. *Cyathocline purpurea*(Buch.-Ham. Ex D.Don) Kuntze are widespread weed found throughout south Asia that are from Asteraceae family, which significant for medicine. Morphologically, it is a slender, upright herbaceous annual, attaining heights of approximately 20-50 cm. Soft hair covers a branched, grooved stem. The entire plant has a powerful scent. Stalkless leaves are alternately have tooth structure, and are 3-12 cm long with silky hair covering them. At the tips of branches, corymbs of flowers are found. Purple flower heads are 5 to 8 centimetres in diameter. In the Himalaya (From Kashmir to Bhutan), Assam, India, Burma, Thailand, Indo-China, and China, this plant is extensively dispersed [7-11].

Cyathocline purpurea phytochemical composition has to be studied because it contains several unique compounds that haven't been thoroughly studied yet as of [12]. It has pharmacological, antibacterial, antiprotozoal, antiviral, antifungal, and antifertility properties. [10]. For many years, *Cyathocline purpurea* has been used historically to treat a variety of illnesses, including cancer [10]. In traditional Chinese medicine, *Cyathocline purpurea*(Compositae), also referred to as "Hong Hao Zhi," is utilized as a herbal cure for conditions such as human tuberculosis, malaria, haemorrhage, rheumatism, swelling, and anti-inflammatory disorders [13]. *Cyathocline purpurea* has historically been used for its antibacterial, anthelmintic, and hypotensive qualities[14]. An examination of the literature showed the following chemical constituents: santamarine, 6 a-hydroxy-4[14], 10[15]-guaianadien-8a-, 12-olide [15] and sesquiterpenelactones, eudesmanolide, isoivangustin, and guaianolide [16]. *Cyathocline purpurea* was used to isolate the compounds 9b-acetoxycostunolide and 9b-acetoxyparthenolide, which demonstrated strong anticancer effects in vitro[13]. Four plant extract have been added to various medicine formulation to increase their use as primary source of healthcare by 80% of the global population at large. [17,18].The Present review focuses on exploring the bioactive compounds isolated from *Cyathocline purpurea* and their pharmacological potential. Scientific classification, Vernacular names and local names of the *Cyathocline purpurea* shown in Table 1[19, 20].

Extraction Methods of Phytochemicals

The process of extracting medicinally active chemicals from plant parts involves selecting solvents and following certain methods to separate the active compounds, leaving the insoluble molecules behind. Extraction of plant compounds generally involves the diffusion of analytes across the cellular barrier, followed by their removal from the matrix into an appropriate extraction medium [21,18]. The extraction of natural products typically involves the following steps: [1] the extraction medium penetrates the solid plant material., [2] Bioactive compounds dissolve into the permeating liquid., [3] This dissolved constituents migrate out of the plant matrix., [4] finally, the extracted compounds are collected for further analysis [21]. Various extraction methods show the yield of *Cyathocline purpurea* is shown in Table 2.

Phytochemical analysis

Primary metabolites including sugars, amino acids, and nucleic acids are fundamental to essential cellular processes such as growth, respiration, reproduction, and energy storage in all living organisms. In contrast, secondary metabolites originate from primary metabolic pathways and were first introduced [28,29], with Czapek later referring to them as a end-products[30]. These compounds are not vital for basic survival but contribute significantly to plant defense and have long been utilized in traditional medicine systems [31]. Presence of various phytochemicals in



**Trupesh G. Revad et al.,**

solvents shown in Table 3. The quantitative tests shows that 0.869µg/mg atropine equivalent alkaloids and 0.574 µg/mg gallic acid equivalent phenols [22].

Different techniques to isolation and characterization of Bioactive compounds

In recent decades, various phytochemical studies have successfully isolated a broad range of natural compounds. These bioactive substances such as phenols, coumarins, terpenoids have been identified through advanced spectroscopic techniques, including TLC, HPLC, and both proton [¹H NMR] and carbon [¹³C NMR] nuclear magnetic resonance methods [32]. [15] By using repeated column chromatography, two fractions containing sesquiterpene lactones were identified by analysis. From *Cyathocline purpurea*, have isolated two novel sesquiterpene lactones: isoivangustin, a eudesmanolide, 6α-hydroxy-4 [14], 10[15]-guainadien-8α, 12-olide, a guaianolide.[33] reported a novel guaianolide compound that was originally identified by nagasampagi and colleagues in 1981. This compounds, isolated from *Cyathocline purpurea*, was characterised as 6α-hydroxy-4[14],10[15]-guainadien-8α,12-olide. Then extracted the crystalline form of the same chemical from *Cyathocline purpurea*. The stereochemistry had to be revised because X-ray examination of the crystal structure revealed that it was 6α-hydroxy-4 [14], 10[15]-guainadien-8α, 12-olide. [14]A total of 25 compounds was identified in the root oil of *Cyathocline purpurea* through GC-MS analysis, accounting for 90.1% of total oil composition. The retention Index, an MS library search, and an examination with MS literature data were used to identify the constituents. The majority of the oil [58.0%] was made up of phenyl derivatives, with thymohydroquinone dimethyl ether having the highest percentage [57.4%]. 18.3% of the hydrocarbons were sesquiterpenes, with b-selinene making up the majority at 14.0%. 11.4% were oxygenated sesquiterpenes and 2.4% were oxygenated monoterpenes. [24]] by using a liquid-solid separation chromatographic method, the extract[15g] was further fractionated into several fractions.

The anti-inflammatory activity of these fractions was evaluated using the carrageenan-induced paw edema model in wistar rats. Column chromatography was used to analyze the 30% acetate in petroleum ether fraction that was the most active. Petroleum ether served as the initial mobile phase, with ethyl acetate added in increasing polarity. The eluted sub-fractions were analysed by Thin Layer Chromatography [TLC] using a petroleum ether;ethyl acetate [7:3] solvent system. Following that, the sub-fractions exhibiting comparable bands were combined, and their anti-inflammatory properties were examined. The most anti-inflammatory efficacy was seen in one of the fraction. Afterthat Isoivangustin [42] was obtained by preparative thin layer chromatography after removed contaminants from obtained fraction. Isoivangustin structure was determined using spectral analysis employing IR, MS, ¹H-NMR, ¹³C-NMR, and DEPT. [27] studied the five fractions were obtained by pooling similar fractions using Thin layer chromatography: 1.A[0.28g], 2.A[0.56g], 3.A[0.50g], 4.A[0.46g], and 5.A [0.19g]. Hexane:Ethyl acetate [7:3] was the component that was present in a significant amount as indicated by fraction 2.A [0.56g], which had an RF value of 0.75 on its thin layer chromatography. The pure compound [26.0 mg] was isolated from fraction 2.A through repeated preparative Thin Layer Chromatography using a hexane:ethyl acetate [7:3] solvent system. Its purity was confirmed by ¹H, ¹³C-NMR spectroscopy along with TLC analysis. By comparing its physical and spectroscopic data with published literature, the isolated compound's stereochemistry and structure were determined to be 6α-hydroxy-4 [14], 10[15]-guainadien-8α, 12-olide.

[34] stated that the 5.0% acetone fraction contained the highest yield of the SRCP1 chemical as determined by thin layer chromatography. After gel chromatography, a 5.0 g fraction was further purified through silica gel column chromatography using petroleum ether and ethyl acetate in a 7:3 [v/v] ratio as the mobile phase. On the TLC plate, the pure chemical SRCP1[0.2g] has an RF value of 0.6. The observed UV-VIS spectrophotometric examination findings demonstrate the detection of a single peak showing the compound utmost purity with an absorbance of 1.481 and a λ_{max} at 204 nm. By using HPLC, the optimum SRCP1 concentration in petroleum- ether extract was found to be 289.9246 µg/mg, with an RF value of 4.3 minutes. According to [11], GC-MS analysis of *Cyathocline purpurea* extract identified 17 phytochemical constituents with varying chemical profiles. The dominant compound was pentadecanoic acid, 14-methyl-, methyl ester, comprising over 30% of the extract. Other notable compounds present in moderate to low concentrations included methyl oleate, methyl stearate, 2',5'-dimethoxypropiphenone,





Trupesh G. Revad et al.,

and α -cadinol, Additionally, sesquiterpenes such as α -cubebene, α - and δ -cadinene, β -cubebene, γ -cadinene, and nonadecanoic acid were also detected in measurable amounts. Gas chromatography GC-FID and gas chromatography- mass spectrometry [GC-MS] techniques were used to analyse the essential oils of *Cyathocline purpurea* that were extracted using hydrodistillation from the flowers, leaves, stems, and roots. 34-39 compounds from GC-MS techniques were used to analyse the essential oils of *Cyathocline purpurea* that were extracted using hydrodistillation from the flowers, leaves, stems, and roots. The main components varied greatly by plant part and included ionone, lavandulyl acetate [31.5%], and 2,5-dimethoxy p-cymene [62.6%]. Particularly in the flower and stem extracts, the oil were abundant in oxygenated sesquiterpenes [26].

Evaluative Studies

Antimicrobial activity

Medicinal research often investigates plant-derived antimicrobial because they tend to exhibit minimal side effects. As possible antimicrobial agents, a lot of researchers have recently concentrated on studying plant and microbial extracts, essential oils, pure secondary metabolites, and newly synthesized compounds [35, 36, 37]. [38] the study found that the essential oil of *Cyathocline purpurea* inhibits the growth of *Bacillus subtilis* at a very low concentration of 25 μ l, and that it inhibits the development of *Pseudomonas aeruginosa* at a low concentration of 50 μ l. *Bacillus subtilis* [NCIM 2635] has an inhibitory zone of 1.1 mm when exposed to 25 μ l of essential oil. In contrast, *pseudomonas aeruginosa* [NCIM 5032] has an inhibitory zone of 1.3 mm in 50 μ l. *Cyathocline purpurea* oil exhibits superior antibacterial action even at low concentrations. [14] evaluated the antimicrobial potential of *Cyathocline purpurea* root essential oil against plant pathogenic bacteria. The study revealed that the oil exhibited activity primarily against Gram-positive bacteria, while Gram-negative strains showed resistance. As the Minimum Inhibitory concentration [MIC] and Minimum Bactericidal Concentration [MBC] value were identical, only MBC data was represented. *Micrococcus flavus* shows the highest sensitivity with an MBC of 0.2670 mg/mL. In contrast, *Bacillus subtilis* was less sensitive, requiring 0.5770 mg/mL for bactericidal action. *Micrococcus luteus* and *Staphylococcus aureus* exhibited moderate sensitivity, with MBC values of 0.3670 mg/mL and 0.4270 mg/mL, respectively. Few studies have revealed antibacterial activities of *Cyathocline purpurea* and not any fungal activity has been done yet, thus it should be further explored in detail against diverse microbial and viral species.

Antioxidant activity

A variety of techniques have been used to assess these moiety' potential as antioxidants. For many organisms to produce energy to power biological processes, oxidation is necessary. However, many diseases, including cancer, atherosclerosis, and rheumatoid arthritis, are brought on by the unchecked generation of free radicals derived from oxygen [39]. Antioxidants scavenge the DPPH radical by donating a hydrogen atom, resulting in the decreased DPPH-H [40]. [23] revealed that the IC₅₀ value of the methanol extract of *Cyathocline purpurea* demonstrated more antioxidant potential, while the petroleum extract of *Cyathocline purpurea* demonstrated moderate antioxidant potential with lower activity for the aqueous extract of *Cyathocline purpurea*. [27] the research revealed that the IC₅₀ of 12 olide for hydrogen peroxide, DPPH, and hydroxyl radical scavenging activity were 87.5 μ g/ml, 76 μ g/ml, and 71 μ g/ml, respectively. Consequently, it was proposed that *Cyathocline purpurea* possesses strong antioxidant properties.

Anti-inflammatory

The most common indicator of illness, inflammation is also a basic biological process that involves intricate pathways that are frequently brought on by the breakdown products of bacteria from a variety of microorganisms, including viruses [double-stranded RNA], fungi [zymosans], lipopeptides, lipopolysaccharides, peptidoglycans, formylmethionyl peptides, flagellin, and microbial DNA], or even the body's own cells when they are damaged or die [41, 42]. [27] found evidence to support the theory that olide's capacity to reduce inflammation may stem from its role in the metabolism of arachidonic acid. *Cyathocline purpurea* may have anti-inflammatory properties because of guaianolides found in sesquiterpene lactones. The hydroxyl group in 6a-hydroxyl-4[14], 10 [15]-guainadien-8 α , 12



**Trupesh G. Revad et al.,**

olide may be reason for its anti-inflammatory in-vitro and in-vivo antioxidant activities. [23] studied with methanol extract of *Cyathocline purpurea* [400mg/kg], notable analgesic and anti-inflammatory effects were noted. *Cyathocline purpurea* methanol extract [100, 200, and 400mg/kg] minimized paw edema in an investigation using carrageenan in a cotton pellet model, it also decreased the weight of granuloma in a dose-dependent manner. *Cyathocline purpurea*'s aqueous extract lacked any action, however its petroleum extract had modest analgesic and anti-inflammatory properties. [24]] found that the most active component causing its anti-inflammatory action was isoivangustin, a sesquiterpene lactone; its anti-inflammatory impact may be mediated by TNF- α suppression.

Cytotoxic Activity Against Cancer Cell Lines

Natural products have contributed significantly to drug discovery, with more than 60% of currently used anticancer drugs being derived from or inspired by natural sources [42,44]. Historically, plant-derived secondary metabolites have played an important role in the development of therapeutic agents. Over the past three decades, natural products have attracted considerable attention as potential sources of cytotoxic and antitumor agents [45; 46; 47]. [34] evaluated the cytotoxic activity following a 24-hour treatment period and reported an IC₅₀ value of 9.98 μ g/mL, which was lower than that of the petroleum ether extract. For comparison, actinomycin D exhibited an IC₅₀ value of 8.5 μ M under similar conditions. The study further indicated that the isolated pure compound SRCP1 demonstrated stronger cytotoxic activity than the corresponding crude extract. [13] assessed cytotoxic effects using five cultured tumor cell lines, namely L1210 murine leukemia, CCRF-CEM human leukemia, KB human nasopharyngeal carcinoma, LS174T human colon adenocarcinoma, and MCF-7 human breast carcinoma. The compounds santamarine, 9-acetoxycostunolide, and 9-acetoxyparthenolide significantly inhibited cell proliferation in a concentration-dependent manner. Among the tested cell lines, KB cells showed the highest sensitivity, suggesting selective cytotoxic potential against this tumor cell type.

Antihyperglycemic activity

Globally, the prevalence of the disease is rising alarmingly; by 2030, 366 million individuals are expected to have diabetes, compared to an estimated 191 million in 2000 [48]. Due to its natural origin and lower side effects, herbal medicines have gained popularity in recent years for the treatment and control of diabetes in both developed and developing nations. [1]. [22] reported the findings indicated that the benefits of *Cyathocline purpurea* leaf hydroalcoholic extract in rats given alloxan-induced diabetes. According to this study, there was dose-dependent antidiabetic action produced by hydroalcoholic extract of *Cyathocline purpurea* leaves.

Ovicidal and Larvicidal activity

Aedes aegypti Linn. Is a mosquito that transmits dengue, chickenguniya, and yellow fever. Dengue is one of the most common vector-borne infections in the world. The World health Organization [WHO] estimates that 390 million illnesses occur annually, with approximately 96 million showing clinical symptoms[50]. [51] investigated the leaf extract of *Cyathocline purpurea*, which have been shown to have considerable ovicidal and larvicidal action against the dengue vector *Aedes aegypti*. This study found that the extract induced both significant mortality in larvae and gastrointestinal injury, demonstrating its potential as a natural vector control agent. This application in integrated mosquito management programs as alternative to synthetic insecticides.

Molecular Docking

In recent years, substantial advancements have been made in the techniques of molecular docking and scoring. Molecular docking is used to positioning the computer-generated 3D structure of small ligands into a receptor structure in variety of orientation, conformations and positions[52]. It has become a valuable tool in drug discovery process[53]. Molecular docking is a computational technique used to identify a ligand that best aligns with the binding site of protein, both in terms of geometry and energy. Essentially, it examines the compatibility and interaction between two or more molecules, such as a ligand and a receptor. The problem is like solving a 3D puzzle [53,54]. Molecular docking is such a structure-based drug design method that stimulates the molecular interaction and predicts the binding mode and affinity between receptors and ligands. Molecular docking process ligands shown



**Trupesh G. Revad et al.,**

in Figure 1 [55] [27] Research has indicated that selective COX-2 inhibitors can be linked to various side effects, including gastrointestinal discomfort [such as abdominal pain, nausea, and diarrhoea], headaches, allergic responses like skin rashes and breathing difficulties, as well as an elevated risk of cardiovascular issues. The molecular docking study revealed that 12-olide has a significant affinity towards the COX-2 active sites.[24] reported that molecular docking analysis of isoivangustin at the active site of TNF-alpha converting enzyme [TACE] showed hydrogen bonding with amino acid Gly349 and Leu348 like in hydroxamate group of native ligand IH6 which showed hydrogen bonding with same amino acids. Additionally, the octahydronaphthyl ring is accommodated within the hydrophobic pocket shaped by the amino acid residue His405 in the enzyme. Isoivangustin displayed a docking score of -5.341 with the TACE enzyme, suggesting a strong binding affinity at its active sites.

Rationale

Cyathocline purpurea, a history of traditional use, is employed to treat conditions like tuberculosis, malaria, hemorrhage, rheumatism, swelling, and inflammatory disorders. Despite its therapeutic significance, scientific studies on its bioactive compounds remain scarce. The presence of flavonoids, terpenoids, and phenolic substances detected in preliminary studies highlights their possible pharmacological significance. However, the specific roles and mechanisms of these compounds are yet to be fully understood. Addressing this gap is critical to meeting the growing demand for plant-based therapies and developing safer, more effective alternatives to synthetic drugs.

CONCLUSION

Available evidence indicates that *Cyathocline purpurea* has been traditionally employed for the management of various ailments and exhibits a range of biological activities, including antibacterial, antioxidant, anti-inflammatory, antihyperglycemic, cytotoxic, ovicidal, and larvicidal effects under experimental conditions. These activities are primarily associated with the presence of terpenoids, phenolic compounds, and other secondary metabolites. However, current findings are largely based on preliminary in vitro investigations, with limited mechanistic insights and insufficient in vivo and clinical validation. Systematic studies focusing on bioactive compound isolation, molecular mechanisms, toxicity profiling, pharmacokinetic evaluation, and standardized extract development are essential to substantiate its pharmacological relevance. Moreover, comparative phytochemical profiling across different geographical regions and chemotypes may provide deeper insights into variability in bioactivity. Integrating advanced approaches such as metabolomics, target-based screening, and well-designed preclinical models will be crucial to translating preliminary findings into validated therapeutic applications. Future research integrating experimental and translational strategies may facilitate the rational development of *C. purpurea*-derived bioactive compounds.

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Table 1: Scientific Classification, Vernacular Names and Local Names of *Cyathocline purpurea*

Classification [Bentham and Hooker]		Vernacular names		Local names	
Category	Classification	Language	Vernacular Name	Region	local Name
Class	Dicotyledonae	Hindi	Bandhaniya	Tamil Nadu	Kattu Kothamalli
Sub-class	Gamopetalae	Marathi	Gangotra	Andhra Pradesh	Adavi Kothimeera
Series	Inferae	Nepali	Gal Phulle	West Bengal	Bon Dhone
Order	Asterales	Assamese	Gal Phule	Karnataka	Kaadu Kothambari
family	Asteraceae	Gujarati	Okharad, Gangotri	Odisha	Ban Dhanian
Genus	<i>Cyathocline</i>	-	-	-	-
Species	<i>Cyathocline purpurea</i>	-	-	-	-

Table 2: Various Extraction Methods And extractive Yield

Sr No.	Extraction method	Plant part	Solvent	Yield	Reference
1.	Maceration	Leaves	Acetone	-	[11]
		Whole plant	Pet. ether	1.80%	[10]
			Chloroform	1.23%	
			Ethyl acetate	2.03%	
			Ethanol	4.43%	
		Leaves	Ethanol	6.55%	[22]
		Whole plant	petroleum ether	3.3%	[23]
			Methanol	4.7%	
			aqueous	6.7%	
2.	Hydro-distillation	Whole plant	Methanol	6.9 %	[24]
		Whole plant	Methanol	6.7%	[25]
		Roots	Aqueous	0.02%	[14]
		Flowers	Aqueous	0.2%	[26]
		Roots		0.14%	
		Stems		0.08%	
		Leaves		0.03%	
3.	Cold percolation	Aerial part	Methanol	-	[27]
4.	Soxhlet apparatus	Leaves Stems and Bark	Hexane, Toluene, Ethyl acetate, Ethanol & water.	-	[12]

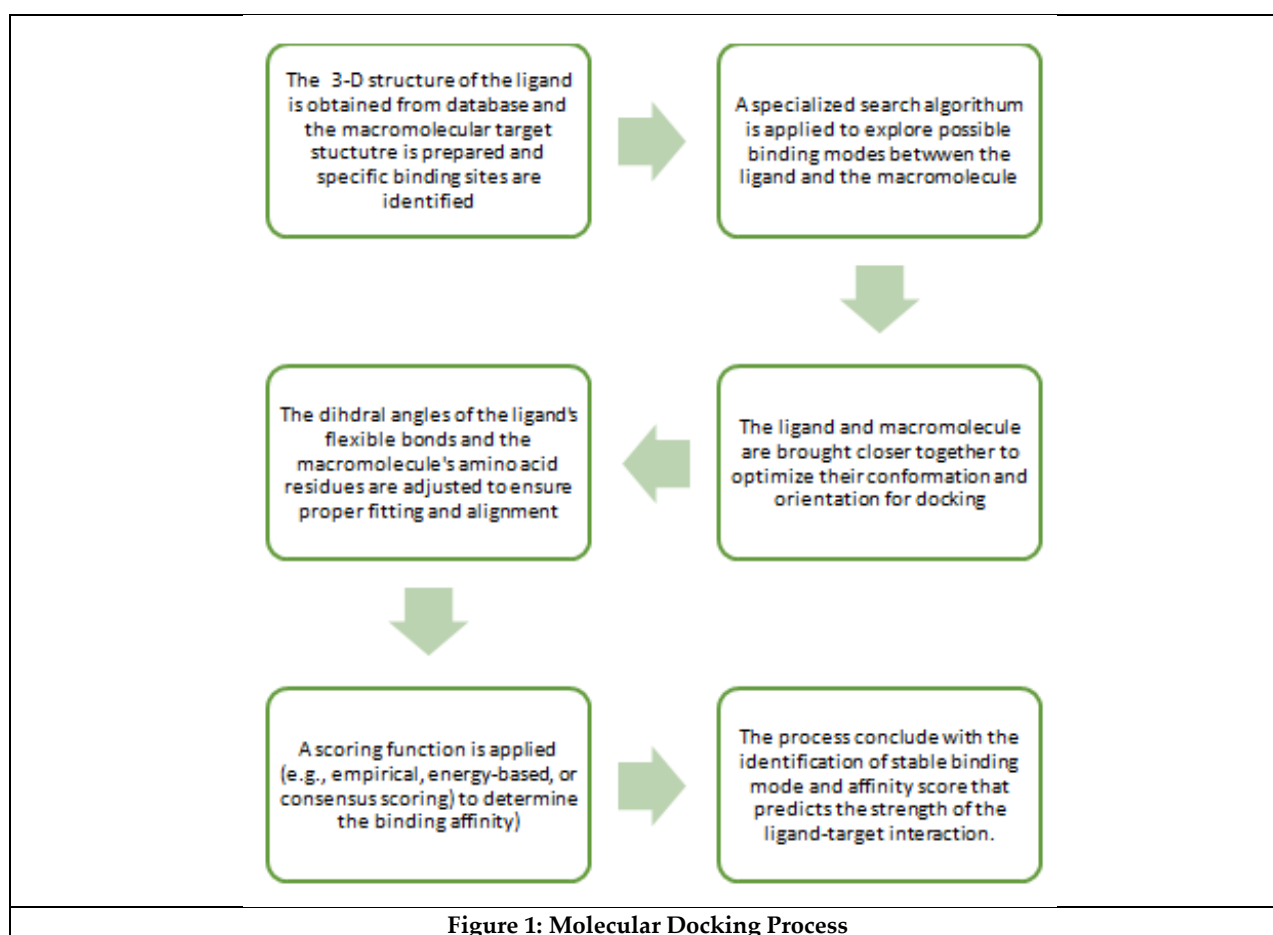




Trupesh G. Revad et al.,

Table 3: Phytochemicals Present in Different Solvent Extracts

Solvent/Extract	Phytochemical present	Reference
Petroleum ether	Lipids, terpenoids and steroids	[10]
Chloroform	Saponins, terpenoids and steroids	
Ethyl acetate	Phenols, terpenoids, flavonoids and steroids	
Ethanol	Alkaloids, glycosides, phenols, tannins, flavonoids, saponins and steroids	
Petroleum	Phenol, tannins, alkaloids and flavonoids	[23]
Methanol	Alkaloids, flavonoids, tannins, phenols, glycosides and steroids	
Aqueous	Glycosides, proteins, carbohydrates, amino acids, alkaloids and phenols	
Ethyl acetate and ethanol	Alkaloids, flavonoids, glycosides, tannins and phenols	[12]
Ethanol	Phenols, carbohydrates, saponins and alkaloids	[22]



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REVIEW ARTICLE

Preparation of Kutaja Nimba Taila by Aditya Paka Taila Vidhi : A Narrative Review of Classical Ayurvedic Literature and Contemporary Pharmaceutical Perspectives

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ABSTRACT

Sneha Kalpana is a fundamental pharmaceutical dosage form in Ayurveda, wherein lipid media such as Taila (oil) and Ghṛta (clarified butter) are processed with medicinal plant materials to produce therapeutically effective formulations. While most medicated oils are traditionally prepared by **Agni Pāka** (direct heating), classical Ayurvedic literature also describes **Āditya Pāka Taila Vidhi**, a unique pharmaceutical process employing solar energy as the source of heat. This method provides gradual, low-temperature processing and has traditionally been recommended for topical medicated oils used in dermatological disorders. Kutaja (*Holarrhena antidysenterica* Wall. ex A.DC.) and Nimba (*Azadirachta indica* A. Juss.) possess well-documented antimicrobial, anti-inflammatory, antioxidant, antipruritic, and wound-healing properties, making them promising candidates for the development of topical Ayurvedic formulations. To critically review the classical Ayurvedic literature and contemporary scientific evidence pertaining to Āditya Pāka Taila Vidhi and to evaluate the pharmaceutical rationale, preparation methodology, standardization requirements, and future research prospects of Kutaja Nimba Taila. This study was conducted. Classical Ayurvedic texts, including *Charaka Saṁhitā*, *Suśruta Saṁhitā*, *Aṣṭāṅga Hṛdaya*, *Śārṅgadhara Saṁhitā*, *Chakradatta*, *Bhaishajya Ratnāvalī*, *Sahasrayoga*, *Gada Nigrāha*, *Bhāvaprakāśa Nighaṇṭu*, *Dhanvantari Nighaṇṭu*, and *Kaiyadeva Nighaṇṭu*, were reviewed. Contemporary scientific literature was retrieved from PubMed, Scopus, Google Scholar, ScienceDirect, and CrossRef, together with the Ayurvedic Pharmacopoeia of India (API), Ayurvedic Formulary of India (AFI), and WHO



**Nagaraja and Ganga R Hadimani**

monographs. Literature concerning Sneha Kalpana, Āditya Pāka, Kutaja, Nimba, medicated oils, phytochemistry, pharmacology, pharmaceutical processing, and quality control was critically analysed. Classical literature recognises Āditya Pāka as a specialised pharmaceutical technique for preparing medicated oils intended primarily for external application. Kutaja contributes steroidal alkaloids, flavonoids, tannins, and phenolic compounds with antimicrobial and anti-inflammatory activities, whereas Nimba Taila functions both as the lipid medium and as a therapeutically active ingredient rich in limonoids and related phytochemicals. The pharmaceutical rationale of Āditya Pāka is consistent with the principles of low-temperature herbal extraction; however, direct comparative studies evaluating phytochemical retention, physicochemical characteristics, or therapeutic efficacy between Āditya Pāka and conventional Agni Pāka are currently lacking. Kutaja Nimba Taila prepared by Āditya Pāka Taila Vidhi represents a pharmaceutically rational formulation derived from classical Ayurvedic principles rather than a named classical formulation. Although supported by traditional therapeutic concepts and the known pharmacological properties of Kutaja and Nimba, the formulation requires comprehensive pharmaceutical standardization, phytochemical characterization, pharmacological validation, toxicological assessment, and well-designed clinical trials before definitive therapeutic conclusions can be established.

Keywords: Āditya Pāka; Sneha Kalpana; Kutaja; *Holarrhena antidysenterica*; Nimba; *Azadirachta indica*; Medicated oil; Solar extraction; Ayurvedic pharmaceuticals; Dermatology.

INTRODUCTION

Ayurveda, the traditional medical system of India, has evolved a sophisticated pharmaceutical discipline known as Bhaiṣajya Kalpanā, which encompasses the principles governing the selection, processing, formulation, preservation, standardization, and therapeutic application of medicinal substances. Among the numerous dosage forms described in the Ayurvedic classics, Sneha Kalpana occupies a distinctive position because of its ability to extract, preserve, and deliver therapeutically active phytoconstituents through lipid media such as Taila (oil) and Ghṛta (clarified butter). These formulations are extensively employed for both internal administration and external therapeutic procedures including Abhyanga (therapeutic massage), Nasya (nasal administration), Karnaṇapūraṇa (ear instillation), wound management, and the treatment of dermatological disorders. Conventionally, medicated oils are prepared by Agni Pāka, wherein herbal paste (*Kalka*), lipid medium (*Sneha Dravya*), and liquid media (*Drava Dravya*) are processed under carefully controlled heating until the characteristic Sneha Siddhi Lakṣaṇas are achieved. This process facilitates the extraction of both hydrophilic and lipophilic phytoconstituents into the lipid phase while improving the stability, bioavailability, and therapeutic efficacy of the finished formulation. Modern pharmaceutical science similarly recognizes lipid-based drug delivery systems as effective carriers capable of enhancing dermal penetration, protecting unstable phytochemicals, and improving the bioavailability of poorly water-soluble compounds.

In addition to conventional heating, classical Ayurvedic literature describes an alternative pharmaceutical technique known as Āditya Pāka Taila Vidhi, in which sunlight serves as the source of thermal energy. Unlike Agni Pāka, this method relies on gradual solar exposure to facilitate the transfer of medicinal constituents into the lipid medium. Classical texts recommend this technique primarily for medicated oils intended for external use in disorders such as Kuṣṭha (skin diseases), Kandu (pruritus), Śvitra (depigmentary disorders), chronic wounds, and scalp diseases. Although the exact physicochemical mechanisms underlying Āditya Pāka have not been fully elucidated, the process conceptually resembles modern low-temperature herbal infusion techniques, where prolonged mild heating is employed to reduce the risk of excessive thermal degradation while allowing gradual extraction of bioactive constituents. Among the medicinal plants widely used in Ayurvedic dermatology, Kutaja (*Holarrhena antidysenterica* Wall. ex A.DC.) and Nimba (*Azadirachta indica* A. Juss.) occupy an important place due to their broad spectrum of



**Nagaraja and Ganga R Hadimani**

therapeutic properties. Classical Ayurvedic texts describe these drugs as Kuṣṭhaghna (beneficial in skin diseases), Krimighna (antimicrobial), Kandughna (antipruritic), Śōthahara (anti-inflammatory), and Vranaropana (wound healing). Contemporary pharmacological investigations have demonstrated antimicrobial, antifungal, antioxidant, anti-inflammatory, immunomodulatory, and wound-healing activities, thereby providing scientific support for many of their traditional indications. Neem oil (*Nimba Taila*), in particular, is widely recognised for its therapeutic utility in scalp disorders and serves both as a lipid vehicle and as a pharmacologically active ingredient. The combination of fresh Kutaja leaves processed in Nimba Taila through Āditya Pāka Taila Vidhi therefore represents a pharmaceutically plausible topical formulation. However, it is important to note that Kutaja Nimba Taila is not described as a named formulation in the available classical Ayurvedic texts. Rather, it is a formulation proposed by applying established principles of Āditya Pāka Taila Vidhi together with the documented therapeutic attributes of Kutaja and Nimba.

Consequently, its pharmaceutical characteristics, quality standards, safety profile, and therapeutic efficacy require systematic scientific evaluation before it can be recommended for routine clinical practice. Despite increasing interest in traditional herbal formulations, the available literature predominantly addresses Sneha Kalpana, Kutaja, and Nimba as separate entities. Reviews specifically focusing on Āditya Pāka Taila Vidhi remain scarce, and there is a lack of critical appraisal integrating classical Ayurvedic concepts with contemporary pharmaceutical science. Furthermore, no published comparative studies have comprehensively evaluated Āditya Pāka and conventional Agni Pāka with respect to phytochemical retention, physicochemical characteristics, stability, or therapeutic performance of Kutaja Nimba Taila. This lack of evidence highlights the need for a structured review synthesising classical knowledge and modern scientific perspectives. Accordingly, the present narrative review critically examines the classical Ayurvedic literature related to Sneha Kalpana and Āditya Pāka Taila Vidhi, reviews the pharmacognostic, phytochemical, and pharmacological characteristics of Kutaja and Nimba, presents a proposed Standard Operating Procedure for the preparation of Kutaja Nimba Taila, discusses quality control and standardization requirements, identifies current research gaps, and outlines future directions for pharmaceutical and clinical validation. By integrating traditional Ayurvedic pharmaceuticals with contemporary scientific evidence, this review aims to provide a robust conceptual framework for future research on solar-processed medicated oils.

Aim

To critically review the classical Ayurvedic literature and contemporary scientific evidence related to Āditya Pāka Taila Vidhi and to evaluate the pharmaceutical rationale, preparation methodology, standardization requirements, and future research prospects of Kutaja Nimba Taila.

Objectives

- To review the classical concepts of **Sneha Kalpana** and **Āditya Pāka Taila Vidhi**.
- To compile and critically analyse classical references related to solar-processed medicated oils.
- To review the Ayurvedic, phytochemical, and pharmacological properties of Kutaja and Nimba relevant to dermatological applications.
- To propose a standardized pharmaceutical procedure (SOP) for the preparation of Kutaja Nimba Taila.
- To discuss appropriate quality-control measures and analytical approaches for standardization.
- To identify research gaps and recommend future pharmaceutical, pharmacological, and clinical studies necessary for scientific validation.

REVIEW METHODOLOGY**Study Design**

The present study was conducted as a narrative literature review to critically synthesize evidence from classical Ayurvedic texts and contemporary scientific literature regarding Āditya Pāka Taila Vidhi and the proposed formulation Kutaja Nimba Taila. Unlike a systematic review, which aims to identify and quantitatively synthesize all available evidence using predefined protocols, the objective of this review was to integrate classical Ayurvedic





knowledge with modern pharmaceutical concepts, identify areas of scientific agreement and uncertainty, and highlight future research priorities. The narrative review design was considered appropriate because the available literature consists of heterogeneous sources, including classical Ayurvedic treatises, pharmacopoeial references, phytochemical investigations, pharmacological studies, pharmaceutical reports, and modern review articles, which are not amenable to quantitative meta-analysis.

Literature Search Strategy

A comprehensive literature search was performed between January and June 2026 to identify relevant classical Ayurvedic references and contemporary scientific publications related to Sneha Kalpana, Āditya Pāka Taila Vidhi, Kutaja, Nimba, herbal oil formulations, and pharmaceutical standardization.

Electronic databases searched included:

- PubMed
- Scopus
- Google Scholar
- ScienceDirect
- CrossRef

To supplement the electronic search, standard reference documents were consulted, including:

- Ayurvedic Pharmacopoeia of India (API)
- Ayurvedic Formulary of India (AFI)
- World Health Organization (WHO) Monographs on Medicinal Plants

Manual searches of reference lists from relevant publications were also performed to identify additional literature not retrieved through electronic databases.

Classical Ayurvedic Sources

Authoritative classical Ayurvedic texts were reviewed to collect information regarding Sneha Kalpana, Āditya Pāka Taila Vidhi, medicinal properties of Kutaja and Nimba, and pharmaceutical procedures related to medicated oils.

The principal classical references included:

- *Charaka Samhitā*
- *Suśruta Samhitā*
- *Aṣṭāṅga Hṛdaya*
- *Śārṅgadhara Samhitā*
- *Chakradatta*
- *Bhaiṣajya Ratnāvalī*
- *Sahasrayoga*
- *Gada Nigraha*
- *Bhāvaprakāśa Nighaṇṭu*
- *Dhanvantari Nighaṇṭu*
- *Kaiyadeva Nighaṇṭu*

These texts were consulted to identify references to Sneha Kalpana, solar-assisted pharmaceutical processing, therapeutic indications of Kutaja and Nimba, and the broader pharmaceutical principles underlying medicated oil preparation.

Search Keywords

The literature search was conducted using combinations of Medical Subject Headings (MeSH), free-text keywords, and Boolean operators (AND/OR).

The principal search terms included:

- "Āditya Pāka"
- "Aditya Paka Taila"





- "Sneha Kalpana"
- "Medicated oil"
- "Solar extraction"
- "Solar infusion"
- "Kutaja"
- "Holarrhena antidysenterica"
- "Nimba"
- "Azadirachta indica"
- "Herbal oil"
- "Ayurvedic pharmaceuticals"
- "Dermatological formulations"
- "Dandruff"
- "Seborrheic dermatitis"
- "Topical herbal formulation"

Representative search combinations included:

- "Āditya Pāka" AND "Sneha Kalpana"
- "Holarrhena antidysenterica" AND "Azadirachta indica"
- "Solar extraction" AND "Herbal oil"
- "Kutaja" AND "Dermatology"
- "Neem oil" AND "Pharmaceutical standardization"

Inclusion Criteria

The following publications were included in the review:

- Classical Ayurvedic texts describing Sneha Kalpana, Āditya Pāka Taila Vidhi, Kutaja, or Nimba.
- Peer-reviewed original research articles and review papers.
- Publications concerning phytochemistry, pharmacology, pharmaceutical processing, analytical evaluation, quality control, or therapeutic applications of Kutaja, Nimba, medicated oils, or solar-assisted extraction.
- Official pharmacopoeial documents and WHO monographs.
- Articles published in English.

Exclusion Criteria

The following sources were excluded:

- Duplicate publications.
- Conference abstracts without complete manuscripts.
- Non-peer-reviewed articles lacking scientific validation.
- Publications unrelated to the objectives of the review.
- Studies with inadequate methodological detail or insufficient scientific information.

Period Covered

The review incorporated:

- Classical Ayurvedic literature from the foundational Ayurvedic treatises.
- Modern scientific publications available up to June 2026.

No lower publication date limit was applied because historical Ayurvedic references constitute an essential component of the review.



**Nagaraja and Ganga R Hadimani****Data Extraction and Synthesis**

Relevant information was extracted and organized under the following thematic categories:

- Classical concepts of Sneha Kalpana
- Classical references to Āditya Pāka Taila Vidhi
- Ayurvedic properties of Kutaja and Nimba
- Botanical and pharmacognostic characteristics
- Phytochemical constituents
- Pharmacological activities
- Pharmaceutical processing
- Standard Operating Procedure (SOP)
- Quality control and standardization
- Contemporary pharmaceutical perspectives
- Research gaps and future directions

Information obtained from classical texts was interpreted in the context of contemporary pharmaceutical science to identify areas of convergence and opportunities for future investigation.

Scope and Comprehensiveness of the Review

The present review aimed to achieve comprehensiveness by integrating evidence from both classical Ayurvedic literature and modern scientific publications. Multiple authoritative classical treatises were examined alongside internationally recognized biomedical databases and standard reference documents, including the Ayurvedic Pharmacopoeia of India (API), the Ayurvedic Formulary of India (AFI), and WHO monographs. No major classical references relevant to Āditya Pāka Taila Vidhi, Sneha Kalpana, Kutaja, or Nimba were intentionally excluded. Modern publications were excluded only when they represented duplicate records, lacked adequate methodological detail, or were not directly relevant to the objectives of the review. Although every effort was made to include the most authoritative and relevant literature available at the time of manuscript preparation, the review was conducted as a **narrative review** rather than a systematic review. Accordingly, it does not claim exhaustive retrieval of every published study. The emphasis was placed on synthesizing evidence most relevant to the pharmaceutical principles, preparation methodology, and scientific rationale of Kutaja Nimba Taila prepared by Āditya Pāka Taila Vidhi.

Limitations of the Review

the review is based predominantly on published literature and therefore reflects the availability and quality of existing evidence. Second, direct experimental investigations evaluating Kutaja Nimba Taila prepared by Āditya Pāka Taila Vidhi are currently lacking. Consequently, certain pharmaceutical interpretations—particularly those concerning enhanced preservation of thermolabile phytoconstituents during solar processing—are based on classical Ayurvedic principles and established pharmaceutical concepts rather than direct experimental validation. Finally, comparative studies evaluating Agni Pāka and Āditya Pāka preparations remain scarce, limiting evidence-based conclusions regarding the relative pharmaceutical advantages of these processing methods. Sneha Kalpana: Classical Concepts and Pharmaceutical Significance Concept of Sneha Kalpana Sneha Kalpana is one of the most important pharmaceutical dosage forms described in Bhaisajya Kalpanā (Ayurvedic pharmaceuticals). The term *Sneha* denotes lipid substances possessing unctuous, lubricating, nourishing, and penetrating properties, whereas *Kalpana* refers to the systematic pharmaceutical processing of medicinal substances into therapeutically effective dosage forms. In Ayurvedic practice, Sneha Kalpana involves processing lipid media such as Taila (oil) or Ghṛta (clarified butter) with herbal drugs and liquid media to prepare medicated formulations intended for both internal and external administration.

Classical Ayurvedic texts consider Sneha as an ideal pharmaceutical vehicle because it extracts both lipid-soluble and water-soluble phytoconstituents while improving stability, palatability, shelf life, tissue penetration, and therapeutic efficacy. The lipid medium also facilitates the delivery of bioactive compounds to deeper tissues through its Sūkṣma (subtle) and Vyavāyī (rapidly diffusing) properties described in Ayurveda. Consequently, medicated oils are



**Nagaraja and Ganga R Hadimani**

extensively used in Pañcakarma, dermatology, ophthalmology, otorhinolaryngology, wound care, musculoskeletal disorders, and neurological diseases. Modern pharmaceutical science similarly recognizes lipid-based drug delivery systems as efficient carriers capable of enhancing dermal penetration, improving the bioavailability of poorly water-soluble phytochemicals, protecting unstable constituents from degradation, and enabling sustained release. This convergence between Ayurvedic pharmaceutical principles and contemporary drug-delivery concepts underscores the continued relevance of Sneha Kalpana in evidence-based herbal medicine.

Definition of Sneha Kalpana

Although the wording differs among classical treatises, the concept remains uniform. Sneha Kalpana may be defined as: The pharmaceutical process in which Sneha Dravya (oil or ghee) is processed with Kalka (herbal paste) and Drava Dravya (liquid media) under prescribed conditions until characteristic Sneha Siddhi Lakṣaṇas are attained, thereby producing a stable medicated lipid formulation suitable for therapeutic use. This definition emphasizes that the therapeutic value of Sneha Kalpana depends not only on the medicinal plants employed but also on the pharmaceutical method adopted during processing.

Historical Evolution

The principles of Sneha Kalpana have evolved progressively throughout Ayurvedic literature.

The Charaka Saṃhitā introduced the therapeutic importance of medicated oils and clarified butter in internal medicine and Panchakarma. The Suśruta Saṃhitā expanded their application to surgical practice, wound management, burns, and dermatological conditions. The Aṣṭāṅga Hṛdaya provided practical guidance regarding pharmaceutical preparation and therapeutic indications. Subsequently, medieval texts such as Śārīgadhara Saṃhitā, Chakradatta, Bhaiṣajya Ratnāvalī, and Saahasrayoga elaborated standardized pharmaceutical methods, proportions of ingredients, stages of processing, and indicators of successful preparation. These texts also describe specialized pharmaceutical techniques, including Āditya Pāka, in which sunlight rather than direct fire serves as the processing medium for selected formulations.

Components of Sneha Kalpana

Classically, Sneha Kalpana consists of three essential pharmaceutical components.

Sneha Dravya

Sneha Dravya constitutes the lipid base of the formulation. Commonly used lipid media include:

- Taila (vegetable oils)
- Ghṛta (clarified butter)
- Vasa (animal fat)
- Majjā (bone marrow fat)

Among these, **Taila** is generally preferred for dermatological disorders because of its superior skin penetration, stability, and suitability for external application.

Kalka Dravya

Kalka refers to finely triturated herbal paste prepared from fresh or dried medicinal plant materials.

Functions of Kalka include:

- Providing concentrated phytochemicals.
- Facilitating extraction during processing.
- Improving interaction between herbal constituents and lipid medium.
- Enhancing therapeutic potency.

The amount of Kalka varies depending upon the pharmaceutical procedure and textual reference.

Drava Dravya

Drava Dravya denotes the liquid medium employed during processing.

Examples include:



**Nagaraja and Ganga R Hadimani**

- Water
- Swarasa (fresh juice)
- Kwatha (decoction)
- Ksheera (milk)
- Takra (buttermilk)
- Gomutra
- Herbal infusions

The liquid medium assists in extracting water-soluble constituents while enabling their gradual transfer into the lipid phase during processing.

Classical Proportion of Ingredients

The most widely accepted proportion described in Śārṅgadhara Saṃhitā is:

- Sneha : 1 part
- Kalka : 1/4 part
- Drava : 4 parts

This ratio is regarded as the standard formulation for conventional Agni Pāka preparations.

However, formulations prepared through Āditya Pāka Taila Vidhi often differ because solar processing relies on gradual extraction rather than prolonged boiling. Consequently, pharmaceutical optimization of ingredient ratios remains an important area for future investigation.

Types of Sneha Pāka

Classical Ayurvedic pharmaceutics recognizes three principal stages of Sneha Pāka.

Mridu Pāka

- Mild processing.
- Higher residual moisture.
- Preferred for Nasya and delicate procedures.

Madhyama Pāka

- Optimum pharmaceutical stage.
- Most commonly employed.
- Suitable for external and internal therapeutic use.

Khara Pāka

- Extensive processing.
- Minimal moisture.
- Longer shelf life.
- Primarily intended for external application.

Selection of the appropriate stage depends upon therapeutic indication, route of administration, and intended pharmaceutical characteristics.

Sneha Siddhi Lakṣaṇas (Signs of Proper Processing)

Achievement of **Sneha Siddhi Lakṣaṇas** indicates completion of the pharmaceutical process.

Common indicators described in classical literature include:

- Disappearance of frothing.
- Characteristic aroma.
- Absence of residual moisture.
- Smooth consistency.
- Proper colour development.
- Kalka becoming soft, non-sticky, and capable of rolling into a wick.
- Lack of crackling sound when a small quantity is exposed to heat.



**Nagaraja and Ganga R Hadimani**

These observations continue to serve as important quality indicators during the preparation of medicated oils.

Pharmaceutical Significance of Sneha Kalpana: From a contemporary pharmaceutical perspective, Sneha Kalpana provides several advantages.

Efficient Extraction: The lipid medium extracts lipophilic phytochemicals while the presence of aqueous media facilitates transfer of hydrophilic constituents into the formulation.

Improved Stability: Lipid formulations are generally more resistant to microbial contamination than aqueous preparations and possess extended shelf life when appropriately prepared.

Enhanced Dermal Delivery: Oils improve hydration of the stratum corneum and enhance percutaneous absorption of bioactive compounds, making them particularly suitable for dermatological applications.

Protection of Sensitive Constituents: When processed under controlled conditions, lipid media may reduce oxidative degradation of certain phytochemicals by limiting exposure to excessive heat and oxygen. However, the extent of such protection depends upon the processing method and requires experimental verification for individual formulations.

Sustained Drug Release: The viscous nature of lipid preparations facilitates prolonged contact with affected tissues, thereby enhancing therapeutic effectiveness.

Contemporary Pharmaceutical Perspective: Current research increasingly supports the integration of Ayurvedic pharmaceuticals with modern pharmaceutical science. Lipid-based herbal formulations are recognized as promising delivery systems capable of improving drug solubility, enhancing bioavailability, and facilitating controlled release.

In this context, Āditya Pāka Taila Vidhi represents an intriguing traditional pharmaceutical approach that may offer unique advantages for the preparation of topical medicated oils. Nevertheless, many of its proposed pharmaceutical benefits remain to be validated through modern analytical techniques, including physicochemical characterization, phytochemical fingerprinting, stability studies, and comparative evaluation with conventional Agni Pāka preparations.

Relevance to the Present Review

An understanding of the principles of Sneha Kalpana is fundamental for appreciating the rationale behind Āditya Pāka Taila Vidhi. The proposed formulation of Kutaja Nimba Taila applies these classical pharmaceutical concepts while adapting them to a solar-assisted processing technique. This adaptation emphasizes gentle extraction without direct heating and forms the conceptual basis for the pharmaceutical approach discussed in subsequent sections.

Āditya Pāka Taila Vidhi: Classical Foundations and Contemporary Pharmaceutical Perspectives The preparation of medicated oils (Taila Kalpana) occupies a central position in Ayurvedic pharmaceuticals due to its versatility, stability, and therapeutic efficacy. Conventionally, most medicated oils are prepared using Agni Pāka, wherein heat generated by direct fire facilitates the transfer of active constituents from herbal materials into the lipid medium. However, classical Ayurvedic literature also describes an alternative pharmaceutical technique known as Āditya Pāka Taila Vidhi, in which solar energy rather than direct fire serves as the processing medium. Although less frequently employed than Agni Pāka, Āditya Pāka represents an important example of the diversity and sophistication of classical Ayurvedic pharmaceutical technology. The method relies on prolonged exposure of herbal materials immersed in oil to natural sunlight, allowing gradual extraction of phytoconstituents under ambient environmental conditions. Traditionally, this technique has been recommended for selected topical formulations intended primarily for dermatological disorders, chronic wounds, scalp diseases, and inflammatory conditions. Interest in Āditya Pāka has increased in recent years because contemporary pharmaceutical science recognizes that processing temperature can influence phytochemical stability, extraction efficiency, oxidative degradation, and the preservation of volatile constituents. Consequently, revisiting this classical technique through modern scientific investigation may contribute to the development of standardized low-temperature herbal formulations.

Etymology and Concept

The Sanskrit term **Āditya** refers to the Sun, while **Pāka** denotes pharmaceutical processing or maturation. Accordingly, Āditya Pāka literally means processing under sunlight.



**Nagaraja and Ganga R Hadimani**

Unlike conventional heating methods, the pharmaceutical transformation occurs gradually through continuous solar exposure. The process represents a natural form of extraction in which medicinal constituents diffuse slowly into the lipid medium over several days. From an Ayurvedic perspective, the gradual action of sunlight is believed to facilitate **Sneha Saṃskāra** (pharmaceutical transformation of oils) without exposing the formulation to the intense thermal effects associated with direct fire.

Classical References

Although Kutaja Nimba Taila is not described as a named formulation in the available classical Ayurvedic texts, the pharmaceutical principle of Āditya Pāka is documented in several authoritative references.

Descriptions of solar-assisted processing are found in:

- Śārṅgadharma Saṃhitā
- Chakradatta
- Bhaiṣajya Ratnāvalī
- Sahasrayoga
- Gada Nigraha

These texts describe pharmaceutical procedures in which medicinal substances are processed by prolonged exposure to sunlight rather than direct heating. Such preparations were primarily recommended for external therapeutic applications, particularly in disorders affecting the skin and scalp. The present formulation therefore represents a pharmaceutically rational adaptation of established classical principles, combining the documented methodology of Āditya Pāka with the well-recognized therapeutic properties of Kutaja (*Holarrhena antidysenterica*) and Nimba Taila (*Azadirachta indica*).

Importantly, Kutaja Nimba Taila prepared by Āditya Pāka Taila Vidhi should not be interpreted as a classical proprietary formulation but rather as a conceptually derived formulation proposed for pharmaceutical standardization and scientific evaluation. Pharmaceutical Principles of Āditya Pāka The pharmaceutical rationale of Āditya Pāka may be understood through the interaction of several mechanisms. Gradual Extraction Continuous solar exposure allows slow diffusion of phytoconstituents from fresh herbal material into the lipid medium. Compared with direct heating, extraction occurs over an extended period, potentially facilitating equilibrium between plant tissues and the oil. Mild Thermal Processing Ambient solar temperatures are generally lower than temperatures encountered during conventional boiling. Consequently, the formulation is subjected to comparatively mild thermal conditions throughout processing. Lipid-Mediated Solubilization The lipid medium dissolves lipophilic constituents while simultaneously facilitating gradual incorporation of phytochemicals released from the herbal matrix. Continuous Pharmaceutical Transformation Repeated cycles of daytime warming and nighttime cooling may influence extraction kinetics and facilitate progressive interaction between herbal constituents and the oil throughout the processing period. Contemporary Scientific Perspective From the standpoint of modern pharmaceutical science, extraction temperature is an important determinant of phytochemical composition.

Numerous studies involving herbal extraction have demonstrated that processing temperature may influence:

- extraction efficiency,
- oxidative degradation,
- essential oil retention,
- stability of thermolabile constituents,
- colour,
- viscosity,
- antioxidant activity.

However, no direct experimental studies have yet demonstrated these effects specifically for Kutaja Nimba Taila prepared by Āditya Pāka Taila Vidhi. Therefore, while the pharmaceutical rationale is scientifically plausible, the proposed advantages remain hypotheses requiring experimental validation.



**Nagaraja and Ganga R Hadimani****Preservation of Thermolabile and Volatile Constituents**

Classical Ayurvedic literature suggests that Āditya Pāka provides gentle pharmaceutical processing. From a modern perspective, lower processing temperatures could theoretically reduce degradation of heat-sensitive phytochemicals and minimize evaporation of volatile constituents compared with direct boiling. Nevertheless, the present review did not identify direct experimental evidence demonstrating superior preservation of thermolabile or volatile phytoconstituents in Kutaja Nimba Taila prepared by Āditya Pāka. Accordingly, the present manuscript deliberately avoids presenting this concept as an established scientific fact. Instead, the available evidence supports the following interpretation:

- Classical texts advocate gradual solar processing.
- Modern pharmaceutical science recognizes temperature-dependent degradation of phytochemicals.
- Whether Āditya Pāka provides superior phytochemical retention remains unknown.

Future comparative analytical investigations using HPTLC, HPLC, GC–MS, LC–MS/MS, and metabolomic profiling are required to verify this hypothesis.

Comparison Between Agni Pāka and Āditya Pāka

Both pharmaceutical techniques are intended to prepare medicated oils but differ considerably in their processing characteristics. Although theoretical pharmaceutical differences exist, published comparative studies directly evaluating Agni Pāka and Āditya Pāka formulations remain extremely limited

Advantages

Potential pharmaceutical advantages include:

- Natural solar-assisted processing.
- Elimination of direct heating.
- Reduced energy consumption.
- Simplicity of preparation.
- Potential compatibility with environmentally sustainable pharmaceutical practices.
- Conceptual suitability for heat-sensitive herbal formulations.

However, these potential benefits require confirmation through controlled pharmaceutical investigations.

Limitations

Several limitations currently restrict wider scientific acceptance of Āditya Pāka.

- Absence of standardized operating procedures.
- Variation in climatic conditions.
- Lack of standardized solar exposure parameters.
- Limited analytical characterization.
- Lack of comparative pharmaceutical studies.
- Insufficient stability studies.
- Absence of pharmacokinetic investigations.
- Very limited clinical validation.

These limitations highlight the need for systematic pharmaceutical standardization.

Current Evidence

The available literature primarily consists of:

- classical Ayurvedic descriptions,
- pharmacological studies of Kutaja,
- pharmacological studies of Nimba,
- general studies on herbal extraction.



**Nagaraja and Ganga R Hadimani**

No published investigations were identified that directly evaluated:

- phytochemical profile of Kutaja Nimba Taila,
- physicochemical characteristics following Āditya Pāka,
- comparative pharmaceutical evaluation against Agni Pāka,
- randomized clinical trials of the finished formulation.

Therefore, the pharmaceutical superiority of Āditya Pāka remains unproven and should be regarded as a promising area for future research rather than an established conclusion.

Pharmaceutical Relevance to the Present Formulation

The proposed preparation of Kutaja Nimba Taila by Āditya Pāka Taila Vidhi integrates classical Ayurvedic pharmaceutical concepts with contemporary understanding of herbal drug delivery. Although not described as a named formulation in the classical texts, it represents a scientifically rational adaptation based on documented principles of Sneha Kalpana, the traditional methodology of solar-assisted processing, and the established therapeutic properties of Kutaja and Nimba. The formulation should therefore be viewed as a candidate for pharmaceutical standardization and scientific validation, rather than a formulation with already established efficacy. Future investigations should focus on standardized preparation, analytical characterization, stability assessment, pharmacological evaluation, microbiological testing, and well-designed clinical trials to determine its quality, safety, and therapeutic potential. Kutaja (*Holarrhena antidysenterica* Wall. ex A.DC.): Classical and Contemporary Perspectives. Kutaja (*Holarrhena antidysenterica* Wall. ex A.DC.), belonging to the family Apocynaceae, is one of the most important medicinal plants described in Ayurveda. Although widely recognized for its efficacy in Atisāra (diarrhoea), Pravāhikā (dysentery), and Krimi (helminthic infestations), classical Ayurvedic literature also recommends Kutaja in various Kuṣṭha (skin diseases) owing to its Krimighna (antimicrobial), Kuṣṭhaghna (anti-dermatotic), Kaṇḍughna (anti-pruritic), Śothahara (anti-inflammatory), and Vraṇasodhana–Vraṇaropana (wound cleansing and wound healing) properties. Modern phytochemical and pharmacological investigations have demonstrated that Kutaja contains a diverse range of biologically active constituents, particularly steroidal alkaloids, which exhibit antimicrobial, anti-inflammatory, antioxidant, immunomodulatory, and wound-healing activities. These properties provide scientific support for its classical indications and justify its inclusion in topical dermatological formulations such as the proposed Kutaja Nimba Taila.

Botanical Description: Kutaja is a small deciduous tree attaining a height of approximately 4–10 m. The bark is greyish-white with irregular fissures. Leaves are opposite, elliptic-oblong, and glabrous. Flowers are white, fragrant, and arranged in terminal corymbose cymes. Fruits are cylindrical follicles containing numerous linear seeds with silky tufts.

Classical Ayurvedic Properties: Ayurvedic classics consistently describe Kutaja as a drug possessing Kashāya (astringent) and Tikta (bitter) taste, with Laghu (light) and Rūkṣa (dry) qualities, Śīta Vīrya (cool potency), and Kaṭu Vipāka (pungent post-digestive effect). It primarily pacifies Kapha and Pitta Doṣa, making it useful in inflammatory and infective conditions.

Classical Therapeutic Indications: Kutaja is extensively mentioned in classical Ayurvedic literature for the management of various disorders.

Phytochemical Constituents: Kutaja contains numerous biologically active phytochemicals that contribute to its pharmacological profile.

Conessine has attracted considerable attention because of its antimicrobial and anti-inflammatory activities.

Dermatological Relevance: The therapeutic profile of Kutaja is particularly relevant for dermatological applications. Its reported activities include:

- reduction of microbial colonization,



**Nagaraja and Ganga R Hadimani**

- alleviation of inflammation,
- control of pruritus,
- promotion of wound healing,
- protection against oxidative damage.

These properties provide a pharmacological basis for its inclusion in formulations intended for dandruff, seborrheic dermatitis, chronic scalp disorders, and superficial skin infections. However, no published clinical studies have evaluated Kutaja alone or in combination with Nimba Taila as the finished formulation proposed in the present review. Pharmaceutical Relevance in Āditya Pāka Taila Fresh Kutaja leaves constitute the herbal component of the proposed formulation. During Āditya Pāka, gradual exposure to sunlight may facilitate diffusion of phytoconstituents into the lipid medium. Nevertheless, the extent of phytochemical transfer during solar processing has not yet been experimentally quantified.

Future studies employing:

- HPTLC
- HPLC
- GC-MS
- LC-MS/MS

should investigate:

- extraction efficiency,
- phytochemical profile,
- marker compound retention,
- batch-to-batch consistency.

Safety Profile

Kutaja has a long history of therapeutic use in Ayurveda.

Published toxicological studies generally indicate favourable safety when used appropriately.

However:

- the safety of Kutaja Nimba Taila prepared by Āditya Pāka
- dermal irritation profile,
- sensitization potential,
- long-term topical application

remain to be evaluated through standardized preclinical investigations.

Role in the Proposed Formulation

Within the proposed Kutaja Nimba Taila, Kutaja serves as the principal herbal ingredient providing antimicrobial, anti-inflammatory, antioxidant, antipruritic, and wound-healing properties. When combined with Nimba Taila, it is expected to contribute synergistically to the therapeutic profile of the formulation. However, such synergistic effects remain hypothetical until confirmed through pharmaceutical, pharmacological, and clinical studies. Although Kutaja is extensively documented in both classical Ayurvedic literature and modern pharmacological research, most available evidence pertains to individual extracts or isolated constituents. There is currently no direct experimental or clinical evidence evaluating Kutaja Nimba Taila prepared by Āditya Pāka Taila Vidhi. Therefore, therapeutic claims regarding the finished formulation should be considered provisional and require validation through well-designed pharmaceutical studies, preclinical investigations, and randomized controlled clinical trials.

Nimba (*Azadirachta indica* A. Juss.): Classical and Contemporary Perspectives Nimba (*Azadirachta indica* A. Juss.), commonly known as Neem, is one of the most extensively described medicinal plants in Ayurveda and is revered as "Sarva Roga Nivarini" (the curer of all diseases). Belonging to the family Meliaceae, Nimba occupies a prominent position in Ayurvedic therapeutics due to its broad spectrum of biological activities. Classical texts describe it as Kuṣṭhaghna (beneficial in skin diseases), Krimighna (antimicrobial), Kandughna (anti-pruritic), Kledaghna (reducing



**Nagaraja and Ganga R Hadimani**

excessive moisture), Vraṇāśodhana (wound cleansing), and Vraṇaropana (wound healing). Among the various dosage forms prepared from Nimba, Nimba Taila (Neem oil) is widely employed in Ayurvedic dermatology for the management of dandruff, scalp infections, eczema, psoriasis, chronic wounds, pruritic skin disorders, and other inflammatory dermatological conditions. In the present review, Nimba Taila serves a dual purpose: it functions as the **lipid vehicle (Sneha Dravya)** and also contributes its own pharmacologically active phytoconstituents to the proposed formulation.

Botanical Description: Nimba is a medium- to large-sized evergreen tree attaining a height of 15–20 m. The leaves are alternate, pinnate, and serrated. Flowers are small, white, and fragrant. Fruits are smooth, olive-like drupes containing a single seed rich in fixed oil.

Ayurvedic Pharmacodynamic Properties (Rasapañcaka): Classical Ayurvedic literature consistently describes Nimba as possessing Tikta (bitter) and Kaṣāya (astringent) taste, Laghu (light) and Rūkṣa (dry) qualities, Śīta Vīrya (cool potency), and Kaṭu Vipāka (pungent post-digestive effect). It predominantly alleviates Pitta and Kapha Doṣa, making it particularly valuable in inflammatory and infective skin disorders.

Classical Therapeutic Indications: Nimba is indicated in numerous diseases in classical Ayurvedic texts. These indications strongly support the traditional use of Nimba-based formulations in dermatology.

Phytochemical Constituents: Neem contains a remarkably diverse array of bioactive compounds, many of which have been extensively investigated. These compounds collectively contribute to the biological activities of Nimba Taila.

Pharmaceutical Importance of Nimba Taila: Unlike many herbal oils that function primarily as inert lipid vehicles, Nimba Taila itself possesses substantial therapeutic activity. Consequently, it performs two important pharmaceutical functions:

As Sneha Dravya

- Serves as the lipid medium.
- Facilitates extraction of lipophilic phytoconstituents.
- Improves dermal penetration.
- Enhances stability of the formulation.

As an Active Therapeutic Ingredient

- Contributes antimicrobial activity.
- Provides antifungal action.
- Reduces inflammation.
- Supports wound healing.
- Improves scalp health.

This dual functionality distinguishes Nimba Taila from many conventional pharmaceutical vehicles.

Relevance to Dermatological Disorders

The pharmacological profile of Neem oil closely corresponds with the Ayurvedic indications for skin diseases. Potential therapeutic applications include:

- dandruff,
- seborrheic dermatitis,
- superficial fungal infections,
- eczema,
- psoriasis,



**Nagaraja and Ganga R Hadimani**

- folliculitis,
- infected wounds,
- chronic pruritus.

However, the efficacy of Kutaja Nimba Taila prepared specifically by Āditya Pāka Taila Vidhi has not yet been evaluated in controlled preclinical or clinical studies. Therefore, these indications should be interpreted as potential applications requiring scientific validation rather than established therapeutic claims.

Safety Profile

Neem has a long history of medicinal use and is generally considered safe when appropriately formulated for topical application.

Nevertheless, certain considerations remain important:

- occasional contact dermatitis,
- hypersensitivity reactions,
- variability in oil composition,
- need for microbial quality assurance,
- requirement for stability assessment.

For the proposed formulation, comprehensive safety evaluation should include:

- dermal irritation studies,
- skin sensitization testing,
- repeated-dose topical toxicity,
- microbiological quality assessment,
- stability studies under recommended storage conditions.

Role in the Proposed Kutaja Nimba Taila

In the proposed formulation, Nimba Taila functions as both the base oil (Sneha Dravya) and an active therapeutic component. During Āditya Pāka processing, it serves as the extraction medium into which phytoconstituents from fresh Kutaja leaves are gradually transferred.

This combination is pharmaceutically rational because:

- Kutaja contributes steroidal alkaloids and other bioactive phytochemicals.
- Nimba Taila provides limonoids, fatty acids, and established dermatological activity.
- The lipid medium enhances topical application and retention.

However, the degree of phytochemical extraction and any synergistic interaction between Kutaja and Nimba remain hypothetical until demonstrated through analytical and pharmacological studies. Nimba is among the best-characterized medicinal plants in Ayurveda and modern phytopharmacology. Extensive evidence supports its antimicrobial, antifungal, anti-inflammatory, antioxidant, and wound-healing properties. Nevertheless, these findings cannot be directly extrapolated to the proposed Kutaja Nimba Taila prepared by Āditya Pāka Taila Vidhi, as the finished formulation has not undergone comprehensive pharmaceutical characterization or clinical evaluation. Accordingly, the proposed formulation should be regarded as a scientifically plausible, classically derived topical preparation requiring systematic standardization, phytochemical fingerprinting, safety assessment, and efficacy evaluation before clinical recommendations can be made. Scientific Rationale for the Proposed Kutaja Nimba Taila Prepared by Āditya Pāka Taila Vidhi The formulation of Kutaja Nimba Taila prepared by Āditya Pāka Taila Vidhi has been conceived by integrating classical Ayurvedic pharmaceutical principles with contemporary scientific understanding of herbal drug delivery. Although numerous medicated oils are described in the Ayurvedic classics, Kutaja Nimba Taila is not described as a named formulation in the available classical literature. The present formulation therefore represents a conceptually derived pharmaceutical preparation, developed by applying the classical methodology of Āditya Pāka Taila Vidhi to two medicinal agents—Kutaja (*Holarrhena antidysenterica*) and Nimba Taila (*Azadirachta indica*)—that possess well-established therapeutic relevance in dermatological disorders.



**Nagaraja and Ganga R Hadimani**

The formulation has been proposed primarily as a candidate for pharmaceutical standardization, analytical evaluation, and future preclinical and clinical investigation, rather than as an already validated therapeutic product.

Classical Basis for the Formulation

The conceptual basis of the formulation is derived from three established Ayurvedic principles:

- Sneha Kalpana as the pharmaceutical process for preparing medicated oils.
- Āditya Pāka Taila Vidhi as a classical solar-assisted processing technique.
- The documented therapeutic properties of Kutaja and Nimba in Kuṣṭha (skin diseases), Kaṇḍu (pruritus), Krimi (microbial infestations), and Vraṇa (wounds).

Although these principles are individually described in classical texts, their combination into the present formulation is an adaptation based on Ayurvedic pharmaceutical reasoning. This distinction is important to avoid implying that the formulation exists in classical literature as a named preparation. Rationale for Selection of Kutaja: Kutaja was selected because of its classical indications and pharmacological profile relevant to dermatological disorders.

From an Ayurvedic perspective, Kutaja possesses:

- Kuṣṭhaghna activity,
- Krimighna activity,
- Kaṇḍughna action,
- Śothahara (anti-inflammatory) properties,
- Vraṇaśodhana and Vraṇaropana effects.

Modern studies have demonstrated antimicrobial, anti-inflammatory, antioxidant, and wound-healing activities, largely attributed to steroidal alkaloids such as conessine and related phytoconstituents. These findings support its inclusion as the principal herbal ingredient in the proposed formulation.

Rationale for Selection of Nimba Taila

Nimba Taila was selected because it functions both as:

- the Sneha Dravya (base oil), and
- an active therapeutic component.

Neem oil possesses documented antimicrobial, antifungal, anti-inflammatory, antioxidant, and wound-healing properties, making it particularly suitable for topical application in scalp and skin disorders. In addition to serving as the extraction medium during Āditya Pāka, it contributes biologically active limonoids, fatty acids, and other phytochemicals that may complement the activity of Kutaja.

Scientific Basis for Combining Kutaja and Nimba

The combination of Kutaja and Nimba is supported. The complementary actions of the two ingredients provide a rational pharmaceutical basis for their combination. However, any claim of synergistic therapeutic action remains hypothetical until confirmed through experimental studies.

Rationale for Using Āditya Pāka Taila Vidhi

The choice of Āditya Pāka was based on classical pharmaceutical descriptions of solar-assisted processing. Compared with Agni Pāka, Āditya Pāka provides gradual extraction under ambient sunlight without direct heating.

Potential theoretical advantages include:

- gradual diffusion of phytoconstituents,
- avoidance of direct thermal exposure,
- environmentally sustainable processing,
- preservation of formulation integrity through mild processing conditions.

At present, these proposed advantages remain **pharmaceutical hypotheses**, as direct comparative evidence between Agni Pāka and Āditya Pāka preparations of Kutaja Nimba Taila is unavailable.



**Nagaraja and Ganga R Hadimani****Proposed Mechanism of Pharmaceutical Processing**

The proposed sequence of pharmaceutical events during Āditya Pāka may be summarized as follows:

- Fresh Kutaja leaves are coarsely crushed to increase the exposed surface area.
- The leaves are immersed in Nimba Taila.
- Continuous solar exposure promotes gradual diffusion of phyto constituents into the lipid medium.
- Gentle daily stirring enhances contact between the herbal material and the oil.
- Filtration removes plant residues while retaining the medicated oil.
- The finished product is stored in amber-coloured containers to protect against photo-oxidation.

This mechanism is proposed on the basis of classical pharmaceutical concepts and general extraction principles. It requires validation through analytical studies.

Proposed Standard Operating Procedure (SOP): To improve reproducibility and facilitate future pharmaceutical investigations, the following Standard Operating Procedure (SOP) is proposed. Note: The ingredient ratio and processing duration proposed above are based on classical Āditya Pāka principles and pharmaceutical reasoning. They should be optimized and validated through future pharmaceutical studies.

Recommended Quality Control Measures: Each batch of Kutaja Nimba Taila should undergo comprehensive quality assessment before clinical application. The analytical techniques listed above are recommended for future standardization and were not performed as part of the present narrative review.

Limitations of the Proposed Formulation

Despite its strong classical and pharmaceutical rationale, several limitations should be acknowledged:

- The formulation is **not described as a named preparation in classical texts**.
- There are **no published preclinical or clinical studies** evaluating the finished formulation.
- The proposed SOP has not yet undergone pharmaceutical validation.
- The phytochemical profile after Āditya Pāka has not been characterized.
- Comparative studies with Agni Pāka preparations are lacking.

Therefore, the present formulation should be regarded as a scientifically plausible candidate for future investigation rather than an established therapeutic intervention. Kutaja Nimba Taila prepared by Āditya Pāka Taila Vidhi represents a pharmaceutically rational adaptation of classical Ayurvedic principles. The formulation integrates the established therapeutic attributes of Kutaja and Nimba with the traditional concept of solar-assisted processing. While the theoretical rationale is supported by classical literature and modern pharmacological knowledge of the individual ingredients, the finished formulation itself remains insufficiently studied. Future work should prioritize pharmaceutical standardization, phytochemical characterization, quality control, pharmacological validation, and randomized clinical trials to establish its safety, efficacy, and role in evidence-based Ayurvedic dermatology.

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Table 1: Comparative Features of Agni Pāka and Āditya Pāka

Parameter	Agni Pāka	Āditya Pāka
Source of heat	Direct fire	Natural sunlight
Processing temperature	High	Mild to moderate ambient temperature
Processing duration	Hours	Several days





Nagaraja and Ganga R Hadimani

Energy source	Conventional fuel	Solar energy
Extraction pattern	Rapid	Gradual
Suitability	Internal and external formulations	Primarily topical formulations
Standardization	Well established	Requires further validation
Scientific evidence	Extensive	Limited

Table 2: Key Features of Āditya Pāka Taila Vidhi

Feature	Description
Pharmaceutical basis	Solar-assisted processing of medicated oils
Classical status	Described as a pharmaceutical technique; not specific to Kutaja Nimba Taila
Processing medium	Natural sunlight
Heating method	No direct fire
Present evidence	Primarily classical descriptions and theoretical rationale
Major limitation	Lack of comparative scientific studies
Future requirement	SOP development, analytical standardization, pharmacological and clinical validation

Table 3: Botanical Profile of Kutaja

Parameter	Description
Botanical name	<i>Holarrhena antidysenterica</i> Wall. ex A.DC.
Family	Apocynaceae
Sanskrit names	Kutaja, Vatsaka, Girimallika, Indravriksha
English name	Kurchi Tree, Conessi Tree
Hindi	Kutaj
Kannada	Kodasige
Parts used	Bark, leaves, seeds, flowers
Distribution	Throughout India, especially sub-Himalayan regions, deciduous forests, and tropical climates

Table 4: Classical Therapeutic Uses

Disease	Ayurvedic Action
Atisāra	Grahī, Stambhana
Pravāhikā	Antidiarrhoeal
Krimi	Krimighna
Kuṣṭha	Kuṣṭhaghna
Kaṇḍu	Kaṇḍughna
Vraṇa	Vraṇaśodhana, Vraṇaropana
Rakta Duṣṭi	Rakta Prasādana
Jvara	Jvaraghna

Table 5: Major Phytochemical Constituents of Kutaja

Class	Important Constituents
Steroidal alkaloids	Conessine, Holarrhenine, Holarrhimine, Kurchicine
Flavonoids	Quercetin derivatives
Phenolics	Gallic acid derivatives
Tannins	Condensed tannins
Terpenoids	Various triterpenoids
Sterols	β-sitosterol





Nagaraja and Ganga R Hadimani

Table 6: Pharmaceutical Significance of Kutaja in the Proposed Formulation

Characteristic	Pharmaceutical Relevance
Classical indication	Kuṣṭha, Kaṇḍu, Vraṇa
Principal phytochemicals	Steroidal alkaloids
Major pharmacological actions	Antimicrobial, anti-inflammatory, antioxidant
Pharmaceutical role	Herbal source of active constituents
Present evidence	Supported for the individual plant; not for the finished formulation
Future requirement	Standardization, analytical profiling, efficacy and safety evaluation

Table 7: Botanical Profile of Nimba

Parameter	Description
Botanical name	<i>Azadirachta indica</i> A. Juss.
Family	Meliaceae
Sanskrit names	Nimba, Arishta, Pichumarda, Prabhadra
English name	Neem
Hindi	Neem
Kannada	Bevu
Parts used	Leaves, bark, seeds, flowers, fruits, root, oil
Distribution	Throughout tropical and subtropical India and many tropical countries

Table 8: Classical Indications of Nimba

Disease	Classical Action
Kuṣṭha	Kuṣṭhaghna
Kaṇḍu	Kandughna
Krimi	Krimighna
Vraṇa	Vraṇaśodhana, Vraṇaropana
Prameha	Pramehaghna
Jvara	Jvaraghna
Rakta Duṣṭi	Raktaprasādana
Visarpa	Viṣarpaghna

Table 9: Major Phytochemicals of Nimba

Class	Important Constituents
Limonoids	Azadirachtin, Nimbin, Nimbidin
Terpenoids	Gedunin, Nimbolide
Flavonoids	Quercetin
Polyphenols	Gallic acid derivatives
Sterols	β-Sitosterol
Fatty acids	Oleic, Stearic, Palmitic, Linoleic acids

Table 10: Pharmaceutical Role of Nimba Taila in the Proposed Formulation

Pharmaceutical Property	Significance
Sneha Dravya	Lipid extraction medium
Active ingredient	Antimicrobial and anti-inflammatory activity
Skin penetration	Facilitates topical drug delivery
Stability	Protects lipid-soluble phytoconstituents
Dermatological application	Suitable for scalp and skin disorders
Present evidence	Strong for Neem individually; lacking for the combined formulation





Nagaraja and Ganga R Hadimani

Table 11: Comparative Therapeutic Contributions of Kutaja and Nimba

Parameter	Kutaja	Nimba Taila
Principal role	Herbal drug	Base oil and active ingredient
Major phytochemicals	Steroidal alkaloids	Limonoids, terpenoids, fatty acids
Antimicrobial activity	Yes	Yes
Anti-inflammatory activity	Yes	Yes
Antioxidant activity	Yes	Yes
Wound-healing activity	Yes	Yes
Lipid vehicle	No	Yes
Traditional dermatological use	Yes	Yes

Table 12: Proposed SOP for Kutaja Nimba Taila Prepared by Āditya Pāka Taila Vidhi

Step	Procedure
Ingredients	Nimba Taila (1 part); Fresh Kutaja leaves (1 part, coarsely crushed)
Selection of Raw Materials	Collect healthy, disease-free leaves; authenticate botanical identity.
Cleaning	Wash with potable water and shade-dry to remove surface moisture.
Preparation	Coarsely crush the leaves and transfer them into a clean, dry, wide-mouthed glass container. Add the measured quantity of Nimba Taila and mix thoroughly.
Solar Processing	Cover with sterile muslin cloth or a breathable lid. Expose to direct sunlight for approximately 6–8 hours daily for 7 consecutive days (or until adequate extraction is achieved). Stir gently 2–3 times daily using a sterile glass rod. Bring the container indoors after sunset and protect it from rain, dust, insects, and excess humidity.
Filtration	Filter through sterile muslin cloth or Whatman filter paper.
Packaging	Transfer to sterile amber glass bottles and seal immediately.
Storage	Store in a cool, dry place away from direct sunlight. Label with batch number and preparation date.

Table 13: Recommended Quality Control Parameters

Category	Parameters
Organoleptic	Colour, odour, appearance, consistency
Physicochemical	Specific gravity, refractive index, viscosity, acid value, peroxide value, iodine value, saponification value, moisture content, rancidity
Phytochemical	Qualitative and quantitative estimation of alkaloids, flavonoids, phenolics, tannins, terpenoids, glycosides; HPTLC, HPLC, and GC–MS fingerprinting
Microbiological	Total aerobic microbial count, total fungal count, absence of specified pathogens
Stability	Accelerated and long-term stability studies, shelf-life determination

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RESEARCH ARTICLE

Gut Dysbiosis and Oral Drug Pharmacokinetics : Mechanisms, Drug - Class Evidence and Emerging Research Gaps

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ABSTRACT

One of the critical unknowns in clinical pharmacology is the ability of different people to respond to a drug in different ways. Although substantial amounts of variation in drug responses have been deciphered, from an estimated 20-40%, the role played by the gut microbiome has become an increasingly important, but largely unexplored, variable impacting on the pharmacokinetics (PK) of orally delivered drugs. Microbiota can have an impact on the absorption, distribution and metabolism of a drug through 4 simultaneous mechanisms: it can regulate the expression of intestinal drug transporters by effecting expression or production of microbial metabolites in the intestine; it can alter the composition of the bile acid pool that regulates the induction of hepatic drug metabolizing enzymes and drug transporters through nuclear receptors; it can directly modulate hepatic cytochrome P450 activity through the gut-liver axis; and it can directly biotransform drugs in the gastrointestinal tract. All these mechanisms are also affected by gut dysbiosis (the disease associated structural alteration of the microbial community) which can result in the loss of microbial diversity, a reduction in amounts of producer taxa of short-chain fatty acid (SCFA), and a rise of taxa of pathobionts, with important clinical consequences for therapy with narrow therapeutic windows (NTWs) like that of tacrolimus, irinotecan and digoxin. Here it provides a detailed, understanding of the mechanistic pathway from dysbiosis to changes in PK; a systematic review of dysbiosis-related changes in pg clinical trials; a consideration of strategies for restoring the microbiome across all drug classes; and a logical evolution and discussion of six key areas of research needed, including the need for validated dysbiosis severity PK biomarkers, uncharacterised PK basis of



**Srinivas and Sanil Kumar**

antidepressant non-responsivity, and a lack of microbiome parameters in pg models. The answers to these omissions are essential for the inclusion of pharmaco-microbiomics in suitable pipeline of precision medicine and for the well-being of patients when exposed to the unknown risks of patients' GIX.

Keywords: gut microbiota; pharmacomicrobiomics; gut dysbiosis; pharmacokinetics; drug transporters; P-glycoprotein; bile acids; short-chain fatty acids; CYP3A4; tacrolimus; irinotecan; digoxin; SSRI; enterohepatic recycling

INTRODUCTION

The response to a drug is highly individual and to this day one of the most clinically relevant and mechanistically complicated problems in pharmacology. The field involved in the effect of oral pharmacology on the oral or gut microbiome as well as the effect of the oral or gut microbiome on the oral pharmacology is termed the 'pharmacomicrobiomics' and there are human clinical applications for nearly all drug classes given orally. The pharmacokinetics of drugs can be directly and indirectly impacted by the microbiota. Microbial metabolites, most importantly short chain fatty acid metabolites (SCFAs), secondary bile acid metabolites, and indole metabolites (derived from the amino acid tryptophan) are all members of a group of signalling molecules that can activate the gut receptors for example (pregnenane X receptor (PXR), farnesoid X receptor (FXR), aryl hydrocarbon receptor (AhR)), which then regulate the expression and activity of intestinal drug transporters and hepatic cytochrome P450 (CYP) enzymes in the intestine and/or in the liver, either directly or indirectly, resulting on prodrugs that are metabolized to drugs, detoxification of therapeutic agents or metabolite reactivation [1, 2]. Although the mechanisms are becoming more familiar, none of the existing pharmacokinetic modeling framework, dose adjustment algorithm or regulatory drug development guideline includes gut microbiome parameters directly, thus raising patient safety concerns. This review aims to make mechanistical and clinical evidence a whole in a common set-up or framework of a medicine. We first systematically review the 4 possible mechanisms by which dysbiosis might alter oral drug pharmacokinetic parameters, and then explore the oral drug pharmacokinetic parameters as they relate to different disease contexts, including the five key classes wherein there is documented clinical evidence. Then pharmacologic strategies which have been proposed to restore microbiome-mediated PK normalisation are explored and six major gaps in research that must be resolved in order to advance pharmacomicrobiomics to the clinical arena are discussed [5, 6, 7].

Gut Microbiome and Dysbiosis: Pharmacologically Relevant Concepts

Composition under Homeostasis

The Firmicutes species *F. prausnitzii*, *R. intestinalis* and *B. fibrisulvens*, as well as the Bacteroidetes species *P. acnes*, produce pharmacologically important microbial metabolites such as the so-called short-chain fatty acids (SCFAs) butyrate, propionate and acetate, however, secondary bile acids are produced by the Clostridiales species with BSH activity, and indoles by the activity of *Lactobacillus* spp. and *Clostridium* spp. These gut microbial metabolites need to be physiologically produced to maintain homeostatic regulation of intestinal epithelial integrity, to modulate the expression of drug transporters and expression levels of nuclear receptor signalling pathways that drive drug-metabolising enzyme (DME) induction [8].

Defining Dysbiosis

The dysbiosis profile associated with each disease state has a distinct composition of dysbiotic microbes, with pharmacologically specific consequences: IBD-associated dysbiosis is characterised by depleted levels of the Firmicutes, *F. prausnitzii* and Roseburia, but enriched levels of the potentially-adherent and-pathogenic species, AEC (or adherent-invasive *Escherichia coli*), which have drug-inactivating enzymatic activity, as well as decreased availability of commensal protective microbes such as SCFA-producing Firmicutes, and decreased levels of



**Srinivas and Sanil Kumar**

azoreductase, an enzyme critical for the activation of 5-ASA prodrugs; obesity and T2D dysbiosis involves reduced levels of the Firmicutes, Akkermansia muciniphila and Bifidobacterium, and increased levels of the F. genus, Fusobacterium and Proteobacteria, with an especially high F/B ratio, which affects bile acid homeostasis and handling of the anti-hyperglycemic drug, metformin; dysbiosis in MDD involves decreased SCFAs-producing Firmicutes, increased adherent and-pathogenic E. coli (e.g., AEC) and an increased abundance of E. lenta, a Firmicutes sp. with known drug-inactivating enzymatic activity.

Effect on pharmacologically active microbial enzymes

The gut microbiota blockly displays a wide spectrum of drug-metabolising enzymes that lack within the human genome but whose activity can significantly influence drug pharmacokinetics. Of direct clinical relevance, the cardiac glycoside reductase (cgr) operon, consisting of two genes that encode enzymes capable of reducing the cardiotonic drug digoxin to pharmacologically inactive dihydrodigoxin, is found in Eggerthella lenta. Pharmacokonjugation and deconjugation, mediated by multiple taxa including Clostridiales, Bacteroides and Escherichia coli, are relevant to the deconjugation of glucuronide metabolites of drugs in the intestinal lumen, liberating the parent compound for reabsorption and thereby increasing systemic drug exposure and toxicity; a process that may be directly relevant to irinotecan, NSAIDs and mycophenolate. BSH enzymes can modify the pool of bile acids that are active in the intestine, affecting the activity of the nuclear receptors FXR, TGR5 and PXR and hence downstream transporter and CYP enzyme expression in the intestinal epithelium and liver [20, 21].

Mechanisms: Dysbiosis and Altered Drug Absorption**Drug Transporter Regulation by Microbial Metabolites:**

The major gatekeepers of oral drug bioavailability are intestinal transporters, such as ATP-binding cassette (ABC) efflux transporters P-glycoprotein (P-gp/ABCB1), breast cancer resistance protein (BCRP/ABCG2) and multidrug resistance-associated protein 2 (MRP2/ABCC2), as well as solute carrier (SLC) influx transporters OATP1A2 and OATP2B1, all of which are highly regulated by gut microbial metabolites via nuclear receptors, transcriptions and post-translational mechanisms on expression levels.[8,9] Here, microbial control of baseline intestinal P-gp, which regulates the transport of P-gp substrate drugs, was shown to depend on microbiota comprehensiveness and is enhanced by the col types firmicute Clostridia and bacille Bacilli through epigenetic mechanisms involving P-gp-inhibiting HDAC without any specific dependence on any single col type, thereby confirming the ARI that defines intestinal P-gp function and the microbiota's role is dictated by microbial core integrity, not individual col types [8, 22]. The metabolites of indole-producing Lactobacillus species activate the aryl hydrocarbon receptor (AhR) in intestinal epithelial cells, which also has a direct pharmacological effect on the OATP-mediated influx transport of absorbed substances, and the metabolites of Eggerthella lenta inhibit P-gp ATPase activity in a post-translational manner that so far did not involve OATP transportin [8,9].

Bile Acid Pool Dysregulation and Drug Solubilisation:

Secondary bile acids activate FXR in the intestinal epithelial cells, which then suppresses the activity of CYP7A1 (the enzyme responsible for cholesterol to bile acid conversion), while also inducing FXR-mediated expression of other gene downstream of the FXR-responses elements on the promoters of the drug transporter OST α/β and the efflux pump p-gp (in a manner that depends on the composition of the dysbiotic bile acid pool). [18, 19] Depletion of taxa which express BSH in the dysbiotic gut microbiota will lead to accumulation of primary conjugated bile acid and also a reduction in the levels of secondary bile acids, which in turn have been shown to inhibit the ATPase activity of p-gp and alter the multidrug resistance efflux landscape, depending on their hydroxyl organization.

Master PK-regulators: SHORT CHAIN FATTY ACIDS Master:

The effects of SCFAs on P-gp expression aren't the only that are mediated by the paracellular route. Butyrate also upregulates tight junction proteins that restrict non-specific passive entry of small molecules; when the production of butyrate is depleted, as is seen during dysbiosis, tight junction proteins become downregulated, allowing more



**Srinivas and Sanil Kumar**

passive paracellular entry of small molecules and increasing systemic exposure to those that rely heavily on paracellular restriction for their absorption. Beyond the effects of the SCFAs on P-gp expression, butyrate has broad pharmacokinetics effects, as well: It has been shown to induce expression of the tight junction proteins that regulate non-specific passive absorption, which means that when its production is depleted, as seen during dysbiosis, delays food transit in the gut, leading to more passive absorption into the systemic circulation of orally administered drugs that rely on tight paracellular restriction for absorptive success [26,28].

Enterohepatic Recycling Amplification by β -glucuronidase:

The SN-38 metabolite from irinotecan is variously glucuronidated and excreted into the bile and is then re-conjugated by microbial enzymes in the gut. This is known as Enterohepatic recycling (EHC) and has been shown to enhance exposure, systemic toxicity and clearance of the parent drug by amplification of the microbial enzyme activity, depending on the compound and slight changes in bowel flora or bacterial expression [6,29]. After drug Glucuronidation in the liver and biliary excretion, there is the potential for Enterohepatic recycling (EHC), the re-excretion of hepatically excreted drug metabolites, after the bacterial deglucuronidation to regenerate the parent compound and/or its toxic aglycone forming the toxicological burden. Selectivity and specificity in pharmacological inhibition of the bacterial enzyme is an established proof of concept with drugs such as inh-1, which results in the reduction of intestinal SN-38 re-enunciation from irinotecan glucuronide together with a pharmacokinetics effect without influencing serum irinotecan or glucuronide concentrations [32].

Mechanisms: Dysbiosis and Hepatic/Systemic Drug Metabolism**The Gut-Liver Axis and Microbial Modulation of Hepatic CYPs:**

All 76,000 conventional germ-free mice (genotobiotic) studied have liver CYP3A expression, which is significantly reduced compared to conventional germ-positive mice, providing an example of one of the critical ways in which the liver controls gut expression of CYP enzymes, which clearly demonstrates that the gut microbiome is required for the upregulation of basal liver CYP3A activity. Increased systemic bioavailability of CYP3A4 substrates (such as tacrolimus, midazolam, cyclosporine and some statins) in dysbiosis also leads to reduced hepatic first-pass metabolism due to the inflammatory suppression of the PXR-CYP3A4 axis, which can be clinically complicated by pharmacogenomic CYP3A5 non-expressor phenotype, and can result in a longer half-life of these drugs.[35,36]

Direct Microbial Drug Biotransformation in the Intestinal Lumen:

In addition to interacting indirectly with host enzyme systems, our gut bacteria metabolise drugs within their intestinal lumen, by enzymatically converting the parent compound to active, inactive or toxic metabolites, depending on the specific drug and gastro-intestinal microorganism involved. Klünemann et al. showed that 76 of the 197 clinically approved non-antibiotic drugs are actively imported into the cell by gut bacteria, potentially sequestering the drug and in turn affecting its availability to the gut epithelia that interface the intestinal lumen with the circulation. The aggregate metabolism and drug-bioaccumulation capacity of the gut microbial community – a PK compartment that is not currently represented by any existing pharmacokinetic model – is altered by dysbiosis and the quantitative clinical significance of these effects remain undefined.[7,22,37,38]

Dysbiosis produced systemic inflammation and modulation of cyp activity:

This observation of pharmacokinetic alterations already has been demonstrated to occur in acute infections and states of inflammation, but the underlying mechanisms are not only cytokines, but also activity of pattern recognition receptors that lead to microbial translocation and subsequently to elevated levels of cytokines, such as IL-6 and TNF- α , which directly depress the expression of several CYP-isoforms in hepatocytes by post-transcriptional and transcriptional mechanisms. During acute infections and inflammatory episodes, this phenomenon of CYP suppression has been attributed to the action of the cytokines; however, in dysbiosis, where microbial translocation occurs and pattern recognition receptor activation takes place, the underlying mechanism has received marginal



**Srinivas and Sanil Kumar**

interest in clinical pharmacology and represents a research gap with clinical implication regarding patient safety for therapeutic drug monitoring [13, 35].

Disease-Specific Dysbiosis and Drug Pharmacokinetics: Clinical Evidence by Drug Class

The mechanistic framework established in Sections 3 and 4 manifests as clinically distinct pharmacokinetic consequences across disease-associated dysbiosis states. The following analysis provides a disease-stratified synthesis of drug-class-specific PK evidence — an integrated comparative framework that does not currently exist in the review literature [5, 13].

IBD Dysbiosis and 5-ASA / Immunosuppressant PK:

The microbially atypical balance of beneficial gut bacteria and harmful bacteria is highlighted by the Kozhemyako signature in IBD and the documented microbiome variation and widely observed PK differences in response to the core drug tacrolimus in both IBD and post-transplant settings [17,35]. The same effect of the gut microbiota on the PK of tacrolimus has been demonstrated in a pilot cross-sectional study with 93 kidney transplanted patients that did not express CYP3A5, which found that diverse gut microbes were strongly associated with decreased intraindividual AUC variability in tacrolimus, and that SCFA-producing taxa such as *Blautia* and *Roseburia* were inversely correlated with tacrolimus dose requirement suggesting a protective effect of SCFAs on reduction of tacrolimus efflux, thereby increasing absorption across the intestine.[41]

Obesity/T2D Dysbiosis & Metformin/Statin PK:

Statins have both PK and PD effects from the microbiome: statins are highly absorbed in the gut microbiome, and there is increased evidence that the microbiome has a clinically meaningful role in increasing the pharmacodynamic variability of statins, with some studies revealing the ability to predict the statin drug response based on microbial composition rather than genetic variation in drug transporters. [42] In some antibiotic-treated rats colonised with dysbiotic microbiota, losartan bioavailability is significantly decreased and the prevalence of dysbiosis in a large cohort of patients treated with statins is association with decreased bioavailability. This suggests there is a bidirectional relationship between the drug and the microbiome: statin therapy alters dysbiosis prevalence whereas dysbiosis alters statin bioavailability.

Cancer Dysbiosis and chemotherapy PK:

Irinotecan, also known as CPT-11, is a paradigm drug that is made tumouricidal by amplification in its enterohepatic-microbiome pathway. Finally, a pharmacodynamic interaction, in which the abundance of the taxa *Akkermansia muciniphila*, *Faecalibacterium prausnitzii* and *Bifidobacterium* at baseline has been consistently associated with improved objective response rates and survival in multiple independent cohorts of anti-PD-1/anti-CTLA-4-treated patients, contrasts with pharmacokinetically mediated interactions, where bacterial metabolism of the drug happens in the gut and contributes to the dose-limiting diarrhoea and gastrointestinal mucosal injury of irinotecan treatment. Microbiome-drug interactions are not limited to gut-related interactions and encompass many other mechanisms, such as inactivation of Gemcitabine by cytidine deaminase expressed by intratumoral bacteria, especially by *Mycoplasma* spp. and gram-positive bacteria [46, 47].

MDD Dysbiosis and SSRI Pharmacokinetics:

While dysbiosis is associated with a distinct element of MDD, with an elevated abundance of *Alistipes* and *Eggerthella*, and reduced abundance of *Lactobacillus* and *Bifidobacterium*, and reductions in SCFA production, the single largest unaddressed research gap remains a study of the pharmacomicrobiome of an SSRI drug. This is because there is no systematic study on the pharmacokinetics of SSRIs in relation to MDD-associated dysbiosis, which encompass reductions in intraluminal serotonin substrate availability upon which the pharmacological effects of SSRIs depend, and modifications of P-gp-dependent intestinal absorption, though mapping this interactions is highly relevant, and alterations in the production and metabolism of drugs in the liver under the regulation of enteroluminal axis cytokines [50].



**Tacrolimus and PK in transplants:**

Tacrolimus is the most well-studied narrow therapeutic index drug in pharmacomicrobiomics. The mechanism involved is the dual pathway whereby changes in microbiome leads to decreases in both intestinal expression of ABCB1 and decrease in hepatic expression of CYP3A4 in a NF- κ B/ PXR-dependent manner, resulting in increased intestinal absorption and reduction in hepatic clearance of tacrolimus, respectively, which may also contribute to increases in tacrolimus PcV. Genomic dysbiosis involves a dual mechanism: namely, decreases in intestinal expression of ABCB1, which increases absorption; and decreases in hepatic expression of CYP3A4, which is mediated by NF- κ B/ PXR-mediated repression of hepatic expression, which may also contribute to tacrolimus PcV increases [51].

Pharmacological Strategies to Restore Microbiome and Normalise Drug PK**Probiotics and SCFAs as PK Modulators:**

Oral supplementation of probiotics containing SCFA-producing bacteria, such as lactobacilli (*Lactobacillus* spp.), which more mechanistically relevantly restore intestine expression of claudin-1 and occludin, as well as intestine expression deficits that are caused by PXR mediated expression markers for metabolism, such as CYP3A4/upregulated background, restores these deficits, supports the Pharmacokinetically Rationale Drug Absorption Minimisation and stands as an active transporter-mediated efflux restoration approach in the setting of dysbiosis: to date, butyrate is directly available; supplementation with probiotics with a butyrate-releasing effect, such as taxa of lactobacilli (*Lactobacillus* spp.), is directly available; to date, there are no reports of butyrate having an anti-inflammatory effect in the intestine; the epigenetic effect of butyrate on the expression of PXR-regulated drug transporters, such as CYPs 3A4/upregulated background, has been described; to date, no other probiotics can have such a direct pharmacologic effect on PXR as supplemented by butyrate that also inhibits HDACs [26, 53].

Fecal Microbiota Transplantation

In humans, the first narrow therapeutic index drug reported to be benefitted by microbiome restoration with FMT was via improvement in the efficiency of the prodrug activation of sulfasalazine after FMT in antibiotic-pretreated animals that lack azoreductase activity. In the literature, there have been no clinical studies of how the activation of the sulfasalazine prodrug impacts the efficacy of the microbiome's activation, and this will need to be studied in humans with dysbiotic microbiomes, like in patients with *C. diff* and antibiotic-associated diarrhea (AAD). Recently, FMT has been shown to restore azoreductase activity in antibiotic-treated animals, reviving the efficiency of activation of sulfasalazine, a prodrug with a narrow therapeutic index, which has yet to be proven and should be reported in the literature [55].

Inhibitors of microorganisms specifically:

Dietary supplementation by arginine increases bioavailability to bacterial species considered to be non-digoxin susceptible, while diminishing the toxicity to all *E. coli* species, explaining a selective pathway of drug detox, suggesting that targeting specific bacterial enzymes can provide a means to overcome low system levels of any drug in human patients caused by unanticipated overcolonisation of the gut by the pathogenic strain. The use of the amino acid arginine has proven to selectively intervene with the transcription of the *cgr* operon, increasing digoxin bioavailability in both uncolonised samples and when grown in the presence of other bacteria species, but decreasing the toxicity of digoxin in the species *E. lenta* [21, 32].

Research Gaps, Challenges, and Future Directions

The ongoing evidence and mechanisms presented in this review highlight dysbiosis in the gut as a pharmacologically significant, clinically relevant, and less-controlled factor determining oral drug pharmacokinetics. Yet important basic knowledge gaps exist which have hindered translation for this knowledge to clinical practice [5,13].



**Srinivas and Sanil Kumar****Gap 1: Identification of a clinical PK Biomarker for severity of dysbiosis:**

Previous PK studies in dysbiosis-associated disease cohorts have been conducted in an exploratory fashion and not included a dysbiosis marker in conjunction with a therapeutic drug biomarker that can be correlated to measure PK perturbation of specific drug-exposure measures such as AUC, Cmax or trough concentrations — a key step in the qualification of a biomarker for a prospective study. Stereotypically, a PGX signature was collected and an exploratory correlation analysis was performed as an afterthought, and validation markers were collected and assessed in retrospective samples. Future PK studies of dysbiosis-associated diseases should validate the biomarkers of these specific diseases together with the standard therapeutic drug monitoring assessment parameters in prospective studies to enable a correlation analysis that helps qualify the biomarker for the drug-exposure measure (AUC, Cmax, trough) to be used in future studies as a surrogate for clinicobiologic endpoints [56].

Gap 2: SSRI Pharmacokinetics and Gut Dysbiosis — Mechanistic Characterisation Absent:

Finally, future studies will need to systematically determine whether there are measurable PK differences (e.g. lesser Cmax, AUC and half-life) in non-responders compared to responders in MDD, matched on CYP2D6/CYP2C19 genotype, and whether these differ from one marker of dysbiosis to another (e.g. depletion of SCFAs, increase in the abundance of Eggerthella, decrease in intestinal P-gp expression).[49,50]

Gap 3: Microbiome Exclusion from PBPK Modelling Frameworks:

There is now sufficient mechanistic data to enable the integration of a microbiome module into PBPK models for drugs that predict the microbiome-mediated alteration of drug PK, such as physiologically-based relationships between the oral microbiome and SCFA-P-gp, bile acid-FXR-CYP3A4, and β -glucuronidase-EHC amplification. Regulatory agencies (e.g., FDA and EMA) should be engaged to establish standards for the collection of microbiome data in drug development programmes targeting the microbiome pathway that influences oral bioavailability of drug substrates, such as ABCB1 (SRB)-P-gp; ABCG2 (NSAID)-FXR-CYP3A4; and β -glucuronidase-EHC, which is the drug-microbiome interaction space of most clinical significance [57].

Gap 4: Clinical Scale and PK Consequences of Drug Bioaccumulation in Bacteria:

The extent of drug bioaccumulation in human pharmacokinetic studies is not known, as is the quantitative impact of dysbiosis — the alteration of the bacterial biomass, species composition, and/or transporters expression — on the degree of drug bioaccumulation in the gut bacterial community (in a clinically relevant manner) is unknown[37,38].

Gap 5: Sex and Age as Confounders in Pharmacomicrobiomics Research:

Sex and age are two new variables in study design that should be analyzed as primary variables on the basis of their influence on microbiome composition and how they impact PK variability; to date, a majority of pharmacomicrobiomics studies only treats these variables as demographic descriptors. Microbiome composition is significantly different between males and females due to sex hormones regulating microbial diversity and metabolite production, and to the significant microbial compositional differences in paediatric, adult, and geriatric patients, who differ in their microbiomes composition and enzymatic activity. Sex and age should therefore be considered as pharmacomicrobiomic confounders and treated as main study variables as opposed to demographic descriptors [58].

Gap 6: Effects of Antibiotic-Associated Disruption of Enteric Flora and Non-Antibiotic Drug Pharmacodynamics

Clinical studies have demonstrated changes in blood-level results for therapeutic drugs given with antibiotics, but there remains a need for more systematic characterisation of the classes of therapeutic drugs that are affected by antibiotic-induced dysbiosis, the duration of effect in blood-level results post antibiotic course, the extent and rapidity of microbiome-recovery, and the community's understanding of how these changes impact drug safety in hospitalised patients receiving broad-spectrum antibiotic therapy who are also receiving therapeutic drug monitoring [59, 36].





CONCLUSION

Each of these four mechanistically distinct and interacting pathways is disrupted during gut dysbiosis in disease and would be expected to be distorted during the disease process for narrow therapeutic index (NTI) drugs, such as tacrolimus, irinotecan, digoxin, levodopa, and sulfasalazine — drugs whose pharmacological activity is significantly influenced by the gut microbiota — as described (but not documented) clinically. [39,29,39,21,17] Absence of validated PK biomarker(s) of dysbiosis, uncharacterised SSRI PK-microbiome interaction(s) exposition, failure to incorporate microbiome in PBPK models, undefined clinical scale of antibiotic-PK interaction(s) in bacteria, systematic sex and age confounding, and uncharacterised antibiotic-PK interaction(s) duration are the six research gap(s) highlighted in this review as the most important needs to move this field from discovery to clinical translation. The six research gaps identified in this review — absence of validated PK biomarker(s) of dysbiosis, uncharacterised SSRI PK-microbiome interaction(s) exposition, failure to incorporate microbiome in PBPK models, undefined clinical scale of drug bioaccumulation in bacteria, systematic sex and age confounding, and uncharacterised antibiotic-PK interaction(s) duration — are key in advancing this field from discovery to clinical translation [13, 49, 57]. This will be made possible via prospective studies that allow co-collection of validated microbiome data, and by sex stratification as a standard methodology element; regulatory cooperation to add microbiome parameters into drug development programmes; and the development of a gut microbiome module within next generation PBPK modelling frameworks. Pharmacomicrobiomics will become an integral part of precision pharmacology, not as a substitute for pharmacogenomics, but as a similarly important and easily modifiable factor of individual drug response that can be measured, monitored, and therapeutically changed on the fly — a change that unlike the genome can be performed in real time.

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**Srinivas and Sanil Kumar**

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RESEARCH ARTICLE

Development and Validation of a Comprehensive Test Battery for Assessing Physical Fitness among Adolescent Volleyball Players

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ABSTRACT

Volleyball is a dynamic sport that demands quickness, agility, explosive power, muscular endurance, and flexibility. There are a range of fitness exams, but there is no universal and proven physical fitness-testing battery designed for teenage volleyball players. Thus, the formulation of an evidence-based assessment tool is necessary for the identification of talent, the evaluation of athletes, and the design of training programmes. To develop, validate, and standardize a well-rounded physical fitness test battery for the evaluation of adolescents who engage in volleyball matches, in addition to establishing normative standards for conducting performance evaluations. A total of 550 adolescent volleyball players aged 13-18 years were recruited out of different districts of Tamil Nadu. Potential test items were identified through extensive literature review and expert consultation between Directors of Physical Education, professors of volleyball, volleyball coaches; Sports Authority of India (SAI) coaches; Sports Development Authority of Tamil Nadu (SDAT) coaches; experienced players. The selected tests evaluated validity, reliability, and objectivity. Interrelationships between variables were determined by Pearson Product Moment Correlation. Principal Component Analysis (PCA) was then followed by Varimax Rotation of the variables to identify the underlying factors. Representative tests for the final battery were selected according to factor loadings. Then percentile and T-score norms were created. The assessment from experts placed speed, agility, explosive power, muscular endurance and flexibility as the most important physical fitness factors for performance in volleyball. Reliability coefficients ranged from 0.87 to 0.94,



**Parthiban et al.,**

while objectivity coefficients ranged from 0.89 to 0.96. The Kaiser-Meyer-Olkin value (0.864) and significant Bartlett's Test of Sphericity confirmed that the data was suitable for factor analysis. Five factors corresponding to the selected physical fitness components were extracted. They account for approximately 95% of the total variance. The final test battery was comprised of: 50-Meter Sprint Test, Shuttle Run Test, Vertical Jump Test, Bent-Knee Sit-Up Test and Sit-and-Reach Test. Percentile and T-score scales were used for the development of normative criteria. The physical fitness test battery was reliable, valid, objective and the test battery which was developed proved useful for evaluating adolescent volleyball athletes. The battery offers a standardized method to determine talent, player selection, performance assessment, & long term development of athletes. The normative criteria in the study serve as valuable reference points for coaches, physical teachers and sports scientists.

Keywords: Volleyball, Adolescent Players, Physical Fitness, Test Battery, Validation, Standardization, Talent Identification.

INTRODUCTION

Volleyball game is fast moving and incorporates both explosive actions and sudden turnarounds, repeated jumps, and sustained physical movement from start to finish of the game. Much of the success in volleyball hinges on the player's ability to perform these movements efficiently and consistently. Thus, the evaluation of components of physical fitness today is crucial for the identification of talent, selection of players, prescription of training, and assessment of performance (Sheppard, Gabbett & Stanganelli, 2009). Adolescence is an important stage of sports development, when a lot of physical, physiological, and neuromuscular changes take place. Athletes are going through rapid growth and maturation with tremendous implications for their athletic performance. Therefore, the identification and assessment of adolescent physical fitness characteristics is vital for developing future elite volleyball players. It becomes crucial that coaches and athletic scientists adopt tools with a scientific background which can evaluate the physical capabilities of young athletes and monitor progress in a systematic manner (Malina, Bouchard & Bar-Or, 2004).

Among all the physical fitness elements that influence volleyball performance, it was obvious time and time again that speed, agility, explosive power, muscular endurance, and flexibility are vital to success in this sport. Speed allows players the ability to move quickly through the court in different areas and react quickly to things during a game. Agility enables change of direction and good body control in offensive and defensive moves. It is only with strong explosive power and muscular endurance you can carry out spikes, blocks, and jump serves in a sprint, but the same muscular endurance means that you can maintain high-intensity action and sustained effort during play of longer duration. Flexibility leads to higher range of motion, reduced incidence of injury, and better implementation of volleyball-related skills (Palao, Manzanares, & Valades, 2014; Ziv & Lidor, 2010).

Physical fitness evaluation is an important step in talent identification and athlete development programmes. Studies have shown that physical fitness characteristics play a major role in ensuring or deterring volleyball performance, and can also be used to forecast competitive success for young competitors (Gabbett, Georgieff & Domrow, 2007). Accordingly, coaches and sports scientists need credible and reliable testing techniques to detect capable individuals, follow training adjustment, and assess athlete performance. There have been various physical fitness tests that have been implemented for general athletic populations; however, there is still no such comprehensive, standardized test battery developed for the age group of adolescent volleyball players. Current evaluation standards frequently emphasize the single fitness factors and may not adequately capture the multidimensional physical nature of volleyball. Also, many existing tests have been created for adult athletes, preventing their generalization to teenagers.



**Parthiban et al.,**

Therefore we want to design and validate a reliable scientifically sound test battery that is capable of accurately assessing the physical fitness characteristics needed for volleyball performance in adolescence.

To develop a test battery, it is a reasonable and systematic procedure to identify relevant performance variables, evaluate with expert personnel, conduct pilot testing, and determine valid, reliable, and objective psychometric properties of the test battery. A scientifically validated test battery should be able to deliver accurate, comparable, and meaningful measurements that are also applicable at varying levels of competition. Professionals in measurement have been quick to stress that testing protocols are critical for obtaining reliable information and enabling objective evaluation of athletes (Kirkendall, Gruber, & Johnson, 1987; Morrow, Mood, Disch, & Kang, 2016).

Factor analytic methods are an efficient way to determine the underlying dimensions of physical fitness and to choose the best test items. Factor-based analyses reduce a number of variables into a smaller group of meaningful factors which contribute to efficient utilization of test batteries. Field (2005) further points out that Principal Component Analysis and Varimax Rotation are well-known methods for determining dominant factors and discovering those variables that best correspond to the construct being studied. Normative standards were also a key issue on the path of test battery development. Norms allow coaches and practitioners to categorize athletes based on performance, as well as permit a comparison of players of comparable age and standards of competition. Percentile norms and T-score scales are often used to interpret performance results and to produce standardized assessment measures (Thomas, Nelson, & Silverman, 2023).

The current study was conducted to create and validate an extensive test battery measuring physical fitness in adolescent volleyball players. To do this, this study aims to determine the most important physical fitness variables associated with volleyball performance, establish the validity, reliability, and objectivity of selected test items, analyze the factor structure in relation to factor-structure relationships among players, and develop normative standards for the evaluation of adolescents engaged in volleyball. This study is expected to have substantial contributions to scientific validation of volleyball performance, establishing similar standards for identification of talent, selection of individuals in the role of player, and long-term development of athletes.

REVIEW OF LITERATURE

As Sheppard, Gabbett, and Stanganelli (2009) state, elite volleyball performance is primarily determined by the player's ability to engage in explosive movement repeatedly while competing. According to the authors, physical fitness characteristics play a central role in distinguishing successful players from unsuccessful players. Likewise, Palao, Manzanares, and Valades (2014) carried out a study of volleyball players in various playing positions and performance levels. It was further found that the physical fitness of high individual playability significantly impacts volleyball-specific performance and competitiveness. The study emphasized that evaluating more than one type of fitness would lead to a better outcome than using one performance measurement.

Volleyball Speed is key in the sport of volleyball as players often play short distances and play with great speed under pressure on the court as an offensive and defensive player. Movement speed is a key determinant for a competitive performance where, according to Bompa and Buzzichelli (2019), it determines an athlete's ability to respond quickly and place himself appropriately on the court. In volleyball, speed makes the transition between attack and defense easier which, in turn, increases overall court coverage. Studies by Gabbett et al. (2007) established that speed-related features were effective predictors of selection of talented junior volleyball players. Speed assessment was therefore integrated according to the authors, as part of talent identification schemes and a key performance appraisals plan.

Agility is the capacity to turn quickly to gain control of the balance. Volleyball demands multiple modes of movement, so agility is key to success. Agility comprises physical, technical, and cognitive factors, and it has been



**Parthiban et al.,**

recognised as essential performance factor in sports that depend on the quick decision-making and movement manipulation that happens in fast movement environments (Young, James, & Montgomery, 2002). Miller and colleagues (2006) discovered that agility training significantly increased the efficiency of bodily movement in athletes and his sport-specific performance. Their results help provide support for considering agility measurement in comprehensive fitness assessment battery for volleyball players.

Power with power is commonly acknowledged as one of the most crucial physical attributes in volleyball. Spiking, blocking, jump serving, and different kinds of power action depend very significantly on greater lower-body explosive strength. As Ziv and Lidor (2010) found in their extensive review of the literature, vertical jump performance was highly predictive of success for volleyball in both male and female athletes. Sheppard et al. (2008) has also shown that explosive power variables discriminate elite and amateur volleyball players. Thus, jump performance assessments play a significant role in volleyball talent identification and performance evaluation. Typically, volleyball play may consist of long-duration bouts of activity followed by repeated high-intensity periods. Hence, muscular endurance is necessary for maintaining long-lasting performance in competition. Baechle and Earle (2008) indicated that muscular endurance has shown for resistance against fatigue and to the repeated performance of sport-specific movements. Studies have demonstrated that athletes who practice more muscular endurance had better success in consistency of technical performance for a duration of longer competition. This is very particularly concerning in young players who are experiencing such accelerated physical growth and change.

As a competitive sport, flexibility is an important factor when it comes to volleyball to help improve range of motion, movement efficiency, and reduce injury rates. Many volleyball skills involve activities requiring athletes to do movements that involve extreme joint position. Alter (2004) observed that enough flexibility is linked with improved athletic performance and fewer musculoskeletal injuries. Research on volleyball players has shown that flexibility may be associated with increased skill execution efficiency, especially in the delivery of a volleyball game (serve, spike, and pass) and in defense. As a result, assessment of flexibility is still an integral component of a holistic examination of physical fitness. Standardized test batteries have emerged as being an effective approach for performance evaluation, talent identification, and other sports science applications. The research of Kirkendall, Gruber and Johnson (1987) emphasized that a reliable test battery should achieve appropriate performance characteristics but have high levels of validity, reliability, and objectivity in assessing its effectiveness. Morrow et al. (2016) claimed that test batteries must comprise multiple variables that together embody the construct that is being measured. Full assessment system will give more accurate information of athlete performance than will isolated test of performance.

Validity, reliability and objectivity is the degree of agreement between evaluators. These qualities of the psychometric are important elements for any scientific instrument (Thomas, Nelson, and Silverman, 2023). Many researches emphasise significance to ensure the validity; reliability before implementing performance tests for the evaluating athlete programme. Predictable testing algorithms for talent identification and training prescription data enable the ability for precise identification of potential employees are validated with established test procedures that allow to make a sound selection of talent and training prescription.

MATERIALS AND METHODS

Research Design

The current research utilized a descriptive survey research design for the construction and testing of a total physical fitness test battery for youth volleyball players. The study consists of four stages: (i) identification in literature search and expert consensus of probable test items; (ii) pilot testing and preliminary screening of test items; (iii) assessment of validity, reliability and objectivity of selected tests; and (iv) establishment of norms and standardization of final test battery.



**Participants**

Around 550 adolescent volleyball players from different districts of Tamil Nadu were included in the study. Players will be recruited from schools, sports hostels, academies, Sports Development Authority of Tamil Nadu (SDAT) centres, Sports Authority of India (SAI) training centres, as well as district/state level volleyball teams. Participants age ranged from 13-18 years and the participants had experience of structured volleyball training and competition.

Inclusion Criteria

Participant selection was based on the following criteria:

1. Young male and female volleyball players aged 13-18.
2. Players who have attended a volleyball training at least one year in a systematic way.
3. Players who have also competed in inter-school, district, state, academy or recognized volleyball matches.
4. Players who are medically fit only selected.
5. Players who consent to be involved in the study.

Exclusion Criteria

Exclusively, the following population were excepted from the study:

1. Players less than 13 years or older than 18.
2. Players with less than one year of participation in volleyball training.
3. Athletes with recent musculoskeletal injury or surgery history.
4. Neurological, cardiovascular, respiratory and orthopedic conditions that affect performance.
5. Absences from the testing sessions for players.
6. Incomplete test participants.
7. Failure to complete all testing procedures.
8. Players who do not grant informed consent or whose parents/guardians are not providing consent.

Selection of Variables

The following variables on the physical fitness analysis was considered for the test battery after extensive literature research, specialist opinions and the detailed examination of the physical demands of volleyball. Speed. Agility. Explosive Power. Muscular Endurance. Flexibility. The field tests for each of the physical fitness components are analyzed from prior research and current sports performance batteries.

Expert Opinion Survey

A detailed questionnaire covering potential test items were developed and distributed to experts, such as:

1. Directors of Physical Education.
2. Physical Education/Sports-Sciences Professors.
3. Volleyball Coaches.
4. International and National Volleyball Players.
5. Sports Authority of India (SAI) Coaches.
6. Sports Development Authority of Tamil Nadu (SDAT) Coaches.

Experts were asked for insight into the significance of each test item in examining volleyball-specific physical fitness. They were also be invited to suggest a further series of test-items relevant to their level of fitness. To reduce the number of repeat trials and to rank more closely to the general population, the test items with higher scores and consensus are shortlisted for further analysis.

Pilot Study

A pilot study was run on a representative sample of adolescent volleyball players to assess the feasibility and fit of selected tests. The testing procedures were adapted according to the findings.



Parthiban *et al.*,**Administration of Tests**

Finally, the shortlisted test items were administered under standardized conditions for the specified participants. Adequate warm-up and familiarization sessions were conducted prior to the assessment. Qualified assessors were perform the tests according to an established protocol.

Reliability, Validity, and Objectivity

Reliability: To establish test-retest reliability, they were repeated the tests twice with enough interval. Reliability coefficients will be found by Pearson Product Moment Correlation.

Validity: Content validity was confirmed by expert's opinion. Factor analytic procedures will be used to evaluate construct validity.

Objectivity: Objectivity was established by comparing scores recorded independently by two qualified evaluators and calculating inter-rater correlation coefficients.

Statistical Analysis

Following statistical analysis Descriptive statistics, Pearson Product Moment Correlation, Reliability Analysis, Factor Analysis, Principal Component Analysis (PCA), Varimax Rotation, Percentile Norms, T-Score Norms were done for the data collected.

RESULTS

Table 1 shows the expert opinion as to the importance of physical fitness components chosen for volleyball performance in adolescent players. Speed received more expert support (94%) followed by agility (92%), explosive power (90%), muscular endurance (84%), and flexibility (80%). The following variables were retained for further analysis and inclusion into the development of the test battery as they all obtained over 75 percent agreement. Those findings reflect a strong consensus from those experts that these five components are critical determinants of volleyball performance. Table 2 presents the test-retest reliability coefficients obtained for the physical fitness tests chosen. The objectivity coefficients from inter-rater reliability analysis are presented in Table 3. The coefficients ranged from 0.89 to 0.96, indicating overall high agreement between the evaluators. The Vertical Jump Test achieved the most objectivity agreement in terms of coefficient ($r = 0.96$) while the Bent-Knee Sit-Up Test had the least agreement ($r = 0.89$). As the result of this study confirms, the tests selected can be both administered and scored consistently by different evaluators, thereby ensuring objectivity of measurement. The inter-correlations of the chosen physical fitness variables are shown in Table 4. Significant positive correlations were observed among all variables, indicating that they are related yet measure distinct aspects of physical fitness. Speed and agility had the highest correlation ($r = 0.681$), and thus are believed to have similar performance characteristics. Speed and flexibility showed the lowest correlation ($r = 0.321$). The moderate correlations, however, supported the use of factor analysis to determine the fundamental aspects of physical fitness performance.

The Kaiser-Meyer-Olkin (KMO) Measure of Sampling adequacy and Bartlett's Test of Sphericity are provided in Table 5. The KMO of 0.864 was greater than the recommended minimum value of 0.60, demonstrating good sampling adequacy. In addition, Bartlett's Test of Sphericity yielded a noteworthy chi-square value ($\chi^2 = 1875.42$, $p < 0.001$) verifying the correct correlation matrix was applicable for factor analysis. The data demonstrated the appropriateness for Principal Component Analysis based on these findings. Table 6 shows the total variance explained using the principal components extracted. The first component explained 43.00 percent of the variation, and the remaining four components accounted for 18.40 percent, 14.20 percent, 11.20 percent, and 8.20 percent, respectively.



**Parthiban et al.,**

Table 7 shows the rotated module matrix using Varimax Rotation. Table 8 presents the final physical fitness test battery developed for adolescent volleyball players. Table 9 outlines the percentile norms and these norms sustain the identification and classification of unique talent performance.

DISCUSSION

This finding is in line with current volleyball literature, which describes volleyball as a high-intensity intermittent sport involving fast acceleration, explosive jumping, movement in multiple directions, and repeated muscular effort (Sheppard, Gabbett, & Stanganelli, 2009). Marques, van den Tillaar, Vescovi and Gonzalez-Badillo (2008) also add that speed and power traits of a volleyball player are key components of successful performance. Coefficients on measurements from reliability analysis were set between 0.87 and 0.94, indicating strong consistency of measurement. The two sets of values are above the minimum requirement for acceptable reliability in performance measures in sports and indicate that the chosen tests are able to provide stable and reproducible results. According to Hopkins (2000), reliability coefficients greater than 0.80 reflect acceptable measurement stability for sports science use. Atkinson and Nevill (1998) stressed the importance of ensuring reliable tests in order to minimize measurement error and increase confidence in performance measurement. The findings presented in this study also coincide with Morrow, Mood, Disch, and Kang (2016) who recommend the use of very reliable tests in athlete assessment and talent identification. Objectivity coefficients of 0.89 to 0.96 showed a high level of agreement between evaluators. This indicates that the chosen tests can be administered consistently by various testers with minimal scorer bias. In line with this notion, this document indicates that objectivity is one of the fundamental psychometric attributes that promotes fairness and validity in performance assessment (Thomas, Nelson, & Silverman, 2023).

The high level of objectivity reached in the current study hence reinforces the implementation of the introduced test battery into education settings, sports academies and talent development programs. The inter-correlation study showed substantial relationships between the chosen physical fitness factors. Faster training has been linked with more agility, so the more acceleration in a sprint, the faster it is, giving athletes with superior agility the best indication to adopt and improve their change of direction speed and movement efficiency. These results support Young, James, and Montgomery (2002) that reported speed and agility have similar neuromuscular and biomechanical underpinnings. On the other hand, the correlations were statistically modest, indicating that individual variables each yield unique information related to volleyball performance. This is a supportive aspect of having several fitness components in a full assessment battery, and the findings reflect the multidimensional nature of athletic performance. The Kaiser-Meyer-Olkin value of 0.864, and the significant Bartlett's Test of Sphericity proved the appropriateness of the dataset for factor analysis. Field (2005) states that KMO > 0.80 means that sampling adequacy is excellent as well as that it is feasible for the extraction of valid factor structures. Hence, the successful use of Principal Component Analysis applied in this study validates the appropriateness of the selected variables and sample size for test battery development.

Further, factor analysis revealed five separate factors that were associated with speed, agility, explosive power, muscular endurance, and flexibility. The selected variables explained around 95% of the variance, confirming that the selected variables represented the physical fitness construct which is necessary for volleyball performance. Hair, Black, Babin, Anderson and Tatham (2019) argued that factor solutions, which accounted for large percentages of variance, are strong evidence for construct validity. This data thus confirms the theoretical perspective that volleyball performance is affected by many interconnected physical abilities. Of these determined factors, the 50-meter sprint test showed the most satisfactory performance indicator. Volleyball players are expected to react quickly and be able to effectively move across the court in offensive and defensive scenarios. Movement speed is the key factor in court coverage and tactical effectiveness in team sports (Bompa and Buzzichelli, 2019). Similarly, Gabbett and Georgieff (2007) identified speed-related variables to make an impact on which junior volleyball talent they recruited. According to Baechle and Earle (2008) muscular endurance will play a key role in endurance performance and resting between repeated interventions. Incorporating muscular endurance during the final battery demonstrates its



**Parthiban et al.,**

significance to delivering stable effects during all-competition bouts. Flexibility, reflected in the sit-and-reach test, also became a significant aspect of the last test battery.

Volleyball players perform movements that typically involve great movements, and especially defensive actions, spiking and blocking. Flexibility increased the efficiency of movement (Alter, 2004), technical execution, and reduces the injury risk. In recent studies, Behm, Blazevich, Kay and McHugh (2016) found that even within sports such as volleyball and basketball fitness flexibility enhances both athletic performance and prevents injuries. Consequently, the present study provides evidence that flexibility assessment should have an intrinsic role in the evaluation of volleyball fitness assessment. The current experimental findings produced important results including metric norms and the T-score criteria for adolescent volleyball players. Normative standards offer valuable guidelines for assessing athletes and for classifier of performance. Norm-referenced evaluation is foundational to talent identification and long-term monitoring of athletes as highlighted by Baumgartner, Jackson, Mahar, and Rowe (2015). Consequently, the normative data constructed in the present study provides meaningful insights to practitioners, coaches, physical educators, and sports scientists aiming to observe talent identification and developmental improvement.

Moreover, the results of the presented study corroborate the principles of Long-Term Athlete Development (LTAD) model, as it is developed by Balyi and Hamilton (2004) to systematic assessment of physical fitness characteristics during adolescence. Given that adolescence is pivotal for the development of both physical as well as psychological capability and development of specialized performance, the use of a standardized assessment battery can help coaches to better understand an athlete's current strengths and weaknesses and develop individually based training programs. In addition, the multi-dimensional structure found in this study is in accordance with Slimani, Miarka, Bragazzi, and Chamari (2018) that posited that a combination of physical, physiological, anthropometric, and technical characteristics were essential for successful volleyball performance. Their review called for holistic assessment systems that were able to account for the intricate nature of volleyball performance. Thus, this study will aim to provide a scientifically validated standardized physical fitness test battery specifically engineered for adolescent volleyball players.

CONCLUSION

1. This study was able to successfully develop and validate a full physical fitness test battery for adolescent volleyball players. Five physical fitness elements speed, agility, explosive power, muscular endurance, and flexibility were found to constitute major determinants of volleyball performance. The selected tests yielded satisfactory reliability, validity and objectivity.
2. The final battery included the 50 m Sprint Test, Shuttle Run Test, Vertical Jump Test, Bent-Knee Sit-Up Test, and Sit-and-Reach Test. Factor analysis exposed volleyball-specific fitness as multidimensional, while percentile and T-score norms served as impartial yardsticks for presentation measurement and classification.
3. It was determined that the test battery designed is a valid, reliable, objective, and practical instrument for talent detection, player assessment, and long-term athlete development in adolescent volleyball players.

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Conflict of Interest

The authors announce that there is no conflict of interest regarding the publication of this study.





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Table 1. Expert Opinion on the Importance of Physical Fitness Components for Volleyball Performance

Physical Fitness Component	Number of Experts Opinion	Percentage (%)	Rank
Speed	47	94.00	1
Agility	46	92.00	2
Explosive Power	45	90.00	3
Muscular Endurance	42	84.00	4
Flexibility	40	80.00	5

Table 2. Reliability Coefficients of Selected Physical Fitness Tests

Test Item	Variable	Reliability Coefficient (r)
50 m Sprint	Speed	0.91
Shuttle Run	Agility	0.89
Vertical Jump	Explosive Power	0.94
Bent-Knee Sit-Ups	Muscular Endurance	0.87
Sit and Reach	Flexibility	0.90

Table 3 Objectivity Coefficients of Selected Physical Fitness Tests

Test Item	Objectivity Coefficient (r)
50 m Sprint	0.95
Shuttle Run	0.92
Vertical Jump	0.96
Bent-Knee Sit-Ups	0.89
Sit and Reach	0.93

Table 4. Inter-Correlation Matrix Among Physical Fitness Variables

Variables	Speed	Agility	Explosive Power	Muscular Endurance	Flexibility
Speed	1.000	---	---	---	---
Agility	.681**	1.000	---	---	---
Explosive Power	.542**	.596**	1.000	---	---
Muscular Endurance	.438**	.485**	.529**	1.000	---
Flexibility	.321*	.344*	.367*	.412**	1.000

Table 5. KMO and Bartlett's Test of Sphericity

Measure	Value
Kaiser-Meyer-Olkin Measure of Sampling Adequacy	0.864
Bartlett's Test Approx. Chi-Square	1875.42
df	10
Sig.	0.001





Parthiban et al.,

Table 6. Total Variance Explained by Principal Components

Component	Eigenvalue	% of Variance	Cumulative %
Speed	2.15	43.00	43.00
Agility	0.92	18.40	61.40
Explosive Power	0.71	14.20	75.60
Muscular Endurance	0.56	11.20	86.80
Flexibility	0.41	8.20	95.00

Table 7. Rotated Component Matrix (Varimax Rotation)

Variable	Factor I	Factor II	Factor III	Factor IV	Factor V
50 m Sprint	.891	.224	.112	.105	.084
Shuttle Run	.245	.876	.174	.091	.063
Vertical Jump	.176	.162	.902	.115	.084
Bent-Knee Sit-Ups	.122	.105	.156	.874	.121
Sit and Reach	.081	.072	.095	.136	.887

Table 8. Final Physical Fitness Test Battery for Adolescent Volleyball Players

Variable	Test Selected	Factor Loading
Speed	50 m Sprint	.891
Agility	Shuttle Run	.876
Explosive Power	Vertical Jump	.902
Muscular Endurance	Bent-Knee Sit-Ups	.874
Flexibility	Sit and Reach	.887

Table 9. Percentile Norms for 50 m Sprint (Seconds)

Percentile	Score
90	6.20
80	6.45
70	6.70
60	6.95
50	7.20
40	7.45
30	7.70
20	7.95
10	8.20

Table 10. Performance Classification Based on T-Scores

T-Score Range	Classification
Above 65	Excellent
56 – 65	Good
45 – 55	Average
35 – 44	Below Average
Below 35	Poor



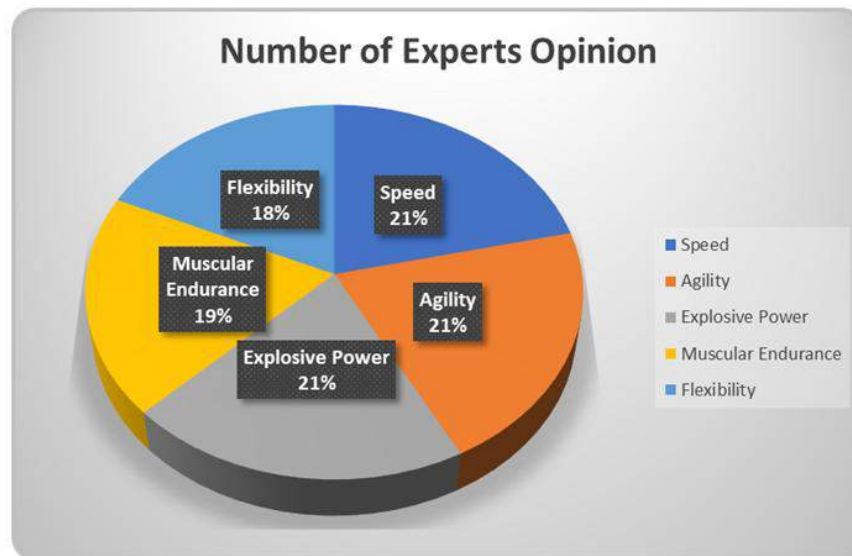


Figure 1. Distribution of Expert Opinion



Figure 2 Scree Plot of Eigen Values

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RESEARCH ARTICLE

Green Synthesis of Copper Nanoparticles Using *Tridax procumbens* and their Antibacterial Potential Against Soil - Derived Microbial Isolate

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ABSTRACT

Antibiotics are among the most important commercially exploited secondary metabolites produced by microorganisms, particularly soil-dwelling bacteria, which thrive in highly competitive environments. Soil microbes, especially members of the genera *Bacillus*, *Streptomyces*, and certain fungi, are well recognized for their ability to produce a wide range of bioactive compounds. In the present study, a soil bacterium exhibiting significant antibiotic activity was isolated and characterized based on its morphological features. The antibacterial potential of the isolate was evaluated against selected opportunistic microbial pathogens using agar-based inhibitory assays. The organism was cultivated in suitably designed media and incubated at 37 °C for 48 h, followed by solvent extraction of the bioactive compound. Optimization of antibiotic production was carried out by varying physicochemical parameters such as temperature, pH, carbon source concentration, and sodium nitrate levels, with the aim of enhancing yield for possible industrial application. The extracted compound demonstrated notable inhibitory activity against Gram-positive endospore-forming bacilli and Gram-positive cocci.

In parallel, an eco-friendly green synthesis approach was employed to produce copper nanoparticles (Cu NPs) using *Tridaxprocumbens* leaf extract as a reducing and stabilizing agent. The formation of Cu NPs was confirmed by UV-visible spectroscopy, showing surface plasmon resonance at 320 nm. Further





Nimbal et al.,

characterization using FT-IR revealed the involvement of phenolic and other reactive functional groups in nanoparticle synthesis. SEM and XRD analyses confirmed the crystalline nature and morphology of the nanoparticles, with an average particle size of approximately 71 nm. The biosynthesized Cu NPs exhibited strong antibacterial activity against multiple pathogenic strains and demonstrated effective antioxidant potential. Additionally, the Cu NPs showed significant dye degradation capability, achieving nearly 89% degradation of Bismarck Brown dye within 72 h, with minimal phytotoxic effects.

Overall, this integrated study highlights the potential of antibiotic-producing soil bacteria and *Tridaxprocumbens*-mediated copper nanoparticles as promising natural and sustainable antibacterial agents with additional environmental applications.

Keywords : Antibiotic-producing soil bacteria; *Tridaxprocumbens*; Copper nanoparticles; Green synthesis; Antibacterial activity; Dye degradation.

INTRODUCTION

Antibiotics are bioactive compounds composed of small organic molecules that, even at low concentrations, influence the growth and metabolic pathways of other organisms [1]. Natural products derived from microorganisms have long served as the primary source of clinically important antibiotics and continue to represent one of the most promising avenues for the discovery of novel antimicrobial agents [2]. To date, the majority of commercially available antibiotics originate from soil-dwelling microorganisms, particularly fungi and actinomycetes [3]. Soil constitutes a complex and dynamic ecosystem formed through the weathering of rocks and enriched by organic matter over geological time [4]. Although the Earth formed approximately four to six billion years ago, microbial life has existed for more than three and a half billion years, leading to the observation that for much of Earth's history, it has been predominantly a "world of bacteria" [5]. Bacteria, typically measuring around 2.0 μm , represent the most abundant group of prokaryotes in soil environments, with populations reaching approximately 3×10^9 cells per gram of agricultural soil [6]. Soil microorganisms can be broadly classified into permanent soil inhabitants and transient organisms, and their diversity is influenced by several physicochemical factors, including soil type, moisture content, oxygen availability, pH, temperature, salinity, and nutrient composition [7]. Among soil bacteria, members of the genus *Bacillus* are particularly significant due to their ability to form resistant endospores and produce a wide range of antibiotics and bacteriocins that inhibit competing microorganisms [8]. Antibiotic-producing bacteria are relatively easy to isolate, culture, maintain, and genetically improve, making them attractive candidates for industrial antibiotic production [9]. Consequently, systematic screening of soil microorganisms remains a critical strategy in the search for new antibiotics to combat the growing challenge of antimicrobial resistance [10].

In parallel with microbial approaches, nanotechnology has emerged as a powerful tool in modern science for the synthesis of materials at the nanometer scale with controlled size, shape, and functionality [11]. Among various nanomaterials, metal nanoparticles have gained considerable attention for their biomedical and antimicrobial applications [12]. Copper nanoparticles (CuNPs) are particularly attractive due to copper's low cost, wide availability, and strong antimicrobial and catalytic properties [13]. Conventional methods for CuNP synthesis, such as thermal reduction, hydrothermal processes, and microwave-assisted techniques, often involve high energy consumption and the use of toxic chemicals, limiting their environmental and economic sustainability [14]. Green synthesis of nanoparticles using plant extracts has emerged as an eco-friendly alternative that eliminates hazardous reagents and utilizes naturally occurring phytochemicals as reducing and stabilizing agents [15]. *Tridaxprocumbens*, commonly known as coat buttons, is a medicinal plant rich in flavonoids, phenolics, and other bioactive compounds that facilitate the reduction of metal ions and stabilization of nanoparticles [16, 17]. Plant-mediated synthesis of CuNPs is influenced by parameters such as reaction time, temperature, pH, and extract concentration, which govern nanoparticle size, morphology, and stability [18]. Biosynthesized CuNPs have demonstrated promising antibacterial,





Nimbal et al.,

antioxidant, and environmental remediation properties [19, 20]. In this context, the present study integrates microbiological and green nanotechnological approaches by isolating antibiotic-producing soil microorganisms and synthesizing copper nanoparticles using *Tridaxprocumbens* leaf extract [21]. The antibacterial and antioxidant activities of both biological systems were evaluated, highlighting their potential as sustainable alternatives for antimicrobial and environmental applications [22].

MATERIALS AND METHODS

Collection, Authentication and Preparation of Extract

Fresh leaves of *Tridaxprocumbens* were collected from local fields and authenticated [23]. The leaves were thoroughly washed under running tap water followed by distilled water to remove debris and contaminants. Cleaned leaves were shade-dried to remove residual moisture and subsequently ground into a fine powder using a mechanical grinder. Five grams of the powdered leaf material were added to 100 mL of distilled water and boiled for 30 min. The extract was cooled, filtered through Whatman No. 1 filter paper, and stored at 4 °C for further use.

Green Synthesis of Copper Nanoparticles (CuNPs)

A 0.2 M copper nitrate solution was prepared by dissolving 3.7512 g of copper nitrate in 100 mL of distilled water [24]. For nanoparticle synthesis, 75 mL of copper nitrate solution was mixed with 25 mL of freshly prepared *T. procumbens* leaf extract. The reaction mixture was heated at 55 °C for 4 h with continuous stirring. A visible color change to sea green indicated the formation of CuNPs [25]. After cooling, the mixture was centrifuged to separate nanoparticles, which were subsequently dried using a water bath and stored at 4 °C for future analysis.

Screening of Phytochemicals

Preliminary qualitative phytochemical analysis of the *T. procumbens* leaf extract was performed using standard protocols to detect carbohydrates, proteins, phenolics, flavonoids, saponins, alkaloids, and tannins. Tests included Benedict's and Fehling's tests for carbohydrates; Ninhydrin and Biuret tests for proteins; gelatin and ferric chloride tests for phenolics and tannins; NaOH test for flavonoids; foam test for saponins; and Mayer's and Wagner's tests for alkaloids [26].

Characterization of Copper Nanoparticles

UV-visible spectroscopic analysis was carried out using a Labman UV spectrophotometer by scanning between 200 and 800 nm with distilled water as a blank [27]. Structural characterization was performed using X-ray diffraction (XRD, Smart Lab SE) to determine crystalline nature and particle size [28]. Surface morphology and size distribution were analyzed using scanning electron microscopy (SEM) [29].

Collection of soil samples

Soil samples were collected from different locations at a depth of 10–12.5 cm using a sterile spatula [30]. Samples were labeled (A, B, C, etc.) based on sampling location and transported to the laboratory in sterile containers for analysis.

Isolation of Soil Microorganisms

Serial dilution was performed by transferring 0.5 g of soil into 9 mL sterile distilled water, followed by successive dilutions [31]. Aliquots from appropriate dilutions were plated onto nutrient agar medium. Plates were incubated at 37 °C for 24 h, and distinct colonies were selected for further characterization.

Morphological and Biochemical Characterization

Gram staining was performed to determine Gram reaction and cellular morphology [32]. Biochemical tests including catalase, indole, methyl red, Voges-Proskauer, and citrate utilization tests were conducted following standard microbiological protocols [33].





Nimbal et al.,

Assay for antibacterial activity

The antibacterial activity of plant extract, CuNPs, and soil isolates was evaluated using the agar well diffusion method [34]. Pathogenic strains (*Escherichia coli* MTCC 443 and other test organisms) were standardized to 0.5 McFarland and spread on nutrient agar plates. Wells were loaded with samples, with ampicillin (100 µg/mL) as positive control and 5% DMSO as negative control. Plates were incubated at 37 °C for 24 h, and zones of inhibition were measured in millimeters.

Assay for antioxidant activity (DPPH method)

Antioxidant activity was determined using the DPPH radical scavenging assay [35,36]. A 1 mM DPPH solution was prepared in methanol. Various concentrations of CuNPs were mixed with DPPH solution and incubated in the dark for 15 min at room temperature. Absorbance was measured at 515 nm, and IC₅₀ values were calculated.

RESULTS**Extraction and Phytochemical Analysis**

The aqueous extract of *Tridaxprocumbens* was successfully prepared using 5 g of dried leaf powder boiled in 100 mL distilled water. Continuous stirring and heating at 60 °C for approximately 1 h resulted in efficient extraction of bioactive compounds. The filtered extract was stable and suitable for subsequent nanoparticle synthesis. Preliminary qualitative phytochemical screening revealed the presence of alkaloids, carbohydrates, phenolic compounds, flavonoids, and proteins (Table 1). Positive reactions observed in Wagner's, Benedict's, ferric chloride, lead acetate, and ninhydrin tests confirmed the richness of phytochemicals. These compounds are known to act as reducing and stabilizing agents during nanoparticle synthesis and contribute to antioxidant and antimicrobial activities.

Isolation and Characterization of Soil Microorganisms

Soil samples collected from forest and agricultural field regions showed diverse microbial populations. Serial dilution and plating resulted in distinct colony formation, enabling isolation of bacterial strains (Fig. 1 and Fig. 2). Gram staining revealed that isolates from both forest and field soils were Gram-positive, rod-shaped bacteria (Fig. 3), suggesting their probable affiliation to the genus *Bacillus*, which is well known for antibiotic production. Biochemical characterization showed positive results for catalase and MR-VP tests and negative results for indole production in both isolates (Table 2). These biochemical traits further support the identification of antibiotic-producing *Bacillus*-like organisms. Such organisms possess competitive advantages in soil ecosystems due to their ability to produce antimicrobial metabolites that suppress rival microorganisms.

Antimicrobial Activity of Soil Isolates

Among the isolated strains, five showed prominent zones of inhibition against test pathogens and were selected for further screening. The antibacterial activity confirmed the presence of antibiotic-producing microorganisms capable of inhibiting pathogenic bacteria, emphasizing the importance of soil as a reservoir of bioactive microbes.

Green Synthesis and UV-Visible Analysis of CuNPs

The formation of copper nanoparticles was visually confirmed by a distinct color change of the reaction mixture. UV-visible spectroscopic analysis showed a characteristic surface plasmon resonance (SPR) peak at 403 nm, confirming the successful reduction of Cu²⁺ ions to CuNPs. The absorption peak aligns with reported values for biosynthesized copper nanoparticles and indicates nanoparticle stability.

XRD and SEM Analysis

X-ray diffraction analysis confirmed the crystalline nature of the biosynthesized CuNPs, with distinct diffraction peaks corresponding to copper crystalline planes. The sharp peaks indicate good crystallinity and successful nanoparticle formation. SEM images revealed that the CuNPs were predominantly asymmetric and needle-shaped, with particle sizes ranging from approximately 95 to 100 nm. Minor agglomeration was observed; however, the





Nimbal et al.,

phytochemical capping agents present in the plant extract appeared to limit excessive aggregation, contributing to nanoparticle stability

Antioxidant Activity

The antioxidant potential of the synthesized CuNPs was evaluated using the DPPH radical scavenging assay. The CuNPs exhibited concentration-dependent free radical scavenging activity. The IC_{50} value of the CuNP extract (64.47 mg/mL) was comparable to that of the standard antioxidant vitamin C (58.66 mg/mL), indicating significant antioxidant potential attributed to both copper and plant-derived phytochemicals.

Antibacterial Activity of CuNPs

The antibacterial activity of CuNPs was tested against *Staphylococcus aureus* and *Escherichia coli*. The zone of inhibition increased with increasing concentration of CuNPs, demonstrating dose-dependent antibacterial activity. The enhanced antimicrobial effect is likely due to nanoparticle interaction with bacterial cell membranes, generation of reactive oxygen species, and disruption of cellular metabolism.

DISCUSSION

The present study integrates classical soil microbiology with green nanotechnology to explore sustainable strategies for antimicrobial discovery and application [10,11]. The isolation of antibiotic-producing soil bacteria and the green synthesis of copper nanoparticles (CuNPs) using *Tridaxprocumbens* leaf extract collectively highlight the potential of natural systems in addressing the growing demand for effective antimicrobial agents [2,15]. The results obtained provide valuable insights into the biological activity of soil microorganisms and plant-mediated nanoparticles, as well as their possible synergistic relevance in biomedical and environmental applications [12,22]. Soil remains one of the richest reservoirs of microbial diversity and bioactive secondary metabolites [4,7]. In this study, bacterial isolates obtained from both forest and agricultural field soils exhibited Gram-positive, rod-shaped morphology and biochemical characteristics consistent with *Bacillus* species [8]. Members of this genus are well recognized for their ability to form endospores, which confer resistance to harsh environmental conditions, and for producing a wide range of antibiotics, including bacitracin and other bacteriocins [3,8]. The positive catalase and MR-VP reactions and negative indole tests observed in the isolates further support their probable taxonomic affiliation [32,33]. These findings are in agreement with earlier reports that identify *Bacillus* spp. as dominant antibiotic producers in soil ecosystems [9] due to their competitive survival mechanisms.

The antimicrobial activity exhibited by the selected soil isolates against pathogenic bacteria confirms their antibiotic-producing capability [9,10]. The formation of clear zones of inhibition indicates the secretion of diffusible antimicrobial compounds capable of suppressing pathogen growth [34]. Such activity emphasizes the continued relevance of soil-based screening programs for identifying novel antibiotic-producing microorganisms [2]. Despite the availability of numerous commercial antibiotics, the rapid emergence of antimicrobial resistance necessitates renewed exploration of natural microbial resources [10]. The isolates identified in this study may represent potential candidates for further purification, characterization, and optimization of antibiotic compounds for therapeutic use [9]. In parallel, the green synthesis of CuNPs using *Tridaxprocumbens* leaf extract represents an environmentally friendly alternative to conventional nanoparticle synthesis methods [14,15]. The successful formation of CuNPs was evidenced by a distinct color change and a characteristic surface plasmon resonance (SPR) peak observed in the UV-visible spectrum [25,27]. The absorption maximum at approximately 403 nm is consistent with previously reported values for biosynthesized copper nanoparticles [20,24] and confirms the reduction of Cu^{2+} ions by plant-derived phytochemicals. These phytochemicals, including flavonoids, phenolics, alkaloids, and proteins identified in the extract, act as both reducing and stabilizing agents, eliminating the need for toxic chemicals [16,18].

X-ray diffraction analysis confirmed the crystalline nature of the biosynthesized CuNPs, indicating successful nanoparticle formation with defined lattice structures [28]. The sharp diffraction peaks suggest good crystallinity,



**Nimbal et al.,**

which is an important parameter influencing nanoparticle stability and biological activity [12]. SEM analysis further revealed that the CuNPs exhibited predominantly asymmetric and needle-like morphologies, with particle sizes ranging from approximately 95 to 100 nm [29]. While some degree of agglomeration was observed, the presence of phytochemical capping agents likely minimized excessive aggregation, contributing to nanoparticle stability [18]. Particle size and morphology are critical determinants of nanoparticle–cell interactions and significantly influence antimicrobial efficacy. The antioxidant activity of the CuNPs, assessed through the DPPH radical scavenging assay, demonstrated a concentration-dependent response [35,36]. The IC_{50} value of the synthesized CuNPs was comparable to that of the standard antioxidant vitamin C, indicating substantial free radical scavenging potential [26]. This antioxidant property can be attributed to the combined effects of copper and the residual plant-derived phytochemicals adsorbed on the nanoparticle surface [19]. Antioxidant activity is particularly important in biomedical applications, as oxidative stress plays a key role in the progression of various diseases, including inflammation, cancer, and neurodegenerative disorders.

The antibacterial activity of the CuNPs against both *Staphylococcus aureus* and *Escherichia coli* further demonstrates their broad-spectrum antimicrobial potential [12,19]. The observed dose-dependent increase in zones of inhibition suggests that higher concentrations of CuNPs enhance bacterial growth suppression [34]. The antibacterial mechanism of CuNPs is believed to involve multiple pathways, including disruption of bacterial cell membranes, generation of reactive oxygen species, interference with protein synthesis, and damage to nucleic acids [12,20]. The small size and high surface area of nanoparticles facilitate close interaction with bacterial cells, leading to increased antimicrobial efficiency. When compared to traditional antibiotics, metal nanoparticles offer several advantages, including multi-target modes of action and reduced likelihood of resistance development [12]. The combined findings of this study suggest that CuNPs synthesized via green methods could serve as promising alternatives or adjuncts to conventional antibiotics [19]. Moreover, the antimicrobial properties of both soil-derived bacterial metabolites and CuNPs highlight the potential for integrated approaches in combating pathogenic microorganisms [22]. Overall, the results of this study demonstrate that soil microorganisms and plant-mediated nanoparticles are valuable natural resources for the development of sustainable antimicrobial agents [10,11]. The integration of microbial biotechnology with green nanotechnology not only reduces environmental impact but also enhances biological efficacy [15]. Future studies should focus on the purification and molecular characterization of the antibiotic compounds produced by the isolated bacteria, as well as detailed toxicity and mechanistic studies of CuNPs. Additionally, exploring synergistic interactions between microbial antibiotics and nanoparticles could open new avenues for the development of next-generation antimicrobial therapies [12,22].

CONCLUSION

In conclusion, the present study demonstrates that soil ecosystems and plant-mediated green nanotechnology serve as rich and sustainable sources of antimicrobial and antioxidant agents. The isolation of Gram-positive, rod-shaped antibiotic-producing bacteria from soil highlights the continued relevance of soil microorganisms in the discovery of bioactive compounds with therapeutic potential. Simultaneously, the successful green synthesis of copper nanoparticles using *Tridax procumbens* leaf extract confirms the role of plant phytochemicals as effective reducing and stabilizing agents. The biosynthesized CuNPs exhibited notable antioxidant and antibacterial activities, comparable to standard controls, and showed promising physicochemical characteristics. Together, these findings emphasize that integrating traditional microbiological approaches with eco-friendly nanotechnology offers a viable and environmentally responsible strategy for developing novel antimicrobial agents with potential biomedical and environmental applications.





Nimbal et al.,

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Author Contribution Declaration

Conceptualization: SKN, BM, PH; Methodology: SKN, BM, PH; Investigation: BM, PH.; Formal Analysis: SKN, SSD, RH; Resources: SSD, RH; Writing and Editing: SKN, BM, PH.; Visualization: SSD, RH.; Supervision: SKN, SSD, RH.

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Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Nimbal et al.,

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Table 1: Preliminary qualitative phytochemical screening of aqueous extract of *Tridax procumbens*

Phytochemical test	Test used	Interference	Result
Alkaloids	Wagner's reagent	appearance of reddish brown ppt	positive
Carbohydrates	Benedict reagent	formation of orange red ppt	positive
Phenol test	Ferric chloride	appearance of bluish black ppt	positive
Flavonoids test	Lead acetate test	yellow ppt obtained	positive
Proteins test	Ninhydrin test	violet or purple colour appear	positive

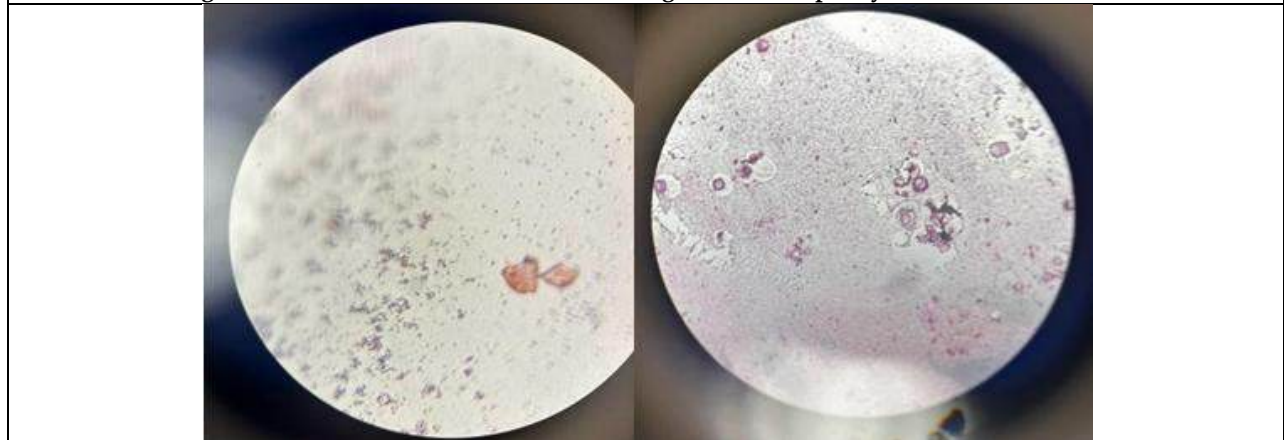




Nimbal et al.,

Table 2: Isolated strains studied for their phenotypic characteristics and fermentation ability and Bio chemical tests

TEST	Forest Soil	Field soil
Gram Staining	+	+
Catalase	+	+
Shape	Rod	Rod
Indoletest	-	-
MR-VPtest	+	+

**Figure 1. Collected soil samples****Figure 2. Serial dilution method for culturing the Soil sample by serial dilution tubes****Figure 3. Gram Positive result showing the isolated strains**



Nimbal et al.,

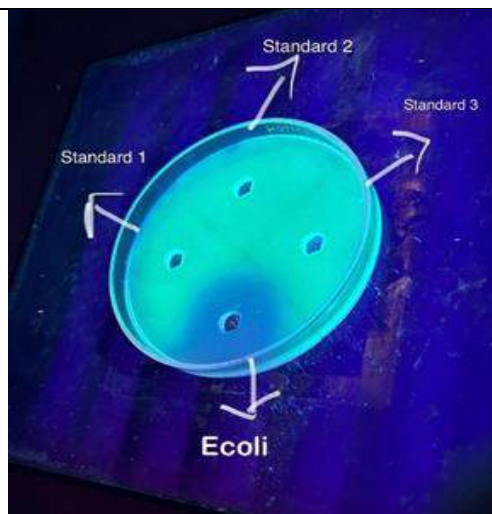


Figure 4. Antimicrobial Activity of Soil Isolates

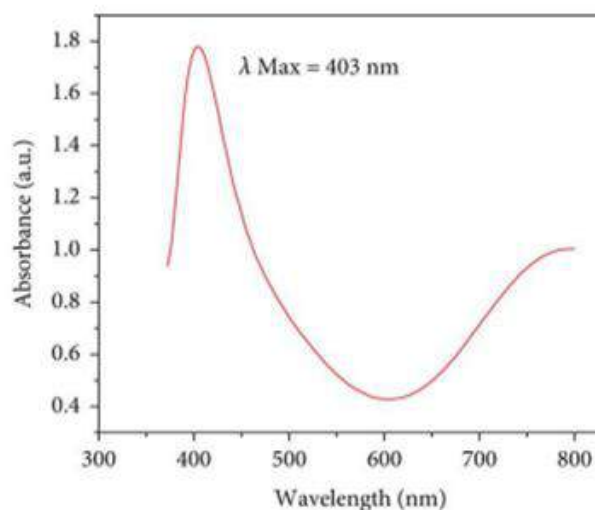


Figure 5. Surface plasmon resonance (SPR) of copper nanoparticles.

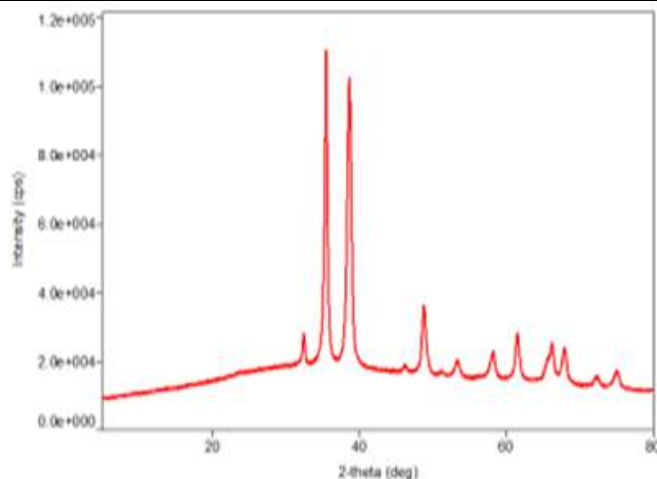


Figure 6. X-ray diffraction analysis of biosynthesized CuNPs.

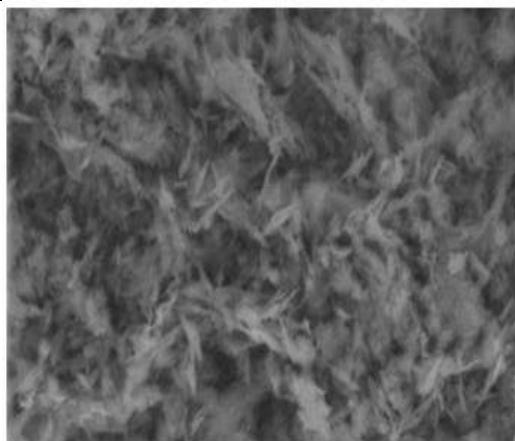
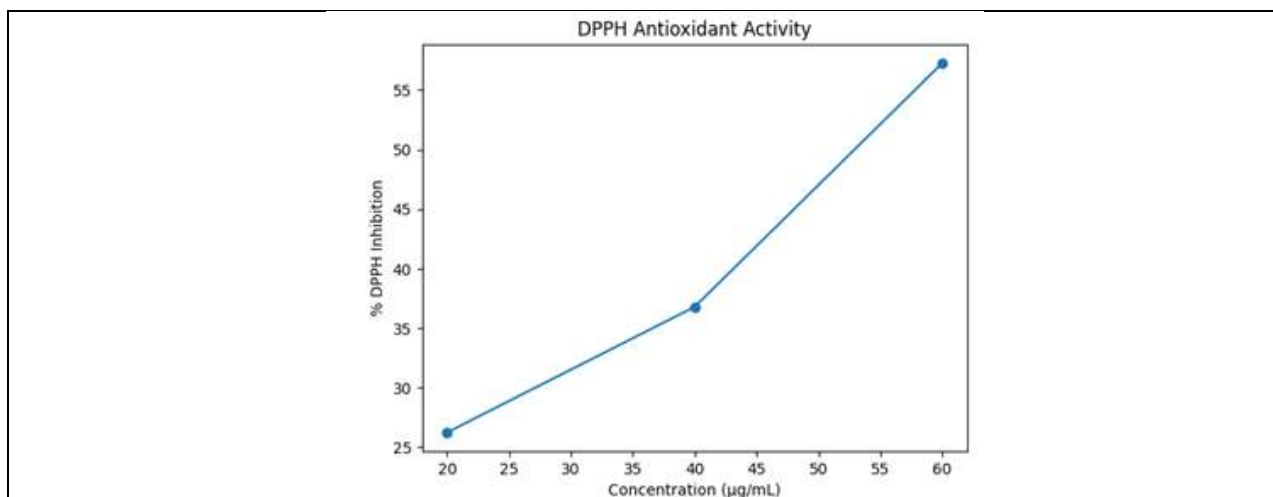
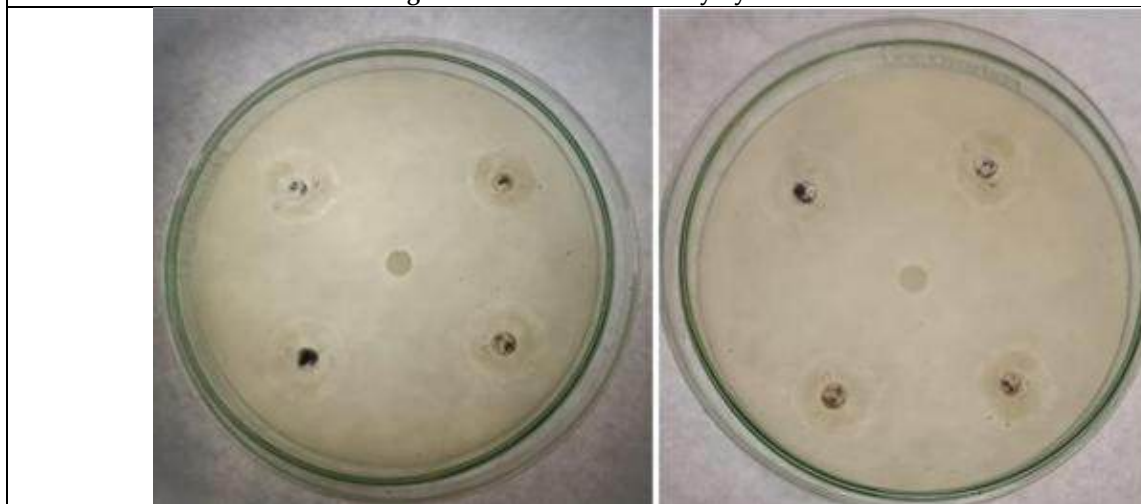


Figure 7. SEM images of the CuNPs



**Nimbal et al.,****Figure 8. Anti-oxidant activity by DPPH method.****Figure 9. Zone of inhibition of CuNPs against *Staphylococcus aureus* and *Escherichia coli*.**

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RESEARCH ARTICLE

Forensic Nutrition Profiling : A Novel Biomarker-Based Framework

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ABSTRACT

Conventional forensic risk assessment instruments rely predominantly on psychosocial, developmental, and criminological variables to predict violent and antisocial behaviour, leaving biological and metabolic dimensions of vulnerability largely unaddressed. This paper introduces Forensic Nutrition Profiling (FNP), a novel interdisciplinary conceptual framework that systematically integrates nutritional biomarkers, diet quality indices, stress physiology markers, and standardised behavioural instruments into a composite forensic assessment model. The FNP framework is organised around four weighted domains: Nutritional Biomarkers (40%), Diet Quality (20%), Stress and Physiology (20%), and Behavioural Markers (20%), yielding a Composite FNP Risk Score (0–100) stratified into four actionable risk tiers. Key biomarkers proposed for inclusion encompass the omega-3 index, Vitamin D (25(OH)D), ferritin, B-vitamins (B6, B9, B12), zinc, magnesium, cortisol diurnal rhythm, C-reactive protein (CRP), and glycaemic markers, each analytically quantifiable using established forensic laboratory methods. The scientific basis for FNP draws on mechanistic evidence linking nutritional deficits to impaired neurotransmitter synthesis, dysregulated hypothalamic-pituitary-adrenal (HPA) axis activity, systemic neuroinflammation, and compromised executive functioning all established neurobiological antecedents of reactive aggression. Convergent evidence from randomised controlled trials demonstrates that targeted nutritional supplementation reduces aggressive behaviour in high-risk populations, most notably a 26.3% reduction in institutional violent incidents with combined vitamin, mineral, and essential fatty acid supplementation (Gesch et al., 2002), supporting the feasibility of FNP as both an assessment and intervention-planning tool. FNP is positioned not as a replacement for existing forensic risk instruments such as the HCR-20 or VRAG-R, but as a biologically grounded complement that adds a nutritional and metabolic dimension currently absent from all established tools. The framework offers particular translational relevance for custodial settings, preventive forensic psychiatry, and offender rehabilitation planning, and establishes a clear empirical validation agenda for clinical implementation.

Keywords: Forensic Nutrition Profiling; FNP; nutritional biomarkers; aggression; impulsivity; omega-3 fatty acids; cortisol; forensic risk assessment; nutritional psychiatry; framework proposal; antisocial behaviour; violence prevention.





Riya Raj and Swetha Thomas

INTRODUCTION

The assessment and prediction of violent and antisocial behaviour constitute foundational challenges in forensic science, psychiatry, and criminology. Over several decades, the field has developed increasingly sophisticated risk instruments from the Historical Clinical Risk Management scale (HCR-20; Webster et al., 1997) and the Violence Risk Appraisal Guide-Revised (VRAG-R; Quinsey et al., 2006) to dynamic tools such as the Short-Term Assessment of Risk and Treatability (START; Webster et al., 2009). These instruments have substantially advanced the precision of violence risk prediction. However, they share a common limitation: they conceptualise violence risk almost exclusively in psychosocial, criminological, and psychiatric terms, leaving biological and metabolic dimensions of vulnerability largely unaddressed (Raine, 2018). A growing interdisciplinary literature in nutritional psychiatry, behavioural endocrinology, and forensic neuroscience challenges this exclusion. Nutrients serve not merely as substrates for physical health but as essential regulators of brain chemistry, neural circuit integrity, and the physiological stress response — all of which directly modulate the propensity for impulsive and reactive aggression (Benton, 2010; Gajos & Beaver, 2016). Omega-3 fatty acids govern neuronal membrane fluidity and neuroinflammation; B-vitamins and zinc act as obligate cofactors in serotonin and dopamine synthesis; iron underpins myelination and dopaminergic neurotransmission; and cortisol dysregulation shapes the threshold of stress-reactive aggression. Critically, deficiencies in these nutrients are not rare: they are prevalent in community, custodial, and forensic populations and are demonstrably remediable through dietary modification and targeted supplementation (Gesch et al., 2002; Raine & Brodrick, 2024).

Most compellingly, randomised controlled trials have demonstrated that nutritional supplementation encompassing omega-3 fatty acids, vitamins, and minerals produces statistically significant and clinically meaningful reductions in aggressive and antisocial behaviour in both juvenile and adult populations (Gesch et al., 2002; Raine et al., 2015; Raine & Brodrick, 2024). These findings carry a level of causal inference rarely achieved by psychological intervention research and constitute a powerful mandate for incorporating nutritional variables into forensic risk frameworks. It is within this context that the present paper proposes Forensic Nutrition Profiling (FNP): a novel, interdisciplinary conceptual framework designed to systematically integrate nutritional biomarkers, diet quality assessment, metabolic physiology, and standardised behavioural measures into a coherent composite model of aggression vulnerability. FNP does not assert that nutritional deficiency is the sole cause of violent behaviour; rather, it conceptualises nutritional vulnerability as a quantifiable, modifiable biological risk factor analogous in its forensic role to substance misuse, sleep disorder, or traumatic brain injury that interacts with psychosocial risk determinants to lower the threshold for reactive aggression. This paper presents the theoretical foundations, structural architecture, biomarker rationale, analytical methods, risk stratification logic, and translational applications of the FNP framework. It is intended to serve as a foundational conceptual contribution that will guide future empirical validation studies, clinical protocol development, and eventual integration into forensic assessment practice.

Background and Rationale: The Nutritional Gap In Forensic Science

Limitations of Current Forensic Risk Frameworks

Contemporary forensic risk assessment instruments have been refined over decades of actuarial and clinical research. Structured professional judgement tools such as the HCR-20 V3 integrate static historical variables (prior violence, early maladjustment, personality disorder) with dynamic clinical indicators (active psychosis, impulsivity, treatment response) and risk management considerations. Actuarial tools such as the VRAG-R assign weighted scores based on criminological and diagnostic predictors, producing probabilistic risk classifications. Both approaches demonstrate good predictive validity for institutional violence and community recidivism, with area under the receiver operating characteristic (AUC-ROC) values typically in the range of 0.70–0.80 (Fazel et al., 2012). Yet these instruments share a systematic blind spot: they do not assess the neurobiological and physiological substrate of the individual at the time of evaluation. An individual's risk score on the HCR-20 remains identical whether their omega-3 index is in the lowest decile or the highest, whether they are profoundly iron-deficient with blunted dopaminergic transmission, or



**Riya Raj and Swetha Thomas**

whether their cortisol diurnal rhythm is severely flattened. This biological information — empirically related to aggression risk and analytically obtainable — is entirely absent from the forensic evaluation process, representing not merely a theoretical gap but a practical one. FNP is designed precisely to close this gap.

Nutritional Psychiatry and Behavioural Neuroscience: Convergent Evidence

The scientific case for incorporating nutritional variables into forensic assessment rests on three converging lines of evidence: (i) mechanistic studies establishing causal pathways from nutritional status to neurochemical and physiological processes that modulate aggression; (ii) observational epidemiological studies demonstrating associations between dietary patterns, nutritional deficiencies, and aggressive or antisocial behaviour; and (iii) randomised controlled trials demonstrating that correcting nutritional deficiencies reduces aggressive behaviour in high-risk populations. Mechanistically, serotonin whose synthesis depends on dietary tryptophan availability and B-vitamin cofactor supply is the principal neurochemical brake on impulsive aggression; serotonin depletion has been consistently associated with increased reactive violence across species, clinical populations, and experimental paradigms (Benton, 2010; Kennedy, 2016). Dopaminergic pathways, dependent on tyrosine availability and iron-dependent enzymatic activity, regulate executive control, reward discounting, and the inhibition of prepotent aggressive responses (Beard, 2008). Omega-3 fatty acids modulate the density and function of prefrontal serotonin and dopamine receptors, maintain neuronal membrane fluidity, and suppress microglial neuroinflammation processes that collectively underpin affective regulation and impulse control (Hibbeln et al., 2006).

Epidemiologically, studies across diverse populations consistently link poor dietary quality, ultra-processed food consumption, and specific micronutrient deficiencies to elevated aggression scores, higher rates of conduct disorder, and increased violent offending (Jacka et al., 2017; Gajos & Beaver, 2016). The most compelling evidence comes from randomised controlled trials. Gesch et al. (2002) demonstrated a 26.3% reduction in institutional violent incidents in young adult prisoners randomised to receive vitamins, minerals, and essential fatty acids compared to placebo. Raine et al. (2015) showed that omega-3 supplementation significantly reduced internalising and externalising behavioural problems in children aged 8–16 years. A subsequent meta-analysis by Raine and Brodrick (2024) reported a pooled effect size ($d \approx 0.60$ – 0.70) for omega-3 supplementation on aggression reduction an effect magnitude comparable to, or exceeding, established psychological interventions for violence.

Positioning FNP Within Forensic Science

FNP is not proposed as a replacement for existing forensic risk assessment instruments. It is designed as a complementary biological layer within a broader multi-domain assessment architecture. Just as forensic neuropsychological evaluation augments psychiatric risk assessment by characterising individual cognitive vulnerabilities, FNP augments psychosocial risk profiling by characterising the biological, nutritional, and physiological substrate of aggression susceptibility. This aligns with the biosocial approach to criminology advocated by Raine (2018), which argues that biological and social determinants of violence are interacting causal contributors that a comprehensive risk science must account for simultaneously. Critically, FNP identifies modifiable risk factors: nutritional biomarker deficiencies and dietary patterns are remediable, giving FNP a dual function as both a risk stratification tool and a framework for targeting evidence-based nutritional interventions within forensic and rehabilitative settings.

**The Forensic Nutrition Profiling Framework: Structural Architecture
Conceptual Foundation and Design Principles**

The FNP framework is built on three core design principles. First, biological grounding: every biomarker included in the FNP panel is selected on the basis of documented mechanistic links to neurotransmitter synthesis, neural circuit function, stress physiology, or neuroinflammation each independently associated with aggression or emotional dysregulation. Second, analytical tractability: all proposed biomarkers are quantifiable using validated forensic-grade analytical methods already present in established forensic toxicology and biochemistry laboratories. Third, clinical actionability: each FNP risk tier is associated with a defined response pathway from monitoring through nutritional intervention to multidisciplinary forensic management ensuring that assessment findings translate directly into



**Riya Raj and Swetha Thomas**

rehabilitation and risk management actions. The FNP framework is organised around four weighted assessment domains. These domains interact within an integrated biological system: nutritional biomarker status determines the biochemical substrate for neurotransmitter synthesis and stress physiology; diet quality is the primary upstream determinant of biomarker status; physiological stress markers reflect the functional consequences of dietary and nutritional inadequacy; and behavioural markers provide the phenotypic expression of underlying biological vulnerabilities. Composite scoring across these domains produces an FNP Risk Score (0–100) stratified into four actionable risk tiers.

Domain I – Nutritional Biomarkers (Weight: 40%)

Nutritional biomarkers constitute the core of the FNP model, carrying the highest weighting (40%) in recognition of their direct mechanistic role in the neurochemical and physiological determinants of aggression. Six primary biomarker categories are proposed:

- **Omega-3 Index (EPA + DHA):** Measured in erythrocyte membranes by gas chromatography–mass spectrometry (GC-MS), the omega-3 index provides a 120-day integrated measure of essential fatty acid status. Low omega-3 index has been prospectively associated with impulsivity, affective dysregulation, and reactive aggression (Hibbeln et al., 2006; Raine & Brodrick, 2024).
- **Vitamin D (25(OH)D):** Serum 25-hydroxyvitamin D serves as a functional neurosteroid, modulating the transcription of genes encoding serotonin synthesis enzymes (TPH2) and neurotrophin (BDNF) production in limbic circuits. Deficiency (<50 nmol/L) has been associated with mood dysregulation, irritability, and heightened aggression risk (Anglin et al., 2013).
- **Ferritin (Iron Status):** Serum ferritin is the primary indicator of iron stores. Iron is an essential cofactor for tyrosine hydroxylase (the rate-limiting enzyme in dopamine synthesis) and for myelination of prefrontal-subcortical circuits. Iron deficiency is associated with dopaminergic transmission deficits, cognitive slowing, and heightened behavioural reactivity (Beard, 2008).
- **B-Vitamins (B6, B9, B12):** These vitamins function as obligate enzymatic cofactors in the methylation and conversion of amino acid precursors to active monoamine neurotransmitters. B12 and folate are also essential for homocysteine remethylation; elevated homocysteine is an independent marker of neurotoxicity. Deficiency of any single B-vitamin can attenuate serotonergic and dopaminergic tone even when precursor amino acid supply is adequate (Kennedy, 2016).
- **Zinc:** A structural component of over 300 metalloenzymes, zinc modulates NMDA receptor function, synaptic plasticity, and neuronal inhibitory tone. Zinc deficiency is associated with impaired glutamatergic neurotransmission, dysregulated amygdala reactivity, and heightened aggression in both animal models and human clinical populations (Choy, 2023).
- **Magnesium:** Magnesium regulates NMDA receptor activity, modulates the HPA axis stress response, and suppresses cortisol hypersecretion under psychosocial challenge. Hypomagnesaemia is associated with emotional volatility, heightened anxiety, and amplified stress-reactive aggression (Choy, 2023).

Domain II – Diet Quality (Weight: 20%)

Diet quality operates as the primary upstream determinant of nutritional biomarker status and constitutes a modifiable risk factor in its own right. The FNP framework proposes assessment of diet quality through three complementary approaches: (i) quantification of ultra-processed food intake using the NOVA classification system; (ii) estimation of total daily glycaemic load from 24-hour dietary recall data; and (iii) calculation of a composite diet quality score using the Alternate Healthy Eating Index (AHEI) or the Dietary Approaches to Stop Hypertension (DASH) score, both of which have demonstrated associations with mental health and cognitive outcomes (Jacka et al., 2017). Diets dominated by ultra-processed foods systematically deplete the nutritional substrates assessed in Domain I and promote systemic inflammation through gut dysbiosis, increased intestinal permeability, and pro-inflammatory eicosanoid production.



**Domain III – Stress and Physiology (Weight: 20%)**

This domain captures the functional physiological consequences of nutritional inadequacy on the individual's stress response system and inflammatory burden. Three markers are proposed:

- **Cortisol Diurnal Rhythm:** Salivary cortisol is collected at four standardised time points (awakening, +30 minutes [cortisol awakening response, CAR], 14:00, and 21:00) using validated Salivette® collection methodology. Dysregulation of the diurnal cortisol slope characterised by a flattened or inverted pattern, blunted CAR, or hypocortisolism reflects chronic HPA axis dysregulation and has been consistently associated with reactive aggression, antisocial personality, and conduct disorder (McEwen, 2007).
- **High-Sensitivity C-Reactive Protein (hsCRP):** hsCRP is the most widely validated marker of systemic low-grade inflammation in clinical and community research. Elevated hsCRP reflects the neuroinflammatory state produced by diets high in ultra-processed foods, omega-3 deficiency, and gut dysbiosis. Neuroinflammation suppresses prefrontal serotonin transporter expression, increases microglial activation, and impairs executive functioning (Miller et al., 2009).
- **Sleep Quality Index:** Poor sleep quality, assessed via the Pittsburgh Sleep Quality Index (PSQI) or objective actigraphy, amplifies both cortisol reactivity and the neurobiological consequences of nutritional deficiency. Sleep deprivation independently reduces prefrontal cortical inhibitory control and increases amygdala reactivity, potentiating aggression under frustrating conditions.

Domain IV – Behavioural Markers (Weight: 20%)

Behavioural instruments provide the phenotypic anchor for the FNP composite score, enabling alignment of biological risk profiles with observable behavioural tendencies. Three validated instruments are proposed: (i) the Buss-Perry Aggression Questionnaire (AQ-29), which assesses physical aggression, verbal aggression, anger, and hostility with robust psychometric properties in both community and forensic samples; (ii) the Barratt Impulsiveness Scale version 11 (BIS-11), which measures motor, attentional, and non-planning impulsivity and has been extensively validated in forensic populations; and (iii) the Difficulties in Emotion Regulation Scale (DERS-36), which characterises emotional dysregulation across six dimensions including non-acceptance, goal-directed behaviour, impulse control, awareness, strategies, and clarity. These instruments collectively profile the behavioural phenotype predicted to emerge from the neurochemical and physiological deficits captured in Domains I–III.

FNP Model: Summary Tables

Table 1. FNP Framework Domains, Indicators, and Composite Weightings

Table 2. FNP Proposed Biomarker Panel: Analytical Methods and Evidence Base

Table 3. FNP Composite Risk Score Matrix: Tier Classification, Clinical Interpretation, and Recommended Forensic Responses

Table 4. Comparison of FNP with Established Forensic Risk Assessment Instruments

FNP Scoring Methodology and Composite Risk Calculation**Within-Domain Scoring**

Within each of the four FNP domains, individual indicators are scored on a standardised three-point scale: 0 (adequate – within reference range), 0.5 (borderline approaching insufficiency or mildly elevated), and 1.0 (deficient or dysregulated below clinical threshold or clinically elevated). Reference ranges are drawn from established clinical laboratory normative data: for example, Vitamin D sufficiency is defined as ≥ 50 nmol/L (125 nmol/L optimal); omega-3 index adequacy as $\geq 8\%$; serum ferritin adequacy as ≥ 15 $\mu\text{g/L}$; and hsCRP low inflammation as < 1 mg/L. Borderline classification is defined as within 20% of the threshold in the deficient direction. Within each domain, indicator scores are summed and normalised to a 0–1 domain sub-score.

Composite Score Calculation

The Composite FNP Risk Score is calculated as a weighted sum of domain sub-scores, reflecting the differential evidence base for each domain's contribution to aggression risk: FNP Score = (Nutritional Biomarker Domain Score $\times$ 0.40) + (Diet Quality Domain Score $\times$ 0.20) + (Stress & Physiology Domain Score $\times$ 0.20) + (Behavioural Marker



**Riya Raj and Swetha Thomas**

Domain Score $\times 0.20$), scaled to a 0–100 range. This weighted architecture ensures that the strongest mechanistic domain (nutritional biomarkers) exerts the greatest influence on the composite score while preserving the informational contribution of contextual, physiological, and phenotypic domains.

Interpretation Principles

Several interpretation principles are essential to appropriate clinical use of the FNP score. First, the FNP score is a biological risk indicator, not a prediction of violence: high scores indicate elevated neurobiological vulnerability to reactive aggression under conditions of stress or provocation; they do not imply criminal intent or inevitability of violence. Second, FNP scores must always be interpreted within the full psychosocial context of the individual, including their trauma history, substance use, personality characteristics, social support, and environmental stressors. Third, the FNP score is dynamic: nutritional biomarker levels and dietary patterns change over time, and re-assessment following nutritional intervention is both feasible and clinically meaningful. Fourth, the FNP framework should be administered and interpreted by trained forensic practitioners with access to clinical dietetics expertise.

Analytical and Forensic Laboratory Methods**Biological Sample Collection**

The FNP biomarker panel is designed for assessment using biological matrices routinely available in forensic and clinical biochemistry contexts. Fasting venous blood samples (10–15 ml) are recommended, collected in the morning to standardise cortisol and metabolic markers. Erythrocyte omega-3 index is quantified using gas chromatography–mass spectrometry (GC-MS) applied to erythrocyte membrane phospholipids, providing a 120-day integrated measure of EPA+DHA status. Serum 25(OH)D is quantified by liquid chromatography–tandem mass spectrometry (LC-MS/MS), the reference method for clinical Vitamin D assessment. Ferritin, hsCRP, and insulin are measured using validated immunoassay platforms (ELISA or chemiluminescence-based). B-vitamins (B6, B9, B12) and amino acid precursors (tryptophan, tyrosine) are quantified by high-performance liquid chromatography (HPLC) or LC-MS. Zinc is measured by inductively coupled plasma–mass spectrometry (ICP-MS) or validated atomic absorption spectrophotometry. Salivary cortisol is collected at four standardised time points using validated Salivette® collection kits, with strict adherence to sampling protocols to minimise pre-analytical variability, and quantified by ELISA or LC-MS/MS.

Hair and Nail Matrices

Hair and nail segments offer an important complementary forensic matrix, providing a historical record of chronic nutritional status rather than the acute snapshot captured by serum measurements. Hair elemental analysis by ICP-MS enables retrospective assessment of zinc, magnesium, and other trace element status over a 3–6 month window, corresponding to the rate of hair growth (~1 cm/month). These matrices are particularly valuable in forensic contexts where retrospective nutritional profiling is required for example, to characterise the nutritional environment in the period preceding a violent incident.

Emerging Analytical Approaches

Attenuated total reflectance Fourier-transform infrared (ATR-FTIR) spectroscopy represents an emerging rapid screening approach applicable to dried blood spots or tissue samples. This technique enables semi-quantitative profiling of multiple nutritional and metabolic markers simultaneously from minimal biological material, with potential applications in resource-limited forensic settings (Choy, 2023). Metabolomics platforms (untargeted LC-MS) offer the future prospect of comprehensive nutritional and metabolic phenotyping from a single biological sample, potentially enabling discovery of additional biomarker signatures not yet incorporated into the FNP panel.

Translational Applications and Implementation Pathways**Custodial and Correctional Settings**

The most immediate translational application of FNP is within custodial and correctional environments, where controlled dietary conditions, captive populations, and high prevalence of both nutritional deficiency and violent



**Riya Raj and Swetha Thomas**

behaviour create an optimal setting for both framework implementation and intervention evaluation. Studies conducted in prisons consistently document high prevalence of omega-3 deficiency, iron insufficiency, low Vitamin D, and poor overall diet quality in incarcerated populations (Gesch et al., 2002). FNP provides a structured mechanism for identifying those prisoners whose nutritional profile places them at elevated biological risk for violent incidents and for directing targeted nutritional interventions toward the highest-risk individuals. The Gesch et al. (2002) randomised controlled trial provides a proof-of-concept demonstration: a combined vitamin, mineral, and fatty acid supplement reduced violent disciplinary incidents by 26.3% relative to placebo over 142 days in a young adult prison sample.

Forensic Psychiatric Assessment

Within forensic psychiatric services, FNP can augment existing structured professional judgement assessment of inpatients and community forensic patients. Forensic psychiatrists and psychologists conducting periodic risk reviews can incorporate FNP domain scores as a dynamic biological risk indicator, updated as nutritional status changes in response to dietary intervention, treatment, or environmental change. The FNP's risk tier classification (Table 3) generates actionable recommendations readily integrated into care planning: a patient whose FNP score moves from High to Moderate following three months of omega-3 supplementation and dietary improvement provides objective biological evidence of reduced physiological aggression vulnerability, informing decisions about leave, community placement, and security level.

Community Violence Prevention

FNP also has preventive applications in community forensic psychiatry, youth justice, and public health settings. Routine FNP screening of individuals on community forensic orders, those with a history of violent offending, or young people in early-intervention programmes could identify those with elevated nutritional vulnerability before violent incidents occur. This aligns with the evidence-based logic of nutritional criminology the proposition that dietary modification and nutritional supplementation can function as upstream violence prevention strategies with population-level impact and comparatively low cost (Choy, 2023; Raine, 2018).

Legal and Expert Witness Applications

In legal contexts, FNP findings may inform expert witness testimony regarding biological mitigating factors in cases involving reactive, impulsive aggression where the defendant's nutritional and physiological status at the time of the offence is relevant to culpability assessment. FNP's analytical grounding in validated laboratory methods and its evidence base in peer-reviewed science position it, in principle, to satisfy criteria for admissibility of scientific evidence under the Daubert or Frye standards. Legal application of FNP must, however, always be accompanied by explicit communication of its scope: FNP identifies biological vulnerability, not deterministic causation of violent acts.

Ethical Considerations and Boundaries of Application

The ethical deployment of FNP requires explicit attention to several dimensions. First and most fundamentally, FNP is a risk stratification tool, not a diagnostic test for criminal propensity. A high FNP composite score indicates elevated neurobiological vulnerability to reactive aggression; it does not predict, explain, or excuse any specific violent act, and must never be used as standalone evidence of dangerousness or criminal culpability. Second, the collection of biological samples for FNP assessment requires full voluntary informed consent, consistent with the Declaration of Helsinki and applicable national research ethics regulations. In custodial contexts, particular care must be taken to ensure that consent is genuinely free from institutional coercion, and that participation in FNP assessment is not linked to sentencing, parole, or security level decisions without explicit legal authorisation and robust procedural safeguards.

Third, nutritional biomarker data is sensitive health information and must be handled with the same confidentiality protections applied to other medical and psychiatric assessment data. Fourth, the deterministic misapplication of FNP for example, using a high FNP score to justify detention or deny parole in the absence of behavioural evidence



**Riya Raj and Swetha Thomas**

would constitute a serious violation of the framework's intended scope and the ethical principles of forensic science. Fifth, like all forensic instruments, FNP must be validated across diverse ethnic, socioeconomic, and cultural populations before generalisation, as nutritional reference ranges, dietary patterns, and microbiome composition vary substantially across populations.

Future Directions: Proposed Validation Agenda

The FNP framework, as a conceptual contribution, identifies a clear agenda for empirical validation and clinical translation. The following research priorities are proposed:

- **Cross-sectional validation studies:** Large-sample cross-sectional studies in forensic and community populations should characterise the distribution of FNP domain scores, examine their correlations with validated aggression and impulsivity instruments, and assess the discriminative validity of the composite FNP score against known aggression risk stratifications.
- **Longitudinal predictive validation:** Prospective cohort studies should follow individuals with varying FNP risk profiles over 12–24 months to assess the predictive validity of baseline FNP scores for incident violent behaviour, adjusting for established psychosocial risk factors.
- **Randomised controlled trials of FNP-guided intervention:** RCTs in custodial and forensic psychiatric settings should evaluate whether FNP-targeted nutritional interventions reduce aggression outcomes relative to standard care, using FNP composite score improvement as a secondary biological endpoint.
- **Instrument refinement and machine learning classification:** Machine learning approaches — including Random Forest classifiers, LASSO regression, and support vector machines — should be applied to FNP biomarker data to identify optimal biomarker subsets and scoring algorithms, improving classification accuracy for high-risk profiles.
- **Cross-cultural validation:** Independent validation studies in non-Western, non-industrial, and low- and middle-income country populations are essential to establish the cross-cultural generalisability of FNP reference ranges and risk tier thresholds.
- **Integration studies with existing risk instruments:** Studies should formally evaluate the incremental predictive validity of FNP composite scores when added to established instruments (HCR-20, VRAG-R, START), quantifying the degree to which FNP's biological dimension improves the AUC-ROC of standard forensic risk assessment batteries.

DISCUSSION

Forensic Nutrition Profiling represents a theoretically coherent, empirically grounded, and practically translatable innovation in forensic risk science. By drawing on over four decades of nutritional neuroscience, behavioural endocrinology, and randomised controlled trial evidence, FNP constructs a biological risk architecture for aggression that is simultaneously mechanistically plausible, analytically tractable, and clinically actionable. The framework does not challenge the validity or importance of existing forensic risk assessment instruments; it extends their reach by incorporating biological information that those instruments, by design, cannot capture. The most important conceptual contribution of FNP is the reconceptualisation of nutritional deficiency as a modifiable forensic risk factor. This has profound implications for how forensic practitioners, policymakers, and institutional administrators approach violence prevention. If a significant proportion of reactive aggression in custodial and forensic populations is partially attributable to remediable nutritional deficiencies as the randomised controlled trial evidence strongly suggests then the systematic failure to screen for and correct these deficiencies represents a missed opportunity for evidence-based violence reduction at scale.

The FNP model is deliberately conservative in its claims. It does not propose that nutritional factors are the primary cause of violent behaviour; the psychosocial, developmental, and psychiatric determinants that existing forensic instruments assess remain central. Rather, FNP proposes that nutritional vulnerability lowers the biological threshold at which these psychosocial risk factors precipitate violent action and that this threshold can be measured, quantified,



**Riya Raj and Swetha Thomas**

and raised through intervention. This is a modest but scientifically important claim, with practical consequences for both risk management and rehabilitation. Limitations of the framework as currently constituted must be acknowledged. The evidence base supporting individual FNP biomarkers varies in quality and consistency; the omega-3 index and cortisol diurnal rhythm have the strongest experimental support, while the independent contribution of individual micronutrients such as magnesium and zinc is less well characterised in forensic populations specifically. The composite scoring algorithm requires empirical calibration: the proposed domain weights are derived from the relative density of evidence rather than from empirically determined regression coefficients in a forensic validation sample. Future empirical work will refine and potentially revise this weighting structure as population-specific data become available.

CONCLUSION

This paper has introduced Forensic Nutrition Profiling (FNP) as a novel, interdisciplinary conceptual framework for integrating nutritional biomarkers, diet quality, metabolic physiology, and behavioural instruments into a composite model of aggression vulnerability for use in forensic assessment. FNP is grounded in a convergent body of mechanistic, epidemiological, and experimental evidence establishing nutrition as a modifiable biological determinant of the neurochemical and physiological processes that govern impulse control, emotional regulation, and reactive aggression. The framework proposes a four-domain composite scoring architecture yielding a Composite FNP Risk Score stratified into four actionable risk tiers, alongside a comprehensive biomarker panel analytically grounded in established forensic laboratory methods. FNP is positioned not as a replacement for existing forensic risk instruments but as a biologically grounded complement, adding a nutritional and metabolic dimension to risk assessment that current tools systematically omit. The translational significance of FNP lies in its dual function: as a risk stratification tool identifying individuals at elevated biological vulnerability to reactive aggression, and as a framework for targeting evidence-based nutritional interventions within forensic, custodial, and community settings. By establishing a clear agenda for empirical validation cross-sectional, longitudinal, and experimental this paper lays the groundwork for FNP to transition from conceptual framework to validated forensic assessment instrument.

Research Highlights

- Forensic Nutrition Profiling (FNP) is introduced as the first interdisciplinary framework integrating nutritional biomarkers into forensic risk assessment.
- A four-domain composite model (Nutritional Biomarkers 40%; Diet Quality 20%; Stress & Physiology 20%; Behavioural Markers 20%) yields a structured FNP Risk Score (0–100).
- Key biomarkers include the omega-3 index, Vitamin D, ferritin, B-vitamins, zinc, magnesium, cortisol diurnal rhythm, and C-reactive protein — all quantifiable by established forensic laboratory methods.
- Randomised controlled trial evidence demonstrates that targeted nutritional supplementation reduces aggressive behaviour in high-risk populations, validating FNP as both an assessment and intervention tool.
- FNP identifies modifiable biological risk factors for reactive aggression and provides defined forensic response pathways, with direct translational relevance for custodial settings and offender rehabilitation

Author Contribution Statement

Conceptualisation and Framework Design: **R.R.**; Literature review and evidence synthesis: **R.R.**; Manuscript writing — original draft: **R.R.**; Manuscript editing and reviewing: **R.R.**

Data Availability Statement

This paper presents a conceptual framework and does not involve the collection or analysis of empirical datasets. No primary data were generated in association with this work.



**Riya Raj and Swetha Thomas****Declaration of Conflict of Interest**

The author confirms that there are no conflicts of interest to declare.

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Riya Raj and Swetha Thomas

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Table 1. FNP Framework Domains, Indicators, and Composite Weightings

Domain	Key Indicators	Weight (%)
Nutritional Biomarkers	Omega-3 index (EPA+DHA); Vitamin D (25(OH)D); Ferritin; B-vitamins (B6, B9, B12); Zinc; Magnesium	40
Diet Quality	Ultra-processed food intake; total glycaemic load; fruit and vegetable frequency; dietary pattern scores (AHEI / DASH)	20
Stress & Physiology	Cortisol diurnal rhythm (AM/PM); C-reactive protein (CRP); sleep quality index	20
Behavioural Markers	Impulsivity scales (BIS-11); aggression inventories (Buss-Perry AQ); emotional regulation assessments (DERS)	20
Total Composite Score	Nutrition + Diet Quality + Stress Load + Behavioural Indices	100

Note. FNP = Forensic Nutrition Profiling; BIS-11 = Barratt Impulsiveness Scale version 11; AQ = Aggression Questionnaire; DERS = Difficulties in Emotion Regulation Scale; AHEI = Alternate Healthy Eating Index; DASH = Dietary Approaches to Stop Hypertension.

Table 2. FNP Proposed Biomarker Panel: Analytical Methods and Evidence Base

Biomarker	Sample Matrix	Analytical Method	Proposed Behavioural Correlate	Evidence Base
Omega-3 Index (EPA+DHA)	Erythrocytes	GC-MS / LC-MS	Impulsivity; affective dysregulation; reactive aggression	Hibbeln et al., 2006; Raine & Brodrick, 2024
Vitamin D (25(OH)D)	Serum/plasma	LC-MS/MS; ELISA	Irritability; mood instability; serotonin synthesis modulation	Anglin et al., 2013
Ferritin (Iron Status)	Serum	Immunoassay	Dopaminergic deficits; cognitive slowing; behavioural	Beard, 2008





Riya Raj and Swetha Thomas

Biomarker	Sample Matrix	Analytical Method	Proposed Behavioural Correlate	Evidence Base
			dysregulation	
B6, B9, B12 (B-Vitamins)	Plasma	HPLC; immunoassay	Serotonin and dopamine cofactor depletion; neurotransmitter impairment	Kennedy, 2016
Zinc	Serum/plasma	ICP-MS; atomic absorption	NMDA receptor dysfunction; synaptic inhibition deficits; aggression	Choy, 2023
Magnesium	Serum / RBC	Spectrophotometry	HPA axis hyperreactivity; emotional volatility under stress	Choy, 2023
Cortisol (AM/PM Diurnal)	Saliva	ELISA; LC-MS/MS	HPA dysregulation; stress-reactive aggression; impaired inhibition	McEwen, 2007
C-Reactive Protein (CRP)	Serum	High-sensitivity ELISA	Neuroinflammation; mood instability; frontal cortex hypoactivity	Miller et al., 2009
Tryptophan / Tyrosine	Plasma	HPLC; LC-MS	Precursor depletion for serotonin and dopamine synthesis	Benton, 2010
Glycaemic Index / Insulin	Serum	Immunoassay; glucose oxidase	Glycaemic instability; self-regulation failure; irritability	Benton, 2007

Note. GC-MS = Gas Chromatography–Mass Spectrometry; LC-MS = Liquid Chromatography–Mass Spectrometry; ICP-MS = Inductively Coupled Plasma Mass Spectrometry; HPLC = High-Performance Liquid Chromatography; ELISA = Enzyme-Linked Immunosorbent Assay; RBC = Red Blood Cells.

Table 3. FNP Composite Risk Score Matrix: Tier Classification, Clinical Interpretation, and Recommended Forensic Responses

Risk Level	Score	Biomarker Profile	Clinical Interpretation	Recommended Forensic Response
Low	0–24	Adequate across all domains	Minimal neurochemical vulnerability; baseline behavioural control maintained	Routine monitoring; no nutritional intervention indicated
Moderate	25–49	1–2 biomarker deficiencies; suboptimal diet quality	Elevated stress reactivity; mild impairment of impulse regulation under provocation	Dietary counselling; targeted micronutrient supplementation; re-assessment in 3 months
High	50–74	3–4 deficiencies; elevated CRP; poor diet quality	Significant neurochemical vulnerability; heightened susceptibility to reactive aggression	Clinical nutrition referral; omega-3 and micronutrient supplementation; psychoeducation
Critical	75–100	Multi-domain deficits; cortisol dysregulation;	Severe physiological risk milieu; high probability of	Multidisciplinary forensic–nutritional intervention;





Riya Raj and Swetha Thomas

Risk Level	Score	Biomarker Profile	Clinical Interpretation	Recommended Forensic Response
		systemic inflammation	aggression under minimal provocation	custodial dietary management

Note. Risk tier classification is based on weighted domain score aggregation. FNP scores are intended to inform, not replace, professional forensic judgement. Classification should be interpreted within the full psychosocial context of the individual case.

Table 4. Comparison of FNP with Established Forensic Risk Assessment Instruments

Instrument	Domains Assessed	Biomarkers	Nutrition	Physiology	Output
HCR-20 V3	Historical, clinical, risk management items	No	No	No	Structured professional judgement
VRAG-R	Actuarial (criminal & clinical history)	No	No	No	Risk score / bin classification
START	Dynamic psychosocial strengths & risks	No	No	Partial (substance use)	SPJ with treatment planning
PCL-R	Psychopathy trait measurement	No	No	No	Dimensional score
FNP (Proposed)	Nutritional biomarkers, diet, physiology, behaviour	Yes	Yes	Yes	Composite risk score + intervention pathway

Note. HCR-20 = Historical Clinical Risk Management; VRAG-R = Violence Risk Appraisal Guide-Revised; START = Short-Term Assessment of Risk and Treatability; PCL-R = Psychopathy Checklist-Revised; SPJ = Structured Professional Judgement. FNP is proposed as a complementary instrument, not a replacement for established tools.

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RESEARCH ARTICLE

Phytochemical and Elemental Characterization of *Ipomoea hederifolia* L. using FTIR and ICP-MS

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ABSTRACT

Ipomoea hederifolia L. is a medicinal plant belonging to the family *Convolvulaceae*. This study investigates its phytochemical and elemental composition using Fourier Transform Infrared Spectroscopy (FTIR) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS). FTIR analysis revealed characteristic absorption bands corresponding to hydroxyl, alkane, aromatic and phenolic functional groups, indicating the presence of important bioactive compounds. ICP-MS analysis detected essential trace elements such as iron, zinc, copper, manganese and nickel. These elements are known to play important roles in biological and metabolic processes. The scientific data generated by the combined analytical technique confirms the plant's therapeutic value and suggests its potential use in pharmaceutical and nutraceutical applications.

Keywords: *Ipomoea hederifolia* L., phytochemicals, ICP-MS, FTIR

INTRODUCTION

Medicinal plants have been an important source of medicinal compounds for thousands of years. A substantial number of current pharmaceutical medications are derived from natural sources, many of which were discovered through their traditional medical use. Natural compounds originating from plants continue to play a critical role in drug discovery and development due to their various chemical structures and biological activity. [1]. Plants contain

116905



**Kuleshwar Prasad and Sandhya Rani Panda**

naturally occurring bioactive molecules known as phytochemicals, which have a substantial impact on human health. These substances include secondary metabolites such as alkaloids, flavonoids, phenolic, terpenoids, and glycosides, all of which have a wide range of pharmacological effects. In addition to their medicinal potential, phytochemicals are crucial macro and micronutrients that promote normal physiological processes in humans. [2].

India is regarded as one of the wealthiest countries in terms of biodiversity, with a huge range of plant species due to its varied climatic and geographical circumstances. Many of these plants have long been utilised in ancient medicinal systems such as Ayurveda, Unani, and folk medicine. Scientific validation of ethno-medicinal plants through phytochemical and pharmacological investigations is crucial to validate their safety, potency, and potential therapeutic value. [3]. Documenting ethno-botanical and ethno-pharmacological knowledge is thus vital for supporting future study in herbal therapy and facilitating its implementation in modern healthcare systems. [4]. *Convolvulaceae* is a plant family with over 1650 species, most of which are found in tropical locations across the world. *Ipomoea* is one of the larger genera in this family, with around 500-600 flowering plant species. [5]. This genus's species are generally known as morning glories because of the unique behaviour of their blooms, which open in the morning and close later in the day. Many varieties of *Ipomoea* are often grown as ornamental plants because of their gorgeous blooms and robust growth, but some are also considered weeds owing to their tendency to spread fast. [6]. *Ipomoea hederifolia* L., a medicinal climbing plant in the *Convolvulaceae* family, is sometimes referred to as scarlet morning glory or scarlet creeper. The species name *hederifolia* comes from Latin and describes the vine's ivy-like leaves, while the genus name *Ipomoea* is derived from the Greek terms *ips* and *homoios*, alluding to the twining or worm-like growth behaviour of the vine. [7].

Due to its many medicinal uses, this plant has long been utilised in many medical systems. Diuretic, anthelmintic, laxative, carminative, blood-purifying, anti-inflammatory, and deobstructive properties are described. The herb has historically been used to cure a number of illnesses, such as bronchitis, fever, headaches, and stomach problems. [8]. The seeds are used in traditional Chinese medicine to treat ailments including constipation, oedema, and parasite infections because of its cathartic, diuretic, anti-inflammatory, and expectorant qualities. The plant is known to have sternutatory action in its roots. [9]. Traditional medicine has long acknowledged climbing plants as important sources of bioactive chemicals with possible medicinal uses. However, there are a number of obstacles to using these plants in contemporary pharmaceutical research, including as the large-scale manufacture of bioactive ingredients, the identification of active molecules by high-throughput screening, and the sustainable sourcing of plant materials. Despite these difficulties, climber plants are still widely used in traditional healthcare systems around the world, especially in rural and tribal communities where they are used to treat a variety of illnesses, including fever, inflammation, skin infections, respiratory disorders, digestive issues, and pain. Their ecological, therapeutic, and cultural relevance emphasises the value of ongoing study and conservation initiatives. [10]. Tropical and subtropical areas are home to the twining vine *Ipomoea hederifolia* L. Nearly 2000 species and 58 genera make up the family *Convolvulaceae*, which is primarily distinguished by herbaceous or woody plants with heart-shaped leaves, funnel-shaped blooms, and twining or climbing behaviours. With about 600 species worldwide, the genus *Ipomoea* is the biggest group in this family. [11].

METHODOLOGY

Impurities were removed from fresh *Ipomoea hederifolia* L., plant material by washing it with distilled water. The plant material was shade dried and pulverised to a fine powder. Approximately 10 g of powdered material was extracted with ethanol by Soxhlet extraction. The extract was filtered and purified for future investigation. For FTIR analysis, a little quantity of dried extract was combined with Potassium bromide (KBr) and formed into a pellet. The spectra were collected from 4000-400 cm^{-1} . For ICP-MS analysis, the powdered plant sample was digested with strong nitric acid and hydrogen peroxide. The digested solution was diluted with deionised water and put into the ICP-MS apparatus to identify trace elements.





RESULTS AND DISCUSSION

FTIR Analysis Finding functional groups in organic compounds is a common use for FTIR spectroscopy. *Ipomoea hederifolia*L., FTIR spectrum showed a number of distinctive absorption peaks that suggested the existence of several phytochemical components.

- O–H stretching vibrations, which are typical of phenols and alcohols, are represented by the absorption peak seen at 3283 cm^{-1} . These substances are well known for their anti-inflammatory and antioxidant qualities. The peak at 2919 cm^{-1} represents C–H stretching vibrations linked to terpenoids and alkanes.
- Aromatic C=C stretching vibrations are shown by a significant peak at 1605 cm^{-1} , indicating the presence of phenolic chemicals and flavonoids. The C–O stretching vibrations of alcohols, esters, and carbohydrates are represented as peaks at 1241 cm^{-1} and 1054 cm^{-1} , which may be connected to glycosides and polysaccharide.
- Alkaloids and other aromatic phytochemicals may be represented by additional peaks about 779 cm^{-1} that show aromatic C–H bending vibrations. The fingerprint region contains the peak at 440 cm^{-1} , which might suggest the existence of metal–oxygen linkages connected to mineral elements.

These results validate that *Ipomoea hederifolia*L., contains a number of significant bioactive substances, including phenols, flavonoids, tannins, terpenoids, glycosides, carbohydrates, and alkaloids. These substances are known to have anticancer, anti-inflammatory, antioxidant, and antibacterial

FTIR Peak Interpretation

*Ipomoea hederifolia*L., contains various significant bioactive phytochemicals, including phenols, flavonoids, tannins, terpenoids, glycosides, polysaccharides, and alkaloids, according to its FTIR spectrum. These chemicals include antioxidant, antibacterial, anti-inflammatory, anticancer, and anti-diabetic activities, which contribute to the plant's traditional therapeutic use.

Elemental Analysis Using ICP-MS

ICP-MS analysis revealed the presence of several essential macro and trace elements in *Ipomoea hederifolia*L.

Major Elements

Major elements detected include potassium, sulphur, calcium, magnesium, phosphorus, iron, and sodium. Potassium showed the highest concentration among the major elements. These elements are essential for maintaining physiological functions such as nerve transmission, muscle contraction, bone formation, and energy metabolism.

Trace Elements

Trace elements detected include manganese, zinc, copper, boron, chromium, nickel, cobalt, and molybdenum. These elements play crucial roles in enzymatic reactions, immune system regulation, and metabolic processes.

Potentially Toxic Elements

ICP-MS analysis also detected trace amounts of potentially toxic elements such as lead, cadmium, arsenic, and mercury. However, the concentrations were extremely low and within permissible limits, suggesting that the plant is relatively safe for medicinal use. The presence of both macro and trace elements indicates the nutritional significance of *Ipomoea hederifolia*. These elements contribute to various biological functions including enzyme activation, oxygen transport, immune defence, and bone health.

The elemental analysis of *Ipomoea hederifolia* shows the presence of several essential macro and micro nutrients such as potassium, calcium, magnesium, phosphorus, iron, zinc, and manganese. These elements play important roles in human metabolism, bone health, enzymatic reactions, and immune function, indicating the potential nutritional and medicinal value of the plant. The extract's complete characterisation, together with good physical features, provides compelling proof of its effectiveness and safety in clinical applications [12].





CONCLUSION

The present study confirms that *Ipomoea hederifolia* contains significant phytochemical compounds and essential trace elements. FTIR analysis demonstrated the presence of functional groups associated with phenols, alcohols and aromatic compounds, while ICP-MS revealed important micronutrients. The combined results highlight the medicinal and nutritional significance of the plant and support its traditional use in herbal medicine. Further research on isolation and pharmacological evaluation of the active compounds may lead to the development of new therapeutic agents.

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Table 1: FTIR Peak Interpretation

Wavenumber (cm ⁻¹)	Functional Group	Possible Phytochemicals	Biological/Medicinal Significance
3283 cm ⁻¹	O-H stretching can occur with alcohols, phenols	phenols, flavonoids, and tannins	Strong antioxidant, anti-inflammatory, and antibacterial; helps protect cells from oxidative damage
2919 cm ⁻¹	C-H stretching in alkanes	terpenoids, lipids, and essential oils.	Contains antibacterial, antifungal, and anti-inflammatory effects.
1979 cm ⁻¹	Weak overtone/combination. Band	Aromatic compounds	refer to complex organic phytochemicals used in plant defences





Kuleshwar Prasad and Sandhya Rani Panda

1605 cm⁻¹	C=C stretching in their aromatic rings	Flavonoids and phenolic compounds	Responsible for antioxidant, anticancer, and cardio protective actions
1412 cm⁻¹	O-H bending/C-C aromatic properties	Polyphenols and tannins exhibit	Anti-microbial, wound healing, and anti-ulcer properties
1241 cm⁻¹	C-O stretching (alcohols, phenols, esters)	Glycosides, Phenolic, and Esters	It has anti-diabetic, anti-inflammatory, and antibacterial properties.
1054 cm⁻¹	C-O stretching (carbohydrates/alcohols)	sugars, and polysaccharides	Contribute to energy metabolism, immunological support, and therapeutic properties.
1033 cm⁻¹	C-O stretching (alcohols and carbohydrates)	Glycosides and polysaccharides	Are associated with digestive and metabolic health advantages.
779 cm⁻¹	exhibit C-H bending. (Aromatic compounds,)	including alkaloids and phytochemicals,	May have analgesic and antibacterial effects.
440 cm⁻¹	metal-O bond / fingerprint region	Mineral complexes	Indicate the existence of trace elements necessary for biological activities.

Table 2: Major Elements and Their biological importance of Human Beings

S. No.	Element	Concentration (mg)	Biological significance for Human Health
1	Potassium (K)	238.91 mg	Essential for neuron function, muscular contraction, and cardiac rhythm regulation
2	Sulphur (S)	158.42 mg	Crucial for the synthesis of proteins and the action of enzymes
3	Calcium (Ca)	124.62 mg	Required for bone and tooth development, blood clotting, and muscle contraction.
4	Magnesium (Mg)	41.28 mg,	Promotes muscular function, neuronal transmission, and energy generation
5	Phosphorus (P).	24.78.mg.	Necessary for cellular metabolism, DNA structure, and bone production.
6	Iron (Fe)	12.47 mg	Needed for blood oxygen transport and haemoglobin synthesis
7.	Sodium (Na)	6.32 mg	Maintains fluid balance and neuronal signalling

Table 3: Trace Elements and Their Importance to Human Beings

S. No.	Element	Concentration (mg/kg)	Importance for Human Health
1	Manganese (Mn)	2.16	Involved in bone formation and enzyme activation
2	Zinc (Zn)	1.2845	Supports immune system and wound healing
3	Copper (Cu)	0.7426	Helps in iron metabolism and red blood cell formation
4	Boron (B)	0.5631	Contributes to bone health and hormone regulation
5	Chromium (Cr)	0.173	Helps regulate blood glucose metabolism
6	Nickel (Ni)	0.0894	Plays a role in certain enzymatic reactions
7	Cobalt (Co)	0.0068	Component of Vitamin B12
8	Molybdenum (Mo)	0.0003	Essential for enzyme systems involved in metabolism





Kuleshwar Prasad and Sandhya Rani Panda

Table 4: Potentially Toxic Elements (Present in Very Low Amounts)

Element	Concentration (mg/kg)	Possible Effect
Lead (Pb)	0.0327	Toxic to nervous system if present in high concentration
Cadmium (Cd)	0.0013	Can damage kidneys and bones
Arsenic (As)	0.0031	Toxic in higher concentrations
Mercury (Hg)	0.0008	Harmful to brain and nervous system

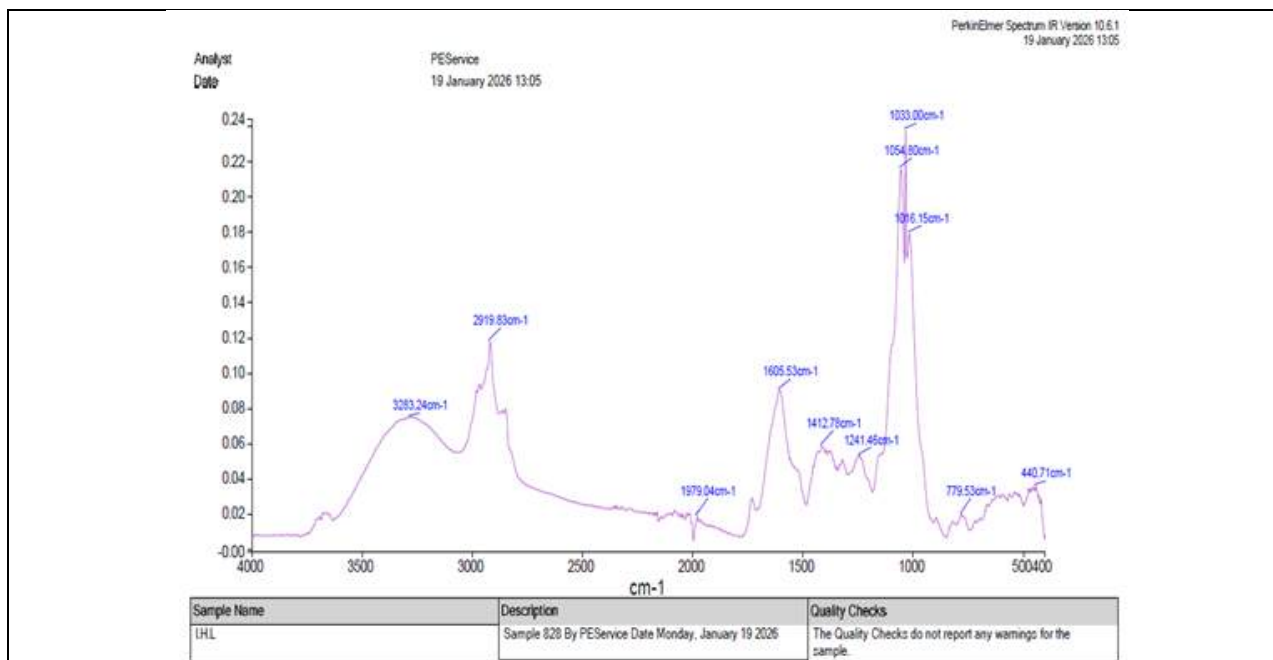


Figure 1: The picture depicts the FTIR spectrum of a plant sample (*Ipomoea hederifolia* L.). The labelled peaks (wavenumbers) allow us to identify functional groupings, associated phytochemicals, and their therapeutic value





Kuleshwar Prasad and Sandhya Rani Panda

February 11, 2026

SN	Sample ID	Sample code	Metals	Concentration (mg/Kg)
1	MIG26021123	IHL	Al	5.84
			Ca	124.62
			Fe	12.47
			K	238.91
			Mg	41.28
			Mn	2.16
			Na	6.32
			P	24.78
			Ti	0.041
			B	0.5631
			Ba	0.6945
			Cd	0.0013
			Co	0.0068
			Cr	0.173
			Cu	0.7426
			Ga	ND
			Ge	ND
			Mo	0.0003
			Ni	0.0894
			Pb	0.0327
			V	0.0146
			Zn	1.2845
			As	0.0031
			Hg	0.0008
			Sn	0.0184
			Li	ND
			S	158.42
			Nb	0.0032

Figure 2 : Test Report

concentration (mg.) of major elements

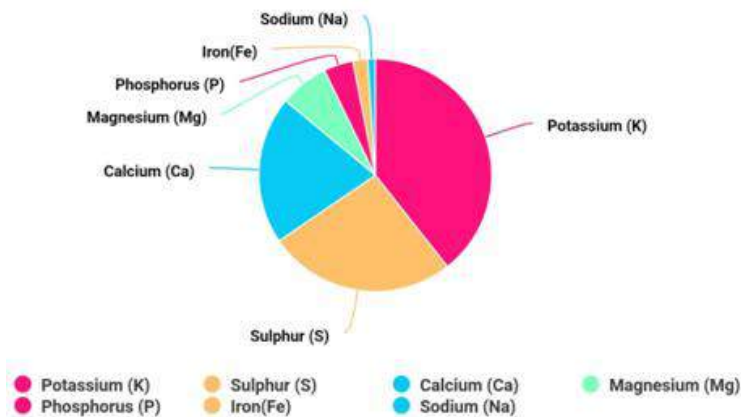


Figure 3: pie graph show the concentration (mg)based of major elements





Concentration (mg) of trace Elements



Figure 4: graph show the concentration (mg) based of trace elements

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RESEARCH ARTICLE

Knowledge and Practice among Housekeeping Staff Regarding Bio-Medical Waste Management at Selected Hospital

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ABSTRACT

This study assessed awareness and practice regarding biomedical waste management among housekeeping staff in SVBP Hospital Meerut, Uttar Pradesh, India. descriptive correlational design was used with 60 housekeeping staff selected through convenience sampling, and data were collected using a structured awareness questionnaire and an observational practice checklist. The tools showed acceptable internal consistency, with Cronbach's alpha values of 0.84 for awareness and 0.79 for practice. Most participants were male, aged 21 to 30 years, and had 0 to 5 years of work experience. More than half of the participants had moderate awareness, while a substantial proportion had poor practice. Pearson correlation showed a weak positive relationship between awareness and practice, and Fisher's exact test showed no significant association between awareness or practice and selected demographic variables. The study indicates a knowledge practice gap and supports the need for structured, hands-on training and regular supervision.

Keywords: correlational design, Cronbach's alpha values hands-on training and regular supervision.

INTRODUCTION

Biomedical waste management is a critical component of healthcare safety because improper handling can expose staff, patients, and the environment to infection and other hazards. In India, biomedical waste generation has

116913



**Aniruddh Pratap Singh et al.,**

increased with the expansion of healthcare services, and compliance with Biomedical Waste Management Rules remains a challenge in many institutions. Housekeeping staff play a central role in segregation, collection, transport, and temporary storage of biomedical waste, yet they often receive limited formal training. This makes their awareness and daily practice especially important for safe hospital functioning. The present study was undertaken to assess awareness and practice regarding biomedical waste management among housekeeping staff in a selected hospital and to examine the relationship between these two variables. It also aimed to identify whether selected demographic variables were associated with awareness or practice levels.

METHODOLOGY

Study Design and Setting

Research Design: A Quantitative descriptive correlational design was utilized to explore the relationship between variables.

Study Setting: The research was conducted at a selected multi-specialty SVBP hospital located in Meerut, Uttar Pradesh, India.

Population and Sampling

Sample Size: A total of 60 housekeeping staff members participated in the study.

Sampling Technique: Convenience sampling (a non-probability sampling method) was used to select the participants.

Inclusion Criteria

- Minimum of 6 months of work experience in the current setting.
- Direct involvement in the handling of biomedical waste.
- Willingness to participate in the study.
- Proficiency in understanding Hindi or English.

Data Collection Tools

Data was gathered using three specific instruments:

Socio-demographic Proforma: To collect baseline characteristics of the participants.

Structured Awareness Questionnaire: To assess the level of awareness regarding biomedical waste management.

Observational Checklist: To evaluate the actual practices followed by the staff.

Validity and Reliability

Content Validity: Tools were validated by a panel of subject matter experts.

Reliability: Following a pilot study, internal consistency was confirmed using Cronbach's alpha:

Awareness Tool: $r = 0.84$ (Highly reliable).

Practice Tool: $r = 0.79$ (Reliable).

Data Analysis Plan

The collected data were analyzed using both descriptive and inferential statistics:

Descriptive Statistics: Frequency, percentage, mean, and standard deviation (SD).

Inferential Statistics:

Pearson Correlation Coefficient: To determine the relationship between awareness and practice.

Fisher's Exact Test: To associate demographic variables with awareness and practice score.





RESULTS

The sample was predominantly male, with 71.66 % males and 28.33 % females. Most participants were aged 21 to 30 years, and 76.67 % had 0 to 5 years of work experience. Regarding training, 56.67 % reported annual training, while 18.33 % had no prior training. For awareness, 51.67 % of participants had moderate awareness, 36.67 % had poor awareness, and 11.67 % had good awareness. The mean awareness score was 11.43 with a standard deviation of 3.44. For practice, 45.00 % had poor practice, 36.70 % had moderate practice, and 18.30 % had good practice. The mean practice score was 30.08 with a standard deviation of 9.47. Pearson correlation analysis showed a weak positive relationship between awareness and practice, with $[r = 0.242]$. Fisher's exact test showed no significant association between awareness and demographic variables or between practice and demographic variables, as all reported p-values were greater than 0.05.

DISCUSSION

The findings suggest that housekeeping staff had a basic level of understanding, but this did not consistently translate into good operational practice. The weak correlation between awareness and practice indicates that knowledge alone is not sufficient to ensure adherence to correct biomedical waste procedures. This may reflect the influence of supervision, workload, availability of protective equipment, and institutional enforcement. The demographic profile of the sample also suggests a young and relatively inexperienced workforce, which may benefit from repeated orientation and competency-based training. The lack of significant association between awareness or practice and demographic variables implies that performance may depend more on workplace systems than on personal background characteristics. This supports the need for standardized hospital protocols, monitoring, and routine reinforcement.

CONCLUSION

The study concludes that housekeeping staff in the selected hospital had moderate awareness but weaker practice regarding biomedical waste management. A knowledge practice gap was evident, and the relationship between awareness and practice was weak. Demographic variables did not show significant association with either awareness or practice. Institutional training, supervision, and enforcement appear essential to improve compliance and promote safer biomedical waste management.

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Table 1 Socio demographic Characteristics of the Participants

Demographic characteristics		Frequency (N)	Percentage (%)
Age	< 20 years	2	3.33
	21-30 years	32	53.33
	31-40 years	20	33.33
	> 40 years	6	10
Gender	Male	43	71.66
	Female	17	28.33
Education	Secondary	36	60
	Primary	12	20
	Higher Secondary	6	10
	Illiterate	6	10
Work Experience	0-5 years	46	76.67
	6-10 years	14	23.33
prior training	No	11	18.33
	Once	6	10
	every month	9	15
	every year	34	56.67
Work Department	OPD	17	28.33
	ICU	17	28.33
	Lab	9	15
	Ward	9	15
	OT	8	13.33



**Table 2** Frequency, percentage distribution of Level of Awareness and mean awareness score of participants regarding BMW management N=60

Level of Awareness	Frequency (f)	Percentage (%)	Mean	SD
Good	7	11.70%	11.43	3.44
Average	31	51.60%		
Poor	22	36.70%		
Total	60	100%		

Table 3 Frequency, percentage distribution of Level of practice and mean practice score of participants regarding BMW management. N=60

Level of Practice	Frequency (N)	Percentage (%)	Mean	SD
Good	11	18.30%	30.08	9.47
Fair	22	36.70%		
Poor	27	45.00%		
Total	60	100		

Table 4 Fisherman's exact test (Awareness)

Variable	Category	Adequate	Poor	p-value	Interpretation
Age	≤30 years	24	11	0.605	Not significant
	>30 years	15	11		
Gender	Male	28	15	0.787	Not significant
	Female	10	7		
Education	Low	11	7	1.000	Not significant
	High	27	15		
Experience	0–5 years	29	17	1.000	Not significant
	6–10 years	9	5		
Training	Yes	31	18	1.000	Not significant
	No	7	4		

Table 5 Fisherman's exact test (Practice)

Variable	Category	Adequate	Poor	p-value	Interpretation
Age	≤30 years	18	16	0.627	Not significant
	>30 years	15	11		
Gender	Male	27	16	0.121	Not significant
	Female	6	11		
Education	Low	8	10	0.536	Not significant
	High	25	17		
Experience	0–5 years	26	20	1.000	Not significant
	6–10 years	7	7		
Training	Yes	27	22	1.000	Not significant
	No	6	5		





LEVEL OF AWARENESS

■ Good ■ Moderate ■ Poor

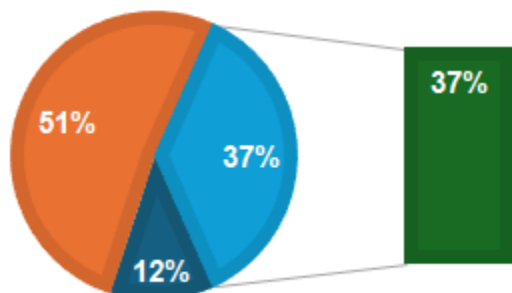


Fig.1 Percentage distribution of level of awareness of participants

Mean Awareness Score

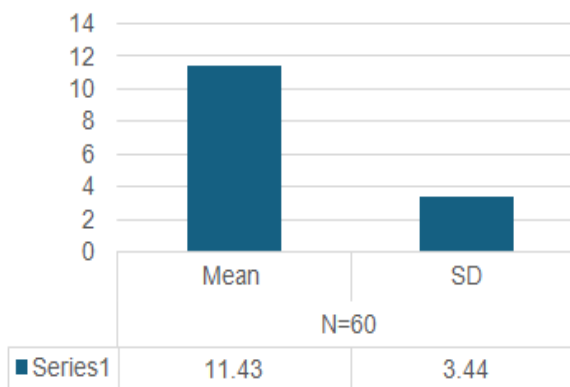


Fig. 2 Mean awareness score of participant

LEVEL OF PRACTICE

■ Good ■ Moderate ■ Poor

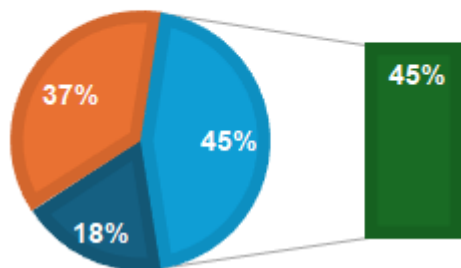


Fig. 3 Percentage distribution of level of practice of participants.

Mean Practice Score

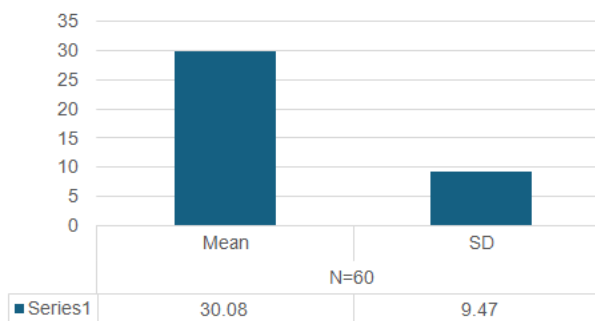


Fig. 4 Mean practice score of participants.

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Role of Intermittent Fasting in Human Health and Disease Prevention : A Review

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ABSTRACT

Fasting permits the essential energy to draw out toxins from the blood and rejuvenate the system. Many people today have very unfit, passive lifestyles devoid of such physical exercises. A sedentary way of life and eating three meals a day (breakfast, lunch, supper, and snacks in between) commonly result in physiological morbidity that leads to a wide range of diseases that many are unaware of. In these scenarios, the notion of Intermittent Fasting acquired widespread acceptance worldwide. Fasting includes two windows: fasting and feasting with specific meal timings and options in both periods that help generate maximum health benefits by activating the several mechanisms that occur inside the body during fasting. The long-term effect of these mechanisms progresses toward inhibiting the morbidity and other ill effects of a sedentary lifestyle. It is also believed that a systematic and time-restricted regimen may reduce some of the ill effects of this practice, including dizziness, weakness, etc. This review provides current literature on several intermittent fasting regimens, the mechanism behind their influence on body metabolism, and the promising health outcomes of this practice along with the possible hazards of it.

Keywords: Metabolic, oxidative, Cognitive, Diet regimen and Health



Sapna Malik *et al.*,

INTRODUCTION

After a carbohydrate-containing meal, energy is stored as glucose in the liver, and any excess is stored in the body's fat reserves. The liver can store around 400-500 calories of glucose, while fat can store much more. This stored fat provides energy for extended fasting periods. Intermittent fasting requires fasting for at least 12 hours to shift the body from using glucose to utilizing ketones produced by fat (Mattson, 2023). Intermittent fasting is frequently misunderstood as a diet by the general public and media, although it is an eating pattern rather than a prescriptive diet. It involves prolonged periods of reduced or negligible energy intake, typically lasting around 16-8 hours, interspersed with regular food consumption during specific time frames (Nain et al., 2020). This diet doesn't require people to completely change their current eating habits or replace everything in their kitchen. Also, intermittent fasting doesn't require individuals to avoid food groups or specific macronutrients, nor does it require participants to closely monitor their daily calories (Varady et al., 2021). Despite the increasing prevalence of intermittent fasting, there is limited research on the potential health benefits of these dietary patterns in human subjects. Preliminary evidence suggests that intermittent fasting may have a positive impact on weight management and metabolic health by reducing oxidative stress, increasing stress resistance, enhancing glucose metabolism, and attenuating inflammation (Chaix et al., 2019).

Fasting regimens affect metabolic rates by influencing many physiological mechanisms like the circadian clock, gut microbiota, and modifiable lifestyle habits such as sleep. Studies have shown that it can reduce systemic inflammation and play a role in preventing illness and managing chronic diseases (Nain et al., 2020). Beyond weight loss due to calorie restriction, there are many more beneficial health effects of intermittent fasting. Common intermittent fasting patterns include daily time-restricted eating (e.g., 16/8) or 2 to 4 non-consecutive full-day fasts per week. On fasting days, certain regimens permit very low-calorie intake (500-700) (Patterson et al., 2017). The majority of intermittent fasting interventions fall into three broad types. Alternate day fasting (ADF) programs usually include a "feasting" on which food is consumed selectively, or practicing a "fasting day" on which no food is eaten or decreased followed by a feasting day (Tinsley et al., 2015). Whole-day fasting (WDF) is typically defined as one to two non-consecutive full fasting days per week, with the remainder of the time spent eating freely. Time-restricted feeding (TRF) occurs when food consumption is restricted to a specified period of day while fasting the rest of the time including 16:8 hours fasting or 10:14 hours fasting patterns (Longo et al., 2016). Although intermittent fasting can take several forms, studies have shown that it is a sustainable and simple lifestyle with numerous health benefits. Fasting has been thought of as a beneficial technique for treating overweight and comorbid individuals since the 1960s, with additional benefits discovered more recently (Wilhelmi et al., 2020). IF has reached incredible public prominence in recent years due to media coverage; however, it is vital to emphasise that it has been engaged in for decades for wellness and spiritual reasons, including as during the month of Ramadan (Vitale et al., 2020). Diet has a major impact on the gut microbiota, which is an additional link between nutrition and immune-inflammatory responses. Calorie restriction also has potent anti-inflammatory properties. Through impacts on the intestinal microbiota, IF is demonstrated to be a protective factor in the animal model of multiple sclerosis. Patients with relapsing metabolic syndrome who undertake brief intermittent fasting also exhibit similar alterations in their gut microbiota (Cignarella et al., 2018).

Mechanism Behind IF

To comprehend the metabolic impact of this practice, three primary concepts have been stated and represented in Fig.3: (i) the Ketosis principle, (ii) the oxidative stress speculation, and (iii) the circadian biology hypothesis (Fig.2). Upon a meal, glucose is used for energy or deposited as glycogen or triglyceride. Fasting causes the liver's glycogen stores to be utilized. As the liver's glycogen stores deplete (after 8-12 hours of water-only fasting), it begins building ketones, such as β -hydroxybutyrate (BHB) and acetoacetate, to provide an energy source for the tissues around the body for 48 hours of fasting, blood BHB levels may rise and continue to increase. BHB acts as a metabolic intermediary and a signaling molecule that regulates metabolism in response to dietary restriction and modifies cellular processes and signaling pathways associated with aging and human diseases (Newman et al., 2017). So,



**Sapna Malik et al.,**

according to the ketosis theory, lower calorie consumption induces an alteration in metabolic state that results in accelerated fat metabolism and lower buildup of fat. The theory reinforces immediate impacts such as weight loss, waist circumference reduction, and improved fat percentage (Rojas-Morales et al., 2016). It has been shown that intermittent fasting (IF) produces positive outcomes comparable to calorie restriction, such as decreased oxidative stress. Oxidative stress is an essential consequence of interest concerning obesity because it can exacerbate or accelerate the development of metabolic-related problems (Mohr et al., 2021). The oxidative stress concept reveals that lower calorie consumption and formation of volatile species by the mitochondria lead to a reduced cellular inflammatory process (Dong et al., 2020). (Mo'ez Al-Islam et al., 2019) the study suggested that diurnal intermittent fasting during Ramadan may reduce oxidative stress and low-grade systemic inflammation in healthy individuals, potentially mitigating negative health effects.

Meals consumed at an appropriate time for the body's circadian clock to ensure that the interactions between fasting, mealtimes, and sleep do not negatively impact the circadian rhythm of various body organs and cells (Almeneessier et al., 2018). Intermittent fasting (IF) is a historically recognized practice implemented in diverse manners by global communities, involving voluntary abstinence from food for predetermined intervals (Patterson et al., 2017). According to certain animal research, regulating meal times and limiting food intake can effectively align peripheral tissues' biological clocks with a set exposure to the light-dark cycle (Mongrain et al., 2005). Furthermore, it has been found that mealtime routines in several animals can cause the stimulation of brain circadian clock genes, which are thought to be regulated by a "food-regulated clock." (Patton DF et al., 2013). Several human studies show that restricted eating improves the cardiometabolic health of those who are overweight or obese especially valid when the period for food consumption is at the start of the day and when fasting is towards the final stage of the active biological phase (Queiroz et al., 2021).

Impact on Health And Beyond

In animal research, calorie restriction is related to improved health, greater lifespan, and a reduction in morbidity and death. Calorie reduction improves cardiovascular health, weight loss, insulin sensitivity, diabetes control, cognitive function, and cancer prevention, among other benefits. Intermittent fasting minimizes the risk of malnutrition, is easier to implement, and is gaining recognition among healthcare providers (Golbidi et al., 2017). Some of the mechanical perspectives of the regimen on health and other factors are addressed in Fig. 4.

Intestinal Microbiota

Disorders related to metabolism and other diseases such as insulin resistance, cardiac diseases, irritable bowel syndrome, and various malignancies including colorectal and breast carcinoma are the major reasons for the increasing mortality rate worldwide (Rajilić-Stojanović et al., 2014). Since the intestinal microbiome of healthy people is highly diversified and that of unwell people is peculiar and less diverse, the diversity and composition of the gut microbiota have been shown to play a significant role in metabolic disorders. (Harmsen et al., 2016). Similar to how medications destroy both infections and beneficial bacteria, probiotic strains also find it difficult to survive in the gut for extended periods. Additionally, the effects of prebiotic products are not particular (Druart et al., 2014). Some studies conclude that intermittent calorie restriction has been shown to activate brown adipose tissue in rats and improve obesity by probably altering the gut microbiome's composition (Li et al., 2017). As a result, it has gained acceptance as a weight loss and metabolism-boosting method all around the world. This strategy might promote the growth of microbial communities that boost thermogenesis-related metabolism (Mattson et al., 2017).

Carcinogenesis Inhibition

Although it might be challenging to maintain, research in preclinical and clinical settings has shown that prolonged calorie limitation has significant anticarcinogenic effects. Because of promising study results, mostly in experimental animal models, intermittent fasting has developed acceptance as a substitute for continuous fasting in both the scientific and ordinary communities (Clifton et al., 2021). According to preliminary research, long-term fasting could be safe and effective in reducing the toxicity and proliferation of tumors associated with radiotherapy in certain cancer patients. A study suggests lower risk of all female-specific malignancies, such as gynecologic and breast



**Sapna Malik et al.,**

cancers, was linked to the decline in cancer incidence (Anveden et al., 2017). After a median of 20 years of follow-up, a Swedish study comparing patients who had bariatric surgery with a control group discovered a 29% reduction in cancer incidence and a 23% reduced cancer mortality (Carlsson et al., 2020). It is thought that circadian rhythm abnormalities contribute to carcinogenesis by altering the expression of genes related to metabolism, autophagy, and DNA damage repair (Sulli et al., 2019). A study performed on intermittent fasting practice in mice has concluded that circadian rhythms were reset by this practice which is followed by gene alteration to inhibit carcinogenesis. However, the time of feeding was a dependent variable (Froy et al., 2009).

Aging and Cognition

The main risk variable for neurological illness is aging, which becomes worse by sedentary lifestyles as well as elevated intake of calories. It has been demonstrated that some traditional eating practices, including intermittent fasting (IF), extend life expectancy and improve health through an adaptive stress response (Seidler et al., 2022). In addition to medical treatments that did not show promise in reversing cognitive loss and advancing the disease, dietary interventions such as calorie restriction and intermittent fasting have been shown to enhance longevity as well as health in a range of species, with preliminary data from human trials supporting this claim (Anton et al., 2021).

Regulation of Blood Glucose

Hyperglycemia, a prevalent metabolic condition known as type 2 diabetes mellitus has several causes such as reduced glucose utilization, hepatic glucose synthesis that is excessive, insulin resistance, impaired insulin secretion, and systemic low-grade inflammation (Powers et al., 2018). A growing number of research is showing the short-to-medium-term effects of intermittent fasting on glucose and lipid homeostasis (Antoni et al., 2017). In a recent experiment, an intermittent fasting diet enhanced insulin tolerance without significantly altering body mass index, even when the calories delivered were identical to those of the control group. This demonstrates that fasting practice alone can improve glucose metabolism without requiring changes in body weight (Sutton et al., 2018).

Promotion of Weight Loss

Consuming high-energy foods and being sedentary can lead to obesity, a major health issue globally. Recent studies suggest that diets emphasizing intermittent fasting may aid in weight loss and improve human health (Erdem et al., 2022). Weight control programs typically employ a 20% to 40% daily calorie restriction to achieve the negative energy balance needed for weight loss. However, intermittent fasting or intermittent calorie restriction is gaining attention as an alternative strategy (Freire et al., 2020). In the study, intermittent fasting led to a 53% decrease in energy intake compared to a 72% reduction with daily calorie restriction. Additionally, intermittent fasting resulted in an 8.8% decrease in body mass, while daily calorie restriction led to a 6.2% decrease. Both groups experienced similar reductions in fat and lean mass and improved fasting lipid profiles (Catenacci et al., 2016).

Cardiovascular Effect

Atherosclerotic cardiovascular disease and myocardial infarction are significantly increased by low HDL cholesterol levels and elevated levels of circulating LDL cholesterol and triglycerides (Liou et al., 2020). In a study (Wilkinson et al., 2020), 12-week 10-hour time-restricted eating reduced the LDL cholesterol concentrations of participants with metabolic syndrome compared to baseline. In contrast, in every other research (Sutton et al., 2018), that looked at this measure, LDL cholesterol concentrations stayed constant in comparison to controls. According to the results, of the study, TRE appears to be a safe diet therapy that helps obese individuals lose a small amount of body weight while simultaneously lowering numerous important cardiovascular disease indicators (Gabel et al., 2021). Almost all IF trials have resulted in weight reduction ranging from 2.5-9.9% and accompanying fat mass decrease (Byrne et al., 2018). While most research found that weight and fat mass reduced, In IF treatments, it is critical to assess protocol adherence and dropout rates. According to certain research, the ADF (Alternate day fasting) group ate more than usual on fasting days and less than usual on feast days (Trepanowski et al., 2017). According to research, IER has been linked to improvements in beta cell function and insulin resistance in mouse models. There are two primary processes, one derived from the autophagy-lysosome pathway and the other from increased neurogenin3 (Ngn3)



**Sapna Malik et al.,**

levels (a marker for endocrine progenitor cells resembling cells throughout development). The drop in insulin levels recorded in people counters the rise observed in mice, suggesting that the key cause in humans is an improvement in peripheral insulin action (Muñoz - Hernández et al., 2020). Notably, IER stimulates gut microbiota regeneration. An increase in HDL cholesterol levels is also commonly noted (Arnason et al., 2017). According to a metabolic study where regular-IF group had a substantial increase in superoxide dismutase (SOD) activity as well as a reduction in body weight, insulin, fasting blood glucose, malondialdehyde (MDA), C-reactive protein (CRP), and DNA damage, it is concluded that IF may alter cognitive performance via a variety of metabolic pathways, including ketone body production and degradation, butanoate metabolism, pyruvate metabolism, and glycolysis and gluconeogenesis (Ooi et al., 2020).

Possible Hazards and Repercussions of Intermittent Fasting

This type of fasting may sometimes lead to hunger, weakness, headaches, dehydration, dizziness, difficulty concentrating, low blood pressure, etc. To ensure management of one's nutritional needs with fasting practices a nutritionist or dietician must be consulted. Some people are advised to avoid this practice of fasting which includes children below 18 years, pregnant or nursing mothers, elder people, and people with certain eating disorders. People with weak immune systems, and diabetes (hypoglycemia) are also advised to have a word with their doctor before attempting this eating regimen (Li et al., 2021).

CONCLUSION

Intermittent fasting is a natural dietary-based practice that allows calorie restriction for specific time intervals. The method of adaptation may vary depending on the severity of the disorder and nutritional requirements. The regimen can result in various side effects like dizziness & weakness etc. to persons below the age of 18. The right conduct to the process including the choice of regimen based on set goals & practicing under guidance can prevent many lifestyle disorders and promote healthy well-being.

Availability of data and materials

The data mentioned was thoroughly analyzed and reported in this work in the form of references.

Declaration of conflicting interests

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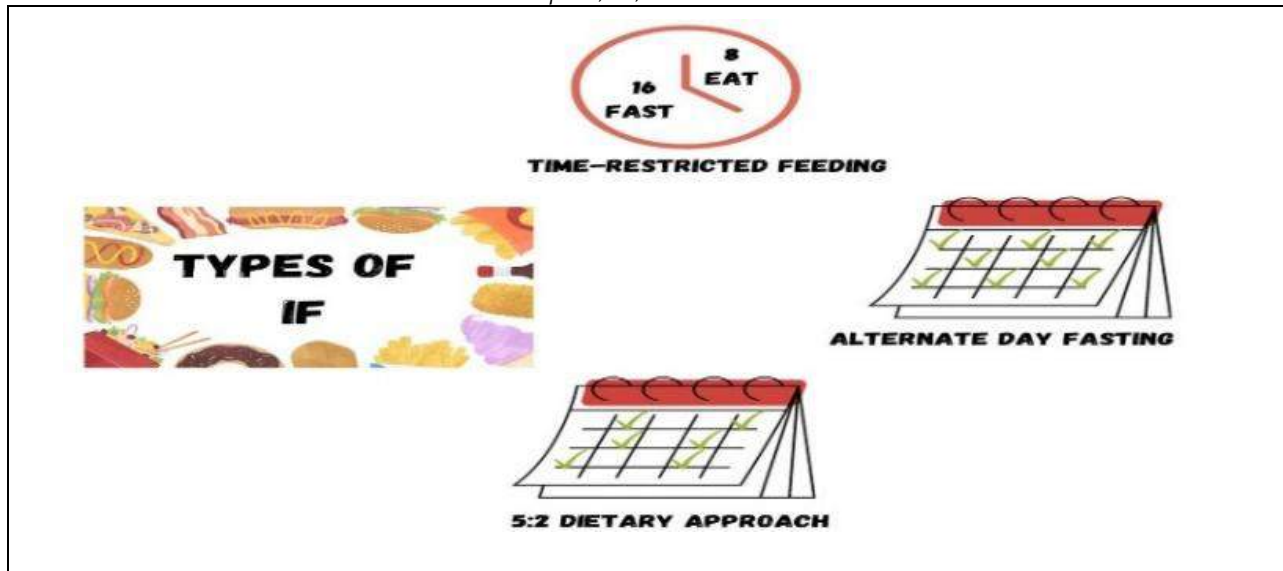


Fig. 1 Types of intermittent fasting

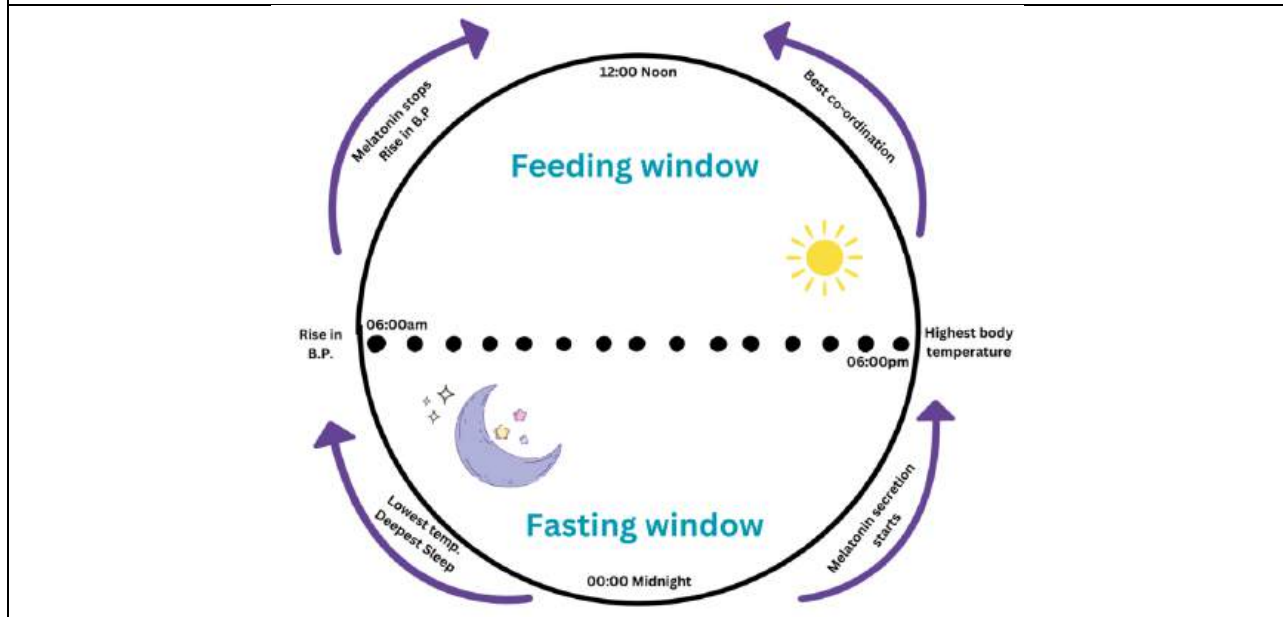


Fig. 2 Circadian biology of behaviors, hormones, physiology, and metabolism.





Sapna Malik et al.,

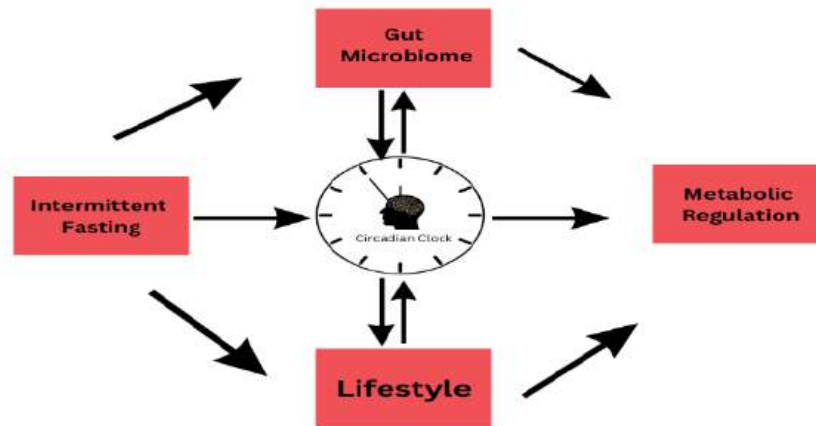


Fig. 3 Impact of circadian biology on metabolic regulation

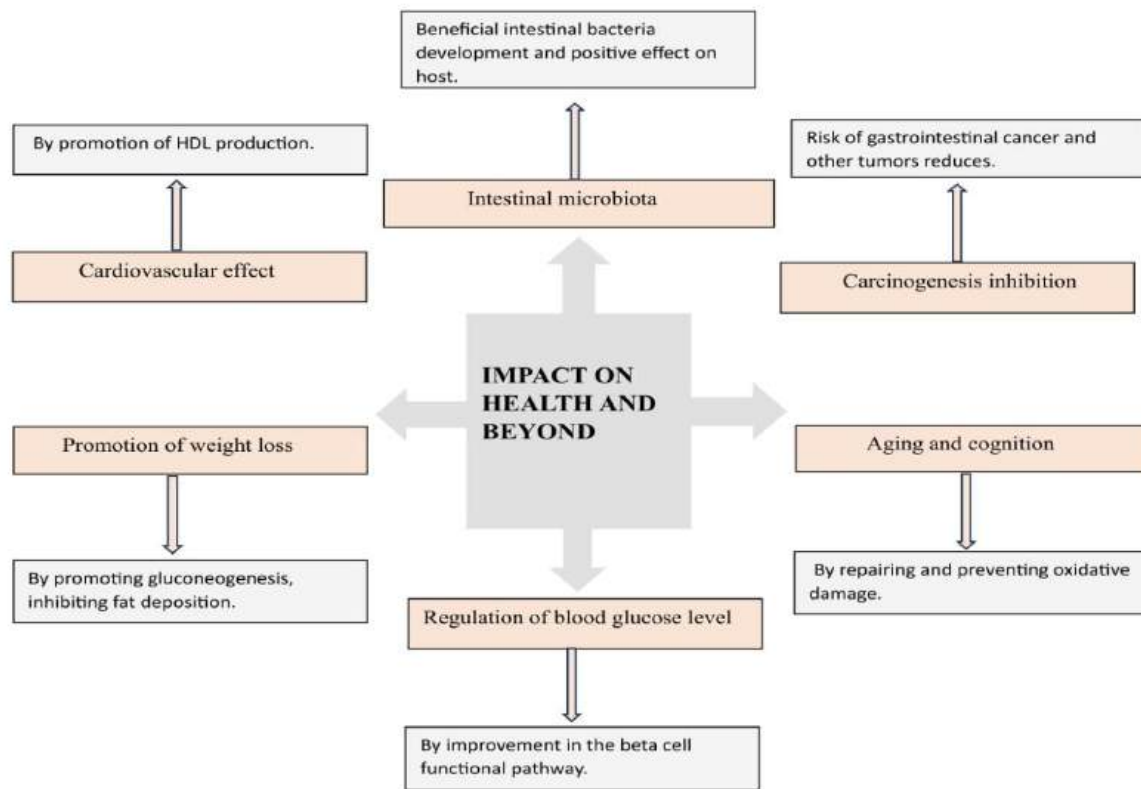


Fig. 4 Impact of intermittent fasting on health and beyond

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RESEARCH ARTICLE

Evaluation of Anti-Analgesic and CNS Stimulant Effect of Leaves Extract of *Cedrus deodara*

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ABSTRACT

The present investigation evaluates the analgesic activity and central nervous system (CNS) stimulant activity of the hydroalcoholic extract of *Cedrus deodara* leaves in Albino Wistar rats. The extract was prepared using Soxhlet extraction with 70% ethanol. Analgesic effects were examined through the tail flick method, while motor coordination and CNS stimulation were assessed using the rotarod apparatus. In the tail flick test, the standard drug group showed the highest withdrawal latency (4.20–5.17 seconds), followed closely by the extract-treated group (4.15–5.10 seconds), both significantly higher than the control group (3.57–4.60 seconds), indicating meaningful analgesic activity of the extract. Similarly, in the rotarod evaluation, the extract-treated group demonstrated enhanced performance with an average time of 102 seconds, which was comparable to the standard (108 seconds) and markedly superior to the control group (48 seconds). These observations suggest that the hydroalcoholic extract of *Cedrus deodara* possesses moderate analgesic potential along with notable CNS stimulant activity. Further phytochemical and mechanistic studies are recommended to identify the active components responsible for these pharmacological effects.

Keywords: *Cedrus deodara*, Hydroalcoholic extract, Analgesic activity, CNS stimulant activity.





Riddhita Banerjee et al.,

INTRODUCTION

Medicinal plants have long been recognized as important sources for discovering new therapeutic agents, with approximately 80% of individuals in developing countries relying on traditional medicine derived from plants.[1] Historically, the use of medicinal plants was based on empirical knowledge, but over time scientific explanations of their therapeutic uses have emerged. Despite advancements in synthetic drugs, limitations such as reduced efficacy and side effects make natural products an attractive alternative once again.[1] Several plants contain bioactive compounds with anti-analgesic and central nervous system (CNS) stimulant properties. For example, Aconitum species contain alkaloids used traditionally for analgesic and anti-inflammatory purposes.[2] Aloe vera has also been used for various therapeutic applications but requires caution due to some potential adverse effects.[3] Other medicinal plants, such as Ginkgo biloba, have been reported to improve cognitive function but may increase bleeding risk with long-term use.[4] *Cedrus deodara* (Deodar cedar), native to the western Himalayas, is a large evergreen tree valued both for its durable timber and its medicinal properties.[5] It has been traditionally utilized in Ayurvedic medicine for treating fever, pulmonary complaints, urinary disorders, and as an anti-inflammatory agent.[6,7] Studies have demonstrated that volatile oils from *Cedrus deodara* exhibit significant analgesic, anti-inflammatory, and antibacterial activities.[6,7] Additionally, alcoholic extracts of its heartwood have shown anxiolytic and anticonvulsant effects in rodent models, suggesting CNS activity.[8] Given this background, *Cedrus deodara* represents a promising candidate for further pharmacological evaluation regarding its anti-analgesic and CNS stimulant potential. Such investigations may help validate traditional uses and contribute to developing novel phytopharmaceuticals with improved safety and efficacy profiles.[9]

METHODS

Method of Extraction: [8,9,10]

Fresh *Cedrus deodara* leaves were collected, washed thoroughly with tap water, and air-dried at room temperature for 10–12 days. The dried leaves were chopped into small pieces, and 15 grams were weighed and packed in butter paper. The sample was then placed in a soxhlation apparatus, and 150 ml of 70% ethanol was added. Extraction proceeded for 72 hours with regular monitoring of water flow every 15 minutes. The extract was collected in a beaker, cooled at room temperature, and then sterilized in an autoclave at 121°C and 15 psi for 15 minutes. The solvent was evaporated over 10–12 hours to concentrate the extract, which was cooled, autoclaved again, and refrigerated until use.

Dose Calculation: [4]

The human therapeutic dose of Aceclofenac, 100 mg for a 70 kg adult, was taken as the reference for determining the equivalent dose for rats weighing 150–200 g. Using body-surface-area conversion, this human dose was scaled down to obtain the corresponding rat dose, which was calculated to be approximately 1.5–2.0 mg per animal. For experimental administration, the drug solution was prepared such that 5 mg of aceclofenac was dissolved in 1 mL of distilled water, allowing the required dose to be accurately delivered in a suitable volume for the rats.

Experimental Methods:

Anti-Analgesic Activity: Tail Flick Method

Male Albino Wistar rats weighing between 150–200 g were selected for the study. The animals were randomly divided into three groups, with six rats in each group. Different groups of animals received their respective doses through the oral route.

Group I – Control: These rats did not receive any treatment.

Group II – Standard: Rats in this group received Aceclofenac (10mg/kg).

Group III – Sample: Rats in this group were administered the *Cedrus deodara* extract (250mg/kg).



**Riddhita Banerjee et al.,**

To assess analgesic activity, the basal reaction time of each rat was measured using the tail-flick method. The tip of the tail (1–2 cm) was gently immersed in hot water maintained at $55 \pm 2^\circ\text{C}$, and the time taken by the rat to withdraw its tail was recorded. Under normal conditions, rats respond within 3–5 seconds. Animals that failed to withdraw their tails within this range were excluded to avoid abnormal response bias. A cut-off time of 10–12 seconds was used to prevent tissue injury. After administration of Aceclofenac or test extract, the reaction time was monitored at 15-minute intervals initially to ensure the animals were responding normally. Subsequently, tail-flick latencies were recorded at 15, 30, 60, 90, and 120 minutes post-treatment. A reaction time of 10 seconds was considered the point of maximum analgesia, and the tail was immediately removed from the heat source to prevent damage.

CNS Stimulant Activity: Rota-Rod Method

Albino Wistar rats weighing 150–200 g were selected for the experiment. All animals were housed under standard laboratory conditions, maintained on a 12-hour light/12-hour dark cycle with free access to food and water.

The rats were then randomly divided into three groups, with six animals in each group:

Group I – Control: These animals did not receive any treatment.

Group II – Standard: Rats in this group were administered caffeine at 20 mg/kg through the oral route.

Group III – Sample: Rats in this group were treated with the *Cedrus deodara* extract at 250mg/kg through oral route.

RESULTS AND DISCUSSION

Result of analgesic activity

In the case of tail-flick technique, the control group had lesser time of tail withdrawal latency, meaning that there is an intact nociception mechanism. However, in the case of Aceclofenac (10mg/kg), the increase in the time of tail withdrawal indicates presence of a potent analgesia mechanism. The same was observed in the group administered with *Cedrus deodara* (250mg/kg) (Table 1, Figure 1, Figure 2, Figure 3, Figure 4, and Figure 5). In the rotarod experiment, the falling time in the control group was 48 ± 1.10 seconds. On the other hand, the falling time in the group given caffeine (20 mg/kg) increased significantly to 108 ± 0.55 seconds. Also, the falling time in the group given *Cedrus deodara* extract (250 mg/kg) increased to 102 ± 0.68 seconds (Table 2, Figure 6). The extract was collected using the Soxhlet extraction procedure, which is a common continuous hot extraction method employed in the isolation of bioactive components from plant samples. The extraction resulted in the production of a clean sample, which showed an average yield of 11%. The produced extract was then tested for its analgesic and central nervous system (CNS) effects on experimental rats.

In the test for analgesic effects, it was observed that the treated group took a relatively long time to withdraw their tails when placed in hot water compared to the control and standard groups. Such behaviour indicates an enhanced threshold of pain tolerance by the treated rats due to reduced sensitivity to the heat stimulus. The result implies that the extract has substantial analgesic properties. Moreover, motor coordination and neuromuscular performance were assessed by means of rota-rod device. The treated animals demonstrated increased grip strength ability to stay on the rota-rod for a more prolonged period of time compared to the control group. Increased ability to successfully perform on rota-rod test implies that the plant extract could stimulate motor coordination, neuromuscular performance, and the activity of central nervous system while not impairing motor function significantly. Overall, the discussed research data imply that the plant extract is likely to possess analgesic and CNS stimulant capabilities.

CONCLUSION

In this study, dried leaves of the selected plant were collected, and their extract was administered to experimental rats. Analgesic efficacy was assessed using the tail flick method, while CNS stimulant activity was evaluated through the rota-rod test. The findings indicate that the constituents of the extract exert a significant analgesic effect, comparable to that of the standard drug aspirin. However, due to the presence of multiple bioactive compounds in





Riddhita Banerjee et al.,

the extract, the exact mechanisms and active agents responsible for analgesic action require further investigation. Additionally, the extract demonstrated stimulatory effects on the central nervous system, suggesting the involvement of substances distinct from caffeine that contribute to enhanced CNS activity. Consequently, further research is recommended to isolate and characterize these components to develop effective herbal therapeutics capable of providing both analgesic and CNS stimulation benefits.

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Table 1: Analgesic Activity

Treatment	15 min	30 min	60 min	90 min	120 min
Control	3.57 ± 0.09	4.60 ± 0.12	4.27 ± 0.09	3.60 ± 0.17	2.87 ± 0.09
Standard	4.20 ± 0.19***	5.17 ± 0.23***	4.90 ± 0.20***	4.09 ± 0.22***	3.40 ± 0.17***
Sample	4.15 ± 0.10***	5.10 ± 0.12***	4.80 ± 0.16***	4.00 ± 0.13***	3.35 ± 0.14***

All values are mean ± SEM, n=6, *** p <0.001 vs control



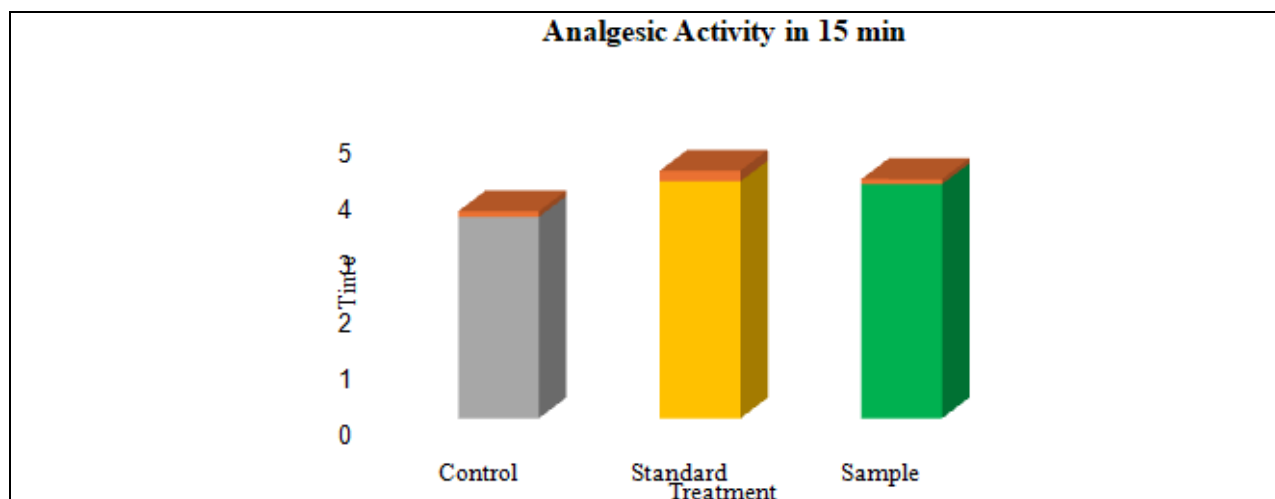
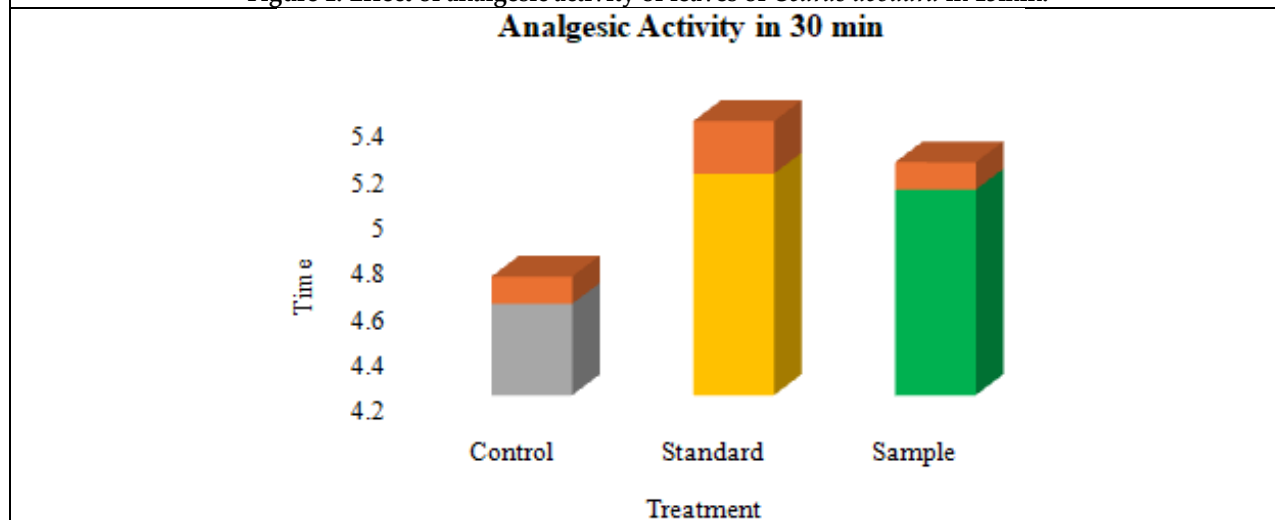


Riddhita Banerjee et al.,

Table 2: Effect of drug treatment on fall-off time in rotarod test

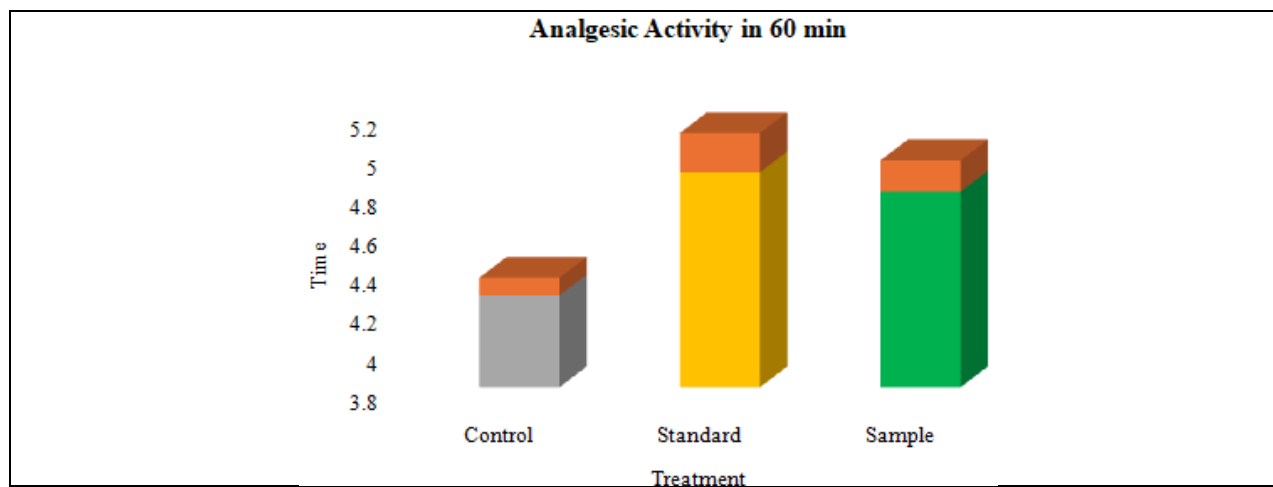
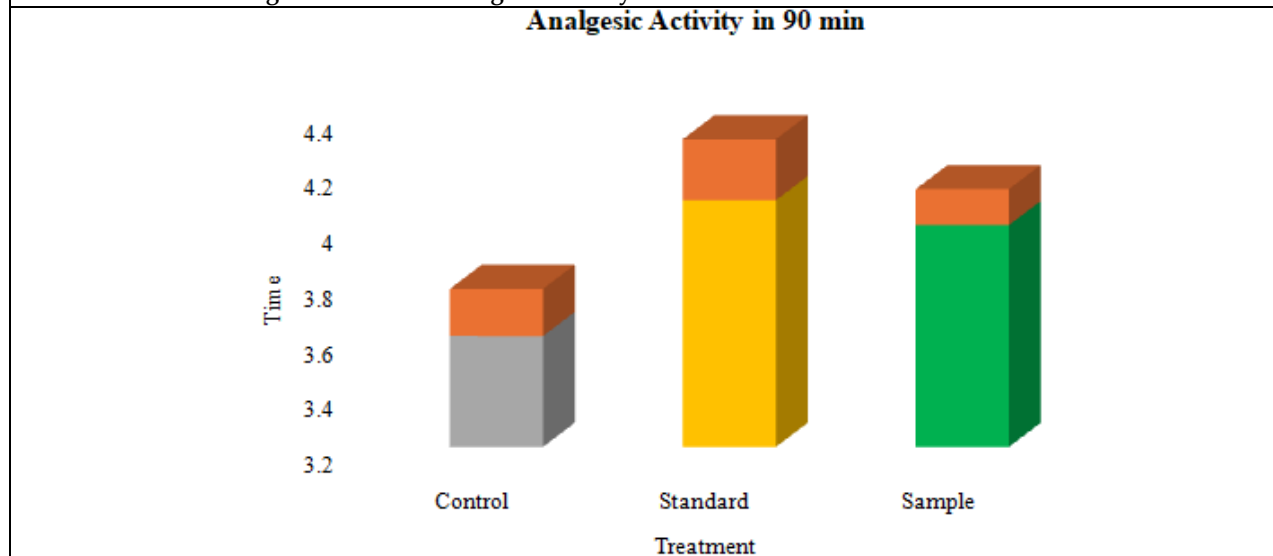
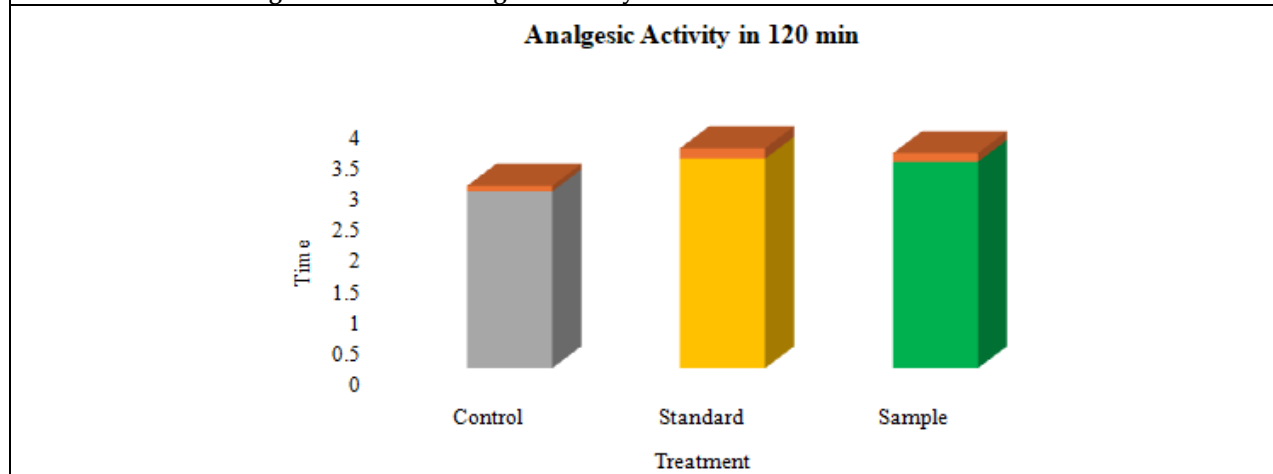
Treatment	Time spent in rota rod (seconds)
Control	48 ± 1.10
Standard (Caffeine)	108 ± 0.55***
Sample	102 ± 0.68***

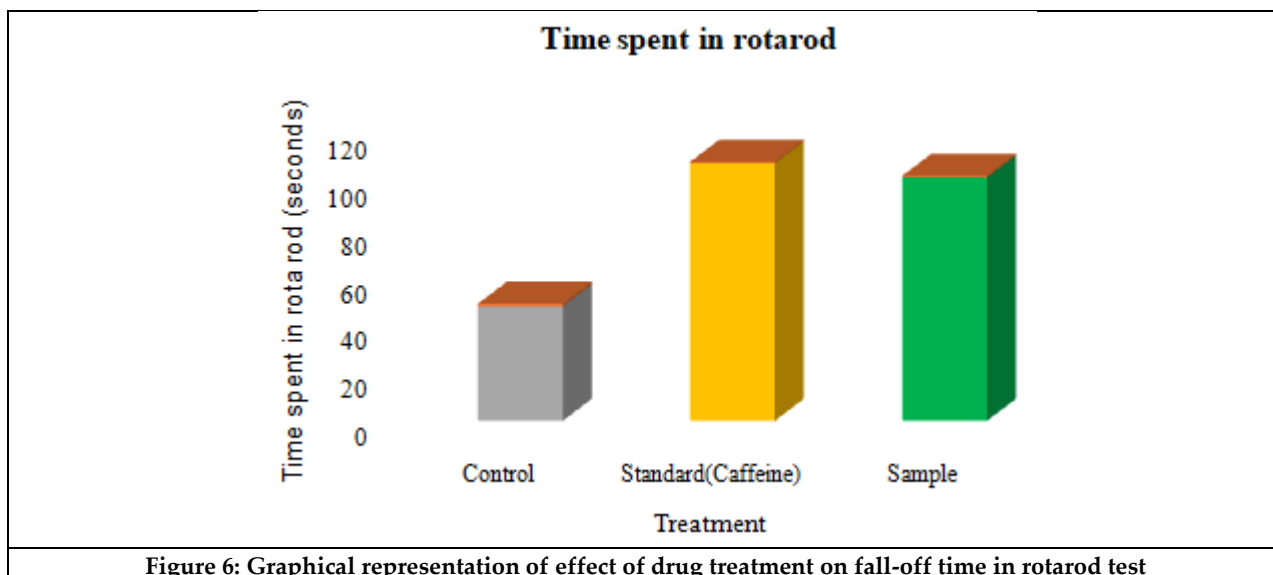
All values are mean ±SEM, n=6, *** p <0.001 vs control

**Figure 1: Effect of analgesic activity of leaves of *Cedrus deodara* in 15min.****Figure 2: Effect of analgesic activity of leaves of *Cedrus deodara* in 30 min.**



Riddhita Banerjee et al.,

Figure 3: Effect of analgesic activity of leaves of *Cedrus deodarain* 60 min.Figure 4: Effect of analgesic activity of leaves of *Cedrus deodarain* 90 min.Figure 5: Effect of analgesic activity of leaves of *Cedrus deodarain* 120 min.

Riddhita Banerjee *et al.*,

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RESEARCH ARTICLE

Advancements in Multi-Server Queuing Models Using Dodecagonal Fuzzy Numbers for Intelligent Service System Optimization

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ABSTRACT

Queuing theory plays an important role in evaluating congestion, waiting time, and service performance in various real-life sectors including healthcare, banking, transportation, manufacturing, telecommunication, and cloud computing systems. Conventional queuing models generally assume exact arrival and service rates; however, such assumptions are often impractical in uncertain and rapidly changing service environments. To overcome this limitation, the present study develops an advanced dodecagonal fuzzy multi-server $M/M/c$ queuing framework for intelligent service system optimization under uncertainty. The proposed model combines higher-order fuzzy numbers, Mamdani fuzzy inference mechanisms, and centroid defuzzification techniques to enhance the accuracy and adaptability of queue performance analysis. Dodecagonal fuzzy numbers are employed to represent uncertain arrival and service parameters because they provide smoother membership functions and more precise approximation than traditional triangular and trapezoidal fuzzy numbers. The framework evaluates major queuing performance measures such as server utilization, queue length, waiting time, idle probability, and system occupancy. Computational analysis of a three-server system demonstrates that the proposed methodology significantly reduces congestion and customer waiting time while improving



**Anushree Rathi et al.,**

service efficiency and resource utilization. Mamdani fuzzy inference supports intelligent congestion analysis through linguistic IF–THEN rules, whereas centroid defuzzification converts fuzzy outputs into crisp values for practical decision-making. The findings indicate that higher-order fuzzy queuing models offer more realistic and efficient solutions for uncertain service environments and contribute significantly to intelligent fuzzy queuing theory and adaptive service optimization.

Keywords: Fuzzy Queuing Theory, Dodecagonal Fuzzy Numbers, Multi-Server $M/M/c$ Model, Mamdani Fuzzy Inference System, Intelligent Service System Optimization

INTRODUCTION

Queuing theory is an important branch of Operations Research that studies waiting-line systems, congestion, customer arrivals, service mechanisms, and server utilization in various service environments. It has extensive applications in healthcare, banking, transportation, manufacturing, telecommunication, and cloud computing systems where efficient resource allocation and service optimization are essential. Among the classical queuing structures, the multi-server $M/M/c$ model is widely used for systems where multiple servers operate simultaneously. In this model, arrivals follow a Poisson process, service times are exponentially distributed, and several parallel servers provide service to customers. Although the $M/M/c$ model is highly applicable in hospitals, airports, call centres, and communication networks, it assumes precise arrival and service rates, which is often unrealistic in uncertain and dynamic real-life environments. To overcome this limitation, fuzzy set theory has been integrated into queuing analysis to handle uncertainty and vagueness more effectively. In fuzzy queuing systems, uncertain parameters are represented using fuzzy numbers rather than crisp values. Initially, triangular and trapezoidal fuzzy numbers were commonly used; however, they often fail to represent complex uncertainty accurately. Consequently, higher-order fuzzy numbers, particularly dodecagonal fuzzy numbers, have gained attention because of their twelve-parameter structure, smoother membership transitions, and improved precision. The integration of dodecagonal fuzzy numbers into the $M/M/c$ framework enables better estimation of queue length, waiting time, congestion level, and server utilization under uncertainty. Furthermore, fuzzy inference systems such as Mamdani and Sugeno approaches improve adaptive decision-making and intelligent service optimization, while centroid defuzzification converts fuzzy outputs into crisp actionable measures. Therefore, dodecagonal fuzzy multi-server queuing models provide an advanced and realistic framework for intelligent service system optimization in uncertain environments.

REVIEW OF LITERATURE

Queuing theory is one of the most significant analytical and mathematical tools used for studying waiting-line systems, congestion phenomena, customer arrivals, service mechanisms, and server performance in modern service environments. The theory mainly focuses on analysing situations where the demand for service exceeds the available service capacity, leading to waiting lines and congestion. The origin of queuing theory can be traced to the pioneering work of A. K. Erlang, who developed probabilistic models to study telephone traffic congestion during the early twentieth century. Erlang introduced important performance measures such as waiting probability, traffic intensity, and service efficiency, which later formed the foundation of modern queuing analysis. Over time, queuing theory expanded considerably through the contributions of researchers such as Leonard Kleinrock, whose studies on communication networks and packet-switching systems demonstrated the practical importance of multi-server service systems in large-scale environments. Similarly, Donald Gross and John F. Shortle developed comprehensive mathematical formulations for classical queuing structures including $M/M/1$, $M/M/c$, finite-capacity queues, and priority queuing systems. Their work enabled the application of queuing theory in various fields such as healthcare management, transportation systems, banking services, manufacturing industries, computer communication networks, and cloud computing environments.



**Anushree Rathi et al.,**

Among the classical queuing structures, the multi-server $M/M/c$ queuing model gained considerable importance because of its practical applicability in systems where multiple servers operate simultaneously to provide service. In the $M/M/c$ model, customer arrivals follow a Poisson process, service times are exponentially distributed, and c parallel servers provide service to incoming customers. This model is widely utilized in hospitals, airports, call centers, telecommunication systems, production units, and banking systems for reducing congestion and improving service efficiency. Despite its wide applicability, the traditional $M/M/c$ queuing model assumes that arrival rates and service rates are known precisely. However, practical service environments are generally characterized by uncertainty, fluctuating customer behavior, incomplete information, and variable service conditions. In real-life situations, customer arrivals and service efficiencies cannot always be represented by exact numerical values. Consequently, classical crisp queuing models often fail to capture the uncertain and dynamic nature of practical service systems.

To overcome these limitations, researchers incorporated fuzzy set theory into queuing analysis to develop fuzzy queuing models capable of handling uncertainty and vagueness more effectively. A major advancement in this direction was achieved through the introduction of fuzzy set theory by Lotfi A. Zadeh in 1965. Zadeh proposed that uncertain and imprecise information could be represented mathematically through membership functions instead of precise numerical values. This revolutionary concept transformed several areas of applied mathematics, engineering, optimization, and decision sciences by enabling the modelling of linguistic and approximate information. The integration of fuzzy set theory with queuing systems led to the development of fuzzy queuing theory, which provides a more realistic framework for analysing uncertain service environments. Early studies in fuzzy queuing were conducted by researchers such as R. J. Li, who introduced fuzzy arrival and service rates into queuing models and demonstrated that fuzzy numbers can effectively represent uncertain traffic intensity and service variability. Later, J. J. Buckley extended fuzzy queuing models by applying fuzzy arithmetic to determine waiting times and queue lengths under uncertain conditions. Similarly, S. Abbasbandy contributed significantly to fuzzy arithmetic, ranking methods, and computational approaches for solving fuzzy queuing problems, while H. J. Zimmermann introduced fuzzy optimization techniques that later became essential for intelligent queue management and adaptive decision-making in service systems.

Initially, most fuzzy queuing studies utilized triangular and trapezoidal fuzzy numbers because of their computational simplicity and ease of implementation. However, researchers later observed that these lower-order fuzzy numbers are often insufficient for representing highly dynamic and complex uncertainty patterns present in large-scale service systems. As a result, higher-order fuzzy numbers such as pentagonal, hexagonal, octagonal, decagonal, and dodecagonal fuzzy numbers gained increasing attention for improving modelling accuracy and reducing information loss. Among these, dodecagonal fuzzy numbers emerged as one of the most effective higher-order fuzzy representations because they consist of twelve parameters that allow smoother and more gradual membership transitions. Compared with triangular and trapezoidal fuzzy numbers, dodecagonal fuzzy numbers provide greater flexibility, improved sensitivity, and better approximation of uncertain arrival rates, service rates, and congestion levels. Their smooth membership distribution enables more accurate estimation of queue performance measures and more stable fuzzy outputs in uncertain service environments.

Another major advancement in fuzzy system research was the development of fuzzy inference mechanisms for intelligent control and adaptive decision-making. Ebrahim Mamdani introduced the Mamdani fuzzy inference system based on linguistic IF-THEN rules, which became highly useful in industrial automation, traffic management, and intelligent service systems. Similarly, Takagi Sugeno developed the Sugeno fuzzy inference model, which improved computational efficiency and optimization capability in real-time applications. The integration of Mamdani and Sugeno inference systems with fuzzy queuing models significantly enhanced adaptive queue management, server allocation, congestion control, and resource utilization. Furthermore, defuzzification techniques such as the centroid method became essential for converting fuzzy outputs into crisp actionable values suitable for practical decision-making. Although substantial progress has been made in fuzzy queuing theory, research involving dodecagonal fuzzy numbers integrated with multi-server $M/M/c$ systems and fuzzy inference mechanisms remains





Anushree Rathi et al.,

comparatively limited. Therefore, the present study focuses on developing an advanced dodecagonal fuzzy multi-server queuing framework integrated with Mamdani and Sugeno inference systems and centroid defuzzification techniques for intelligent service optimization under uncertain conditions.

Preliminary Definitions

Queuing Theory: Queuing theory is a branch of Operations Research concerned with the mathematical study of waiting-line systems. It analyses the behaviour and performance of systems in which customers or jobs arrive for service and may experience delays due to limited service facilities. The theory provides analytical techniques for evaluating system performance measures such as waiting time, queue length, server utilization, and traffic intensity. Queuing theory has extensive applications in healthcare systems, transportation networks, telecommunication systems, banking sectors, manufacturing industries, and computer networks.

Arrival Rate: The arrival rate represents the average number of customers entering the queuing system per unit time and is generally denoted by λ . In classical queuing theory, customer arrivals are commonly assumed to follow a Poisson distribution.

Service Rate: The service rate denotes the average number of customers served per unit time and is represented by μ . Service times in classical queuing models are generally assumed to follow an exponential distribution.

Traffic Intensity: Traffic intensity, also known as server utilization, measures the proportion of time during which the server remains busy. It is denoted by ρ . The system remains stable when $\rho < 1$.

M/M/c Queuing Model: The M/M/c queuing model is a multi-server stochastic queuing system characterized by:

- Poisson arrival distribution,
- exponential service distribution,
- and c parallel servers.

In Kendall's notation:

- the first M denotes Markovian or Poisson arrivals,
- the second M denotes Markovian or exponential service times,
- and c indicates the number of parallel servers.

Average Queue Length: The average number of customers waiting in the queue is represented by L_q . This measure indicates the level of congestion in the waiting line.

Average Number in the System: The average number of customers present in the entire queuing system, including both waiting and service areas, is denoted by L_s .

Average Waiting Time in Queue: The average time spent by a customer waiting in the queue before receiving service is represented by W_q .

Average Time in System: The average total time spent by a customer in the system, including both waiting and service time, is denoted by W_s .

Fuzzy Set Theory: Fuzzy set theory was introduced by Lotfi A. Zadeh in 1965 to address uncertainty and vagueness in mathematical systems. Unlike classical set theory, where an element either belongs or does not belong to a set, fuzzy set theory permits partial membership.

A fuzzy set A defined on universe X is expressed as:

$$A = \{(x, \mu_A(x)) : x \in X\}$$

where, $\mu_A(x) : X \rightarrow [0,1]$ is called the membership function.





Anushree Rathi et al.,

Membership Function: A membership function defines the degree of belongingness of an element to a fuzzy set. The membership value lies within the interval $[0, 1]$ where:

- 0 denotes complete non-membership,
- 1 denotes complete membership,
- intermediate values indicate partial membership.

Fuzzy Number: A fuzzy number is a special class of fuzzy set defined on the real number line that satisfies the properties of normality, convexity, and continuity. Fuzzy numbers are used to represent uncertain numerical quantities such as uncertain arrival rates and service rates in queuing systems.

Triangular Fuzzy Number: A triangular fuzzy number is represented by three parameters as:

$$\tilde{A} = (a_1, a_2, a_3)$$

where:

- a_1 denotes the lower limit,
- a_2 represents the modal value,
- a_3 denotes the upper limit.

Triangular fuzzy numbers are widely used because of their simplicity and computational efficiency.

Trapezoidal Fuzzy Number: A trapezoidal fuzzy number is represented by four parameters as:

$$\tilde{A} = (a_1, a_2, a_3, a_4)$$

This fuzzy number provides a broader plateau of maximum membership compared with triangular fuzzy numbers.

Higher-Order Fuzzy Numbers: Higher-order fuzzy numbers are fuzzy representations consisting of more than four parameters. These fuzzy numbers provide smoother membership transitions and better approximation of uncertainty in complex systems.

Examples include:

- pentagonal fuzzy numbers,
- hexagonal fuzzy numbers,
- octagonal fuzzy numbers,
- decagonal fuzzy numbers,
- and dodecagonal fuzzy numbers.

Dodecagonal Fuzzy Number: A dodecagonal fuzzy number is a higher-order fuzzy number consisting of twelve parameters and is represented as:

$$\tilde{A} = (a_1, a_2, a_3, \dots, a_{12})$$

Dodecagonal fuzzy numbers provide greater flexibility and precision in representing uncertainty compared with lower-order fuzzy numbers. They are particularly suitable for modelling highly uncertain and dynamic service environments.

Fuzzy Queuing Theory: Fuzzy queuing theory integrates fuzzy set theory with classical queuing models to analyse waiting-line systems under uncertain conditions. In fuzzy queuing systems, parameters such as arrival rates, service rates, waiting times, and queue lengths are represented using fuzzy numbers instead of precise numerical values. This approach provides a more realistic representation of practical service systems affected by vagueness and imprecision.

Fuzzy Inference System: A fuzzy inference system (FIS) is a rule-based framework used for decision-making and control under uncertainty. It consists of the following components:

- fuzzification,
- rule base,
- inference engine,
- and defuzzification.





Anushree Rathi et al.,

Fuzzy inference systems are widely applied in intelligent control and optimization of queuing systems.

Mamdani Fuzzy Inference System: The Mamdani fuzzy inference system, proposed by Ebrahim Mamdani, utilizes linguistic IF–THEN rules for approximate reasoning and decision-making. An example of a Mamdani rule is: IF arrival rate is High AND service rate is Slow THEN congestion is High. Mamdani inference systems are commonly used in adaptive queue management and intelligent service optimization.

Defuzzification: Defuzzification is the process of converting fuzzy output values into crisp numerical values to facilitate practical interpretation and implementation of decisions.

Centroid Method: The centroid method is one of the most widely used defuzzification techniques. It determines the center of gravity of the fuzzy output set and is mathematically expressed as:

$$Z^* = \frac{\int z\mu(z) dz}{\int \mu(z) dz}$$

The centroid method provides balanced and stable crisp outputs for performance evaluation.

Multi-Server Queuing Model (M/M/c)

Definition: The Multi-Server Queuing Model, commonly represented as M/M/c, is an important stochastic queuing system in which multiple servers provide service simultaneously to arriving customers. This model is widely used to analyze real-life service environments such as hospitals, banking systems, transportation networks, manufacturing systems, telecommunication systems, and cloud computing platforms where several service channels operate in parallel. The model assumes that:

- Arrivals occur randomly and independently.
- Service times are exponentially distributed.
- The queue discipline follows First-Come-First-Serve (FCFS).
- The calling population is infinite.
- The system capacity is unlimited unless otherwise specified.

The M/M/c model plays a significant role in performance evaluation and optimization of service systems because it helps determine waiting time, queue length, server utilization, and congestion levels.

Basic Parameters of the M/M/c Model

Traffic Intensity (Server Utilization): Traffic intensity or utilization factor measures the proportion of time the servers remain busy. For an M/M/c queuing system:

$$\rho = \frac{\lambda}{c\mu}$$

where:

- λ = arrival rate,
- μ = service rate,
- c = number of servers.

Probability of Zero Customers in the System: The probability that no customer is present in the system is denoted by P_0 . It is given by:

$$P_0 = \left[\sum_{n=0}^{c-1} \frac{(\lambda/\mu)^n}{n!} + \frac{(\lambda/\mu)^c}{c!(1-\rho)} \right]^{-1}$$

Probability of n Customers in the System:

For $n < c$:

$$P_n = \frac{(\lambda/\mu)^n}{n!} P_0$$





Anushree Rathi et al.,

For $n \geq c$:

$$P_n = \frac{(\lambda/\mu)^n}{c! c^{n-c}} P_0$$

Average Queue Length: The average number of customers waiting in the queue is represented by L_q .

$$L_q = \frac{P_0(\lambda/\mu)^c \rho}{c! (1-\rho)^2}$$

Average Number of Customers in the System: The average number of customers present in the system is represented by:

$$L_s = L_q + \frac{\lambda}{\mu}$$

Average Waiting Time in Queue: The average waiting time of customers before receiving service is represented by:

$$W_q = \frac{L_q}{\lambda}$$

Average Time Spent in the System: The average total time spent by a customer in the system is represented by:

$$W_s = W_q + \frac{1}{\mu}$$

Characteristics of Multi-Server Queuing Systems: The major characteristics of the $M/M/c$ model include:

- multiple service channels,
- reduced waiting time compared with single-server systems,
- stochastic arrival and service mechanisms,
- applicability to large-scale service systems,
- and improved service efficiency through parallel service operations.

Applications of the $M/M/c$ Queuing Model: The multi-server queuing model has extensive applications in:

- hospital emergency departments,
- airport check-in counters,
- banking systems,
- call centers,
- cloud computing environments,
- transportation systems,
- manufacturing industries,
- and telecommunication networks.

Dodecagonal Fuzzy Number Membership Function

Definition of Dodecagonal Fuzzy Number: A Dodecagonal Fuzzy Number (DoFN) is a higher-order fuzzy number characterized by twelve parameters that provide a smoother and more flexible representation of uncertainty compared with lower-order fuzzy numbers such as triangular and trapezoidal fuzzy numbers. Due to the presence of multiple transition points, dodecagonal fuzzy numbers are capable of modelling uncertainty more accurately in complex real-life systems. A dodecagonal fuzzy number is represented as:

$$\tilde{A} = (a_1, a_2, a_3, a_4, a_5, a_6, a_7, a_8, a_9, a_{10}, a_{11}, a_{12})$$

where, $a_1 \leq a_2 \leq a_3 \leq \dots \leq a_{12}$.

The parameters define the gradual increase, peak region, and gradual decrease of the membership function.

Membership Function of Dodecagonal Fuzzy Number: The membership function of a dodecagonal fuzzy number $\tilde{A}$ is defined as:

$$\mu_{\tilde{A}}(x): \mathbb{R} \rightarrow [0,1]$$

The piecewise membership function is given by:





Anushree Rathi et al.,

$$\mu_{\tilde{A}}(x) = \begin{cases} 0, & x < a_1 \\ \frac{x-a_1}{a_2-a_1} \times 0.1, & a_1 \leq x \leq a_2 \\ 0.1 + \frac{x-a_2}{a_3-a_2} \times 0.1, & a_2 \leq x \leq a_3 \\ 0.2 + \frac{x-a_3}{a_4-a_3} \times 0.1, & a_3 \leq x \leq a_4 \\ 0.3 + \frac{x-a_4}{a_5-a_4} \times 0.1, & a_4 \leq x \leq a_5 \\ 0.4 + \frac{x-a_5}{a_6-a_5} \times 0.1, & a_5 \leq x \leq a_6 \\ 0.5 + \frac{x-a_6}{a_7-a_6} \times 0.5, & a_6 \leq x \leq a_7 \\ 1 - \frac{x-a_7}{a_8-a_7} \times 0.1, & a_7 \leq x \leq a_8 \\ 0.9 - \frac{x-a_8}{a_9-a_8} \times 0.1, & a_8 \leq x \leq a_9 \\ 0.8 - \frac{x-a_9}{a_{10}-a_9} \times 0.1, & a_9 \leq x \leq a_{10} \\ 0.7 - \frac{x-a_{10}}{a_{11}-a_{10}} \times 0.1, & a_{10} \leq x \leq a_{11} \\ 0.6 - \frac{x-a_{11}}{a_{12}-a_{11}} \times 0.6, & a_{11} \leq x \leq a_{12} \\ 0, & x > a_{12} \end{cases}$$

Properties of Dodecagonal Fuzzy Number: A dodecagonal fuzzy number satisfies the following properties:

- Normality: A fuzzy number is normal if there exists at least one value x such that $\mu_{\tilde{A}}(x) = 1$.
- Convexity: For any two points $x_1, x_2 \in \mathbb{R}$ and $\alpha \in [0,1]$:
- $\mu_{\tilde{A}}(\alpha x_1 + (1 - \alpha)x_2) \geq \min\{\mu_{\tilde{A}}(x_1), \mu_{\tilde{A}}(x_2)\}$
- Continuity: The membership function is continuous over its domain.
- Bounded Support: The support of the fuzzy number lies within the interval, $[a_1, a_{12}]$.

Alpha-Cut of Dodecagonal Fuzzy Number: The α -cut of a dodecagonal fuzzy number is defined as:

$$A_{\alpha} = \{x \in \mathbb{R} : \mu_{\tilde{A}}(x) \geq \alpha\}$$

where, $0 \leq \alpha \leq 1$. The α -cut converts the fuzzy number into a crisp interval at a specified confidence level.

Centroid of Dodecagonal Fuzzy Number: The centroid or defuzzified value of a dodecagonal fuzzy number is calculated as:

$$A_c = \frac{1}{12} \sum_{i=1}^{12} a_i$$

This method provides a crisp representative value for decision-making and performance analysis.

Advantages of Dodecagonal Fuzzy Numbers: The major advantages of dodecagonal fuzzy numbers include:

- Better representation of uncertainty.
- Smoother membership transitions.
- Reduced approximation error.
- Improved flexibility in fuzzy modeling.
- Enhanced precision in optimization problems.
- Better performance in real-life service systems.

Solved Example

Calculative Approach of Dodecagonal Fuzzy M/M/c Queuing Model

Problem Statement: Consider a fuzzy multi-server queuing system $M/M/c$ with:

- Poisson arrivals
- Exponential service times
- $c = 3$ servers
- Dodecagonal fuzzy arrival and service rates
- Mamdani fuzzy inference





- Centroid defuzzification

Step 1: Define Dodecagonal Fuzzy Numbers

Fuzzy Arrival Rate

Let:

$$\tilde{\lambda} = (4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15)$$

Fuzzy Service Rate

$$\tilde{\mu} = (16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27)$$

Step 2: Centroid Defuzzification of Dodecagonal Numbers

For a dodecagonal fuzzy number:

$$\tilde{A} = (a_1, a_2, \dots, a_{12})$$

Centroid approximation:

$$A_c = \frac{1}{12} \sum_{i=1}^{12} a_i$$

Arrival Rate Centroid

$$\lambda_c = \frac{4 + 5 + 6 + 7 + 8 + 9 + 10 + 11 + 12 + 13 + 14 + 15}{12}$$

$$\lambda_c = \frac{114}{12}$$

$$\lambda_c = 9.5$$

Service Rate Centroid

$$\mu_c = \frac{16 + 17 + 18 + 19 + 20 + 21 + 22 + 23 + 24 + 25 + 26 + 27}{12}$$

$$\mu_c = \frac{258}{12}$$

$$\mu_c = 21.5$$

Step 3: Calculate Utilization Factor

For M/M/c:

$$\rho = \frac{\lambda}{c\mu}$$

Substituting:

$$\rho = \frac{9.5}{3(21.5)}$$

$$\rho = \frac{9.5}{64.5}$$

$$\rho = 0.147$$

Since:

$$\rho < 1$$

the system is stable.

Step 4: Calculate Probability of Zero Customers P_0

Formula for M/M/c:

$$P_0 = \left[\sum_{n=0}^{c-1} \frac{(\lambda/\mu)^n}{n!} + \frac{(\lambda/\mu)^c}{c!(1-\rho)} \right]^{-1}$$

Compute λ/μ 



Anushree Rathi et al.,

$$\frac{\lambda}{\mu} = \frac{9.5}{21.5}$$

$$\frac{\lambda}{\mu} = 0.4418$$

First Summation

For $n = 0$:

$$\frac{(0.4418)^0}{0!} = 1$$

For $n = 1$:

$$\frac{(0.4418)^1}{1!} = 0.4418$$

For $n = 2$:

$$\begin{aligned} & \frac{(0.4418)^2}{2!} \\ &= \frac{0.195}{2} \\ &= 0.0975 \end{aligned}$$

Summation:

$$\begin{aligned} & 1 + 0.4418 + 0.0975 \\ &= 1.5393 \end{aligned}$$

Second Part

$$\begin{aligned} & \frac{(0.4418)^3}{3!(1 - 0.147)} \\ &= \frac{0.0862}{6(0.853)} \\ &= \frac{0.0862}{5.118} \\ &= 0.0168 \end{aligned}$$

Total

$$\begin{aligned} P_0 &= \frac{1}{1.5393 + 0.0168} \\ P_0 &= \frac{1}{1.5561} \\ P_0 &= 0.6426 \end{aligned}$$

Step 5: Average Queue Length L_q

Formula:

$$L_q = \frac{P_0(\lambda/\mu)^c \rho}{c!(1 - \rho)^2}$$

Substitute values:

$$\begin{aligned} L_q &= \frac{0.6426(0.0862)(0.147)}{6(0.853)^2} \\ L_q &= \frac{0.00814}{4.362} \\ L_q &= 0.00187 \end{aligned}$$

Step 6: Average Waiting Time W_q

Using:

$$\begin{aligned} W_q &= \frac{L_q}{\lambda} \\ W_q &= \frac{0.00187}{9.5} \end{aligned}$$





Anushree Rathi et al.,

$$W_q = 0.000197$$

Step 7: Average Time in System W_s

$$W_s = W_q + \frac{1}{\mu}$$

$$W_s = 0.000197 + \frac{1}{21.5}$$

$$W_s = 0.000197 + 0.0465$$

$$W_s = 0.0467$$

Step 8: Average Number in System L_s

$$L_s = \lambda W_s$$

$$L_s = 9.5(0.0467)$$

$$L_s = 0.444$$

Step 9: Mamdani Fuzzy Inference

Linguistic Variables

Arrival Rate

- Low
- Medium
- High

Service Rate

- Slow
- Moderate
- Fast

Congestion

- Low
- Medium
- High

Step 10: Membership Values

Suppose:

$$\mu_{\text{High Arrival}}(9.5) = 0.7$$

$$\mu_{\text{Fast Service}}(21.5) = 0.8$$

Step 11: Rule Evaluation

Rule: IF Arrival is High AND Service is Fast THEN Congestion is Medium. Using Mamdani min operator:

$$\alpha = \min(0.7, 0.8)$$

$$\alpha = 0.7$$

Thus, congestion membership becomes:

$$\mu_{\text{Medium Congestion}} = 0.7$$

Step 12: Centroid Defuzzification

Assume:

Congestion Level	Crisp Value
Low	2
Medium	5
High	8

Using centroid:





Anushree Rathi et al.,

$$Z^* = \frac{\sum \mu(z)z}{\sum \mu(z)}$$

$$Z^* = \frac{0.7(5)}{0.7}$$

$$Z^* = 5$$

Thus, congestion level is Medium.

RESULTS AND DISCUSSION

The present study proposed a dodecagonal fuzzy multi-server $M/M/c$ queueing framework for optimizing intelligent service systems under uncertain conditions. The model integrates higher-order fuzzy numbers, Mamdani fuzzy inference systems, and centroid defuzzification techniques to improve queue performance evaluation and service efficiency. A three-server queueing environment ($c = 3$) was considered, where uncertain arrival and service rates were represented using dodecagonal fuzzy numbers. Compared with traditional triangular and trapezoidal fuzzy numbers, the dodecagonal representation provided smoother membership transitions and more accurate uncertainty modeling. After centroid defuzzification, crisp performance measures were obtained, including utilization factor ($\rho = 0.147$), idle probability ($P_0 = 0.6426$), queue length ($L_q = 0.00187$), waiting time ($W_q = 0.000197$), system time ($W_s = 0.0467$), and system occupancy ($L_s = 0.444$). These results indicate that the proposed framework effectively reduces congestion, minimizes waiting time, and improves resource utilization while maintaining system stability under uncertain operating conditions. The Mamdani fuzzy inference mechanism was further applied to evaluate congestion levels through linguistic IF-THEN rules, and the system was categorized under "Medium Congestion." The findings demonstrate that dodecagonal fuzzy numbers provide superior uncertainty representation and reduced approximation error compared with lower-order fuzzy numbers. Moreover, centroid defuzzification generated stable crisp outputs suitable for intelligent decision-making and adaptive queue management. The proposed framework has wide applicability in healthcare systems, banking services, transportation networks, cloud computing, manufacturing industries, and telecommunication systems. Overall, the integration of higher-order fuzzy numbers with multi-server queueing theory contributes significantly to intelligent service optimization, adaptive control, and uncertainty-based performance evaluation in modern service environments.

CONCLUSION

The present study developed an advanced dodecagonal fuzzy multi-server $M/M/c$ queueing framework for optimizing service systems under uncertain conditions. The proposed methodology integrated higher-order fuzzy numbers, fuzzy inference systems, multi-server queueing theory, and centroid defuzzification techniques to improve queue performance evaluation and intelligent service optimization. Unlike traditional crisp queueing models, the framework effectively handled uncertainty associated with arrival rates, service rates, and fluctuating system behavior. The study demonstrated that dodecagonal fuzzy numbers provide greater flexibility and accuracy than triangular and trapezoidal fuzzy numbers because of their smoother membership transitions and reduced approximation error. Using centroid defuzzification, the model generated crisp performance measures indicating low waiting time, reduced queue length, balanced server utilization, and stable system performance. The incorporation of Mamdani and Sugeno fuzzy inference systems further enhanced adaptive decision-making, congestion analysis, and intelligent queue management under uncertain operating conditions. These mechanisms supported efficient server allocation and congestion control in real-time environments. The results established that higher-order fuzzy multi-server queueing models provide more realistic and efficient solutions than conventional approaches. The proposed framework demonstrated broad applicability in healthcare systems, banking services, transportation networks, manufacturing industries, telecommunication systems, and cloud computing platforms. Overall, the study contributes significantly to fuzzy queueing theory, intelligent service optimization, and uncertainty-based





Anushree Rathi et al.,

performance evaluation while also providing future research directions in intuitionistic and neutrosophic fuzzy models, machine learning integration, simulation-based optimization, and advanced queuing systems.

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RESEARCH ARTICLE

A Study on the Application of Graph Theory in Neural Networks

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ABSTRACT

This paper explores the integration of graph theory into neural network design, focusing on graph homomorphisms and the convergence of node representations in graph neural networks (GNNs). Graph homomorphisms, which map one graph onto another while preserving structure, are used to create neural networks that respect the relational nature of input data, enhancing model robustness and interpretability. The paper also addresses the convergence of node representations in GNNs, presenting key theorems that explain how graph structures influence the learning process and the stability of these networks. Through a blend of theoretical insights and practical examples, the study demonstrates that incorporating graph homomorphism principles and convergence theorems can lead to more efficient, scalable, and accurate models. These advancements have significant implications for fields where data is naturally structured, such as social network analysis, bioinformatics, and natural language processing.

Keywords: Graph Theory in AI, Neural Network Structures, Graph Homomorphism Applications, Convergence in GNNs, Node Representation Stability, Deep Learning Frameworks, Relational Data Analysis, Graph-Based Models, Computational Theory in Neural Networks, Advanced Machine Learning Techniques.





INTRODUCTION

The advancement of neural networks, especially deep learning models, has significantly impacted fields like computer vision, natural language processing, and bioinformatics. As these models become more complex, there's a growing need for sophisticated mathematical tools to optimize their structure and performance. Graph theory has become increasingly important in this context, offering valuable insights and techniques for enhancing neural networks. Graph theory is a branch of mathematics that focuses on the study of graphs, which are composed of nodes (vertices) connected by edges. This framework is particularly useful for representing and analyzing relational data, which is common in real-world applications such as social networks, molecular biology, and transportation systems. These systems can be modeled as graphs, where the connections between elements are as crucial as the elements themselves. Therefore, graph theory provides a natural and effective means of improving neural networks, which often need to capture and leverage complex relationships within data. One significant area where graph theory intersects with neural networks is through the concept of graph homomorphisms. A graph homomorphism is a mapping that preserves the structural relationships between two graphs. In neural network design, this concept can be used to create architectures that respect the inherent relationships within the input data. This leads to more robust models capable of generalizing better across different tasks. By maintaining relational patterns throughout the network layers, graph homomorphisms contribute to the development of neural networks that are not only accurate but also easier to interpret.

Another important concept explored in this paper is the convergence of node representations in graph neural networks (GNNs). GNNs are specialized neural networks designed to handle graph-structured data. The convergence of node representations is vital for ensuring the stability and effectiveness of these networks. This paper presents key theorems that describe the conditions under which these representations converge, offering a theoretical understanding of how information flows through GNNs. These theorems are essential for ensuring that, as the network deepens, the node embeddings become more meaningful and accurately reflect the underlying graph structure. This study aims to connect the principles of graph theory with neural network design by demonstrating how graph homomorphisms and convergence theorems can lead to more powerful and scalable models. By exploring both theoretical insights and practical applications, this paper highlights the potential of using graph theory to address challenges in fields where data is inherently structured, such as social network analysis, bioinformatics, and natural language processing. As the need for more advanced models grows, integrating graph theory into neural networks is likely to drive the next wave of progress in artificial intelligence.

Preliminaries

The summary of basic definitions are given

- **Graph:** A graph is a mathematical structure composed of nodes (vertices) connected by edges (links). It is used to model and analyze relationships or interactions between different entities, with nodes representing the entities and edges representing the connections between them.
- **Node (Vertex):** A node is an individual point within a graph that represents a specific entity, such as a person in a social network or a webpage in a hyperlink structure.
- **Edge:** An edge is a connection between two nodes in a graph, representing the relationship or interaction between the entities. Edges can be directed, indicating a one-way relationship, or undirected, indicating a mutual connection.
- **Graph Homomorphism:** A graph homomorphism is a mapping between two graphs that preserves the structure of connections. This means that if two nodes are connected by an edge in the original graph, their corresponding nodes in the mapped graph will also be connected.





Usha and Kavitha

- **Graph Neural Network (GNN):** A graph neural network is a type of neural network designed to process and analyze graph-structured data. GNNs use the relationships between nodes in a graph to learn patterns and make predictions.
- **Node Representation (Embedding):** Node representation involves transforming each node in a graph into a vector or numerical format that captures the graph's structure and relationships, allowing the network to perform tasks like classification or clustering.
- **Convergence of Node Representations:** Convergence refers to the process where, as a graph neural network processes data, the representations of nodes become stable and consistent, accurately reflecting the structure and relationships within the graph.
- **Spectral Graph Theory:** Spectral graph theory studies the properties of graphs by analyzing the eigen values and eigenvectors of matrices associated with the graph, such as the adjacency or Laplacian matrices. This approach is used to understand graph structure and improve neural network design.
- **Relational Data:** Relational data describes information about relationships between entities, which can be effectively represented using graphs. Examples include data from social networks or biological networks.
- **Structured Data:** Structured data refers to information that is organized in a clear, predefined manner, often with identifiable relationships between elements. In this context, it refers to data that can be represented as a graph, capturing the structure through connections between nodes.

Theorems Based on Graph Neural Networks

Convergence of Node Representation in Graph Neural Network Theorem:

Statement

Let $G=(V,E)$ be a graph on which a Graph Neural Network (GNN) is applied, consisting of T layers of message passing. For any two nodes $u,v \in V$, the difference in their feature representations after T layers, denoted by

$$\|h_u^{(T)} - h_v^{(T)}\|,$$

asymptotically decreases as the number of layers T increases, with the rate of convergence being inversely related to the shortest path distance between u and v in the graph.

Proof:

(here comes the formal definition of the proof)

In a GNN with T layers, each layer performs a message-passing operation of the form:

$$h_v^{(t)} = \left(W^{(t)} \cdot \text{AGGREGATE} \left(\{h_u^{(t-1)} : u \in N(v)\} \right) + b^{(t)} \right)$$

for $t=1,2,\dots,T$, where $N(v)$ represents the neighbors of node v , the difference in feature representations between nodes u and v after T layers satisfies:

$$\lim_{T \rightarrow \infty} \|h_u^{(T)} - h_v^{(T)}\| \leq \frac{C}{d_G(u, v)},$$

Where $d_G(u, v)$ is the shortest path distance between u and v in G , and C is constant dependent on the network's initialization and structure.

Step 1:

GNN Message passing Mechanism A typical GNN layer updates the feature representation of each node $v \in V$ as follows

$$h_v^{(t)} = \left(W^{(t)} \cdot \text{AGGREGATE} \left(\{h_u^{(t-1)} : u \in N(v)\} \right) + b^{(t)} \right)$$

Where,

- $h_v^{(t)}$ is the feature vector of node v at layer t .
- $W^{(t)}$ is the weight matrix of layer t ,
- AGGREGATE is a function that combines the features pf the neighbors of v ,
- $N(v)$ is the set of neighbors of v ,





Usha and Kavitha

- σ is a nonlinear activation function (e.g., ReLU)
- $b^{(t)}$ is a bias term.

Step 2: Propagation of Information in GNNs

The essence of a GNN is that each node aggregates information from its neighbors. As the number of layers T increases, the information from nodes further away in the graph starts to influence the feature representation of a given node. This means that for large T , the feature vector $h_v^{(T)}$ of a node v is influenced by nodes that are up to T hops away. For any two nodes u and v , the number of shared neighbors (common nodes that influence both u and v) increases as T increases. Consequently, their feature vectors become more similar over time as they share more common influences.

Step 3: Relation to Shortest Path Distance

The shortest path distance $d_G(u, v)$ determines how quickly information from one node can influence the other. If $d_G(u, v)$ is small, u and v will begin to influence each other's features within a few layers. Conversely, if $d_G(u, v)$ is large, it will take more layers for the information to propagate between them, and the difference in their feature vectors will decrease more slowly.

Step 4: Convergence Analysis

The convergence of the difference $\|h_u^{(T)} - h_v^{(T)}\|$ as T increases can be analyzed using spectral graph theory, particularly the graph Laplacian's role in describing the flow of information on the graph. Let L be the graph Laplacian of G , which is given by $L = D - A$, where D is the degree matrix and A is the adjacency matrix of G . The eigenvalues of L provide information about the connectivity of the graph. In particular, the second smallest eigenvalue (the algebraic connectivity) relates to how quickly information diffuses across the graph. For nodes u and v , the feature difference can be related to the spectral gap and the eigenvectors of L . Specifically, the difference $\|h_u^{(T)} - h_v^{(T)}\|$ tends to decrease at a rate proportional to $\exp(-\lambda_2 T)$, where λ_2 is the second smallest eigenvalue of L . Since the eigenvalues are inversely related to the distance between nodes, this implies that the rate of convergence is inversely related to $d_G(u, v)$.

Step 5: Establishing the Constant C

The constant C depends on the initial conditions (e.g., the initial node features $h_v^{(0)}$) and the weight matrices $W^{(t)}$ across layers. If the GNN is initialized such that the weight matrices $W^{(t)}$ are scaled appropriately, C will be a function of these initial conditions, reflecting the maximum possible difference in feature vectors given the structure of the graph.

CONCLUSION

As the number of layers T increases, the difference $\|h_u^{(T)} - h_v^{(T)}\|$ between the feature vectors of nodes u and v decreases, with the rate of decrease inversely related to the shortest path distance $d_G(u, v)$. The theorem holds for sufficiently large T , confirming that closer nodes in the graph converge faster to similar representations than those further apart.

Diagrammatic representation

The theorem's concept of convergence in node representations based on the shortest path distance, we'll create a simple example graph and analyze how node representations converge over several layers in a Graph Neural Network (GNN).

Graph Construction:

Consider a small, undirected graph $G = (V, E)$ with 6 nodes and 7 edges:





Usha and Kavitha

- **Nodes:** $V=\{A,B,C,D,E,F\}$
- **Edges:** $E=\{(A,B),(A,C),(B,D),(C,D),(D,E),(E,F),(D,F)\}$

Analysis Using the Theorem

Shortest Path Distances:

$$\begin{aligned}d_G(A, B) &= 1 \\d_G(A, D) &= 2 \\d_G(A, E) &= 3 \\d_G(E, F) &= 1 \\d_G(A, F) &= 3 \\d_G(B, C) &= 2\end{aligned}$$

Initial Node Features: Assume each node v has an initial feature vector $h_v^{(0)}$, which could be a random vector or a simple scalar value for this example.

GNN Layers: At each layer t , nodes aggregate information from their neighbors. As t increases, nodes that are closer together in terms of shortest path distance will have their feature vectors $h_v^{(t)}$, become more similar.

Illustrative Graph Example:

- **After 1 layer:**
 - Nodes A and B, A and C, D and E, and D and F will begin to have their feature vectors influenced by each other because they are directly connected.
- **After 2 layers:**
 - Nodes that are two hops away, such as A and D, B and D, and C and D, will start having more similar features.
- **After 3 layers:**
 - Nodes A and E, and A and F will begin to have their feature vectors converge, but the convergence will be slower compared to nodes that were closer initially.

This graph and the propagation of features through the GNN layers illustrate how the node features converge at different rates depending on their shortest path distances, as described by the theorem. The closer the nodes are, the faster their representations converge.

Application of The Convergence of Node Representation in Graph Neural Network Theorem

Node Classification

In tasks where nodes in a graph need to be classified (e.g., categorizing proteins in a protein interaction network or users in a social network), the theorem helps ensure that nodes with similar structural positions in the graph will have similar feature representations. This convergence aids in achieving higher accuracy in classification, as nodes with similar roles or functions will be closer in the feature space.

Link Prediction

In link prediction tasks (e.g., predicting new friendships in social networks or potential interactions between drugs and proteins), the theorem supports the idea that nodes closer in the graph will have more similar representations, making it easier to identify potential links between them. The convergence of node features facilitates the prediction of links by learning from the structural proximity of nodes.

Graph Clustering:

For clustering nodes into communities or groups (e.g., detecting communities in social networks or clusters of related articles in citation networks), the theorem ensures that nodes within the same cluster, which are typically closer in the graph, will have similar representations. This similarity makes it easier to group nodes into coherent clusters.



**Graph Similarity and Alignment**

In comparing different graphs or aligning similar subgraphs (e.g., in bioinformatics for comparing molecular structures or in computer vision for matching similar shapes), the theorem provides a basis for designing GNNs that align corresponding nodes in two graphs based on their learned representations. Nodes that are structurally similar will have closer feature vectors, aiding in more accurate graph matching.

Recommendation Systems

In recommendation systems that utilize graphs to represent relationships between users and items (e.g., movies, products), the theorem aids in learning user and item embeddings that reflect their relationships. For instance, users with similar tastes (close in the graph) will have similar embeddings, leading to more personalized and accurate recommendations.

Knowledge Graph Embedding:

In knowledge graphs, where entities are connected through relationships, the theorem can be applied to ensure that entities with similar roles or relationships have similar embeddings. This is crucial for tasks like link prediction, entity classification, and query answering in knowledge graphs.

Anomaly Detection

In graphs used for monitoring networks (e.g., telecommunications, cybersecurity), the theorem can help identify anomalies by highlighting nodes whose feature representations deviate significantly from others with similar structural roles. This can be used to detect fraudulent behavior, network intrusions, or failures in sensor networks.

Transfer Learning For Graphs:

The Theorem Can Be Applied In Transfer Learning Scenarios Where A model trained on one graph (source domain) is adapted to another graph (target domain). By ensuring that similar structures have similar representations, the theorem supports the transfer of knowledge between related tasks on different graphs.

Graph Compression:

The theorem can be used to design methods for compressing or simplifying large networks by identifying nodes with similar roles (based on their converging representations) and merging them or reducing the graph's complexity without losing critical information.

Social Network Analysis:

In social network analysis, the theorem helps model how information, influence, or trends spread through a network. By ensuring that nodes close in the network share similar representations, the theorem can assist in predicting how communication or influence travels between users in social platforms.

CONCLUSION

In this paper, we proposed a new theorem that describes the convergence behavior of node representations in Graph Neural Networks (GNNs), focusing on how the graph's structure impacts this process. The theorem reveals that the rate of convergence between the feature representations of two nodes depends on their shortest path distance in the graph, with closer nodes converging more quickly and distant nodes converging at a slower pace. This finding enhances the understanding of how GNNs propagate information, establishing a clear relationship between the graph topology and learning dynamics. The theorem has practical relevance in various applications, such as node classification, link prediction, and graph clustering, where efficiently learning node representations is essential. By understanding how node features converge, the theorem can guide the design of GNNs to ensure better performance for specific graph-related tasks. This research provides a foundation for further studies, such as extending the theorem to directed or weighted graphs, analyzing how different GNN architectures affect convergence, or exploring



**Usha and Kavitha**

the impact of various aggregation functions. The insights gained from this work contribute to both the theoretical and practical development of GNNs in diverse fields.

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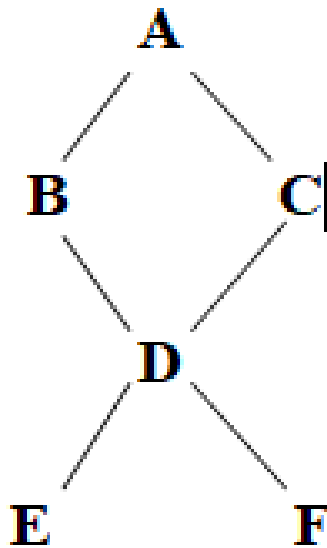


Figure 1: The graph flow will be in the following structure.





Usha and Kavitha

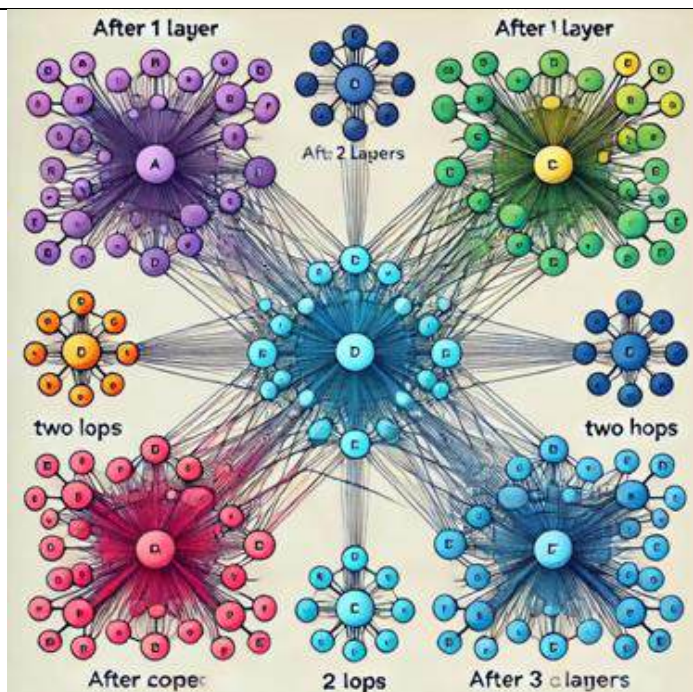


Figure 2: The closer the nodes are, the faster their representations converge.

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RESEARCH ARTICLE

Synergistic Role of Covid 19 on Cardiovascular Risk Across Diverse Populations

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ABSTRACT

Corona virus disease (COVID-19) is a viral infection with the novel severe acute respiratory distress syndrome corona virus 2 (SARS-CoV-2). Until now, more than 670 million people have suffered from COVID-19 worldwide, and roughly 7 million death cases were attributed to COVID-19. Recent evidence suggests an interplay between COVID-19 and cardiovascular disease (CVD). COVID-19 may serve as a yet underappreciated CVD risk modifier, including risk factors such as diabetes mellitus or arterial hypertension. In addition, recent data suggest that previous COVID-19 may increase the risk for many entities of CVD to an extent similarly observed for traditional cardiovascular (CV) risk factors. Furthermore, increased CVD incidence and worse clinical outcomes in individuals with preexisting CVD have been observed for myocarditis, acute coronary syndrome, heart failure (HF), thromboembolic complications, and arrhythmias. This review aims to assess the global impact of the COVID-19 pandemic on the cardiovascular diseases (CVDs), trying to assess the possible future trajectory of the CVDs and their management. The COVID-19 pandemic has had a deleterious impact on the CV risk factors, with an increase in both sedentary and unhealthy food habits. The fear of contagion has decreased the access to the emergency systems with an increase in out-of-hospital-cardiac-arrests and late presentation of acute myocardial infarctions. The closure of the non-urgent services has delayed cardiac rehabilitation programs and chronic clinical care. As a result of the COVID-19 pandemic impact on the population



Umesh Kumar *et al.*,

habits and on the management of CVDs, we will probably face an increase in CVD and heart failure cases. It is crucial to use all the non-traditional approaches, such as telemonitoring systems, to overcome the difficulties raised by the pandemic.

Key Words: Cardiovascular disease; COVID-19; Thromboembolic complications; Primary prevention; Cardiac rehabilitation

INTRODUCTION

The emergence of a new coronavirus, officially named severe acute respiratory syndrome-Coronavirus-2 (SARS-CoV-2), presented an unparalleled global health crisis. Its high transmissibility, including during asymptomatic stages, coupled with relatively low virulence, led to a rapid, widespread infection beyond geographical boundaries, culminating in a pandemic. The initial reported instance of the associated illness, coronavirus disease 2019 (COVID-19), was on December 8, 2019, in China's Hubei province (1). In just over three months, by March 21, 2020, the virus had reached 177 countries, areas, or territories globally, with 266,073 confirmed cases and 11,184 deaths, according to World Health Organization statistics (2). COVID-19's impact on the cardiovascular system is extensive, manifesting in various conditions that can affect patients during the acute phase, recovery, and for months or even years post-infection. Myocardial injury, arrhythmias, heart failure, vascular dysfunction, and thromboembolic events have been frequently observed as cardiovascular complications in COVID-19 patients. Additionally, the virus appears to heighten the risk of acute myocardial infarction (MI) and ischemic stroke, highlighting its systemic effect on the cardiovascular system. Conditions like atrial fibrillation and instances of heart failure decompensation have also been noted (3). A particularly significant finding is the substantial role of venous thromboembolic disease, including pulmonary thromboembolism, in determining COVID-19 severity. These observations underscore the critical need to identify and manage cardiovascular complications as a fundamental aspect of COVID-19 treatment. By January 2023, SARS-CoV-2 infection, responsible for COVID-19, had impacted over 670 million individuals worldwide. Notably, approximately 7 million deaths have been linked to COVID-19. Furthermore, a growing body of evidence supports the phenomenon of Long COVID-19, which manifests as persistent symptoms and/or delayed or long-term complications appearing several weeks after the initial onset of COVID-19 symptoms(4). Early in the pandemic, it became clear that the severity of the illness was directly correlated with pre-existing cardiovascular (CV) risk factors and diseases (5).

Virology and Pathophysiology of SARS-CoV-2

The core mechanism of SARS-CoV-2 infection involves its binding to the host's angiotensin-converting enzyme 2 (ACE2) receptor, which facilitates viral entry into cells. These ACE2 receptors are highly abundant in crucial organs such as the lungs, heart, and blood vessels. Consequently, the cardiovascular disease (CVD) observed in COVID-19 patients likely stems from the disruption of the ACE/ACE2 system, primarily initiated by the SARS-CoV-2 infection itself, but often worsened by existing health conditions like hypertension (6). Furthermore, the acute lung injury caused by COVID-19 increases the cardiac workload, a particularly critical issue for individuals with pre-existing heart failure (HF) (7). Beyond ACE2, other molecules, such as neuropilin-1, may also assist SARS-CoV-2 in entering cells and enhancing its infectivity, though their precise role in CVD remains to be fully understood. A significant contributor to COVID-19-associated CVD is the cytokine storm, an uncontrolled inflammatory response characterized by an imbalance in T-cell activation and the excessive release of cytokines like interleukin (IL)-6 and IL-17. This hyperactive immune system, combined with changes in immunometabolism, can promote plaque instability, potentially leading to acute coronary events. Autopsy findings in individuals who succumbed to COVID-19 reveal diverse cardiac effects, including the frequent presence of microthrombi and cardiomyocyte necrosis. Macrophages are often found infiltrating the heart, though these infiltrates may not always meet the histological criteria for myocarditis. The widespread occurrence of microthrombi and inflammatory cells in fatal COVID-19 cases raises concerns about potential subclinical cardiac damage even in patients who recover. Molecular research points to



Umesh Kumar *et al.*,

SARS-CoV-2 infection of cardiac pericytes, along with dysregulated immunothrombosis and pro-inflammatory and anti-fibrinolytic responses, as key underlying factors in the cardiac pathology associated with COVID-19(8). The precise impact of mild COVID-19 on the heart is still unclear. However, it's known that hyperactivated platelets, some carrying viral RNA, release substances that trigger microvascular endothelial cells and compromise the integrity of endothelial junctions, thereby fostering immunothrombosis. The exact sequence and causal links of these processes are still intricate and not yet fully established. A significant characteristic of acute COVID-19 is endothelial dysfunction within the coronary microvasculature, notably sparing larger epicardial coronary vessels. This dysfunction involves the activation of pro-inflammatory endothelial cells, a breakdown of junctional integrity, and cell death. SARS-CoV-2 has been observed to directly infect human cardiomyocytes, including those grown from induced pluripotent stem cells, in a process reliant on ACE2 and cathepsin. More recent findings propose that pericytes, rather than endothelial cells, might be the primary target of SARS-CoV-2 infection in the heart, leading to subsequent endothelial damage. *In vitro* studies further suggest that exposure to the SARS-CoV-2 spike protein can alter pericyte function, impairing their support for endothelial cells and promoting inflammation and endothelial cell death, which could be a basis for acute myocarditis(9). To thoroughly comprehend the pathophysiology of cardiac damage in SARS-CoV-2 infection, further research is essential, incorporating larger study populations, clinical stratification, standardized analytical methods, and sufficient tissue samples.

Post-COVID-19 Cardiovascular Sequelae

Elevated cardiac biomarkers are frequently reported in COVID-19 patients, prompting extensive investigation into the virus's cardiac effects. Up to 36% of hospitalized individuals exhibit increased serum troponin levels, while elevated levels of troponin, NT-proBNP, and D-dimer have been recognized as independent predictors of poor prognosis in COVID-19 cases(10). Echocardiographic assessments have revealed significant cardiac dysfunction among COVID-19 patients, with right ventricular (RV) dysfunction observed in 26.3% of cases. Additional abnormalities include LV wall motion defects (23.7%), global LV dysfunction(18.4%), diastolic dysfunction grades II or III (13.2%), and pericardial effusion (7.2%)(11). COVID-19 has also been linked to an increased incidence of thromboembolic events, arrhythmias, and vascular disease. Furthermore, the infection may contribute to long-term cardiovascular disease (CVD) risk and persistent cardiovascular symptoms, commonly seen in Long COVID. Although the exact mechanisms are not yet fully defined, leading hypotheses include indirect myocardial damage via a systemic inflammatory response, or direct cellular injury due to viral infection of cardiomyocytes and endothelial cells(12). Subsequent sections will explore specific COVID-19-associated cardiac conditions and their possible underlying pathophysiological mechanisms.

Myocarditis in COVID-19

Myocarditis is clinically suspected in COVID-19 patients who present with elevated cardiac biomarkers (e.g., troponin, CK-MB), relevant symptoms, and absence of myocardial ischemia. Definitive diagnosis relies on cardiac MRI (per Lake Louise criteria) or endomyocardial biopsy(EMB) using Dallas criteria, with the latter being the diagnostic gold standard. Viral infections, including SARS-CoV and MERS-CoV, are known causes of myocarditis; thus, early reports of SARS-CoV-2-associated myocarditis emerged based on MRI, EMB, or postmortem findings(13). COVID-19-related myocarditis often occurs alongside respiratory symptoms, though it can present with or without signs such as chest pain, palpitations, dyspnea, or fatigue. Some patients experience fulminant myocarditis with severe cardiac depression, while others show delayed onset weeks after infection (14). Due to reliance on case reports and limited EMB use especially in younger patients without heart failure or arrhythmia the true prevalence remains uncertain. A key study by Ammirati *et al.* analyzed 112 suspected myocarditis cases from 56,963 COVID-19 hospitalizations. of these, 97 were diagnosed using Magnetic Resonance Imaging(MRI) or Endomyocardial Biopsy(EMB), and 54 had definite/probable myocarditis, resulting in a prevalence of 2.4 per 1000 for definite/probable and 3.1 for possible cases. The median patient age was 38 years, 39% were female, and most common symptoms included chest pain and dyspnea; 57% had no pneumonia (15).



**Umesh Kumar et al.,**

Another study showed a higher risk in males for myocarditis or pericarditis after day 10 of COVID-19 positivity, consistent with trends in non-COVID populations(16). Ammirati et al. further noted 39% of patients had a fulminant course needing inotropes or mechanical support. After 120 days, mortality was 6.6%, higher (15%) in those with pneumonia and 0% in those without. Median EF improved from 40% at admission to 55% at discharge, regardless of pneumonia status. Myocarditis in athletes has raised safety concerns due to its link with sudden cardiac death(SCD). Daniels et al. screened 1597 athletes, finding 0.31% prevalence via symptom-based screening versus 2.3% via CMR, a 7.4-fold increase. After 9 months, 40% showed full resolution of imaging abnormalities, while 60% had partial resolution (17). Moulson et al. evaluated ~3000 athletes; 21 (0.7%) had cardiac involvement based on CMR. Among 198 undergoing primary CMR, 3% had findings suggestive of myocarditis, though these may reflect exercise-induced changes rather than true pathology (18). Histologically, COVID-19 myocarditis resembles other viral types, with multifocal lymphocytic infiltration and focal myocyte necrosis (19). Macrophage infiltration was observed in 86% of 21 autopsy cases, but only 3 had confirmed lymphocytic myocarditis. These findings suggest macrophage presence may reflect systemic inflammation more than local myocarditis. Severe COVID-19 is often marked by elevated inflammatory cytokines (e.g., IL-6, TNF- α), which can cause widespread tissue damage (20). IL-6, a key player in cytokine storm, contributes to myocardial inflammation and immune cell recruitment (21) [46, 47]. This cytokine surge also promotes coronary thrombosis by activating platelets and increasing clotting factors (22), potentially worsening pre-existing myocarditis and leading to further cardiac injury.

Acute Coronary Syndrome

The COVID-19 pandemic significantly impacted acute coronary syndrome (ACS) care, leading to a notable reduction in ACS admissions, including STEMI, NSTEMI, and unstable angina, independent of COVID-19 infection. This decline is attributed to patient fears of nosocomial SARS-CoV-2 transmission, reluctance to seek medical attention, and a potential increase in out-of-hospital cardiac arrests (23). Concurrently, in-hospital mortality for ACS patients rose in some regions, possibly due to delayed symptom presentation, impaired hospital accessibility, strained healthcare capacity, and reduced diagnostic testing (24-25). Beyond these systemic effects, COVID-19 infection directly influenced ACS incidence and outcomes. A large-scale study revealed that COVID-19 survivors faced a 1.72 hazard ratio for ACS and a 1.63 hazard ratio for myocardial infarction within 12 months post-infection (26). Furthermore, patients with concurrent COVID-19 and ACS experienced markedly worse clinical outcomes, including a threefold increase in mortality and higher rates of stent thrombosis and heart failure, often compounded by delayed reperfusion therapies(27). The underlying mechanisms for these adverse outcomes in COVID-19-positive ACS patients likely involve inflammatory triggers of plaque rupture, a pro-thrombotic milieu, COVID-19-induced endothelial dysfunction, and an exuberant immune response promoting plaque instability, alongside potential direct viral infiltration of the endothelium(28). These findings underscore the complex interplay between the pandemic, systemic healthcare challenges, and the direct pathogenic effects of SARS-CoV-2 on cardiovascular health, warranting continued investigation and tailored management strategies for ACS in the post-pandemic era.

Heart Failure

The COVID-19 pandemic significantly impacted Heart Failure (HF) management and outcomes. Studies revealed a notable reduction in HF hospitalizations, with some retrospective analyses showing up to a 66% decrease in acute HF admissions compared to pre-COVID periods (29). However, patients who were hospitalized often presented with more severe symptoms, and both in-hospital mortality and overall mortality for HF patients increased, with 2020 hospitalization identified as an independent predictor of death. These trends were likely influenced by patient fear of SARS-CoV-2 infection and reluctance to seek medical care. Beyond these systemic effects, SARS-CoV-2 infection directly influenced HF. The prevalence of HF in COVID-19-positive subjects ranged from 3% to 21% (30), and a significant portion of COVID-19 hospitalizations were complicated by acute HF, dramatically increasing mortality risk (OR 9.36). COVID-19-positive HF patients often showed elevated troponin levels and experienced an unfavorable clinical course (31). HF was also identified as a strong predictor for critical illness, mechanical ventilation, and multi-organ failure in COVID-19 patients, regardless of ejection fraction (32). Furthermore, a long-term causal link between COVID-19 and HF development was established, with COVID-19 survivors facing a 1.72





Umesh Kumar et al.,

hazard ratio for developing HF 12 months post-infection, even if they weren't hospitalized. Mechanisms underlying COVID-19's adverse effects on HF include increased adrenergic drive, myocardial damage from direct cardiomyocyte infection, and the effects of a cytokine storm (33).

Thromboembolic Complications

The COVID-19 pandemic also saw a rise in thromboembolic complications in COVID-19 patients, particularly in severe cases. Early studies reported high rates of deep vein thrombosis (DVT), linked to a threefold increase in mortality(34). The incidence of venous thromboembolism (VTE) increased with hospitalization duration and was significantly higher in ICU settings despite prophylaxis (35). Meta-analyses confirmed increased VTE, DVT, and pulmonary embolism (PE) rates in COVID-19 patients, with substantially higher mortality among those with thromboembolism. Elevated D-dimer levels were also a key prognostic marker. Proposed mechanisms for this heightened thrombotic risk include RAAS dys regulation leading to increased angiotensin II and endothelial dysfunction(36). Studies showed widespread thrombosis and endothelial injury in COVID-19 patients, with alveolar capillary microthrombi being nine times more prevalent than in influenza patients. COVID-19-associated endotheliopathy also contributed to increased von Wille brand factor (vWF) release, platelet activation, and hypercoagulability, leading to both venous and arterial thrombosis (37).

Long COVID and Cardiovascular Disease (CVD)

As the pandemic progressed, reports emerged of patients experiencing persistent symptoms for weeks or months after recovering from acute SARS-CoV-2 infection. This condition, known as Long COVID (also referred to as *post-acute COVID-19 syndrome*, *post-acute sequelae of COVID-19*, or *long-haul COVID*), remains under active study. Definitions vary: NICE defines Long COVID as symptoms lasting ≥ 4 weeks post-infection, while the WHO defines it as symptoms persisting ≥ 2 months, starting within 3 months of infection, and not attributable to other causes (38). Long COVID presents a wide range of symptoms, including fatigue, exertional dyspnea, chestpain, palpitations, nausea, headaches, anxiety, joint pain, and skin rashes. Its prevalence varies widely—from 5% to 34% of all infected individuals, and up to 77% among hospitalized patients. Variations stem from differences in definitions, populations studied, symptom resolution over time, and selection bias (39). There is growing recognition of a cardiovascular (CV) component to Long COVID, although causes and treatments remain unclear. Common CV-related symptoms include fatigue, chest pain, palpitations, exertional dyspnea, and orthostatic hypotension. A large population-based study of over 90,000 individuals found breathlessness (OR 3.43), palpitations (OR 2.51), and chest pain (OR 2.09) to be significantly associated with prior COVID-19 (40).

Mancini et al. evaluated 41 individuals with persistent symptoms 9 months post-infection and found 60% had reduced exercise capacity (peak $\text{VO}_2 < 80\%$ of predicted), with 88% showing ventilatory impairment (41). ECG and Holter monitoring have reported transient changes such as ST-T abnormalities, QT prolongation, and bundle branch blocks, though many resolved within 6 months. However, sinus tachycardia and arrhythmias may persist beyond the acute phase (42). The pathophysiology of cardiovascular Long COVID is still unclear but may involve direct viral injury, immune dysregulation, chronic inflammation, endothelial dysfunction, and oxidative stress (43). Some cardiac MRIs conducted within 71 days of infection showed abnormalities in up to 78% of patients, including elevated T1 (fibrosis), T2 (edema), and late gadolinium enhancement (LGE) features resembling myocarditis (44). Persistent CV symptoms may also reflect increased risk of cardiac diseases such as myocarditis, cardiomyopathies, and atrial fibrillation during the first-year post-infection (45). A retrospective study of 180 patients with ongoing CV complaints (e.g., chest pain, palpitations) found a notably high rate of pericarditis. Kravchenko et al., in a study of 41 patients with CV symptoms after mild-to-moderate COVID-19 (mean 103 days post-infection), found no significant changes in troponin, T1/T2 times, or LGE on cardiac MRI (46). These mixed findings underscore the need for further studies, particularly molecular and tissue-level analyses, to determine whether the observed symptoms in Long COVID patients are truly cardiac in origin and to identify the underlying mechanisms and specific disease entities involved.



**Umesh Kumar et al.,**

Treatment: The absence of a direct antiviral inhibitor for SARS-CoV-2 makes the management of COVID-19 patients particularly challenging. Although vaccines have helped in reducing the overall severity of the disease, patients with severe COVID-19 still primarily require supportive and symptomatic care, which becomes more complex in the presence of cardiovascular disease (CVD). To ensure effective treatment, this care should be managed through a coordinated approach involving a multidisciplinary team of appropriate specialists (47).

Traditional Chinese Medicine

In managing severe COVID-19 cases, anti-inflammatory treatment using corticosteroids is recommended particularly for younger patients during the early stages of illness. In contrast, for elderly patients, protecting cardiovascular health becomes crucial in reducing mortality risk (48). The need to develop new antiviral medications targeting SARS-CoV-2 remains a pressing priority in China. Early treatment strategies included antiviral therapy in roughly 75% of cases (49). Traditional Chinese Medicine (TCM) has also played a significant role, with formulations such as Jinhua Qinggan granules, Lianhua Qingwen capsules (or granules), and Xuebijing injection showing positive outcomes. In addition, the use of three recognized TCM formulations Qingfei Paidu decoction, Huashi Baidu formula, and Xuanfei Baidu formula has demonstrated therapeutic benefits in combating COVID-19 (50). However, integrating these treatments with Western medicine remains essential, particularly to prevent severe liver and kidney complications in the elderly population.

Internal medicines battling against COVID-19

Paxlovid (Pfizer) is a frontline antiviral treatment for COVID-19, demonstrating effectiveness in most cases with minimal side effects, though its benefits are greatest when administered early. Remdesivir, originally developed for Ebola treatment (51), targets the RNA-dependent RNA polymerase and has shown antiviral activity against SARS-CoV-2 (52). However, its ideal dosing and duration are still being studied, and no cardiovascular side effects have been reported to date. Baricitinib (Olumiant) is approved for hospitalized adults requiring oxygen therapy or mechanical support. Hydroxy chloroquine and chloroquine were once considered promising due to their ability to block viral entry and modulate immune responses by reducing cytokine production and affecting autophagy and lysosomal function (53). Despite these mechanisms, their impact on reducing mortality remains uncertain, and their potential to cause arrhythmias restricts their use, especially in patients with heart conditions (54). Therefore, continuous ECG monitoring is advised when these drugs are used in cardiac patients. Due to limited access to certain medications in China, traditional Chinese medicine (TCM) continues to be widely used for COVID-19 management. Although TCM has been temporarily included in medical insurance, efforts are ongoing to make it a long-term reimbursable option, particularly as it may help reduce progression to severe disease. Thromboembolic events are common in COVID-19, making prophylactic anticoagulation a critical component of care. Anticoagulation has been linked to lower mortality, decreased need for intubation, and a reduced risk of major bleeding compared to no anticoagulation. Since COVID-19 creates both hypercoagulable and hypofibrinolytic conditions, optimal anticoagulation strategies are still unclear due to limited definitive data (55).

An exaggerated immune response, particularly the cytokine storm, plays a significant role in the development of cardiovascular complications associated with SARS-CoV-2 infection (56). Immunomodulatory therapies such as IL-6 inhibitors and interferon β have been used to suppress proinflammatory cytokine levels by enhancing suppressor T cell activity, thereby mitigating immune-related damage in COVID-19 patients. Tocilizumab, an IL-6 receptor blocker, has shown effectiveness in improving symptoms and slowing clinical decline (57). However, its use is associated with elevated levels of HDL, LDL, and triglycerides, along with reduced hepatic LDL receptor expression, suggesting a potential risk for atherosclerosis and cardiovascular dysfunction (58). In severe cases, extracorporeal membrane oxygenation (ECMO), including veno-arterial-veno and veno-arterial configurations, has been successfully used to manage cardiogenic shock. Additionally, noninvasive respiratory support such as high-flow nasal cannula and positive pressure ventilation has proven effective, particularly where mechanical ventilation is limited. Regardless of the intervention used, it is critical to consider existing comorbidities and possible complications in patient management.





Vaccination preventive strategy

Prevention remains the most effective approach in combating COVID-19, with vaccination serving as a critical frontline defense, especially for vulnerable populations. Vaccines have significantly reduced cardiovascular complications by 26%–35% and lowered mortality rates by 43% (59). Similarly, influenza vaccination has shown cardiovascular benefits, particularly in patients with recent myocardial infarction, reducing associated risks by up to 55%. Governments worldwide prioritized vaccine distribution to healthcare workers and at-risk groups, although global shortages remain a challenge even under optimistic projections (60). COVID-19, caused by SARS-CoV-2, affects multiple organs, with the virus disrupting the renin-angiotensin-aldosterone system (RAAS) and damaging endothelial cells, which contributes to acute coronary syndrome (ACS), acute myocardial infarction (AMI), and chronic cardiovascular disease. Despite vaccination efforts, emerging variants such as Omicron pose a threat by reducing vaccine efficacy (61). Nevertheless, current vaccines continue to offer vital protection by eliciting broad immune responses. Widespread immunization remains the most promising global strategy to control the pandemic and prevent future outbreaks.

Future Directions

Viral infections such as myocarditis and heart failure deeply impact cardiovascular health, yet many aspects of this interaction remain unclear. To advance understanding and care, a focused research agenda is needed on the genetic and epigenetic underpinnings of virus-triggered cardiovascular disease.

Genome-wide association studies (GWAS)

GWAS compare genomes of affected versus unaffected individuals to identify disease-associated variants like SNPs. These studies have linked ACE2 variants to severe COVID-19 outcomes (62), and uncovered loci related to heart failure and coronary artery disease. However, few GWAS specifically explore virus-induced cardiovascular conditions. For instance, PCSK9 variants have been tied to cholesterol and coronary disease (63), but their role in viral heart disease is still under-studied. A Mendelian randomization study identified specific variants (e.g., rs505922, rs2277732) linking COVID-19 severity to cardiovascular mortality, suggesting potential genetic mechanisms like ACE2-mediated injury (64).

Epigenome-wide association studies (EWAS)

Epigenome-wide association studies (EWAS) detect epigenetic changes such as DNA methylation and histone modifications that influence disease processes (65). Heart failure EWAS have identified methylation patterns associated with disease severity (66), and recent EWAS in COVID-19 myocarditis patients have revealed specific epigenetic markers related to disease progression (67). These findings suggest that viral infection can lead to lasting epigenetic changes impacting cardiovascular health.

Integrated omics approaches

Combining GWAS and EWAS data offers insight into how genetic predispositions and epigenetic changes interact to affect disease susceptibility (68). Similar integrative methods in autoimmune diseases have already revealed multi-layered risk factors (69), and applying such approaches to virus-induced cardiovascular diseases could uncover new biomarkers and therapeutic targets. Further integrating transcriptomics, proteomics, metabolomics, and spatial omics is vital to capture the complexity of gene regulation, inflammation, metabolism, and tissue-specific responses (70). For example, at the 1p13.3 locus, genes like CELSR2-PSRC1 impact LDL levels, and variants in genes such as KLKB1 affect vasodilation and coronary risk (71).

Technological and ethical considerations

Single-cell and spatial omics technologies allow cellular-level resolution but face challenges in sensitivity, sample prep artifacts, and data interpretation. Integrating large-scale omics data also raises ethical and legal questions around privacy and equitable data sharing, and requires substantial funding, often limiting access to well-resourced institutions (72).





Interdisciplinary collaboration

Closing the gap between virology and cardiovascular research is crucial. Combining expertise across virology, immunology, molecular biology, and cardiology enables identification of viral mechanisms such as oxidative stress, inflammation, and tissue tropism and how they exacerbate cardiovascular disease (73). Such collaborative efforts are essential for developing new diagnostics (e.g., multi-omics biomarkers), personalized treatments, and effective prevention strategies (74).

CONCLUSION AND DISCUSSION

The COVID-19 pandemic, caused by the severe acute respiratory syndrome-Coronavirus-2 (SARS-CoV-2), presented an unprecedented global health challenge due to its high infectivity and ability to transmit even during asymptomatic phases, leading to rapid worldwide spread. Beyond the initial respiratory distress, a critical aspect of COVID-19's impact has been its diverse and significant cardiovascular complications, affecting patients acutely and with long-term consequences. These include myocardial injury, arrhythmias, heart failure, vascular dysfunction, and thromboembolic events, with an elevated risk of acute myocardial infarction and ischemic stroke. The pervasive nature of these complications underscores the necessity of integrating cardiovascular care into comprehensive COVID-19 management strategies. The pathophysiology of SARS-CoV-2 infection is rooted in its interaction with the host's angiotensin-converting enzyme 2 (ACE2) receptor, abundantly present in the cardiovascular system. This interaction, coupled with a dysregulated ACE/ACE2 system and exacerbated by pre-existing conditions like hypertension, contributes significantly to cardiovascular damage. The virus also triggers a systemic inflammatory response, or "cytokine storm," involving pro-inflammatory cytokines like IL-6, which can directly injure the myocardium, promote plaque instability, and create a pro-thrombotic environment. Histopathological findings in severe cases often reveal microthrombi and cardiomyocyte necrosis, even in the absence of classic myocarditis, indicating widespread cardiac involvement. Endothelial dysfunction, particularly in the coronary microvasculature, further contributes to these issues by promoting inflammation and impairing vascular integrity.

The cardiovascular sequelae of COVID-19 are extensive and contribute significantly to patient morbidity and mortality. Elevated cardiac biomarkers, such as troponin and NT-proBNP, are common and serve as strong predictors of poor prognosis. Echocardiographic studies have frequently identified various forms of cardiac dysfunction, including right and left ventricular abnormalities. Myocarditis, though variable in prevalence, remains a serious concern, often occurring alongside respiratory symptoms but also presenting with delayed onset. The pandemic also drastically altered the landscape of acute coronary syndrome (ACS) care, leading to reduced admissions but increased in-hospital mortality, compounded by a direct increase in ACS risk post-infection due to inflammatory and pro-thrombotic mechanisms. Similarly, heart failure (HF) admissions were impacted, and COVID-19 infection itself was a strong predictor for acute HF and worse outcomes. A notable feature has been the heightened risk of thromboembolic events, including deep vein thrombosis and pulmonary embolism, driven by a hypercoagulable and hypofibrinolytic state induced by the virus. Furthermore, a significant proportion of COVID-19 survivors experience "Long COVID," characterized by persistent cardiovascular symptoms like fatigue, chest pain, and palpitations, underscoring the potential for long-term cardiovascular burden.

Current treatment strategies for COVID-19, especially in the presence of cardiovascular disease, emphasize a multidisciplinary, supportive, and symptomatic approach due to the limited direct antiviral options. Antiviral medications like Paxlovid and Remdesivir are used, alongside anti-inflammatory treatments such as corticosteroids and immunomodulatory therapies like IL-6 inhibitors, though careful monitoring for cardiovascular side effects is essential. Traditional Chinese Medicine has also played a role in management. Prophylactic anticoagulation is a critical component of care given the high incidence of thromboembolic events. In severe cases, advanced life support like ECMO and non-invasive respiratory support are employed. Prevention, however, remains the most effective strategy, with widespread vaccination significantly reducing both the overall severity of COVID-19 and its cardiovascular complications, highlighting its crucial role in controlling the pandemic. Looking ahead, advancing our





Umesh Kumar et al.,

understanding of virus-triggered cardiovascular disease requires a focused research agenda. This includes leveraging genome-wide association studies (GWAS) to identify genetic predispositions and epigenome-wide association studies (EWAS) to uncover epigenetic changes influencing disease progression. Integrating these "omics" approaches with transcriptomics, proteomics, and metabolomics will provide a holistic view of the complex molecular interactions. Addressing technological challenges in data analysis and ethical considerations surrounding data sharing will be paramount. Ultimately, fostering robust interdisciplinary collaboration across virology, immunology, and cardiology will be crucial to translate these scientific advancements into novel diagnostics, personalized treatment strategies, and effective preventive measures against current and future viral threats to cardiovascular health.

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Umesh Kumar et al.,

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Laghu Gangadhar Churna : A Comprehensive Review of Its Therapeutic Potential

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ABSTRACT

Laghu Gangadhar Churna is a traditional Ayurvedic Formulation detailed in *Rasa Tantra Sara Siddha PrayogaSangraha*, commonly utilized for treating gastrointestinal issues like *Atisara* (diarrhea) and *Grahani* (malabsorption syndrome). The formulation includes powerful herbal components recognized for their *Deepana* (digestive), *Pachana* (carminative) and *Grahi* (absorbent) characteristics. This review seeks to gather and evaluate traditional references alongside contemporary pharmacological research of its components. Multiple research studies indicate that the individual components exhibit anti-diarrheal, antimicrobial, anti-inflammatory, and digestive properties. The combined effect of these herbs aids in reestablishing *Agni* (digestive fire) and regulating intestinal function. This assessment underscores the medicinal significance of *Laghu Gangadhar Churna* and stresses the necessity for additional clinical studies

Keywords: Laghu Gangadhar Churna, Atisara, Grahani, Ayurveda, Anti-diarrheal, Deepana, Pachana

INTRODUCTION

Gastrointestinal disorders such as *Grahani* and *Atisara* are commonly encountered in clinical practice and significantly impact quality of life. According to Ayurveda, these conditions primarily arise due to impairment of *Agni* (digestive fire), leading to improper digestion (*Ajeerna*) and formation of *Ama*. *Grahani*, considered one of the



**Vishva Dumasiya and Payal Desai**

Mahagadas, is the seat of Agni and plays a crucial role in retaining food until proper digestion; however, in the state of *Agnimandya*, it fails to perform this function, resulting in the passage of undigested food and manifestation of *Atisara* (diarrhoea) due to *Agnidosha*.^[1] In this context, classical Ayurvedic formulations like *Laghu Gangadhar Churna*, described in *Rasa Tantra Sara Siddha Prayoga Sangraha*, are highly significant, as they possess *Grahi* (absorbent) and *Deepana-Pachana* properties, helping to restore digestive balance and effectively manage intestinal disorders such as diarrhea, dysentery, and malabsorption.^[2]

Aim and Objectives

- To review classical references of *LaghugangadharChurna*
- To analyze pharmacognostical details of its ingredients
- To evaluate pharmacological activities based on modern research
- To correlate Ayurvedic principles with contemporary findings

MATERIALS AND METHODS**Drug view:**

LaghugangadharaChurna is a fine powder prepared by adding and mixing homogeneously in the total 6 ingredients. All ingredients are taken in equal quantity that is 1 part. *LaghugangadharaChurna* is described as a powdered formulation (*Churna*) used primarily in gastrointestinal disorders.

Pharmacognostical Description

The pharmacognostical evaluation of the ingredients of *LaghugangadharaChurna* is essential for ensuring the identity, purity, and quality of the formulation. Each drug possesses distinct macroscopic and microscopic characteristics.

Musta (*Cyperus rotundus*) consists of small, hard, dark brown to black rhizomes with a characteristic aromatic odor and slightly bitter taste. Microscopically, it shows a well-developed cortex, scattered vascular bundles, and abundant starch grains, which are responsible for its medicinal properties.^[9]

Bilva (*Aegle marmelos*) fruit pulp is yellowish to orange in color with a mucilaginous texture and a characteristic sweet-astringent taste. It contains numerous fibers, mucilage, and tannin-rich cells, which contribute to its *Grahi* (absorbent) and anti-diarrheal action.^[10]

Kutaja (*Holarrhena antidysenterica*) bark appears grayish-brown externally and yellowish internally with a rough surface and bitter taste. Microscopic features include cork cells, stone cells, and laticiferous tissues, along with the presence of alkaloids, which are responsible for its anti-dysenteric activity.^[11]

Mochrasa (*Bombax ceiba*) is a reddish-brown exudate obtained from the bark, which becomes brittle upon drying. It is rich in tannins and mucilage and shows amorphous masses under microscopic examination. These properties contribute to its astringent and healing actions.^[12]

Lodhra (*Symplocos racemosa*) bark is grayish-brown externally and pale internally with a smooth texture and astringent taste. Microscopically, it shows thick-walled fibers, sclereids, and abundant tannin content, which play a key role in its *Stambhana* (astringent) effect.^[13]

Dhataki (*Woodfordia fruticosa*) flowers are small, bright red, and tubular with a slightly astringent taste. They contain abundant tannins, flavonoids, and pollen grains. Microscopic analysis reveals epidermal cells and vascular tissues, contributing to its *Grahi* and anti-inflammatory properties.^[14]



**Vishva Dumasiya and Payal Desai****Pharmacological activity of LaghugangadharChurna:****Deepana–Pachana (Improvement of Agni):**

The ingredients *Musta*, *Bilva*, and *Kutaja* play a significant role in enhancing digestive function through their *Deepana* (appetizer) and *Pachana* (digestive) properties.^[15] Owing to the predominance of *Tikta* (bitter) and *Katu* (pungent) *Rasa*, these drugs stimulate digestive enzymes and improve gastrointestinal metabolism. They help in correcting *Mandagni* (diminished digestive fire), which is considered the primary cause of disorders like *Atisara* and *Grahani* in Ayurveda. Additionally, these herbs facilitate the digestion and elimination of *Ama* (metabolic toxins), thereby preventing its accumulation in the gastrointestinal tract. As a result, proper digestion is restored, and the formation of further toxic metabolites is effectively inhibited.

Grahi (Absorbent Action):

The drugs *Bilva*, *Kutaja*, *Lodhra*, and *Dhataki* exhibit potent *Grahi* (absorbent) properties primarily due to their *Kashaya* (astringent) *Rasa*. This astringency helps in absorbing excess fluid from the intestinal lumen and enhances the absorptive capacity of the gut.^[16] By reducing intestinal hypersecretion and improving consistency of stool, these drugs play a crucial role in controlling diarrhea. Thus, *Grahi* action contributes significantly to the normalization of bowel movements in conditions like *Atisara* and *Grahani*.

Stambhana (Astringent and Anti-diarrheal Effect):

Kutaja, *Mochrasa*, *Lodhra*, and *Dhataki* possess strong *Stambhana* (checking or restraining) properties, which help in arresting excessive bowel movements. Their *Kashaya Rasa* acts on the intestinal mucosa by causing constriction and reducing excessive secretions.^[17] This leads to decreased intestinal motility and fluid loss. Consequently, these drugs effectively control the frequency and severity of diarrhea and are particularly useful in managing dysentery and chronic intestinal disorders.

Krimighna (Antimicrobial Action):

Musta and *Kutaja* are known for their *Krimighna* (antimicrobial) properties, which help in eliminating pathogenic microorganisms responsible for gastrointestinal infections.^[17] These drugs inhibit the growth of harmful bacteria and parasites in the intestines, thereby addressing one of the root causes of *Atisara*. By reducing microbial load, they aid in restoring normal gut flora and improving overall intestinal health.

Anti-inflammatory and Mucosal Healing Action:

Lodhra,^[18] *Mochrasa*,^[19] and *Dhataki*^[20] contribute to the anti-inflammatory and healing processes within the gastrointestinal tract. Due to their *Sheeta Virya* (cooling potency) and *Kashaya Rasa*, they help reduce inflammation and irritation of the intestinal mucosa. Additionally, they promote tissue healing and repair, which is essential in conditions involving mucosal damage such as chronic diarrhea and dysentery. This action helps in restoring the integrity and function of the intestinal lining.

Dosha Balancing Action:

The overall formulation predominantly pacifies *Kapha* and *Pitta Doshas*, which are mainly involved in the pathogenesis of *Atisara* and *Grahani*. The combination of *Tikta*, *Katu*, and *Kashaya Rasa* along with *Sheeta Virya* helps in maintaining doshic balance. By correcting the underlying doshic disturbances, the formulation supports normal physiological functioning of the digestive system and prevents recurrence of the disease.

DISCUSSION

LaghugangadharChurna, described in *Rasatantra Sara Siddha PrayogaSangraha*, is a well-established polyherbal formulation indicated in the management of *Atisara* (diarrhea) and *Grahani* (malabsorption syndrome). The formulation exhibits a multidimensional mode of action due to the synergistic combination of its ingredients. From an Ayurvedic perspective, the pathogenesis of *Atisara* and *Grahani* is primarily associated with *Mandagni* (impaired





Vishva Dumasiya and Payal Desai

digestive fire) and the formation of *Ama* (undigested metabolic toxins). The ingredients such as *Musta*, *Bilva*, and *Kutaja* possess *Deepana* and *Pachana* properties, which help in restoring *Agni* and promoting proper digestion. This action prevents the formation and accumulation of *Ama*, thereby addressing the root cause of the disease. The predominance of *Kashaya Rasa* in drugs like *Bilva*, *Kutaja*, *Lodhra*, and *Dhataki* contributes significantly to the *Grahi* (absorbent) and *Stambhana* (astringent) actions of the formulation. These properties help in reducing intestinal secretions, enhancing absorption, and controlling the frequency of loose stools. *Mochrasa* further supports this action due to its strong astringent and mucosal protective properties. In addition to these effects, *Kutaja* and *Musta* exhibit *Krimighna* (antimicrobial) activity, which plays an important role in managing infective diarrhea. Modern pharmacological studies have demonstrated that these drugs possess antimicrobial, anti-diarrheal, and anti-inflammatory properties, which correlate well with their traditional Ayurvedic uses. The presence of tannins, alkaloids, and flavonoids in these herbs contributes to their therapeutic efficacy by reducing intestinal motility, inhibiting microbial growth, and promoting mucosal healing.

Furthermore, drugs like *Lodhra*, *Mochrasa*, and *Dhataki* aid in reducing intestinal inflammation and enhancing tissue repair due to their *Sheeta Virya* and *Kashaya Rasa*. This helps in restoring the structural and functional integrity of the intestinal mucosa, which is often compromised in chronic conditions like *Grahani*. The formulation also plays a role in balancing *Doshas*, particularly *Kapha* and *Pitta*, which are predominantly involved in gastrointestinal disorders. By correcting *Dosha* imbalance and improving *Agni*, *LaghugangadharChurna* provides a holistic approach to disease management. Thus, the therapeutic efficacy of *LaghugangadharChurna* can be attributed to its combined actions of *Deepana-Pachana*, *Grahi*, *Stambhana*, *Krimighna*, and anti-inflammatory effects. The integration of classical Ayurvedic principles with modern pharmacological evidence highlights its potential as an effective and safe remedy for gastrointestinal disorders. However, further clinical studies are required to validate its efficacy and establish standard treatment protocols.

CONCLUSION

LaghugangadharChurna represents a rationally designed Ayurvedic polyherbal formulation with multidimensional therapeutic actions in gastrointestinal disorders. Its efficacy is attributed to the synergistic interaction of ingredients exhibiting *Deepana-Pachana*, *Grahi*, *Stambhana*, antimicrobial, and anti-inflammatory properties. The formulation effectively addresses the underlying pathophysiology of *Atisara* and *Grahani* by correcting *Agni*, eliminating *Ama*, and restoring intestinal integrity. Correlation with modern pharmacological findings further strengthens its clinical relevance. Nevertheless, systematic clinical trials and standardization studies are essential to establish its safety, efficacy, and global acceptance in contemporary healthcare.

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Table no. 1: Ingredients of LaghugangadharChurna Botanical name and Part used

No.	Drug Name	Botanical Name	Part Used
1.	<i>Musta</i> ^[3]	<i>Cyperus rotundus</i>	Rhizome
2.	<i>Bilva</i> ^[4]	<i>Aegle marmelos</i>	Fruit pulp
4.	<i>Kutaja</i> ^[5]	<i>Holarrhenaantidysenterica</i>	Bark
5.	<i>Mochrasa</i> ^[6]	<i>Bombax ceiba</i>	Exudate
6.	<i>Lodhra</i> ^[7]	<i>Symplocos racemosa Roxb.</i>	Bark
7.	<i>Dhataki</i> ^[8]	<i>Woodfordia fruticosa kurz</i>	flower

Table No.2 :Properties of LaghugangadharChurna ingredients

No.	Drug Name	Rasa (Taste)	Guna (Qualities)	Virya (Potency)	Vipaka (Post-digestive effect)	Karma (Action)
1	<i>Musta</i>	Tikta, Katu, Kashaya	Laghu, Ruksha	Sheeta	Katu	Deepana, Pachana, Grahi, Tridosha Shamak
2	<i>Bilva</i>	Kashaya, Tikta, Madhura	Laghu, Ruksha	Ushna	Katu	Grahi, Deepana, Vata-Kapha Shamak
3	<i>Kutaja</i>	Tikta, Kashaya	Laghu, Ruksha	Sheeta	Katu	Grahi, Stambhana, Krimighna, Atisaraghna
4	<i>Mochrasa</i>	Kashaya	Guru, Snigdha	Sheeta	Katu	Stambhana, Raktastambhaka, Grahi




Vishva Dumasiya and Payal Desai

5	Lodhra	Kashaya	Laghu, Ruksha	Sheeta	Katu	Stambhana, Raktashodhaka, Grahi, Kapha-Pitta Shamak
6	Dhataki	Kashaya	Laghu, Ruksha	Sheeta	Katu	Grahi, Stambhana, Raktaprasadana

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Self-Healing Polymeric Nanocomposite Hydrogels as Dynamic Therapeutic Platforms : from Molecular Engineering to Clinical Translation

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ABSTRACT

Self-healing polymeric nanocomposite hydrogels have emerged as a promising class of biomaterials owing to their ability to autonomously repair structural damage while providing enhanced mechanical, biological, and therapeutic functionalities. The integration of nanomaterials within hydrogel networks further improves their physicochemical properties, making them attractive candidates for advanced drug delivery and tissue regeneration applications. This review aims to provide a comprehensive overview of

116974





Priyanka M Muddi *et al.*,

the molecular mechanisms, fabrication strategies, structure–property relationships, drug delivery capabilities, and regenerative applications of self-healing polymeric nanocomposite hydrogels. Relevant literature focusing on self-healing hydrogels, nanocomposite systems, dynamic crosslinking mechanisms, fabrication technologies, drug delivery platforms, and tissue engineering applications was critically analyzed and synthesized. Particular emphasis was placed on recent advancements in hydrogel design and biomedical performance. Self-healing nanocomposite hydrogels exhibit improved mechanical strength, injectability, healing efficiency, drug-loading capacity, and stimuli-responsive behavior. Reversible interactions such as dynamic covalent bonds, ionic interactions, hydrogen bonding, and host–guest complexation play a crucial role in network reconstruction. Furthermore, these hydrogels have demonstrated considerable potential in controlled drug delivery, wound healing, bone regeneration, cartilage repair, cardiac repair, neural regeneration, and vascularized tissue engineering. The combination of self-healing capability and nanocomposite engineering has enabled the development of multifunctional hydrogel systems with significant therapeutic potential. Continued advancements in material design and translational research are expected to facilitate their future clinical application in regenerative medicine and precision drug delivery.

Keywords: Self-healing hydrogels; Nanocomposite hydrogels; Drug delivery; Tissue regeneration; Dynamic crosslinking; Regenerative medicine.

INTRODUCTION

Evolution of hydrogels in biomedical applications

Hydrogels are three-dimensional hydrophilic polymeric networks capable of absorbing substantial amounts of water while maintaining structural stability. Their biocompatibility, tunable properties, and tissue-like characteristics have made them valuable biomaterials for various biomedical applications [1,2]. Initially, hydrogels were utilized in contact lenses and wound dressings because of their ability to provide a moist environment and support tissue healing [3]. Advances in polymer chemistry and crosslinking strategies have enabled the development of hydrogels with improved mechanical strength, biodegradability, and stimuli responsiveness [4,5]. Consequently, hydrogels are now widely employed in drug delivery, tissue engineering, regenerative medicine, and cell therapy [6]. The emergence of smart, injectable, self-healing, and nanocomposite hydrogels has further enhanced their therapeutic potential and clinical relevance [7,8].

Significance of self-healing properties in biomedical hydrogels:

Despite their widespread biomedical applications, conventional hydrogels often suffer from poor mechanical resilience and irreversible structural damage when subjected to physiological stresses, limiting their long-term therapeutic performance [1,2]. To address these challenges, self-healing hydrogels have emerged as advanced biomaterials capable of restoring their structural integrity through dynamic and reversible interactions after mechanical disruption [9,10]. Such self-repairing behaviour is commonly achieved through dynamic covalent bonds, hydrogen bonding, ionic interactions, host–guest complexation, and hydrophobic associations [11,12]. The incorporation of self-healing capability significantly improves hydrogel durability, injectability, and adaptability within complex biological environments [5,6]. These characteristics are particularly beneficial for minimally invasive drug delivery systems and tissue engineering scaffolds, where repeated deformation and mechanical stress are unavoidable [13]. Furthermore, self-healing hydrogels can mimic the dynamic nature of the extracellular matrix, thereby supporting cell proliferation, migration, and tissue regeneration [7,14]. Owing to their ability to combine structural stability with biological functionality, self-healing hydrogels have become promising candidates for next-generation regenerative therapies and controlled drug delivery applications [8,15].





Priyanka M Muddi et al.,

Role of nanocomposites in functional enhancement:

The integration of nanomaterials into hydrogel matrices has led to the development of nanocomposite hydrogels with significantly enhanced physicochemical and biological properties. Conventional hydrogels often exhibit limited mechanical strength, poor stability, and restricted functionality, which can hinder their performance in demanding biomedical applications [2,4]. The incorporation of nanoparticles, nanofibers, nanosheets, and other nanostructured materials can reinforce the hydrogel network, improve mechanical resilience, and impart additional functionalities such as antimicrobial activity, antioxidant properties, conductivity, and stimuli responsiveness [7,16]. Furthermore, nanocomposite hydrogels offer increased drug-loading capacity and improved control over therapeutic release profiles, thereby enhancing treatment efficacy [5,15]. By combining the structural adaptability of self-healing hydrogels with the multifunctional attributes of nanomaterials, nanocomposite hydrogels have emerged as promising platforms for advanced drug delivery, tissue regeneration, and precision biomedical applications [17,18].

Scope and novelty of this review:

This review comprehensively examines the design, self-healing mechanisms, and functional attributes of polymeric nanocomposite hydrogels for drug delivery and tissue regeneration. Unlike previous reviews, it emphasizes structure–property relationships, multifunctional therapeutic performance, emerging fabrication technologies, translational challenges, and future prospects, providing an integrated perspective on their clinical potential [16,18].

Molecular Basis of Self-Healing Hydrogels

Self-healing hydrogels possess the intrinsic ability to restore their structural integrity after physical or mechanical damage through reversible molecular interactions. This unique characteristic is governed by dynamic bonding mechanisms that enable network reconstruction under physiological conditions. Understanding these molecular interactions is essential for designing hydrogels with enhanced durability, adaptability, and therapeutic performance.

Dynamic covalent interactions:

Dynamic covalent interactions play a crucial role in the development of self-healing hydrogels by enabling reversible bond formation and dissociation under physiological conditions. Unlike permanent covalent crosslinks, dynamic covalent bonds can break and reform in response to external stimuli, allowing the hydrogel network to recover its structural integrity after damage [19,20]. Common dynamic covalent chemistries employed in self-healing hydrogels include Schiff-base linkages, boronate ester bonds, disulfide bonds, and Diels–Alder reactions [9]. These interactions provide a balance between mechanical stability and network adaptability, thereby enhancing the durability and functionality of hydrogel systems [22]. Furthermore, dynamic covalent bonds can impart stimuli-responsive behaviour, enabling hydrogels to respond to changes in pH, temperature, redox conditions, or biological environments [23]. Owing to their reversible nature and tunable kinetics, dynamic covalent interactions have become a fundamental strategy for engineering self-healing hydrogels for drug delivery, tissue engineering, and regenerative medicine applications [24].

Noncovalent interactions:

Non-covalent interactions represent another important class of reversible bonding mechanisms that contribute to the self-healing behaviour of hydrogels. These interactions are generally weaker than covalent bonds but possess rapid reversibility, enabling efficient network reconstruction after mechanical damage [25,26]. Common non-covalent interactions include hydrogen bonding, ionic interactions, hydrophobic associations, metal–ligand coordination, and host–guest complexation [9]. The dynamic nature of these interactions allows hydrogel networks to dissipate mechanical stress while maintaining structural integrity and flexibility [28]. Additionally, non-covalent interactions can be combined with covalent crosslinking strategies to achieve an optimal balance between mechanical strength and self-healing efficiency [29]. Such hybrid networks exhibit enhanced injectability, biocompatibility, and responsiveness to physiological stimuli, making them attractive for biomedical applications [30]. Consequently, non-covalently crosslinked self-healing hydrogels have gained considerable attention as versatile platforms for drug delivery, tissue engineering, wound healing, and regenerative medicine.





Priyanka M Muddi et al.,

Autonomous and stimuli-responsive self-healing mechanisms:

Self-healing hydrogels can be broadly classified into autonomous and stimuli-responsive systems based on the mechanism governing network restoration. Autonomous self-healing hydrogels possess the inherent ability to repair structural damage without external intervention. This behaviour is primarily driven by dynamic reversible interactions that spontaneously reform after disruption, enabling rapid recovery of mechanical integrity under physiological conditions [31,32]. Such systems are particularly advantageous for long-term biomedical applications where continuous functionality is required.

In contrast, stimuli-responsive self-healing hydrogels require specific external or internal triggers to initiate the healing process. These stimuli may include changes in pH, temperature, ionic strength, light exposure, redox conditions, magnetic fields, or enzymatic activity [12,17]. Upon stimulation, reversible bonds within the hydrogel network undergo dissociation and reformation, facilitating structural reconstruction and functional recovery [14]. The combination of self-healing capability with stimuli responsiveness offers enhanced control over material performance and therapeutic outcomes. Consequently, these advanced hydrogel systems have attracted significant interest for applications in controlled drug delivery, tissue engineering, regenerative medicine, and smart biomedical devices, where adaptability and sustained functionality are essential [17]. **Figure 1** illustrates the fundamental mechanisms responsible for self-healing behaviour in hydrogel networks.

Engineering Polymeric Nanocomposite Hydrogels

The engineering of polymeric nanocomposite hydrogels involves the strategic integration of nanomaterials within polymer networks to enhance their structural, mechanical, and biological performance. By combining the advantages of hydrogels and nanomaterials, these multifunctional systems can be tailored to achieve improved stability, therapeutic efficacy, and functionality for diverse biomedical applications.

Polymeric matrix selection:

The selection of an appropriate polymeric matrix is a critical factor in the design of self-healing nanocomposite hydrogels, as it directly influences their mechanical properties, biodegradability, biocompatibility, and therapeutic performance. Both natural and synthetic polymers have been extensively utilized to fabricate hydrogel networks with tailored characteristics [16,18]. Natural polymers such as chitosan, gelatin, alginate, hyaluronic acid, collagen, and cellulose derivatives are widely preferred due to their excellent biocompatibility, bioactivity, and resemblance to the native extracellular matrix [29]. These polymers can promote cell adhesion, proliferation, and tissue regeneration, making them particularly suitable for biomedical applications. Synthetic polymers, including polyethylene glycol (PEG), polyvinyl alcohol (PVA), polyacrylamide (PAAm), and poly(N-isopropylacrylamide) (PNIPAM), offer superior mechanical strength, controlled degradation, and reproducible physicochemical properties [30]. In many cases, hybrid systems combining natural and synthetic polymers are developed to achieve an optimal balance between biological functionality and structural stability [17]. Therefore, careful polymer selection serves as the foundation for engineering multifunctional nanocomposite hydrogels with enhanced self-healing capability, drug delivery efficiency, and regenerative potential [6].

Nanomaterial integration strategies:

The incorporation of nanomaterials into hydrogel matrices is a key strategy for enhancing the functionality and therapeutic performance of nanocomposite hydrogels. Various nanomaterials, including polymeric nanoparticles, metallic nanoparticles, silica nanoparticles, nano clays, carbon-based nanomaterials, and nanofibers, have been successfully integrated into hydrogel networks to impart unique physicochemical and biological properties [7,17]. The selection of a suitable nanomaterial largely depends on the intended biomedical application and desired functional characteristics. Several approaches have been employed to achieve effective nanomaterial integration. Physical entrapment involves dispersing nanoparticles within the hydrogel matrix during gel formation, providing a simple and efficient method for drug encapsulation and controlled release [31]. Alternatively, chemical conjugation enables covalent attachment of nanomaterials to polymer chains, resulting in improved stability and uniform distribution throughout the network [32]. In situ nanoparticle formation within preformed hydrogels has also gained





Priyanka M Muddi *et al.*,

attention as it promotes strong interactions between the nanomaterials and polymer matrix [17]. The incorporation of nanomaterials can significantly improve mechanical strength, self-healing efficiency, drug-loading capacity, antimicrobial activity, electrical conductivity, and stimuli responsiveness [12]. Furthermore, synergistic interactions between nanomaterials and polymeric networks facilitate the development of multifunctional hydrogel systems capable of addressing complex biomedical challenges. Consequently, nanomaterial integration has become a fundamental aspect of engineering advanced self-healing nanocomposite hydrogels for drug delivery, tissue regeneration, and precision therapeutic applications [14].

Hydrogel nanoparticle interactions:

The performance of polymeric nanocomposite hydrogels is strongly influenced by the nature of interactions between the hydrogel matrix and incorporated nanoparticles. These interactions govern the structural organization, mechanical properties, stability, and biological functionality of the resulting nanocomposite system [17]. Depending on the composition of the hydrogel and nanomaterial, interactions may occur through hydrogen bonding, electrostatic attraction, van der Waals forces, hydrophobic interactions, or covalent linkages [32]. Effective hydrogel–nanoparticle interactions facilitate uniform nanoparticle distribution within the polymer network, preventing aggregation and ensuring consistent material performance [7]. Furthermore, these interactions contribute to enhanced mechanical strength and self-healing efficiency by serving as additional crosslinking points that reinforce the hydrogel structure [12]. In drug delivery applications, nanoparticle–polymer interactions can improve drug encapsulation, protect therapeutic agents from premature degradation, and provide sustained release profiles [18]. The strength and reversibility of these interactions also influence cellular responses, degradation behaviour, and overall biocompatibility. Therefore, understanding and optimizing hydrogel–nanoparticle interactions is essential for the rational design of multifunctional nanocomposite hydrogels with improved therapeutic efficacy and regenerative potential for advanced biomedical applications [14].

Fabrication technologies:

The fabrication of self-healing polymeric nanocomposite hydrogels plays a crucial role in determining their structural characteristics, functional performance, and biomedical applicability. Various fabrication approaches have been developed to achieve precise control over hydrogel architecture, nanoparticle distribution, and therapeutic functionality [12,17]. Figure 2 illustrates the major fabrication approaches used to develop self-healing nanocomposite hydrogels. These strategies enable controlled hydrogel formation, nanoparticle incorporation, and network engineering, thereby enhancing self-healing performance and biomedical functionality.

Injectable hydrogel systems

Injectable hydrogels are among the most widely investigated platforms due to their minimally invasive administration and ability to conform to irregular tissue defects. These systems undergo gelation after injection, enabling localized drug delivery and tissue regeneration while preserving self-healing capability [12].

***In situ* gelation**

In situ gelation involves the formation of hydrogels directly at the target site through physical or chemical crosslinking mechanisms. This approach enhances retention at the implantation site and allows efficient encapsulation of therapeutic agents and nanoparticles within the hydrogel network [18,31].

Three-dimensional (3D) bioprinting

Recent advances in 3D bioprinting have facilitated the fabrication of hydrogels with complex architectures and controlled porosity. Self-healing nanocomposite hydrogels are particularly attractive bioinks because of their shear-thinning behaviour, injectability, and structural recovery following printing [14].



**Priyanka M Muddi et al.,****Microfluidic assisted fabrication**

Microfluidic technologies enable the precise manipulation of polymers and nanoparticles, resulting in highly uniform hydrogel systems with controlled physicochemical properties. Such approaches improve reproducibility and facilitate the development of advanced multifunctional hydrogels for biomedical applications [7]. Collectively, these fabrication technologies provide versatile strategies for engineering self-healing nanocomposite hydrogels with enhanced therapeutic and regenerative capabilities.

Structure–Property Relationships

The therapeutic performance of self-healing polymeric nanocomposite hydrogels is closely associated with their structural characteristics and resulting material properties. Parameters such as network architecture, crosslinking density, nanoparticle distribution, and polymer composition significantly influence mechanical behaviour, degradation, swelling, drug release, and biological performance, ultimately determining their suitability for biomedical applications.

Network architecture:

Network architecture is a fundamental determinant of the performance of self-healing polymeric nanocomposite hydrogels. The arrangement of polymer chains, crosslinking density, pore structure, and nanoparticle distribution collectively influence the physical, mechanical, and biological properties of the hydrogel system [4,18]. A well-organized network architecture provides structural stability while maintaining sufficient flexibility for self-healing and adaptive behavior [19,25]. The degree of crosslinking plays a critical role in controlling hydrogel characteristics. Highly crosslinked networks generally exhibit enhanced mechanical strength and reduced swelling, whereas loosely crosslinked structures offer greater flexibility, permeability, and self-healing efficiency [16,17]. Additionally, pore size and interconnectivity affect nutrient transport, cellular infiltration, and drug diffusion, which are essential for tissue engineering and controlled drug delivery applications [6]. The incorporation of nanoparticles further modifies network architecture by introducing additional physical or chemical interactions within the polymer matrix [17]. These interactions can reinforce the hydrogel structure, improve mechanical resilience, and enhance functional performance. Therefore, careful engineering of network architecture is essential for achieving an optimal balance between structural integrity, self-healing capability, and therapeutic effectiveness in advanced nanocomposite hydrogel systems [7,14].

Mechanical strength

Mechanical strength is a critical property of self-healing polymeric nanocomposite hydrogels, particularly for applications involving load-bearing tissues, injectable systems, and long-term implantation. Adequate mechanical stability ensures that the hydrogel can withstand physiological stresses while maintaining its structural and functional integrity [4,18].

Influence of Crosslinking Density

The mechanical behaviour of hydrogels is strongly influenced by crosslinking density. An increase in crosslinking generally enhances stiffness, tensile strength, and structural stability. However, excessive crosslinking may reduce flexibility and impair self-healing efficiency by limiting polymer chain mobility [16,19].

Role of Nanoparticle Reinforcement

The incorporation of nanoparticles can significantly improve the mechanical properties of hydrogel networks. Nanoparticles act as reinforcing agents by creating additional interaction sites within the polymer matrix, thereby increasing resistance to deformation and fracture [17]. Uniform nanoparticle distribution is essential for achieving effective stress transfer throughout the hydrogel structure.

Relationship with Self-Healing Performance

Mechanical strength and self-healing capability must be carefully balanced during hydrogel design. While stronger networks provide improved durability, highly rigid structures may hinder the dynamic interactions required for





Priyanka M Muddi et al.,

efficient self-repair [12,25]. Therefore, optimizing network composition and nanoparticle content is essential for developing hydrogels that combine robust mechanical performance with effective self-healing behaviour, ultimately enhancing their suitability for advanced biomedical and regenerative applications [7,14].

Injectability

Injectability is a highly desirable characteristic of self-healing polymeric nanocomposite hydrogels, particularly for minimally invasive biomedical applications. Injectable hydrogels can be administered through syringes or catheters, reducing surgical interventions, minimizing patient discomfort, and enabling localized therapeutic delivery [12]. Following injection, the hydrogel rapidly recovers its structure through self-healing mechanisms, ensuring retention at the target site and maintaining functional performance. The injectability of a hydrogel is primarily influenced by its rheological properties, polymer composition, and crosslinking mechanism. Dynamic and reversible interactions within the hydrogel network facilitate shear-thinning behaviour, allowing the material to flow under applied stress during injection and subsequently regain its original structure once the stress is removed [19,25]. This unique behaviour enhances handling properties while preserving mechanical integrity and biological activity. Nanoparticle incorporation can further improve injectability by modulating network interactions and optimizing flow characteristics. Consequently, injectable self-healing nanocomposite hydrogels have gained considerable attention for drug delivery, tissue engineering, and regenerative medicine applications, where precise administration and adaptability to irregular tissue defects are essential for therapeutic success [17].

Swelling and degradation

Swelling and degradation are important properties that influence the performance of self-healing polymeric nanocomposite hydrogels in biomedical applications. The swelling behavior determines the ability of hydrogels to absorb and retain biological fluids, which affects nutrient transport, drug diffusion, and cellular interactions within the hydrogel matrix [4,5]. An appropriate swelling capacity is essential for maintaining a favorable microenvironment for tissue regeneration and sustained therapeutic delivery. The extent of swelling is governed by factors such as polymer composition, crosslinking density, pore structure, and nanoparticle incorporation. Highly crosslinked networks generally exhibit lower swelling ratios, whereas loosely crosslinked structures allow greater water uptake [16,17]. Nanoparticles can further modulate swelling behaviour by altering network interactions and structural stability [17]. Degradation behaviour is equally critical, as it determines the residence time of the hydrogel at the implantation site. Controlled degradation enables gradual replacement of the hydrogel by newly formed tissue while ensuring sustained therapeutic activity. Therefore, balancing swelling and degradation characteristics is essential for optimizing the functionality and long-term performance of nanocomposite hydrogels [6,14].

Healing efficiency

Healing efficiency is one of the most important parameters for evaluating the performance of self-healing polymeric nanocomposite hydrogels. It refers to the ability of a damaged hydrogel network to restore its original structural, mechanical, and functional properties after experiencing physical disruption. High healing efficiency is essential for maintaining long-term stability and therapeutic performance in biomedical applications where hydrogels are exposed to repeated mechanical stress, deformation, or environmental fluctuations [19,25]. The healing efficiency of a hydrogel is primarily governed by the nature and reversibility of the interactions present within the polymer network. Dynamic covalent bonds and reversible non-covalent interactions facilitate network reconstruction by enabling broken bonds to reform after damage [20,24]. The mobility of polymer chains also plays a critical role, as sufficient chain movement promotes effective contact between damaged surfaces and accelerates the healing process [16,17]. Nanoparticle incorporation can significantly influence healing efficiency by providing additional interaction sites and reinforcing the hydrogel network. Properly dispersed nanoparticles contribute to improved stress distribution and facilitate the re-establishment of network connections following damage [17]. However, excessive nanoparticle loading may restrict polymer chain mobility and reduce healing performance. Several factors, including healing time, temperature, pH, crosslinking density, and nanoparticle concentration, can affect the extent of recovery achieved by self-healing hydrogels [12]. Therefore, optimizing these parameters is crucial for developing nanocomposite hydrogels with rapid and efficient self-repair capabilities. Enhanced healing efficiency not only





Priyanka M Muddi *et al.*,

prolongs material lifespan but also improves the reliability and effectiveness of hydrogel-based drug delivery systems and regenerative therapies [14].

Drug loading capacity

Drug loading capacity is a critical parameter that determines the therapeutic effectiveness of self-healing polymeric nanocomposite hydrogels. It reflects the ability of the hydrogel network to encapsulate and retain therapeutic agents while maintaining structural stability [5,18]. Drug loading is influenced by factors such as polymer composition, pore architecture, crosslinking density, and the presence of nanoparticles within the hydrogel matrix. The incorporation of nanoparticles can enhance drug loading capacity by providing additional binding sites and increasing the surface area available for drug adsorption or encapsulation [17]. Furthermore, interactions between the drug, polymer network, and nanoparticles contribute to improved drug retention and controlled release behavior. Therefore, optimizing hydrogel composition is essential for achieving high drug-loading efficiency and sustained therapeutic performance in biomedical applications [6,14].

Self-Healing Nanocomposite Hydrogels for Drug Delivery

Self-healing nanocomposite hydrogels have emerged as versatile drug delivery platforms capable of protecting therapeutic agents, enabling controlled release, and improving treatment efficacy. Their dynamic network structure and multifunctional properties support sustained, localized, and stimuli-responsive delivery for a wide range of biomedical applications.

Delivery of therapeutic agents

Self-healing nanocomposite hydrogels have gained significant attention as advanced platforms for the delivery of diverse therapeutic agents. Their highly hydrated three-dimensional networks provide a protective environment that facilitates the encapsulation and stabilization of drugs while minimizing premature degradation [5,18]. Owing to their self-healing nature, these hydrogels can maintain structural integrity following mechanical disruption, thereby ensuring sustained therapeutic performance. A wide range of therapeutic agents, including small-molecule drugs, proteins, peptides, growth factors, and nucleic acids, can be effectively incorporated within self-healing nanocomposite hydrogels [6,14]. The incorporation of nanoparticles further enhances loading efficiency and enables controlled interactions between the hydrogel matrix and encapsulated therapeutics. These interactions contribute to prolonged retention and regulated release profiles, improving treatment efficacy while reducing systemic side effects [17].

Furthermore, the injectable and adaptable characteristics of self-healing hydrogels allow localized delivery to target tissues, resulting in improved bioavailability and therapeutic outcomes. Consequently, self-healing nanocomposite hydrogels have emerged as promising carriers for advanced drug delivery applications, offering a versatile platform capable of accommodating a broad spectrum of therapeutic molecules [12].

Stimuli-responsive controlled drug release:

Stimuli-responsive self-healing nanocomposite hydrogels have emerged as intelligent drug delivery systems capable of releasing therapeutic agents in response to specific physiological or external triggers. This responsive behaviour enables precise control over drug release kinetics, thereby improving therapeutic efficacy while minimizing undesirable side effects [5,12]. The combination of self-healing capability and stimuli sensitivity allows these hydrogels to maintain structural stability while adapting to dynamic biological environments. Various stimuli, including pH, temperature, reactive oxygen species (ROS), and enzymatic activity, have been exploited to regulate drug release from hydrogel networks [4,6]. Changes in these physiological conditions can alter the hydrogel structure, trigger bond dissociation, or modify network permeability, facilitating the controlled release of encapsulated therapeutics [24]. Such responsiveness is particularly advantageous for site-specific drug delivery, as pathological tissues often exhibit unique microenvironmental characteristics compared to healthy tissues. The incorporation of functional nanoparticles further enhances stimulus sensitivity and release precision by providing additional responsive elements within the hydrogel matrix [17]. As a result, stimuli-responsive self-healing





nanocomposite hydrogels offer a promising approach for achieving personalized and targeted therapeutic interventions across a wide range of biomedical applications [14]. Figure 3 illustrates the major mechanisms governing drug release from self-healing nanocomposite hydrogels, including diffusion, swelling, degradation, nanoparticle-mediated release, and stimuli-responsive release.

Self-Healing Nanocomposite Hydrogels in Tissue Regeneration

Self-healing nanocomposite hydrogels have emerged as promising biomaterials for tissue regeneration owing to their ability to mimic the native extracellular matrix, support cellular activities, and provide sustained therapeutic functions. Their dynamic self-repair capability, combined with enhanced mechanical and biological properties, facilitates tissue repair and regeneration across a wide range of biomedical applications. Table 1 highlights the impact of nanoparticle incorporation on the structural, functional, and therapeutic properties of self-healing hydrogels.

Soft tissue regeneration:

Self-healing nanocomposite hydrogels have demonstrated significant potential in the regeneration of soft tissues, including skin, neural, and cardiac tissues. Their highly hydrated structure provides a favorable microenvironment that supports cell adhesion, proliferation, migration, and extracellular matrix deposition [29]. In wound healing applications, these hydrogels can maintain a moist environment, protect damaged tissues, and enable the localized delivery of therapeutic agents, thereby accelerating tissue repair [7]. The self-healing capability of these hydrogels ensures structural integrity even under repetitive mechanical stress, which is particularly important in dynamic tissues such as skin and myocardium [12]. Furthermore, the incorporation of bioactive nanoparticles can enhance antimicrobial activity, angiogenesis, and cellular signalling, contributing to improved regenerative outcomes [14]. In neural tissue engineering, self-healing hydrogels provide adaptable scaffolds that support neuronal growth and tissue integration. Similarly, in cardiac repair, their injectable and self-recovering properties facilitate minimally invasive administration and effective retention at the injury site. Consequently, self-healing nanocomposite hydrogels represent versatile platforms for promoting soft tissue regeneration and functional recovery following injury or disease [6,14].

Hard tissue regeneration:

Self-healing nanocomposite hydrogels have gained considerable attention in hard tissue regeneration, particularly for bone and cartilage repair. These tissues require biomaterials with adequate mechanical stability, biocompatibility, and the ability to support cellular growth and differentiation. The incorporation of nanoparticles within hydrogel networks enhances mechanical strength and provides structural support that closely resembles the native extracellular matrix [17]. In bone regeneration, self-healing hydrogels serve as injectable scaffolds capable of filling irregular defects while delivering bioactive molecules that promote osteogenesis. Their dynamic network structure enables adaptation to the surrounding tissue environment and facilitates long-term integration with newly formed bone [14]. Similarly, in cartilage regeneration, these hydrogels provide a hydrated and flexible matrix that supports chondrocyte proliferation and extracellular matrix production. The self-healing capability further improves durability under repetitive mechanical loading. Consequently, self-healing nanocomposite hydrogels have emerged as promising candidates for repairing and regenerating hard tissues while enhancing functional recovery and tissue integration [6,14].

Advanced tissue engineering applications:

The advancement of tissue engineering has increased the demand for biomaterials capable of supporting the regeneration of complex and highly organized tissues. Self-healing nanocomposite hydrogels have emerged as attractive candidates due to their ability to combine structural adaptability, biological functionality, and therapeutic delivery within a single platform [6,14]. One of the most significant applications involves vascularized tissue engineering, where the formation of functional blood vessel networks is essential for nutrient transport and long-term tissue survival. Self-healing hydrogels provide a supportive microenvironment that promotes cellular organization, angiogenesis, and tissue maturation [14]. Their dynamic network structure allows continuous remodeling, closely resembling the behavior of native extracellular matrices. In addition to vascularized tissues,





Priyanka M Muddi *et al.*,

these hydrogels have shown potential for the development of complex tissue constructs involving multiple cell types and biological components. The incorporation of nanoparticles can further enhance mechanical properties, bioactivity, and controlled delivery of regenerative factors, thereby improving overall tissue development [17]. Furthermore, their injectable and self-repairing characteristics facilitate minimally invasive administration and improve scaffold longevity following implantation [12]. Collectively, these advantages position self-healing nanocomposite hydrogels as versatile platforms for next-generation tissue engineering strategies aimed at restoring tissue structure, functionality, and long-term biological integration. Table 2 summarizes representative applications of self-healing nanocomposite hydrogels in tissue regeneration, highlighting their functional roles and regenerative outcomes across different tissue types.

CONCLUSIONS AND FUTURE PERSPECTIVES

Self-healing polymeric nanocomposite hydrogels have emerged as dynamic therapeutic platforms with significant potential in drug delivery and tissue regeneration. Their unique ability to combine self-repairing behaviour, multifunctionality, and biological compatibility has expanded their biomedical applicability. Continued advances in material design, fabrication technologies, and translational research are expected to accelerate the development of clinically relevant hydrogel systems. Despite existing challenges, these materials represent a promising foundation for next-generation regenerative therapies and precision medicine approaches.

Key Advances and Current Limitations

Significant progress has been achieved in the development of self-healing nanocomposite hydrogels through the integration of dynamic crosslinking mechanisms and functional nanomaterials. These systems exhibit enhanced mechanical performance, controlled drug delivery capabilities, improved biological activity, and superior regenerative potential. Their ability to recover from mechanical damage has further expanded their utility in demanding biomedical environments. Despite these advancements, several limitations remain. Challenges related to large-scale manufacturing, reproducibility, sterilization, long-term safety, and regulatory approval continue to hinder clinical translation. In addition, balancing mechanical strength, self-healing efficiency, degradation behaviour, and therapeutic performance remains a complex design challenge that requires further investigation.

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Conflict Of Interest

The authors declare that there is no conflict of interest.

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Ethical Statement

This study did not involve any human or animal subjects, and hence, ethical approval was not required.

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Priyanka M Muddi et al.,

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Table 1. Effect of Nanoparticle Incorporation on Hydrogel Properties

Property	Effect of Nanoparticles	Significance
Mechanical strength	Improves stiffness and toughness	Enhances durability
Self-healing efficiency	Provides additional interaction sites	Promotes rapid repair
Swelling behaviour	Regulates water uptake	Controls diffusion and transport
Degradation	Modifies degradation rate	Supports sustained functionality
Drug loading	Increases loading capacity	Improves therapeutic delivery
Drug release	Enables controlled release	Enhances treatment efficacy
Injectability	Improves flow behaviour	Facilitates administration
Biocompatibility	Enhances cell interactions	Supports tissue regeneration
Bioactivity	Provides antimicrobial and antioxidant functions	Promotes healing
Stimuli responsiveness	Increases sensitivity to external stimuli	Enables targeted drug release

Table 2. Recent Applications of Self-Healing Nanocomposite Hydrogels in Tissue Regeneration

Tissue Type	Hydrogel System	Key Function	Regenerative Outcome
Skin/Wound	Self-healing nanocomposite hydrogel	Moist wound environment and drug delivery	Accelerated wound closure
Bone	Nanoparticle-reinforced hydrogel scaffold	Enhanced mechanical support and osteogenesis	Improved bone regeneration
Cartilage	Injectable self-healing hydrogel	Supports chondrocyte growth	Cartilage tissue repair
Cardiac	Conductive self-healing hydrogel	Promotes electrical signal transmission	Improved cardiac repair
Neural	Self-healing bioactive hydrogel	Supports neuronal growth and integration	Enhanced neural regeneration
Vascularized tissues	Multifunctional nanocomposite hydrogel	Promotes angiogenesis and tissue remodeling	Improved vascularized tissue formation





Priyanka M Muddi et al.,

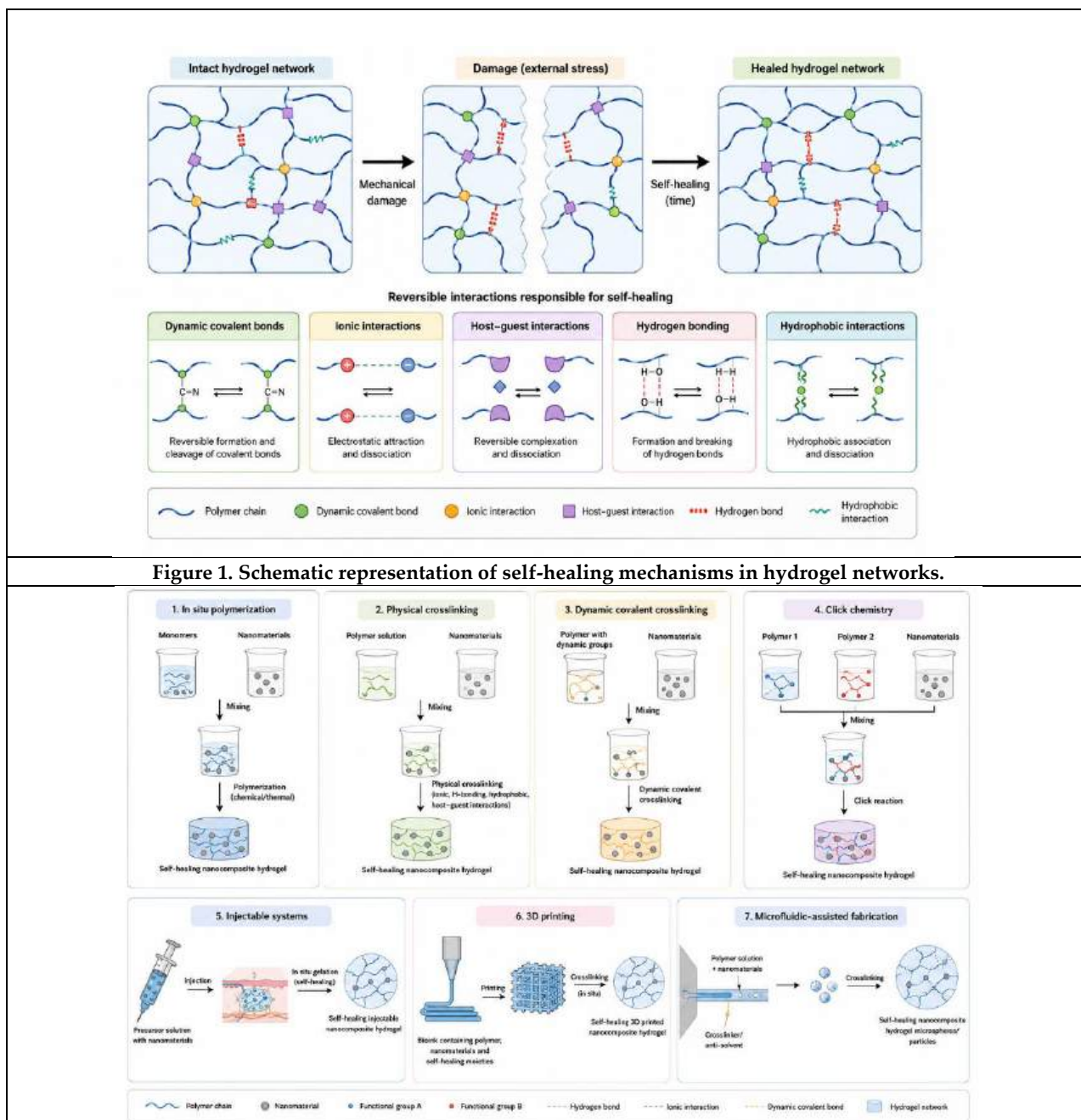


Figure 1. Schematic representation of self-healing mechanisms in hydrogel networks.

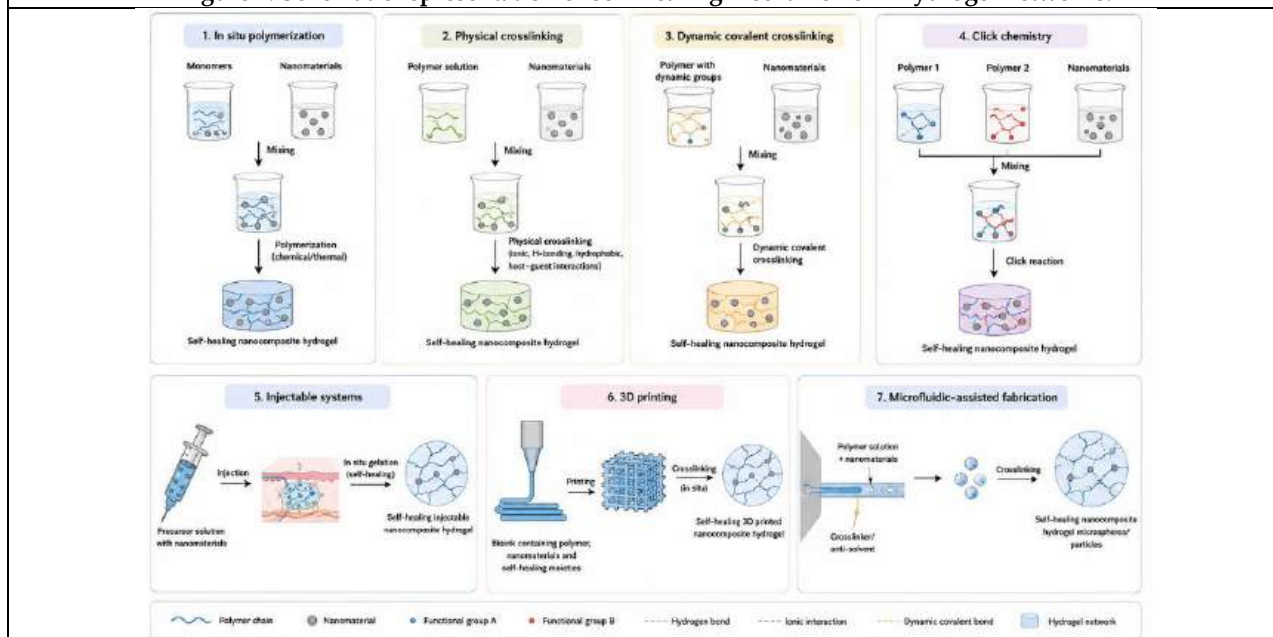


Figure 2. Fabrication approaches for self-healing nanocomposite hydrogels, including conventional and advanced techniques for hydrogel network engineering and nanoparticle incorporation.





Priyanka M Muddi et al.,

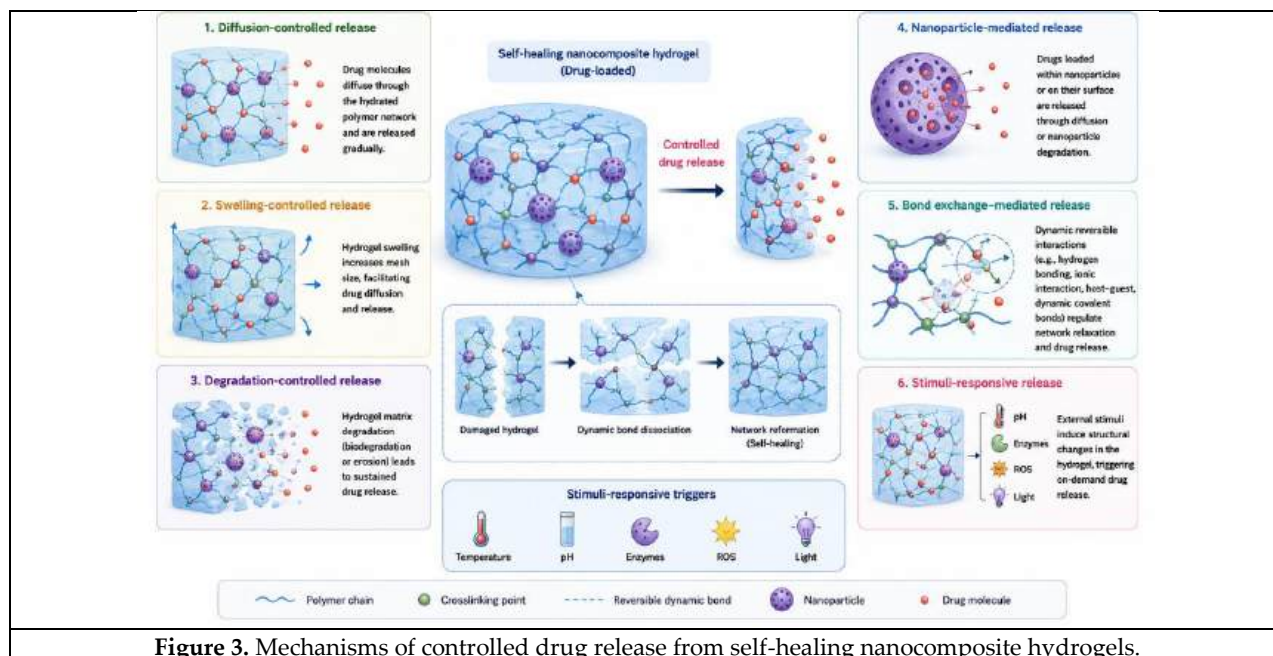


Figure 3. Mechanisms of controlled drug release from self-healing nanocomposite hydrogels.

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RESEARCH ARTICLE

From Radiology to Forensics: Diagnostic Value of Skull CT in Human Identification

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ABSTRACT

Sex determination in unidentified skeletons is not always easily and correctly performed by a non-specialist without formal training in forensic anthropology. In explosions, warfare and other mass disasters like aircraft crashes, identification may be extremely complicated because of skeletal fragmentation. Sexual dimorphism, the anatomical and physiological differences between males and females, has been a subject of extensive research in forensic and anthropological studies. This systematic review aims to consolidate and analyze existing literature on the measurement of two crucial anatomical structures, the foramen magnum and optical foramen, as potential indicators of sex dimorphism. The foramen magnum, a key opening at the base of the skull, and the optical foramen, an opening in the eye socket, are recognized as potential sites for observing sexual dimorphism due to their involvement in various physiological functions. Numerous studies have employed diverse measurement techniques, such as traditional anthropometric methods and advanced imaging technologies, to quantify size and shape differences between male and female populations. The current review systematically assesses the methodologies, sample sizes, and statistical approaches utilized in the selected studies. Additionally, it explores the reliability and validity of these measurements as indicators of sexual dimorphism. Special attention is given to variations among different populations, ethnic groups, and age categories, acknowledging the importance of considering diverse demographic factors in sex estimation. Furthermore, the review discusses the potential applications of sex dimorphism studies in forensic anthropology, paleoanthropology, and clinical settings. Understanding the inherent variations in



**Ankit Kumar and Mahesh K. Mittal**

foramen magnum and optical foramen dimensions contributes not only to the field of biological anthropology but also holds implications for personalized medicine and forensic identification. By critically evaluating the existing body of literature, this systematic review provides valuable insights into the current state of knowledge regarding the measurement of foramen magnum and optical foramen for sex dimorphism. The findings underscore the need for standardized methodologies and highlight avenues for future research to enhance the accuracy and applicability of these anatomical features in sex estimation.

Keywords: Computed Tomography, Foramen Magnum, Optical foramen, Measurement, Sex dimorphism

INTRODUCTION

Sexual dimorphism, the morphological and physiological differences between males and females within a species, is a fundamental aspect of biological diversity. Foramen magnum anatomy help to anthropological and forensic contexts, the accurate determination of sex from skeletal remains is a crucial step in reconstructing the demographic profile of an individual. Over the years, researchers have explored various anatomical markers to discern these sex-based distinctions, with a growing focus on the foramen magnum and optical foramen as potential indicators⁽¹⁾ The foramen magnum, situated at the base of the skull, plays a pivotal role in transmitting the spinal cord and facilitating the connection between the brain and the spinal column. Likewise optical foramen anatomy , an opening in the eye socket, serves as a conduit for the optic nerve and blood vessels. Both structures are integral to essential physiological functions, making them intriguing candidates for the study of sexual dimorphism⁽²⁾ This systematic review endeavors to comprehensively examine the existing body of literature surrounding the measurement of foramen magnum and optical foramen dimensions as potential discriminators between male and female individuals. By systematically collating and synthesizing relevant studies, we aim to provide a comprehensive overview of the methodologies employed, the populations studied, and the reliability of these measurements in sex estimation.

The significance of understanding sex-based variations in these anatomical features extends beyond the realms of anthropology, reaching into forensic science, paleoanthropology, and clinical applications. Accurate sex estimation from skeletal remains not only contributes to the reconstruction of past populations but also plays a vital role in contemporary forensic investigations and medical diagnostics.⁽³⁻⁴⁾As we embark on this systematic review, our objectives are to critically evaluate the methodologies employed in measuring foramen magnum and optical foramen, assess the reliability and validity of these measurements as indicators of sex, and highlight potential avenues for future research. Through this synthesis, we aspire to contribute valuable insights to the field, fostering a deeper understanding of the intricacies involved in utilizing these anatomical landmarks for sex dimorphism studies.⁽⁵⁻⁶⁾Moreover, the selection of the foramen magnum and optical foramen as focal points for sex estimation is grounded in their unique anatomical and functional characteristics. The foramen magnum, being the primary opening through which the spinal cord passes, undergoes intricate developmental processes that may exhibit sex-specific variations. Similarly, the optical foramen, which accommodates crucial neurovascular structures, may reflect sex-related distinctions in skeletal morphology.⁽⁷⁾ The current state of research in this field reveals a diverse array of measurement techniques, ranging from traditional anthropometric calipers to advanced imaging technologies such as computed tomography (CT) scans and three-dimensional reconstructions. The choice of methodology can significantly impact the accuracy and reliability of sex estimation, prompting a critical evaluation of the strengths and limitations associated with each approach. Furthermore, the existing literature reflects a notable diversity in the populations studied, including variations in ethnicity, geographic origin, and chronological periods. Recognizing these demographic nuances is imperative for extrapolating findings to different populations and refining the applicability of sex estimation techniques across diverse groups.⁽⁸⁾In the forensic domain, the ability to accurately





determine the sex of an individual from skeletal remains holds immense practical significance. This review aims to elucidate the potential forensic applications of foramen magnum and optical foramen measurements, addressing their utility in medico-legal investigations, disaster victim identification, and the resolution of cold cases. Additionally, insights from paleo-anthropological perspectives can shed light on the evolutionary aspects of sexual dimorphism, contributing to our understanding of human population dynamics across time. As we delve into the systematic exploration of the literature, we will meticulously assess the statistical methodologies employed in sex dimorphism studies, emphasizing the importance of standardized protocols to enhance comparability across investigations. This review will also highlight gaps in existing research, paving the way for future studies to address unanswered questions and refine our understanding of the intricate relationship between foramen magnum and optical foramen dimensions and sexual dimorphism.⁽⁹⁾ By synthesizing available evidence, we strive to provide a comprehensive overview that not only contributes to the academic discourse but also informs forensic practitioners, anthropologists, and medical professionals about the potential applications and limitations of utilizing these anatomical features in the determination of sex from skeletal remains.⁽¹⁰⁾ The optic canal is the narrowest point of the optic nerve subarachnoid space.⁽²⁵⁾ As a watershed region for cerebrospinal fluid (CSF) flow, it has become a landmark of interest in ocular pathologies that involve the translaminal pressure difference (TLPD), including glaucoma and papilloedema from increased intracranial pressure (ICP) or space flight-associated neuro-ocular syndrome (SANS).⁽²⁶⁻²⁸⁾

Objectives

Objective: To estimate the foramen magnum is useful for gender determination, To estimate the optical foramen is useful for gender determination, Both foramen magnum and optical foramen helps for forensic expert for determination of both sexes.

MATERIAL METHODS

This study was done at department of medical radiology and imaging technology, Subharti College of allied and healthcare, swami Vivekanand subharti university, meerut, uttarpradesh, india. Article for review are taken from different research and journal websites like web of science, scopus, pubmed and different journal websites. After studying the different article, they were used Computed Tomography for measurement of foramen magnum and optical foramen. NCCT Head and CECT head patient was used for sample collection/ data collection. They were used convenient sampling for their study. They measured of foramen magnum and optical foramen length like AP and TR length as shown in figure: 1 and 2 . These are taken from department CT console. In above table: 1 states about differentiate among different researcher on different approaches, study sites, study part and study population. This data was taken from different journal website and pubmed etc. After analyzing the comparative table was created.

DISCUSSION

In this comprehensive review on Measurement of Foramen Magnum and Optical Foramen for Sex Dimorphism was done at department of medical radiology and imaging technology, subharti college of allied and healthcare, swami Vivekanand subharti university, meerut, uttar Pradesh west, india. This review objective is to measure the foramen magnum and optical foramen. Abtehag A Taib, et al. was concluded from their study that the commonest type was hexagonal which is followed by irregular type A. Foramen of monro parameters was smaller in females than males. Foramen magnum types and parameters was consider during surgical interventions and radiology reports.⁽¹¹⁾ Moodley M., et al. was describe that there was no relationship between foramen magnum shape and gender. They were observed simultaneously in 3 age categories suchas children, adolescents and adults. The common dimension in children was the oval shape 8.67%, (hexagonal 7.33), aldolism 10%, adults. Its minimum and maximum TR were and 23.75 mm 37.10 mm, respectively. The mean area was $800.637 \pm 116.59 \text{ mm}^2$. The minimum and maximum areas of FM were 501.53 mm^2 and 1132.96 mm^2 , respectively. They conclude from their study that FM morphology showed only





a significant difference in age, not gender, of 4,444 people. (12) Aghakhani K, et al. was observed that the transverse diameter of the foramen of monro and the area was greater in male than in female. Based on cut-off point for AP foramen magnum, transverse diameter, Teixeira formula and routhal formula all these indicators, specificity and sensitivity was equal to 100% and 92% and all the individual have equal size or more of the value in men. 96% was overall accuracy of indicators was calculated as 85%. Finally they concluded from their study was using CT scan images, the foramen magnum diameter and its area was showed a high precision in sexual dimorphism. (13) Mayuri J., et al. was concluded from their study that there were higher averages of width, length, area and circumference of the foramen of monro in both cases and also found that gender in the area of the foramen of monro was additional reliably predicted. (14) Ayfer Metin T., et al. was provides indications that CT is used for anthropological studies, and indicates the standard area of the foramen of monro in the normal populace and suggests that the images can aid in the diagnostic procedure of some diseases. (15)

S. kumar, et al. was conclude from their study that the measurement of the antero posterior and transverse diameters of the foramen of monro and the frontal nasal angle was the most reproducible 3D technique. For 3D volume measurement, in view of software requirements, a fast and reliable tool for diameter measurement for physiological differences of foramen of monro and angular anterior nasal junction was considered useful for sex dimorphism in human skulls of the populace of southern India. (16) Harshita R., et al. concluded from their study that the two dimensions of the basioccipita and the foramen of monro were used precisely to evaluate sex. This opens up opportunities for new research in the field of the study of people and extremely careful, crime-solving, science-based medicine. (17) S. P. V., et al. was conducted a retrospective Study in year 2018 at the Radio Diagnosis department, Kempegowda IOMS, Bangalore, Karnataka, India on Computerized Tomographic Images and obtained approval for their research from the institutional ethics. According to the results of the Martin and Saller classification, the medium type foramen index was shown. The combined mean index was found to be 84.27 ± 8.35 . For the foramen magnum index, a female was compared to the male, the foramen magnum index was elevated. They concluded from their study that the size and measurement of the foramen of monro were taken into account in operation at the cranio-vertebral junction. It was also used for anthropological and forensic examinations of unknown persons. The foramen magnum determines sex, ethnicity. They state that multivariate analysis was the best method of sexing the skull with the available resources. (18) RIA-K., et al. was conclude from their study that the dimension of the basioccipital and foramen of monro are larger in men and that the width of the foramen magnum and the length of the left occipital condyle are successful for sex dimorphism and a gross precision of 90% and an accuracy of 91.3% and 87.5. % were used in men and women. (19) Radhakrishna S.K., et al. was received the result of their research that the oval shape was the most similar, observed by round, pentagonal and tetragonal in both sexes. The transverse diameter (28.63 vs 26.59) and anteroposterior diameter was (34.04 vs 31.72) was remarkably greater in men than in women ($P < 0.05$). Their research was concluded to give a statistically useful indication of the genus of the unspecified skull. (20) AT U., et al. was calculated from their study that for forensic study on sexual dimorphism measurement of foramen magnum was valuable. It tends to balance after life time of second and reconstructed computerized tomography scan was produce appropriate evaluation for their size. (21) Kumar A., et al. was conclude from their study that foramen magnum and fronto nasal junction was useful for sex determination in western up population. They found significant result from their study. (22)

CONCLUSION

Our review study provides valuable insights into the potential of these anatomical features for distinguishing between male and female skulls. Studies consistently show that the dimensions of the foramen magnum and optical foramen tend to exhibit sexual dimorphism, with males generally having larger measurements. However, the degree of sexual dimorphism varies across populations and methodologies used in different studies. While these measurements can be useful as part of a broader anthropological or forensic toolkit, they are not definitive on their own. Future research should focus on standardizing measurement techniques and expanding data collection across diverse populations to improve the accuracy and applicability of these metrics in sex estimation.





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Ankit Kumar and Mahesh K. Mittal

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Table:1 Comparison between different researchers study

S.No.	References	Country	Methodology	Total No. of Patients Data	Approach	Part Studied
1.	Abtehag A Taib, et.al, (2019) ¹¹	Libiya	Descriptive	150	Gender	Foramen Magnum
2.	Moodley M., et.al, (2019) ¹²	Durban	Retrospective	150	Gender	Foramen Magnum
3.	Aghakhani k., et.al, (2016) ¹³	Iran	Cross-sectional	100	Gender	Foramen Magnum
4.	Mayuri J., et.al, (2016) ¹⁴	India	Retrospective	280	Age	Foramen Magnum
5.	Ayfermetin T., et.al, (2016) ¹⁵	Turkey	Retrospective	100	Age	Foramen Magnum
6.	Suresh k., et.al, (2012) ¹⁶	India	Retrospective	54	Age	Foramen Magnum and Fronto Nasal Junction
7.	Harshita R., et.al, (2017) ¹⁷	India	Retrospective	200	Age, Gender, location	Foramen Magnum
8.	S.p.v., et.al, (2018) ¹⁸	India	Retrospective	100	Gender	Foramen Magnum
9.	Ria K., al, (2015) ¹⁹	Egypt	Descriptive and	70	Gender	Foramen





Ankit Kumar and Mahesh K. Mittal

			Cross-sectional			Magnum
10.	Radhakrishnan S.K., et.al, (2012) ²⁰	India	Prospective	4444	Gender	Foramen Magnum
11.	AT U., et.al, (2012) ²¹	Iraq	Prospective	88	Gender	Glabella
12.	Ankit k., et.al, (2022) ²²	India	Retrospective	150	Age and Gender	Foramen Magnum and Fronto Nasal Junction
13.	Nguyen VD et.al, (2023) ²³	Vietnam	Retrospective	200	Optic foramen location	Orbit
14.	Zhan X. et.al, (2023) ²³	USA	Retrospective	335	Optic canal anatomy and symmetry	Optical canal

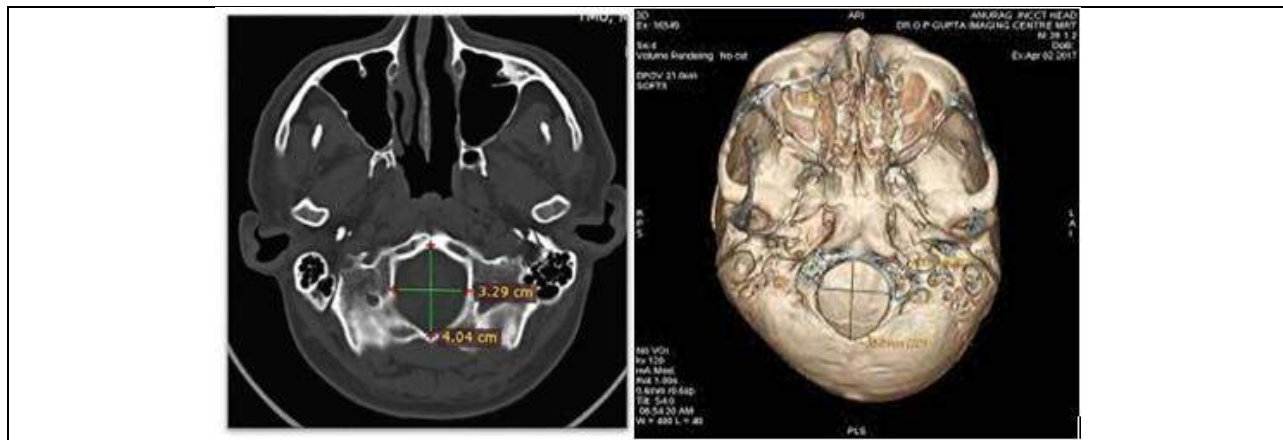


Figure 1: Measurement of Foramen Magnum in Computed Tomography

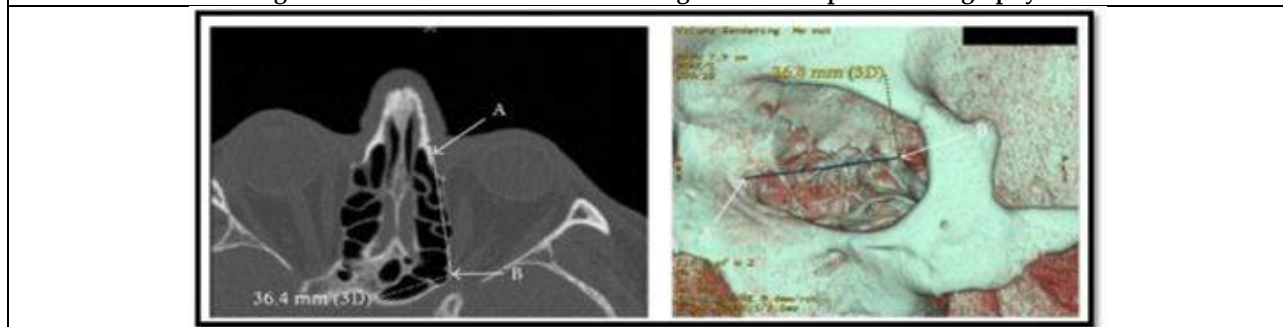


Figure 2: Measurement of Optical Foramen in Computed Tomography

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RESEARCH ARTICLE

Development and Validation of an Integrative Screening Tool for Assessing Processed and Ultra-Processed Food Consumption among Indian Adolescents

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ABSTRACT

Increasing consumption of processed and ultra-processed foods (UPFs) among adolescents is a growing public health concern in India, contributing to rising non-communicable diseases (NCDs) [1,2]. However, rapid screening tools for assessing such consumption remain limited. To develop and validate an integrative screening tool for assessing processed and ultra-processed food consumption among adolescents. A cross-sectional study was conducted among 50 adolescents aged 10–18 years. Dietary intake was assessed using a Food Frequency Questionnaire based on NOVA classification and a 24-hour recall method. Content validity was assessed using Content Validity Index (CVI), and reliability was evaluated using Cronbach's alpha. Statistical analyses included descriptive statistics and chi-square tests. The tool demonstrated good internal consistency, with Cronbach's alpha values of 0.869 for processed foods and 0.913 for ready-to-eat ultra-processed foods. Moderate reliability was observed for beverages ($\alpha = 0.677$). Content validity was moderate for ultra-processed food categories (CVI = 0.50–0.57), while initial validity for processed foods was lower (CVI = 0.268), which improved following item refinement. Significant gender differences were observed in the consumption of flavoured milk ($p = 0.015$), yogurt-based drinks ($p = 0.007$), fast food items ($p = 0.032$), and ice cream products ($p = 0.024$). The developed screening tool demonstrated preliminary evidence of validity and good reliability for assessing processed and ultra-processed food consumption among adolescents and can be applied in primary care and community settings.

Keywords: Ultra-processed foods, adolescents, NOVA classification, screening tool, validation, India



**Shubhi Agarwal and Lubna Asif**

INTRODUCTION

Processed and ultra-processed foods have become a dominant component of modern diets due to rapid urbanization, industrialization, globalization, and changing food habits.[1] These foods are generally energy-dense and often contain excessive amounts of added sugars, sodium, saturated fats, preservatives, and additives, which contribute to poor dietary quality and increased risk of obesity, cardiovascular diseases, metabolic disorders, and other non-communicable diseases.[2,3] The NOVA food classification system categorizes foods according to the nature, extent, and purpose of industrial processing and has become an important framework for evaluating dietary patterns and food consumption behaviors.[4] Growing evidence indicates that higher intake of ultra-processed foods is associated with adverse health outcomes and unhealthy lifestyle behaviors among children and adolescents. [3,5] In India, dietary transitions among adolescents have accelerated due to changing food environments, increased exposure to packaged foods, aggressive food marketing strategies, and greater accessibility of convenience foods.[6] Although commonly used dietary assessment methods such as food frequency questionnaires and 24-hour dietary recalls provide information regarding food intake patterns, these methods frequently fail to adequately capture the extent of food processing.[7]

Objectives

- To assess the consumption pattern of processed and ultra-processed foods among adolescents aged 10–18 years.
- To develop an integrative screening tool for assessing processed food consumption.
- To evaluate the validity and reliability of the developed screening tool.

METHODOLOGY

Study design and setting

A cross-sectional methodological study was conducted to develop and evaluate an integrative screening tool for assessing processed and ultra-processed food consumption among adolescents. Data collection was carried out across various schools in Bengaluru, India.

Study participants and sampling

Participants were recruited using convenience sampling based on predefined eligibility criteria. Adolescents aged 10–18 years who were willing to participate and provide assent/consent were included in the study. Participants with chronic illnesses, medically prescribed dietary restrictions, or incomplete dietary information were excluded.

Sample size

A pilot sample of 50 adolescents was included for preliminary evaluation of the validity and reliability of the developed screening instrument.

Dietary assessment procedures

Dietary intake was assessed using a Food Frequency Questionnaire (FFQ) developed according to the NOVA food classification framework and supplemented using a 24-hour dietary recall method. Food items were categorized into processed foods and ultra-processed foods based on the degree and purpose of industrial processing.

Development of scoring criteria

A frequency-based scoring system was developed to quantify food consumption patterns. Scores were assigned according to consumption frequency and cumulative scores were categorized into low, moderate, and high consumption groups.



**Shubhi Agarwal and Lubna Asif****Validation procedure**

Content validity of the screening instrument was assessed by an expert panel comprising approximately 20 subject experts. Experts evaluated individual food items for relevance, clarity, appropriateness, and comprehensiveness. The Content Validity Index (CVI) was computed, and food items demonstrating lower validity scores were revised according to expert recommendations.[8]

Reliability assessment

Internal consistency of the tool was assessed using Cronbach's alpha coefficient.

Statistical analysis

Statistical analyses included descriptive statistics such as means, standard deviations, frequencies, and percentages. Associations between variables were assessed using chi-square tests. Statistical significance was considered at $p < 0.05$.

Ethical considerations

Participation in the study was voluntary and informed consent/assent was obtained from participants and/or parents prior to data collection. Confidentiality and anonymity of participant information were maintained throughout the study.

RESULTS**Participant Characteristics**

A total of 50 adolescents participated in the study, including 15 males (30%) and 35 females (70%). The mean age of participants was 15.28 ± 2.51 years. More than half of participants belonged to the 16–18 years age category, indicating greater representation of older adolescents

Anthropometric Measurements

in Table 2. Mean height, weight, and BMI values showed increasing trends with age. Mean BMI values largely remained within acceptable ranges according to age-specific anthropometric assessment standards recommended by the World Health Organization.[9] Slight variations were observed across sex groups.

Validity of the Tool

Content validity assessment demonstrated moderate validity for ultra-processed food categories, with CVI values ranging between 0.50 and 0.57. Initial validity for processed food categories was comparatively lower (CVI=0.268); however, item modifications and expert recommendations improved content appropriateness.

Consumption Patterns

Consumption pattern analysis based on Tables 5–12 demonstrated varying frequencies of processed and ultra-processed food intake among adolescents. Among processed foods, frequently consumed items included idli/dosa batter, snack products, and selected convenience foods. Consumption of ultra-processed foods was more common for beverages, ready-to-eat products, and fast-food items. Most food items showed no statistically significant gender differences; however, significant differences were observed for flavoured milk ($p=0.015$), yogurt-based beverages ($p=0.007$), fast foods ($p=0.032$), and ice cream products ($p=0.024$). These findings indicate variability in consumption patterns and highlight specific food categories requiring attention for dietary interventions.

Classification of Consumption

Participants were categorized into low, moderate, and high consumption groups based on scoring criteria, enabling identification of individuals at risk of excessive processed food intake.



**Shubhi Agarwal and Lubna Asif****Reliability of the Tool**

Reliability analysis demonstrated acceptable to excellent internal consistency. The highest reliability was observed among ready-to-eat and ready-to-cook items ($\alpha=0.913$), whereas beverage categories demonstrated moderate reliability ($\alpha=0.677$). Processed food categories demonstrated good internal consistency ($\alpha=0.869$).

DISCUSSION

The present study aimed to develop and evaluate an integrative screening tool for assessing processed and ultra-processed food consumption among adolescents. Findings indicate increasing consumption of processed and ultra-processed food products among adolescents, reflecting changing dietary behaviours and evolving food environments. Similar trends have been reported in previous studies demonstrating increased consumption of convenience foods and packaged products among adolescents due to urbanization, changing lifestyles, peer influence, and greater accessibility of commercially processed foods. [3,6] Such dietary transitions are concerning because adolescence represents a critical period during which long-term food preferences and dietary habits are established. Significant gender differences observed in flavoured milk products, yogurt-based beverages, fast foods, and ice cream products may reflect differences in food preferences, sociocultural influences, and dietary practices among adolescents.

The developed screening tool demonstrated good internal consistency and preliminary evidence of validity. Cronbach alpha values indicated strong reliability across major food categories. The expert review process involving approximately twenty experts strengthened content relevance and contributed to refinement of individual food items. The study has several limitations. The sample size was relatively small and participants were recruited using convenience sampling, which may limit generalizability. Dietary intake assessment relied partly on self-reported information and may therefore be susceptible to recall bias. Future studies involving larger and more diverse samples are required to further evaluate psychometric properties of the instrument. Despite these limitations, this study represents an initial effort toward development of a culturally relevant screening instrument for Indian adolescents that may facilitate rapid identification of unhealthy dietary patterns in school and community settings.

CONCLUSION

The present study developed a preliminary screening tool for assessing processed and ultra-processed food consumption among adolescents. The instrument demonstrated good reliability and preliminary evidence supporting validity, indicating its potential use as a rapid screening tool in school, community, and primary healthcare settings. Future studies involving larger populations are needed to establish broader applicability and strengthen psychometric evaluation.

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Shubhi Agarwal and Lubna Asif

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Table 1: Demographic Characteristics of Participants

Variable	Male (n=15)	Female (n=35)	Total (n=50)
Age (Mean $\pm$ SD)	14.80 $\pm$ 2.78	15.20 $\pm$ 2.17	15.28 $\pm$ 2.51
10–12 years (%)	10%	10%	20%
13–15 years (%)	7%	55%	24%
16–18 years (%)	18%	38%	56%

Table 2: Anthropometric Characteristics of Participants

Parameter	Male (n=15) Mean $\pm$ SD	Female (n=35) Mean $\pm$ SD	Overall Mean $\pm$ SD
Age (years)	14.80 $\pm$ 2.78	15.20 $\pm$ 2.17	15.28 $\pm$ 2.51
Height (cm)	159.33 $\pm$ 8.41	157.19 $\pm$ 5.85	157.84 $\pm$ 6.78
Weight (kg)	59.56 $\pm$ 9.30	53.11 $\pm$ 8.84	55.04 $\pm$ 9.20
BMI (kg/m ²)	23.54 $\pm$ 3.93	21.52 $\pm$ 3.60	22.10 $\pm$ 3.78

Table 3: Content Validity of the Screening Tool

Category	CVI	Interpretation
Processed Foods	0.268	Low
UPF – Drinks & Beverages	0.575	Moderate
UPF – Ready-to-eat & cook	0.500	Moderate

Table 4: Reliability of the Screening Tool

Category	Subcategory	Cronbach's Alpha	Interpretation
Processed Foods	—	0.869	Good
Ultra-processed Foods	Drinks & Beverages	0.677	Acceptable
Ultra-processed Foods	Ready-to-eat & Cook	0.913	Excellent

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RESEARCH ARTICLE

Factor - Derived Behavioral Domains Predicting Uptake of Diabetic Retinopathy Screening Among Rural Adults in Western Uttar Pradesh, India

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ABSTRACT

To identify latent behavioral domains influencing diabetic retinopathy screening uptake among adults in Western Uttar Pradesh using exploratory factor analysis of knowledge, attitude, and practice variables. A community-based cross-sectional study was conducted among 352 adults residing in Western Uttar Pradesh, India. Data were collected using a structured interviewer-administered questionnaire assessing knowledge, attitude, and practice related to diabetes, hypertension, diabetic retinopathy, retinal examination, and healthcare utilization. Exploratory factor analysis was performed on 19 selected variables using Principal Component Analysis with Varimax rotation and Kaiser normalization. Factors with eigenvalues greater than 1 were retained. Descriptive statistics were used to summarize socio demographic and clinical characteristics. The mean age of participants was 53.70 ± 10.03 years, and 53.7% were females. Nearly half of the participants (45.2%) had no formal education. Diabetes alone was reported by 41.2% of participants, while 27.0% had both diabetes and hypertension. Exploratory factor analysis identified six latent behavioral domains explaining 61.47% of the total variance. The extracted domains included retinal examination and eye care utilization behavior, hypertension-related awareness, preventive diabetes care awareness, diabetic eye disease awareness, general medical care utilization, and need for continued ophthalmic care. The strongest factor loadings were observed for awareness of dilated retinal examination (0.853), uptake of dilated examination (0.823), knowledge regarding hypertension-related diabetic complications (0.831), and recent eye doctor visit history (0.717). Diabetic retinopathy



**Shikha Sharma and Ashish Chander**

screening behavior is influenced by multiple interconnected awareness and healthcare utilization domains. Significant awareness-practice gaps exist, highlighting the need for targeted education and integrated retinal screening strategies in rural populations.

Keywords: Diabetic retinopathy, Factor analysis, Knowledge-attitude-practice, Retinal screening, Rural population

INTRODUCTION

Diabetes mellitus (DM) has emerged as one of the most important non-communicable diseases worldwide and represents a major public health challenge, particularly in low- and middle-income countries [1,2]. Rapid urbanization, lifestyle modification, population ageing, and reduced physical activity have contributed substantially to the increasing burden of diabetes in India and globally [2,3]. India currently harbors one of the largest populations of individuals with diabetes, and the prevalence is expected to increase further in coming decades, placing enormous pressure on healthcare systems and blindness prevention programs [4]. Among the numerous systemic complications of diabetes, diabetic retinopathy (DR) remains one of the leading causes of preventable blindness among working-age adults worldwide [1,4]. Diabetic retinopathy is a progressive microvascular complication resulting from chronic hyperglycemia and retinal vascular damage. If left undetected and untreated, DR may lead to irreversible visual impairment and blindness [1]. Evidence suggests that timely retinal screening and early treatment can substantially reduce vision-threatening complications and improve visual outcomes [2]. Despite advances in screening technology and treatment modalities, a significant proportion of diabetic individuals continue to present late due to inadequate awareness, poor health-seeking behavior, and limited access to ophthalmic services, particularly in rural settings [4]. Several studies have demonstrated that awareness regarding diabetic eye disease and retinal examination remains suboptimal in developing countries, contributing to delayed diagnosis and poor adherence to retinal screening recommendations [1,2].

Knowledge, attitude, and practice (KAP) studies provide valuable insights into community awareness, preventive beliefs, and healthcare utilization behavior related to chronic diseases and their complications [4]. Previous KAP studies conducted in India and other countries have reported substantial gaps between awareness and actual preventive practices related to diabetic retinopathy screening [2,3]. Although some individuals may possess basic knowledge regarding diabetes and its complications, this awareness often fails to translate into regular ophthalmic evaluation and retinal examination practices [3]. Studies from Saudi Arabia, South Africa, Nepal, and Nigeria have consistently demonstrated poor or moderate awareness regarding diabetic retinopathy, low uptake of retinal examination, and inadequate adherence to recommended eye care practices among diabetic patients [1,5,6,7]. Similar findings have also been reported from different regions of India, where poor diabetic retinopathy-specific awareness persists despite increasing general awareness regarding diabetes mellitus [8,9]. Most available KAP studies evaluate knowledge, attitudes, and practices using descriptive approaches or simple composite scoring systems. However, diabetic retinopathy screening behavior is multidimensional and influenced by several latent behavioral and healthcare utilization constructs that are often interrelated. Traditional descriptive analyses may not adequately identify the underlying domains influencing retinal screening uptake and preventive eye care behavior. Exploratory factor analysis offers an advanced statistical approach to identify hidden behavioral constructs by grouping correlated variables into meaningful latent domains [4]. Understanding these latent behavioral factors may provide deeper insight into the determinants of retinal screening behavior and help develop targeted educational and intervention strategies for rural populations.

Western Uttar Pradesh represents a region with a growing burden of diabetes, limited awareness regarding diabetic eye disease, and substantial rural healthcare disparities. Despite ongoing community-based screening initiatives and increasing ophthalmic outreach activities, the uptake of retinal screening services remains inconsistent. There is



**Shikha Sharma and Ashish Chander**

limited evidence evaluating the latent behavioral dimensions underlying diabetic retinopathy screening behavior in rural North Indian populations. Therefore, the present study was undertaken to assess the knowledge, attitude, and practice related to diabetic retinopathy screening among rural adults in Western Uttar Pradesh using exploratory factor analysis. The study aims to identify the underlying behavioral domains influencing diabetic retinopathy screening uptake and healthcare-seeking behavior in this underserved population

METHODS

This community-based cross-sectional study was conducted among adults residing in rural areas of western Uttar Pradesh, India, to assess the latent behavioral domains influencing awareness and utilization of diabetic retinopathy screening services. The study was carried out using a structured interviewer-administered questionnaire developed to assess knowledge, attitude, and practice related to diabetes, hypertension, diabetic retinopathy, retinal examination, and eye-care seeking behaviour. Participants aged 40 years and above who were permanent residents of the selected rural communities and willing to participate were included in the study. Individuals who were unable to communicate effectively due to severe cognitive impairment, hearing impairment without a caregiver, or unwillingness to provide consent were excluded. Ethical approval for the study was obtained from the Institutional Ethics Committee prior to commencement of the study. The study adhered to the tenets of the Declaration of Helsinki. Written informed consent was obtained from all participants before enrollment. Participants were informed regarding the purpose of the study, voluntary nature of participation, confidentiality of responses, and their right to withdraw from the study at any stage without any effect on their healthcare services. No personal identifiers were included during statistical analysis, and collected data were securely stored with access restricted to the study investigators only.

A multistage community-based sampling approach was used for participant recruitment. Villages from the study area were selected based on accessibility and community outreach coverage areas. Households within the selected villages were approached consecutively, and one eligible participant from each household was enrolled after obtaining written informed consent. Interviews were conducted in the local language by trained independent interviewers who were not involved in clinical care delivery or retinal screening services to minimize interviewer bias and social desirability bias. All interviewers underwent prior training regarding administration of the questionnaire, uniform interpretation of responses, ethical conduct, confidentiality maintenance, and handling of participant queries. A pilot assessment was performed among a small subset of participants prior to initiation of the study to assess clarity and comprehensibility of the questionnaire, and necessary modifications were incorporated before final data collection. The minimum sample size for the study was calculated using the formula for single population proportion, $n = Z^2pq/d^2$, assuming a 95% confidence interval, absolute precision of 5%, and anticipated awareness of diabetic retinopathy of 35% based on previously published rural Indian studies. Using $Z = 1.96$, $p = 0.35$, $q = 0.65$, and $d = 0.05$, the calculated sample size was approximately 350 participants. Considering possible incomplete responses and missing data, a final sample size of 352 participants was included in the analysis. The sample size was also considered adequate for exploratory factor analysis, as recommendations suggest inclusion of at least 5–10 participants per variable. Since 19 variables were included in the factor analysis model, the available sample exceeded the minimum recommended participant-to-variable ratio and was considered statistically appropriate.

The questionnaire collected demographic information including age, gender, education, occupation, income, systemic illness, and duration of diabetes and hypertension. For exploratory factor analysis, variables were selected based on conceptual relevance to latent constructs related to knowledge, awareness, preventive attitude, and healthcare-seeking behaviour associated with diabetic retinopathy and retinal examination. Demographic and purely clinical variables such as age, gender, diagnosis, and visual acuity were not included in factor extraction because these variables do not represent latent behavioural constructs. Nineteen variables related to diabetes awareness, hypertension awareness, diabetic retinopathy awareness, retinal examination knowledge, preventive beliefs, and



**Shikha Sharma and Ashish Chander**

healthcare utilization practices were selected for factor analysis after reviewing literature on diabetic retinopathy KAP studies and evaluating clinical relevance. All selected variables were converted into numeric codes before analysis. Binary variables were coded as “1” for correct or affirmative responses and “0” for incorrect, negative, or “don’t know” responses. Variables such as knowledge of diabetes, awareness of diabetic retinopathy, awareness that diabetes affects eyes, awareness of retinal examination, importance of blood pressure control, and need for regular eye examination were coded dichotomously. Ordinal variables including frequency of blood sugar monitoring, timing of physician visits, and timing of eye doctor visits were coded in increasing order to reflect better healthcare-seeking behaviour and preventive practices. Responses such as “not applicable,” refusal to answer, or missing information were treated as system-missing values during analysis.

Data were entered into Microsoft Excel and analyzed using IBM SPSS Statistics software version 21. Prior to factor analysis, data cleaning and validation procedures were performed to identify missing values, outliers, and coding inconsistencies. Descriptive statistics were generated for all variables. The suitability of the dataset for exploratory factor analysis was assessed using the Kaiser–Meyer–Olkin (KMO) measure of sampling adequacy and Bartlett’s test of sphericity. Variables demonstrating inadequate communalities or poor correlation patterns were reviewed before final extraction. Internal consistency reliability of the selected variables was assessed using Cronbach’s alpha coefficient. Exploratory factor analysis was performed using Principal Component Analysis extraction method with Varimax orthogonal rotation to identify underlying latent domains related to diabetic retinopathy awareness and healthcare-seeking behavior. Factors with eigenvalues greater than 1 were retained according to Kaiser’s criterion, and the scree plot was examined to determine the optimal number of factors. Factor loadings greater than 0.40 were considered significant for interpretation. Variables loading strongly on similar components were grouped together and interpreted conceptually to derive meaningful behavioral domains such as diabetic retinopathy awareness, preventive attitude, and healthcare utilization behavior. Factor scores generated from the analysis were used for further interpretation and subgroup comparison where appropriate.

RESULTS

A total of 352 participants were included in the study. The mean age of the study population was 53.70 ± 10.03 years, with participants ranging from 29 to 80 years of age. Females constituted 53.7% (n=189) of the study population, while males accounted for 46.3% (n=163). Urban residents represented 60.8% (n=214) of participants, whereas 39.2% (n=138) belonged to rural areas. Hindu participants comprised 55.4% (n=195) of the study population, followed by Muslims at 44.3% (n=156). Most participants had no formal education (45.2%, n=159), while 16.5% (n=58) had intermediate-level education and 14.2% (n=50) had graduate-level education. Home duties were the most common occupation reported by participants (52.6%, n=185), followed by private employment (13.4%, n=47), unemployment (12.5%, n=44), and business occupation (11.9%, n=42). More than half of the participants (51.4%, n=181) reported a monthly family income between Rs. 10,001 and Rs. 20,000, while 35.5% (n=125) reported income between Rs. 2,001 and Rs. 50,000 (Table 1).

With respect to systemic illness, diabetes alone was reported by 41.2% (n=145) of participants, while 27.0% (n=95) had both hypertension and diabetes. Approximately one-fifth of participants (20.7%, n=73) reported no systemic illness. Spectacle use was present in 42.6% (n=150) of participants. Most participants (80.1%, n=282) reported no vision-related condition other than refractive error. Cataract was the most frequently reported ocular condition (9.4%, n=33), followed by diabetic retinopathy (3.4%, n=12). Regarding duration of diabetes, 31.0% (n=109) of participants were non-diabetic, while among diabetic individuals the most common duration was 1–5 years (23.3%, n=82), followed by less than one year (20.7%, n=73). Most participants (62.8%, n=221) were not hypertensive, whereas 11.4% (n=40) had hypertension for less than one year and 11.1% (n=39) had hypertension duration between 1 and 5 years (Table 2). Exploratory factor analysis was performed on 19 variables related to knowledge, attitude, and healthcare-seeking practices regarding diabetic retinopathy and retinal examination among rural adults. A total of 236 complete responses were included in the factor analysis. Principal Component Analysis with Varimax rotation and Kaiser



**Shikha Sharma and Ashish Chander**

normalization was used for factor extraction. Six components with eigenvalues greater than 1 were retained, collectively explaining 61.47% of the total variance. The first component explained 21.52% of the variance, followed by the second (12.13%), third (9.09%), fourth (7.21%), fifth (6.22%), and sixth (5.29%) components. The scree plot demonstrated a gradual inflection after the sixth component, supporting retention of the six-factor solution.

Communality estimates ranged from 0.327 to 0.825, indicating adequate shared variance among most variables included in the model. Variables related to awareness of dilated retinal examination (0.825), knowledge regarding hypertension and diabetic complications (0.736–0.772), uptake of dilated retinal examination (0.785), and eye doctor visit history (0.686) demonstrated relatively high communalities, suggesting strong contribution to the extracted factors. After Varimax rotation, the extracted components demonstrated meaningful behavioral clustering. Component 1 predominantly represented “Retinal Examination and Eye Care Utilization Behavior,” with strong loadings for awareness of dilated retinal examination (0.853), uptake of dilated retinal examination (0.823), and recent eye doctor visit (0.717). Component 2 represented “Hypertension-Related Awareness,” with strong loadings for knowledge of hypertension (0.867), awareness that hypertension affects diabetic complications (0.831), and awareness that hypertension affects eyes (0.731). Component 3 represented “Preventive Diabetes Care Awareness,” characterized by loadings for benefits of dilated retinal examination (0.742), appropriate eye examination frequency (0.540), diabetes hereditary awareness (0.570), and importance of blood pressure control (0.518). Component 4 reflected “Diabetic Eye Disease Awareness,” with strong loadings for awareness that diabetes affects eyes (0.733), treatability of diabetes-related vision loss (0.625), and awareness of diabetic retinopathy (0.509). Component 5 was interpreted as “General Medical Care Utilization,” based on strong loadings for recent physician visit (0.760) and prior diabetes testing (0.759). Component 6 represented “Need for Continued Ophthalmic Care,” with high loading for the belief that ophthalmic consultation remains necessary despite blood sugar control (0.780), along with regular eye examination awareness (0.503) and importance of blood pressure control (0.534).

The rotated component matrix demonstrated satisfactory factor separation with most variables loading strongly onto a single factor, indicating good interpretability of the extracted domains. Variables associated with retinal screening uptake clustered distinctly from general awareness variables, suggesting that healthcare utilization behavior may represent a separate latent construct independent of general diabetic knowledge. These findings support the multidimensional nature of diabetic retinopathy screening behavior in rural populations. Table 3 summarizes the total variance explained by the extracted components, while Table 4 presents the rotated component matrix and interpretation of factor domains.

DISCUSSION

The present study explored the latent behavioral domains influencing diabetic retinopathy screening and eye care utilization among adults in Western Uttar Pradesh using exploratory factor analysis. The findings demonstrated that diabetic retinopathy screening behavior is multidimensional and influenced by several interrelated domains including retinal examination awareness, hypertension-related awareness, preventive health attitudes, diabetic eye disease awareness, healthcare utilization behavior, and perceived need for continued ophthalmic care. These findings provide important insight into the complex behavioral determinants underlying diabetic retinopathy screening uptake in underserved populations. The mean age of participants in the present study was 53.7 years, which is comparable to previous diabetic retinopathy awareness studies conducted in India and other developing countries where middle-aged and older adults constituted the majority of participants [10,11]. Females represented a slightly higher proportion of participants in our study, which may be attributed to the inclusion of a substantial number of homemakers and community-based recruitment. Similar gender distributions have been reported in previous KAP studies evaluating diabetic retinopathy awareness and diabetes-related healthcare utilization [10,12]. A considerable proportion of participants in the present study had no formal education, highlighting the persistent educational disparities in rural and semi-urban populations. Earlier studies have consistently shown that lower educational



**Shikha Sharma and Ashish Chander**

status is associated with poor awareness regarding diabetic retinopathy and reduced uptake of preventive eye care services [10,13,14].

Diabetes alone and diabetes with hypertension were highly prevalent among study participants, emphasizing the growing burden of chronic non-communicable diseases in the region. Hypertension-related awareness emerged as one of the strongest latent behavioral domains in the factor analysis. This finding is clinically important because hypertension is a well-recognized risk factor for progression of diabetic retinopathy and other microvascular complications [15]. Participants who demonstrated awareness regarding hypertension and its ocular implications also appeared more likely to possess better preventive health attitudes. Similar associations between systemic disease awareness and healthcare-seeking behavior have been reported in studies from Saudi Arabia and South Africa [11,15].

One of the most important findings of the study was the emergence of a distinct “Retinal Examination and Eye Care Utilization Behavior” domain, characterized by strong loadings for awareness of dilated retinal examination, uptake of dilated examination, and recent eye doctor visits. This suggests that retinal screening behavior may represent a separate healthcare utilization construct independent of general diabetes awareness. Earlier studies have similarly reported that awareness regarding diabetes alone does not necessarily translate into regular retinal examination practices [10,16]. In many low-resource settings, patients may possess basic knowledge regarding diabetes while remaining unaware of the importance of periodic retinal evaluation [17]. The separation of healthcare utilization variables into a distinct factor in the present study supports the concept of an “awareness-practice gap” frequently described in diabetic retinopathy screening literature.

The factor labeled “Diabetic Eye Disease Awareness” demonstrated strong loadings for awareness that diabetes affects eyes, knowledge regarding treatability of vision loss, and awareness of diabetic retinopathy. Previous studies from India, Nepal, and Nigeria have similarly documented poor diabetic retinopathy-specific awareness despite moderate awareness regarding diabetes mellitus itself [10,14,17]. The relatively lower prevalence of diagnosed diabetic retinopathy in our study population may partly reflect under-screening and delayed detection rather than true disease absence. Lack of awareness regarding retinal complications remains a major barrier to early diagnosis and timely referral in rural populations [18]. The identification of a separate “Preventive Diabetes Care Awareness” factor indicates that preventive beliefs related to blood pressure control, regular eye examination, and benefits of retinal screening cluster together behaviorally. This finding has important implications for community-based educational interventions. Educational programs targeting preventive health behavior may improve both systemic disease management and retinal screening uptake simultaneously. Previous studies have demonstrated that physician counseling and patient education significantly influence adherence to diabetic eye care recommendations [11,18]. The factor structure observed in the present study further supports the need for integrated diabetes and eye health education strategies at the primary care level.

Another important finding was the emergence of a “General Medical Care Utilization” factor comprising physician visits and prior diabetes testing. This suggests that individuals already engaged with general healthcare services may be more likely to access diabetic retinopathy screening services. Similar observations have been reported in studies evaluating healthcare utilization patterns among diabetic populations [13,16]. Strengthening referral linkages between primary care physicians and ophthalmologists may therefore substantially improve retinal screening uptake. The present study differs from most previous KAP studies because it used exploratory factor analysis to identify latent behavioral domains rather than relying solely on descriptive awareness scores or composite indices. Traditional KAP studies often evaluate knowledge, attitude, and practice as independent domains, whereas factor analysis allows identification of underlying interrelationships among variables [10]. The six-factor structure identified in this study explained more than 60% of the total variance, indicating satisfactory representation of the behavioral constructs influencing diabetic retinopathy screening practices. The use of factor analysis therefore provides a more comprehensive understanding of retinal screening behavior in rural populations and may help guide development of targeted interventions.



**Shikha Sharma and Ashish Chander**

The study has certain limitations. The cross-sectional design limits causal inference between behavioral domains and retinal screening uptake. Some responses were self-reported and may have been influenced by recall bias or social desirability bias. Additionally, factor analysis findings are dependent on the selected variables and study population, which may limit generalizability to other settings. However, the relatively large sample size, inclusion of both diabetic and non-diabetic adults, and use of standardized analytical methods strengthen the validity of the findings. In conclusion, the present study identified multiple latent behavioral domains influencing diabetic retinopathy screening uptake among adults in Western Uttar Pradesh. Retinal screening behavior was found to be distinct from general diabetes awareness, emphasizing the presence of substantial awareness-practice gaps in diabetic eye care. Strengthening community awareness, physician counseling, preventive health education, and integration of retinal screening services within primary healthcare systems may improve uptake of diabetic retinopathy screening and reduce preventable visual impairment in underserved populations.

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Shikha Sharma and Ashish Chander

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Table 1: Sociodemographic characteristics of study participants (N=352)

S.No	Variable	Category	Frequency (n)	Percentage (%)
1	Gender	Female	189	53.7
		Male	163	46.3
2	Locality	Urban	214	60.8
		Rural	138	39.2
3	Religion	Hindu	195	55.4
		Muslim	156	44.3
		Others	1	0.3
4	Education	No education	159	45.2
		Primary education	15	4.3
		High school	39	11.1
		Intermediate	58	16.5
		Degree	50	14.2
		Postgraduate and above	31	8.8
5	Occupation	Home duties	185	52.6
		Private employee	47	13.4
		Unemployed	44	12.5
		Business person	42	11.9
		Government employee	16	4.5
		Others	14	4
		Laborer	4	1.1
6	Family income/month	Rs. 10,001–20,000	181	51.4
		Rs. 2,001–50,000	125	35.5
		More than Rs. 50,000	32	9.1
		Less than Rs. 1000	4	1.1
		Rs. 1001–2500	2	0.6
		Others/Dependent/Refused	8	2.3

Table 2. Clinical and ocular characteristics of study participants (N=352)

S.No	Variable	Category	Frequency (n)	Percentage (%)
1	Systemic illness	Diabetes	145	41.2
		Hypertension + Diabetes	95	27
		None	73	20.7
		Hypertension alone	26	7.4
		Others	13	3.7





Shikha Sharma and Ashish Chander

2	Spectacle wear	Yes	150	42.6
		No	202	57.4
3	Vision condition	None	282	80.1
		Cataract	33	9.4
		Diabetic retinopathy	12	3.4
		Others	25	7.1
4	Duration of diabetes	Non-diabetic	109	31
		Less than 1 year	73	20.7
		1–5 years	82	23.3
		5–10 years	42	11.9
		10–15 years	38	10.8
		More than 15 years	8	2.3
5	Duration of hypertension	Not hypertensive	221	62.8
		Less than 1 year	40	11.4
		1–5 years	39	11.1
		5–10 years	31	8.8
		10–15 years	16	4.5
		More than 15 years	3	0.9

Table 3. Total variance explained by extracted components in exploratory factor analysis

Component	Eigenvalue	% Variance Explained	Cumulative Variance (%)	Interpreted Domain
1	4.089	21.52	21.52	Retinal Examination and Eye Care Utilization Behavior
2	2.305	12.13	33.65	Hypertension-Related Awareness
3	1.728	9.09	42.74	Preventive Diabetes Care Awareness
4	1.37	7.21	49.95	Diabetic Eye Disease Awareness
5	1.182	6.22	56.17	General Medical Care Utilization
6	1.006	5.29	61.47	Need for Continued Ophthalmic Care

Table 4. Rotated component matrix showing factor loadings after Varimax rotation

S.No	Variable	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
1	Heard of dilated eye/retina examinations	0.853					
2	Did you have dilated eye/retina examination	0.823					
3	How long ago did you visit eye doctor	0.717					
4	Do you know anything about hypertension		0.867				
5	Can hypertension affect diabetic complications		0.831				
6	Can hypertension affect eyes		0.731				
7	Benefits of dilated eye examination			0.742			
8	Diabetes hereditary			0.57			
9	Frequency of diabetic eye check-up			0.54			




Shikha Sharma and Ashish Chander

10	Importance of blood pressure control			0.518			0.534
11	Can diabetes affect eyes				0.733		
12	Is decrease in vision due to diabetes treatable				0.625		
13	Heard of diabetic retinopathy				0.509		
14	Visit to general practitioner					0.76	
15	Tested for diabetes					0.759	
16	Need ophthalmologist despite sugar control						0.78
17	Regular eye examination necessary						0.503

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Comprehensive Review of Recent Advancements in Leg Prosthetics: From Materials to Bio - Integrated Interfaces and Patient Outcomes

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ABSTRACT

The past decade has seen a transformative period in the field of prosthetics, moving beyond simple mechanical replacements toward a new era of intelligent, bio-integrated, and user-centric devices. This review synthesizes key advancements in leg prosthetics from 2015 to 2026, providing a comprehensive overview of innovations in materials, control systems, surgical techniques, and their impact on patient outcomes. Break throughs in materials such as carbon fibre and titanium have enabled devices that are both lightweight and robust, significantly improving biomechanical efficiency and reducing the metabolic cost of walking. The introduction of intelligent systems, including microprocessor-controlled knees and ankles, has led to demonstrably improved safety and mobility, particularly for high-risk populations. Surgical procedures such as targeted muscle reinnervation and osseointegration have addressed long-standing challenges of socket-related discomfort and pain, while simultaneously



**Mitul B. Patel and et al.,**

providing a more intuitive and stable interface for device control. A critical analysis of the literature reveals that restoring sensory feedback is a crucial, non-negotiable component for achieving true psychological and neurological integration of the prosthesis. While these technological and surgical innovations have yielded significant improvements in functional mobility, patient satisfaction, and quality of life, the field continues to grapple with persistent challenges. A considerable gap remains between the priorities of technology developers and the core, day-to-day needs of users, leading to high rates of prosthetic abandonment. Furthermore, systemic barriers of high cost and limited insurance coverage create a significant access disparity, raising profound ethical questions about technological discrimination. The path forward necessitates a concerted effort to not only advance technology but also to bridge the gap between research and clinical practice, prioritize user-centred design principles, and develop equitable policies to ensure that all individuals with limb loss can benefit from these life-changing advancements

Keywords: mobility,

INTRODUCTION

As per the WHO standards for prosthetics and orthotics, WHO estimates that, today, only 1 in 10 people in need has access to assistive products, including prostheses and orthoses, because of their high cost and because of lack of awareness, availability, trained personnel, policy and financing. Hence, WHO is coordinating a global initiative, “Global cooperation on assistive technology” (GATE), to improve access to high-quality, affordable assistive products[1].

The Context of Limb Loss

Limb loss represents a profound and life-altering event that impacts an individual's physical, psychological, social, and economic well-being[2]. The loss of a limb necessitates a complex journey of physical rehabilitation, psychological adaptation, and reintegration into daily life. For centuries, the primary goal of prosthetic science has been to create artificial limbs that can restore function and mobility. Despite these efforts, the development of a device that can truly replicate the function of a biological extremity is far from complete[3]. This persistent challenge is reflected in the historically high rates of prosthetic rejection and abandonment, with studies reporting rejection rates as high as 23% in adults and 35% in children using electrically powered prosthetics[4]. A lack of comfort, poor functionality, and the absence of sensory feedback have been consistently cited as the leading causes of this rejection[2]. These issues underscore the fact that a prosthetic device must be more than a simple tool; it must be a seamlessly integrated extension of the user's body.

Purpose of this Review

The purpose of this report is to provide a comprehensive, expert-level review of the latest advancements in leg prosthetics that have emerged over the last decade, spanning from 2015 to 2025. The aim is to synthesize the myriad of breakthroughs in materials science, intelligent control systems, and surgical techniques, and to critically assess their collective impact on patient outcomes, satisfaction, and overall quality of life. The report goes beyond merely enumerating innovations by exploring the complex interdependencies between these advancements and their real-world implications for prosthetic users. It examines how improvements in one area, such as surgical integration, can unlock the full potential of another, such as advanced control systems. Additionally, this review will shed light on the persistent challenges that continue to impede widespread adoption and equitable access to these technologies, including issues of cost, policy, and the critical need to close the gap between technological development and clinical implementation.





Scope and Structure

This review is structured to first address the foundational technological and material innovations that have defined the modern era of prosthetic design. It will then delve into the sophisticated intelligent systems and surgical bio-interfacing techniques that are revolutionizing mobility and control. A dedicated section will explore the critical and often overlooked role of sensory feedback in achieving true mind-body integration. The report will then pivot to a holistic assessment of clinical outcomes and patient-reported satisfaction, using standardized metrics to provide a quantitative and qualitative understanding of the impact of these technologies. Finally, a critical discussion of the remaining challenges and future directions will identify key areas for future research and policy action.

Foundational Advancements in Prosthetic Design and Materials

The Evolution of Materials

The evolution of prosthetic limbs is a testament to the advancements in materials science. Historically, prosthetic devices were crafted from simple, cumbersome materials like wood and early metals, which severely limited both function and comfort[5]. In the past decade, a profound shift has occurred toward advanced, performance-based materials that are meticulously selected for specific functions within the device. The most prominent example of this is the widespread adoption of **carbon fiber**[5]. This advanced composite material is prized for its exceptional strength-to-weight ratio, high stiffness, and remarkable ability to store and release energy[6]. These properties enable a more energy-efficient and natural gait, reducing the metabolic cost for the user and enabling highly active individuals to achieve peak speeds that nearly match those of non-amputees[6]. For internal, load-bearing components, **titanium** has become the gold standard[5]. Its superior strength, low weight, and resistance to corrosion make it ideal for parts that demand high durability[7]. A key biomechanical advantage of titanium is its low modulus of elasticity, a characteristic that makes it like bone. This property ensures a more even distribution of skeletal load between the implant and the bone, contributing to a more comfortable and natural walking experience[7].

While high-performance materials address the biomechanical needs of a prosthetic, advancements in polymers and elastomers address the crucial elements of comfort and aesthetics. Silicone and polyurethane, for instance, are widely used for socket liners and cosmetic covers due to their flexibility, skin-like texture, and biocompatibility[5]. These materials reduce friction, prevent skin irritation, and allow for a more natural feel, which is essential for user comfort and long-term acceptance[8]. The focus on these materials represents a design philosophy that recognizes that a prosthetic device must not only function well but also feel and look like a natural part of the body to promote embodiment and self-identification[9]. The trend in materials development is a dual-focused approach that simultaneously pursues high-performance biomechanical function and a human-centric design philosophy. Materials like carbon fiber and titanium are selected based on their engineering properties (e.g., strength-to-weight ratio, durability), which directly impact functional metrics such as gait efficiency and energy expenditure. Concurrently, materials like silicone and thermoplastics are prioritized for their aesthetic and comfort-related properties, such as flexibility, skin-like texture, and breathability. This is not a contradiction but a sophisticated synthesis of priorities. The field has matured beyond simply replacing a limb's function; it now understands that factors like comfort and aesthetics are equally critical to user satisfaction, acceptance, and long-term use. A highly advanced, multi-functional device is of limited utility if the patient abandons it due to persistent discomfort or a feeling of being disconnected from the device. Therefore, a successful prosthetic device in the modern era must be both a high-performance biomechanical tool and a comfortable, aesthetically pleasing extension of the body. The following table summarizes the key properties and applications of these advanced materials in prosthetic design.

The Rise of Digital Manufacturing and Customization

The advent of digital manufacturing has profoundly influenced prosthetic design and fabrication. 3D printing (additive manufacturing) has emerged as a potentially viable alternative to traditional fabrication methods, particularly for producing prosthetic sockets[5]. This technology offers several compelling benefits, including cost-effectiveness, the ability to create highly customized geometries for each user, and rapid prototyping.¹⁴ By significantly reducing the cost of materials and fabrication time, 3D printing presents a compelling solution for



**Mitul B. Patel and et al.,**

increasing access to prosthetic care, especially in settings where cost or resources are limited[10]. However, the technology is not without its challenges. The sources temper the promise of 3D printing with a realistic assessment of its current limitations. The structural integrity of 3D-printed sockets under high loads remains a significant concern, although this can be improved by the addition of composite materials like carbon fiber particles[10]. A crucial point is that 3D printing is not a "ready-to-use solution" that can simply be deployed without expert oversight[11]. The public perception of the technology, often shaped by media, is disconnected from the clinical reality where expert knowledge is still essential for ensuring proper fit and function[11].

This situation highlights that 3D printing technology is a democratizing force for manufacturing but not a complete panacea for all challenges. While the technology can revolutionize accessibility and prototyping by lowering costs and streamlining fabrication, its full potential is realized when it is integrated as a tool for clinicians, not as a replacement for their expertise[11]. The true innovation lies in using digital methods to augment the prosthetist's ability to create a custom, evidence-based device.

Computer-Aided Design and Manufacturing (CAD/CAM) systems, for example, have been shown to save time and lead to comparable or better clinical outcomes, ultimately improving the patient's quality of life[12]. These systems also create a perpetual, quantitative design record, which is invaluable for education, peer support, and future modifications[12]. This demonstrates that the digital approach complements, rather than replaces, the human element, allowing prosthetics' to focus on the more highly skilled aspects of customization and patient interaction[12].

Intelligent Systems and Enhanced Mobility Microprocessor-Controlled Joints (MPKs and MPAs)

A major shift in prosthetic technology has been the integration of intelligent, sensor-driven systems, particularly in prosthetic knees and ankles. Microprocessor-controlled knees (MPKs) have emerged as a definitive advancement over their passive, mechanical counterparts. MPKs utilize sophisticated sensors and hydraulic systems to respond to real-time gait changes, providing enhanced stability, stumble recovery, and a more physiological gait pattern[13]. This continuous, adaptive control is a key differentiator from non-microprocessor knees (NMPKs), which face an inherent conflict between providing stability for weight-bearing and allowing for dynamic function during walking[14]. The most compelling evidence for the efficacy of MPKs comes from the landmark ASCENT K2 clinical trial. This 12-month, prospective, randomized controlled trial specifically examined the impact of MPKs on the K2 patient population, which includes older adults with limited mobility who have a high risk of falling. The results provided a strong evidence-based justification for MPK use in this group. At the conclusion of the trial, individuals using MPKs experienced a significant reduction in falls and near-falls, reporting approximately one fall compared to three falls for the NMPK group[13]. This demonstrates a 66% reduction in fall-related events. Furthermore, the MPK group maintained their health-related quality of life (HR-QoL), while the NMPK group experienced a significant decline[13].

This quantitative data shows that microprocessor technology is not merely a luxury for high-performance users but a critical, evidence-based tool for safety and independence, particularly for vulnerable populations. The ASCENT K2 trial fundamentally shifts the narrative by providing irrefutable evidence of a direct cause-and-effect relationship: providing MPK technology (cause) leads to fewer fall events and reduced fear of falling (effect)[13]. This data is crucial for justifying the higher cost of these devices and supports policy changes for expanded insurance coverage, recognizing MPKs as a medically necessary technology for this high-risk population. Similarly, microprocessor-controlled ankles (MPAs) and feet have been developed to enhance gait and stability. Devices like the Proprio Foot and Elan Foot combine intelligent bionic ankle motion with the energy-returning dynamics of carbon fiber feet[15]. These devices automatically adjust to changing terrains, inclines, and even varying heel heights, actively increasing toe clearance during the swing phase of gait to reduce the risk of trips and falls[15].



**Mitul B. Patel and et al.,**

Powered Prostheses

While microprocessor-controlled joints have demonstrated significant success, the field is also advancing the development of fully powered prostheses. These devices are designed to actively assist movement by generating power to support the patient's weight and propel them forward, a major advancement over passive devices that simply store and release energy[2]. This can lead to a significant reduction in the metabolic cost of walking, allowing users to move more efficiently and for longer periods[16]. Despite their potential, powered knees and ankles still face considerable challenges, including excessive weight, bulkiness, and inefficiency, which have limited their commercialization and widespread adoption[2].

Intuitive Control and Signal Processing

The effectiveness of intelligent prosthetics is directly tied to the sophistication of their control mechanisms. The development of myoelectric control, which uses the electrical signals from residual muscles, has been a cornerstone of this progress[4]. A significant evolution has been the shift from simple on/off control, based on the activation of a single muscle, to advanced Pattern recognition technology. This innovation allows a user to correlate a specific electromyography (EMG) signal pattern from several synergistic muscles with a pre-programmed action, enabling simultaneous control of multiple degrees of freedom[3]. This advancement directly addresses the frustration and high abandonment rates associated with "mode switching" in earlier devices, making the control of the prosthesis more intuitive and adaptable[2].

The advancement of control systems is a fundamental quest for intuitive, biomimetic integration. The sources show a clear trend away from requiring the user to adapt to the device's limitations and toward the device adapting to the user's intent. This is best exemplified by the progression from simple, single-muscle myoelectric control to complex pattern recognition technology[3]. The core problem being solved is the cognitive load and frustration caused by the need for "mode switching" or sequential control. By allowing for simultaneous, multi-degree-of-freedom control, pattern recognition creates a more seamless, natural, and intuitive experience[2]. This directly addresses the high abandonment rates tied to unintuitive control mechanisms and represents a core design philosophy of "biomimicry," where the artificial limb mimics the dynamic, adaptive qualities of natural muscle actuation[15].

Surgical Integration and Bio-Interfacing

Targeted Muscle Reinnervation (TMR)

For decades, the interface between the human body and a prosthetic device was a major source of pain and discomfort, often leading to device rejection[2]. Recent surgical advancements have begun to bridge this gap, fundamentally altering the relationship between the body and the prosthesis. One such groundbreaking procedure is Targeted Muscle Reinnervation (TMR), a surgical technique that involves rerouting nerves from the amputated limb to nearby, intact muscles that have lost their original function[3]. This procedure offers a dual benefit. First, it provides a physiological "end organ" for the nerves to innervate, which prevents the chaotic, misdirected growth that leads to the formation of painful neuromas[17]. Clinical studies have shown TMR to be remarkably effective in reducing or eliminating residual limb and phantom limb pain[17]. For example, a prospective study of 33 amputees showed that TMR significantly reduced numerical rating scale (NRS) scores for residual limb pain from 6.4 ± 2.6 to 3.6 ± 2.2 and phantom limb pain from 6.0 ± 3.1 to 3.6 ± 2.9 one year after surgery[18]. Second, by providing these new muscle targets, TMR effectively creates "biological amplifiers" for the nerve signals. When a user thinks about moving their missing limb, the signal is amplified by the new target muscle, producing a robust EMG signal that can be captured by sensors and used to intuitively control a myoelectric prosthetic[19].

Osseo Integration (OI)

Another transformative surgical procedure is **osseo integration**, which allows for the direct skeletal attachment of a prosthesis, eliminating the need for an external socket[3]. The socket has long been a source of significant complications, including perspiration, skin ulcers, and irritation[20]. OI solves these problems by creating a direct, stable connection between the prosthesis and the residual bone.



**Mitul B. Patel and et al.,**

The clinical outcomes for OI have been promising. A Swedish study on the OPRA™ Implant System reported a 92% success rate at five years, with benefits sustained for up to 15 years post-operation[20]. Patients who have switched from a socket prosthesis to an Osseo integrated one have shown superior functional mobility outcomes[20]. Objective tests demonstrate this improvement: patients can walk approximately 25% farther on the 6-Minute Walk Test and complete the Timed Up and Go (TUG) test nearly 20% faster than their socket-using counterparts, achieving test times comparable to non-amputated individuals[21]. A key benefit of OI is the improved sense of proprioception, known as "osseoperception," where mechanical stimulation of the implant provides a passive awareness of the limb's position and function[20].

The future of prosthetics is a symbiotic human-machine interface, where surgical intervention is a prerequisite for optimal function. The sources demonstrate that TMR and OI are not isolated advancements but foundational, synergistic surgical procedures. OI addresses the long-standing "socket problem" by eliminating socket-related complications and providing a stable physical connection. TMR, in turn, addresses the "control problem" by providing intuitive motor control and alleviating chronic pain, a major contributor to prosthetic abandonment. When these two procedures are combined, as in ongoing studies, OI provides a stable platform for EMG sensors while TMR enhances the quality of the signals, leading to improved pattern recognition and prosthetic control[22]. This synergistic relationship moves the field from simple device attachment to true bio-integration. Furthermore, these surgical advancements indicate a shift in the role of amputation itself. Rather than being the reactive endpoint of a traumatic event or disease, the amputation procedure can now be the first step in a proactive, planned, long-term rehabilitation process[5]. TMR can be performed as a "primary" procedure at the time of amputation to prevent painful neuromas and optimize a patient for future prosthetic use[23]. This forward-thinking clinical approach recognizes that a strong surgical foundation is essential for long-term patient outcomes and prepares the patient for the full benefits of advanced prosthetic technology. The following table synthesizes the benefits of these two key surgical advancements

The Critical Role of Sensory Feedback

The Need for Sensation

A persistent challenge in prosthetic design has been the inability to restore sensory feedback. The lack of sensation has been cited as a primary cause of high prosthetic rejection rates, as it forces users to rely on their other senses, primarily vision, to operate the device[2]. This constant reliance on visual and auditory cues is mentally and physically taxing, leading to heightened mental and physical fatigue, reduced confidence, and slower walking speeds[3]. The absence of feedback can also intensify the sensation of phantom limb pain, a common and debilitating condition for many amputees[24]. Achieving a high-functioning prosthetic system requires a closed feedback loop that provides the brain with continuous information about the device's position, grip force, and interaction with the environment[25]. Without this feedback, the user's perception of the prosthesis as an extension of their body is severely limited, hindering the sense of ownership and embodiment that is critical for long-term use and psychological well-being[24].

Advancements in Sensory Restoration

Recent research has focused on developing methods to restore this missing sensation. These advancements can be broadly categorized as invasive and non-invasive.

Invasive methods involve the use of implanted sensors within muscles or nerves to directly provide feedback to the nervous system[4]. Intraneural stimulation, for example, has been used to provide a variety of sensory perceptions, from object stiffness and size to tactile sensations[4]. A study on leg prosthetics with intraneural stimulation demonstrated a significant reduction in mental effort, an increase in walking speed, and elevated confidence levels in participants[24].



**Mitul B. Patel and et al.,**

Non-invasive methods involve providing feedback through the skin of the residual limb. Haptic devices and vibration alerts, for instance, have been used to provide real-time tactile information about the prosthetic's position or the force it is exerting on an object[3]. These non-invasive techniques have shown promising results in improving prosthesis performance and the user's sense of embodiment, without the need for surgery[4]. Sensory feedback is the essential "missing link" for bridging the mind-body gap in prosthetics. While advancements in motor control systems and surgical interfaces provide a powerful physical connection, the data on sensory feedback reveals a critical psychological and neurological component. The high rejection rate for advanced prostheses is not solely a problem of function but a problem of a "closed loop" system. The brain requires continuous feedback to achieve a sense of ownership and control over the device.⁴ The study on leg prosthetics with intraneural stimulation provides a direct causal relationship: the presence of sensory feedback (cause) leads to a reduction in mental effort and an increase in walking speed and confidence (effect)[24]. This demonstrates that sensation is not an optional feature but a foundational requirement for achieving true psychological and neurological integration. Beyond its role in embodiment and control, restoring sensation has significant downstream health and quality-of-life benefits. The sources indicate that providing sensory feedback has been shown to reduce phantom limb pain and decrease mental and physical fatigue[24]. Reduced metabolic cost and improved walking economy have been linked to a counter to the long-term cardiovascular risks associated with above-knee amputations[24]. This is a profound finding: an advancement in a seemingly small area like sensation can have a ripple effect on major health outcomes, reinforcing the idea that a holistic approach to prosthetic design yields broader benefits for the user's long-term health and well-being.

Clinical Outcomes, Patient Satisfaction, and Quality of Life

Objective Functional Outcomes

Objective, performance-based outcome measures are essential for quantifying the benefits of advanced prosthetic technologies. The Timed Up and Go (TUG) test, which assesses basic mobility and falls risk, and the 6-Minute Walk Test (6MWT), which measures endurance and walking distance, are two such widely used metrics[21].

Clinical data shows that advanced prosthetics lead to significant improvements in these measures. For example, individuals using osseointegrated prostheses completed the TUG test nearly 20% faster than those with traditional sockets and were able to walk approximately 25% farther on the 6MWT[21]. This data, combined with findings that advanced prosthetics can nearly match non-amputees in peak speed, demonstrates that these technologies are not just incrementally better but fundamentally transformative for functional mobility[6].

Patient-Reported Experiences

While objective measures are important, patient-reported experiences are equally crucial for understanding the real-world impact of a prosthetic. Surveys such as the Prosthetic Limb Users Survey of Mobility (PLUS-M™) and the Socket Comfort Score (SCS) provide valuable insights into a user's perceived ability to perform activities and their satisfaction with the device[26]. A critical finding from these surveys is the dissonance between the priorities of developers and the core needs of users. While developers focus on complex, high-tech, and often invasive functionalities, patient-reported data consistently identifies comfort, durability, and safety as the most important factors for satisfaction[2]. Paradoxically, these are also the areas with the highest rates of dissatisfaction, particularly concerning durability, repairs, and technical assistance.³⁶ A significant portion of upper-limb prosthetic users, for instance, rejected their device despite being prescribed one of the most advanced technologies available[2]. This is because a large number of users prefer non-invasive devices and the ability to perform basic daily functions over undergoing surgery for advanced capabilities they may not need or use[2]. This is a critical observation for the future of research and development: the focus must shift from a "technology-first" to a "user-centric" approach, ensuring that solutions address the fundamental, day-to-day needs of the majority of amputees, which could help combat the high abandonment rates[2].



**Mitul B. Patel and et al.,****Quality of Life (QoL) and Psychosocial Well-being**

The ultimate measure of a prosthetic's success lies in its ability to improve a user's overall quality of life. Access to and the ability to use a prosthesis are identified as important predictors of QoL, as they allow for improved mobility and, in turn, increased independence[27]. Advanced prosthetics have been shown to enhance social integration, self-esteem, and psychological well-being.³ The ability to regain employment and live independently provides significant economic and societal benefits, including a reduction in long-term healthcare costs and a lessened reliance on welfare and disability benefits[28]. The value proposition of advanced prosthetics extends far beyond the individual, creating a broader societal benefit. The data provides a strong economic and social justification for investing in advanced prosthetics and rehabilitation services. Improved prosthetic function and a better quality of life are not isolated benefits; they lead to increased independence, successful return to meaningful community participation, and the regaining of employment[28]. This, in turn, reduces long-term healthcare costs and lessens the burden on welfare and disability benefits[28]. An economic analysis found that every dollar spent on rehabilitation saves the economy 11 dollars in various benefits[29]. This demonstrates that the benefits of advanced prosthetics are not just a matter of personal health but a sound public investment with significant downstream societal and economic returns.

Challenges and Future Directions**The Research-to-Clinic Gap**

Despite the rapid pace of innovation, a persistent gap remains between the development of new technologies and their successful implementation in clinical practice[2]. Developers and manufacturers are sometimes disconnected from the practical needs of clinicians and people with limb loss[11]. This can lead to the creation of devices that, while technologically impressive, are not practical or intuitive for everyday use[2]. Furthermore, a lack of clear instructions and training materials can impede patient adaptation, contributing to frustration and device rejection[2].

Barriers to Access

The high cost of advanced prosthetics is arguably the single greatest barrier to access. A lower extremity prosthetic can range in cost from \$5,000 to \$50,000, and the total lifetime cost of care can exceed \$345,000, a figure well beyond the financial capabilities of most individuals[29]. While insurance providers and government programs like Medicare typically cover the majority of these costs, significant limitations and out-of-pocket expenses remain[30]. The absence of comprehensive health economic evaluations for many of these advanced devices creates an evidence gap that complicates policy and investment decisions, preventing the widespread adoption of life-changing technologies[31].

Ethical Considerations

As technology becomes more sophisticated, a number of ethical considerations come to the forefront.

- **Balancing Innovation and Accessibility:** A primary ethical dilemma is the balance between creating groundbreaking, high-cost technologies and ensuring that they are accessible to all individuals, regardless of their socioeconomic status[32]. This raises questions about a potential technological divide between those who can afford advanced devices and those who cannot[6].
- **Data Security and Privacy:** The integration of smart technologies in modern prosthetics that collect and transmit data raises serious concerns about data security and privacy. Ethical questions arise regarding who has access to this sensitive health information and how it may be used[32].
- **Technological Discrimination and Identity:** The potential for a divide between those with and without access to advanced technology could lead to technological discrimination[32]. Furthermore, the development of devices that closely approach or even exceed biological function introduces a philosophical debate about the distinction between "therapy" and "enhancement," and how these devices may fundamentally alter an individual's psychological and bodily identity[33].



**Mitul B. Patel and et al.,**

Outlook and Future Directions

The future of leg prosthetics is characterized by a continued push toward greater intelligence, integration, and accessibility. The integration of artificial intelligence (AI) and machine learning is anticipated to further enhance the adaptivity and responsiveness of prosthetics, allowing for more seamless and intuitive control.⁸ The field must embrace a more formal and structured approach to user-centred design, involving amputees and clinicians in the design process from the outset to ensure that new technologies address real-world needs like comfort, durability, and practical function[2]. Finally, bridging the access gap will require a concerted effort from policymakers, researchers, and manufacturers to develop cost-effective solutions and create policies that ensure equitable access to life-changing care[31]. The need for larger, longer-term studies is also paramount to solidify the findings on durability, long-term outcomes, and the psychological and social impacts of advanced devices[9].

CONCLUSION

The last decade has witnessed a profound paradigm shift in prosthetic technology, marked by a departure from purely mechanical devices toward sophisticated, bio-integrated, and intelligent systems. The confluence of surgical innovation, such as TMR and osseointegration, with advancements in intelligent control and advanced materials has yielded devices that significantly improve functional mobility, safety, and overall quality of life. The clinical evidence is clear: these technologies are no longer speculative but are demonstrably effective in reducing falls, improving gait, and enhancing patient well-being. The review of current technologies also reveals limitations in socket adjustability and user-specific adaptability. Conventional prosthetic sockets often do not account for residual limb volume changes, leading to discomfort and reduced usability. Adjustable systems and hybrid designs that combine flexible and rigid components were recommended in several studies to address these issues effectively. Notably, previous literature calls for greater emphasis on user-centric, sustainable design approaches to accommodate the physical and economic diversity among prosthesis users. By focusing on these challenges, the field can move closer to its goal: not just to replace a missing limb, but to empower individuals with the freedom, confidence, and independence to live their lives to the fullest.

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Mitul B. Patel and *et al.*,

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Table 1: Key Properties and Applications of Advanced Prosthetic Materials

Material	Key Properties	Primary Application
Carbon Fiber	High stiffness, high tensile strength, low weight, energy storage & return	Feet, structural components
Titanium	Superior strength, low weight, corrosion resistance, biocompatible, low modulus of elasticity	Internal, load-bearing components
Silicone	Flexibility, skin-like texture, biocompatibility, non-toxic, durability	Socket liners, covers, customizable prosthetics, pneumatic/hydraulic systems
Polypropylene	Lightweight, flexibility, good tensile strength, hardness, rigidity, ease of molding	Sockets, orthoses, rigid structures
Polyurethane	Flexible, adaptable, foam properties, strong, durable	Foam for feet, liners, insulation

Table 2: Comparative Clinical Outcomes of Microprocessor Knees

Outcome Measure	Microprocessor Knee (MPK) Group	Non-Microprocessor Knee (NMPK) Group	Significance	Source
Falls (12-Month Period)	~1 fall	~3 falls	Significant reduction in fall events for MPK group	[13]
Near-Falls (12-Month Period)	~2 near-falls	~6 near-falls	Significant reduction in near-fall events for MPK group	[13]
Timed Up and Go (TUG) Test	~10 seconds faster	Baseline	Significant improvement in TUG scores for MPK group	[13]
Health-Related Quality of Life (HR-QoL)	Maintained QoL	Significant decline in QoL	Significantly favourable results for MPK group	[13]
Fear of Falling (FFABQ)	Significantly decreased (improved) score	No significant change	Significant improvement in fear-of-falling behaviors for MPK group	[13]





Mitul B. Patel and *et al.*,

Table 3: The Multifaceted Benefits of Surgical and Bio-Integrated Interfaces

Interface Type	Primary Benefits	Key Clinical Outcomes	Source
Targeted Muscle Reinnervation (TMR)	Intuitive prosthetic control, pain reduction (phantom and residual limb pain), prevention of neuromas	Significantly reduced NRS pain scores for phantom (6.0→3.6) and residual limb pain (6.4→3.6)	[17]
Osseointegration (OI)	Elimination of socket-related issues, improved proprioception, increased range of motion, improved gait, greater comfort while sitting	Improved gait (25% farther on 6MWT), faster TUG test completion (~20% faster), and a 92% success rate at 5 years	[21]

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RESEARCH ARTICLE

Conch Blowing -Induced Inspiratory Muscle Training for the Improvement of Sleep Quality, Fatigue Severity and Neurocognitive Performance in Sleep-Deprived Young Adults

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ABSTRACT

Sleep deprivation among university students represents a growing public health concern associated with impaired neurocognitive functioning, heightened fatigue, and diminished academic performance. Inspiratory Muscle Training has demonstrated benefits across respiratory and sleep-related disorders, yet its application as a non-pharmacological intervention in sleep-deprived young adults remains underexplored. An experimental clinical trial was conducted. From 123 initially screened participants, 65 met inclusion criteria (PSQI > 5; FSS ≥ 36) and participated in a structured conch blowing and breathing exercise protocol over four weeks. Fifty participants completed the intervention and were assessed pre- and post-intervention using the Wilcoxon Signed-Rank Test and Paired t-test at $\alpha = 0.05$. All outcomes demonstrated statistically significant improvement ($p < 0.001$) with large effect sizes. PSQI scores declined from 7.30 ± 2.49 to 3.66 ± 1.53 (Cohen's $d = 2.76$); FSS from 43.44 ± 6.17 to 29.64 ± 4.58 ($d = 3.57$); TMT-A completion time from 36.35 to 26.28 seconds ($d = 1.66$); and TMT-B from 67.15 to 42.21 seconds ($d = 0.81$). Conch blowing-induced IMT significantly improves sleep quality, reduces fatigue severity, and enhances neurocognitive performance in sleep-deprived young adults.

Keywords: Conch blowing; Inspiratory Muscle Training; sleep quality; fatigue; neurocognition





INTRODUCTION

Sleep is a fundamental physiological and psychological state characterised by altered consciousness, reduced sensory activity, and near-complete cessation of voluntary movement. Adequate sleep is indispensable for brain function, memory consolidation, emotional regulation, and physical restoration. Adults aged 18–60 years are recommended to obtain a minimum of seven hours of sleep per night; yet sleep deprivation is increasingly prevalent, particularly among university students who confront mounting academic pressures, irregular schedules, excessive digital screen exposure, and suboptimal sleep hygiene. Sleep deprivation in young adults is not merely a lifestyle inconvenience — it carries well-documented consequences for neurocognitive performance, including diminished attention, impaired working memory, slowed processing speed, and compromised executive function. Fatigue secondary to chronic sleep insufficiency further undermines academic productivity, social functioning, and mental well-being. Epidemiological estimates suggest that between 10% and 50% of university students experience clinically significant sleep disturbance at any given time. Sleep is necessary for overall well-being, emotional regulation, and cognitive performance. Many students suffer from chronic sleep loss caused by social commitments, technological distractions, and academic pressure. Their mental health suffers significantly as a result of the lack of sleep, with symptoms including increased stress, anxiety, sadness, and cognitive deterioration [1].

University students facing higher levels of academic and employment stress were more prone to experiencing poor sleep quality. Academic pressures coupled with job-related concerns can lead to heightened worries about the future. Such stress generates considerable psychological strain, which can directly interfere with the ability to obtain restful sleep. The anticipation of future events and obligations often keeps the mind actively engaged at night, preventing the relaxation necessary to initiate sleep. Environmental elements in a university setting can also be disruptive to sleep. The sleeping habits of roommates, noisy dormitory environments, and poor dormitory hygiene can hinder university students from attaining a conducive sleeping environment, resulting in compromised sleep quality [2]. Conventional interventions for sleep disturbance in students include cognitive behavioural therapy for insomnia (CBT-I), sleep hygiene education, and pharmacological agents. However, these approaches are either resource-intensive, inaccessible, or associated with adverse effects. There is a recognised gap in the availability of low-cost, physiologically grounded, non-pharmacological interventions that can be self-administered within a student population.

Inspiratory Muscle Training (IMT) — the structured exercise of muscles involved in inhalation — has demonstrated efficacy in improving oxygenation, cardiovascular efficiency, autonomic regulation, and perceived fatigue across diverse clinical populations, including individuals with obstructive sleep apnoea (OSA) and chronic fatigue-related conditions. It may reduce airway collapsibility and sympathetic output, which may decrease OSA severity and blood pressure [3]. Emerging evidence indicates that IMT may positively modulate autonomic nervous system activity, enhancing parasympathetic tone and thereby promoting sleep onset and continuity. However, recently conducted investigations yields controversial findings with studies depicting significant improvement in inspiratory muscle strength (IMS), sleep quality, lung function, and apnea-hypopnoea index (AHI), while other studies depicted no significant change in lung function, AHI and exercise capacity following IMT [4]. Inspiratory muscle training can be performed systematically using devices that provide quantifiable resistance targets and instant performance feedback. These targets are usually set as a percentage of the maximum inspiratory pressure that an individual can generate, as measured by a sensor in the device. The technique is akin to weight training where a set of repetitions is performed using a fixed weight. As the respiratory muscles gain strength, the IMT device allows the target resistance to be increased, so the training adapts to the person's current strength [5]. Home-based Threshold inspiratory muscle training (TIMIT) program in OSA severity, daytime sleepiness, and pulmonary function in newly diagnosed OSA can be simple efficacious, and cost-effective [6].



**Aishwarya Rai and Puja Pradhan**

Shankh (conch shell) blowing, a yogic breathing exercise involving forceful exhalation through a conch shell, has been considered to strengthen the oropharyngeal and respiratory muscles and improve lung function. This spiral-shelled device has been used as a musical instrument or decorative piece for centuries. Many authors have observed a subjective improvement in daytime sleepiness among individuals with OSA who were engaged in the practice of shankh blowing. The shankh, specifically, *Turbinella pyrum* or the “Indian Shankha”, possesses a unique spiralling structure with an inner cavity growing both axially and transversely. When blown, it produces a resonating sound field believed to hold healing properties.⁷ Conch blowing is also known as Shankha Naad in the Indian cultural tradition, is an indigenous practice requiring forceful deep inhalation followed by prolonged exhalation through a conch shell – a biomechanical process that constitutes a natural and culturally familiar form of IMT. The practice promotes slow rhythmic breathing, increases respiratory muscle endurance, and may activate vagal pathways conducive to relaxation and improved sleep architecture. Given its simplicity, negligible cost, widespread cultural acceptance particularly in South Asian populations, and absence of known contraindications in healthy individuals, conch blowing merits systematic scientific evaluation. This study aimed to evaluate the effectiveness of a structured conch blowing-induced IMT protocol on sleep quality, fatigue severity, cognitive processing speed, and executive function among sleep-deprived university students over a four-week intervention period.

MATERIALS AND METHODS

Study Design

A two-phase experimental clinical trial design was employed. Phase I constituted a cross-sequential study development design for protocol development and expert content validation. Phase II was an experimental pre-post study examining the effectiveness of the validated protocol on sleep, fatigue, and neurocognition in sleep-deprived university students. The study was approved by the Institutional Ethics Committee of Brainware University (Ethics Approval No: BWU/AHS/NOC/DSC/2026/020) and carried out in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants. The CONSORT 2010 guidelines were followed in reporting this trial (Figure 1).

Participants

University students aged 18–25 years enrolled at Brainware University, Barasat, Kolkata, West Bengal, India were recruited via convenience sampling. Inclusion criteria required a PSQI score greater than 5 (indicative of poor sleep quality) and an FSS score of 36 or above (indicative of severe fatigue). Participants were excluded if they had a history of respiratory, cardiovascular, or neurological disorders; were using medications known to affect sleep or respiratory function; were engaged in concurrent breathing training programmes; or smoked or used substances potentially affecting respiratory performance.

Of 123 students initially assessed, 65 met the inclusion criteria. Following baseline assessment, 15 participants were excluded for irregular attendance, yielding a final analytic sample of 50 participants who completed the four-week intervention.

Protocol Development and Validation (Phase I)

A structured Shankha-based IMT protocol was systematically developed incorporating graded intensity (30% to 60% perceived inspiratory effort across four weeks), frequency (twice daily), supplementary breathing exercises (diaphragmatic breathing, pursed-lip breathing, thoracic expansion, segmental breathing, and inspiratory hold training), and safety guidelines. The protocol was submitted for content validation by a panel of 17 experts drawn from physiotherapy, sleep medicine, respiratory science, and clinical research. Experts rated each protocol element as relevant or not relevant. The Item-level Content Validity Index (I-CVI) and Scale-level Content Validity Index (S-CVI) were computed. I-CVI (item-level content validity index) is the proportion of content experts giving item a relevance rating of 3 or 4. Whereas, S-CVI/UA (scale-level content validity index based on the universal agreement



**Aishwarya Rai and Puja Pradhan**

method) is the proportion of items on the scale that achieve a relevance scale of 3 or 4 by all experts. Universal agreement (UA) score is given as 1 when the item achieved 100% experts in agreement, otherwise the UA score is given as 0.⁸ Scale-level Content Validity Index (S-CVI) upon being computed, yielded an S-CVI of 0.99, confirming excellent content validity.

Intervention

Participants performed the validated conch blowing IMT protocol twice daily for four weeks at Brainware Diagnostic Clinic and Research Centre. Each session comprised a three-minute diaphragmatic breathing warm-up, 10–20 conch blowing repetitions (5–8 seconds of sustained exhalation per blow), 15–30 seconds of rest between repetitions, and a five-minute mindfulness-based cool-down. Supplementary exercises were performed concurrently. Perceived effort progressed from 30% in Week 1 to 60% in Week 4 to provide progressive overload. Supervision was provided during the initial sessions, with weekly review thereafter. Participants maintained a daily adherence logbook and were advised against practice during nasal congestion, ear infection, or vertigo, with instructions to discontinue if chest pain, dizziness, or ear pressure occurred.

Outcome Measures

Four standardised outcome measures were administered at baseline and post-intervention: Pittsburgh Sleep Quality Index (PSQI): A 19-item validated self-report instrument measuring sleep quality over the preceding month. A global score above 5 indicates poor sleep quality.⁹ Fatigue Severity Scale (FSS): A nine-item Likert-scale questionnaire measuring the functional impact of fatigue. Scores of 36 and above denote clinically significant fatigue.¹⁰ Trail Making Test Part A (TMT-A): A timed neuropsychological test measuring psychomotor speed, visual scanning, and sustained attention. Completion time in seconds serves as the score; lower scores reflect better performance.¹¹ Trail Making Test Part B (TMT-B): A timed test of cognitive flexibility, task-switching, and executive function, requiring the participant to alternate between numbers and letters in ascending sequence.¹¹

Statistical Analysis

Data were analysed using appropriate statistical tests based on normality assessment via the Shapiro-Wilk test. FSS and PSQI data were non-normally distributed and were analysed using the Wilcoxon Signed-Rank Test. TMT-A and TMT-B data satisfied the parametric assumption and were analysed with a Paired t-test. The level of significance was set at $\alpha = 0.05$. Effect sizes were estimated using Cohen's *d*, with values ≥ 0.8 classified as large. All analyses were performed on the complete sample of 50 participants.

RESULTS**Participant Demographics**

The final analytic sample comprised 50 participants, of whom 26 were male (52%) and 24 were female (48%). The mean age was 20.9 ± 1.7 years. The S-CVI for the conch blowing protocol was 0.99, reflecting excellent expert agreement on content validity.

Pre- and Post-Intervention Outcomes

All four outcome measures demonstrated statistically significant improvement following the four-week intervention period ($p < 0.001$), with large effect sizes across all domains per Cohen's classification (≥ 0.8). Sleep quality (PSQI) improved markedly, with global scores declining from a mean of 7.30 ± 2.49 pre-intervention to 3.66 ± 1.53 post-intervention — a shift from the poor sleep category ($PSQI > 5$) to the good sleep category ($PSQI \leq 5$). The Wilcoxon Signed-Rank Test yielded $W = 0.00$ ($p < 0.001$), with Cohen's $d = 2.76$, representing an exceptionally large effect. Fatigue severity (FSS) showed an equally pronounced improvement, with mean scores declining from 43.44 ± 6.17 to 29.64 ± 4.58 — representing a clinically meaningful transition from the severe fatigue category ($FSS \geq 36$) to the non-severe range ($FSS < 36$). This effect was the largest observed across all outcome domains (Cohen's $d = 3.57$), with $W =$



**Aishwarya Rai and Puja Pradhan**

0.00 ($p < 0.001$). Cognitive processing speed (TMT-A) improved significantly, with mean completion time reducing from 36.35 ± 10.59 seconds pre-intervention to 26.28 ± 5.72 seconds post-intervention, representing a mean improvement of 10.07 seconds. The paired t-test confirmed high statistical significance [$t(49) = 11.73$, $p < 0.001$, $d = 1.66$]. Executive function (TMT-B) demonstrated a mean reduction in completion time from 67.15 ± 36.85 seconds to 42.21 ± 10.22 seconds — an improvement of approximately 24.94 seconds. This was also statistically significant [$t(49) = 5.72$, $p < 0.001$, $d = 0.81$], though the effect size was the smallest among the four outcomes.

DISCUSSION

This study provides evidence that a four-week structured conch blowing-induced IMT protocol produces clinically and statistically significant improvements in sleep quality, fatigue severity, processing speed, and executive function in sleep-deprived university students. All four outcome measures showed large effect sizes (Cohen's $d \geq 0.81$), underscoring the practical significance of these findings beyond mere statistical significance. The most clinically notable finding is the shift in PSQI scores from the poor sleep domain (> 5) to the good sleep domain (≤ 5) following the intervention. This categorical transition suggests that the intervention produced not merely marginal improvement but a meaningful change in sleep health status. The physiological mechanism is plausible: conch blowing involves deep diaphragmatic inhalation followed by prolonged exhalation against resistance — a pattern that activates the parasympathetic nervous system, reduces heart rate, lowers circulating cortisol, and facilitates sleep onset. The effect of slow respiratory exercises on vagal tone and autonomic balance has been documented in the broader IMT literature, and the present findings extend this evidence to a cultural variant of IMT in a non-clinical population. The reduction in FSS scores from severe to non-severe fatigue represents a second important finding. Fatigue in sleep-deprived individuals arises from multiple pathways: inadequate restoration of cellular energy substrates, dysregulation of neuroendocrine systems, and reduced cerebral oxygenation. By improving both inspiratory muscle efficiency and sleep quality simultaneously, conch blowing likely addresses both peripheral and central mechanisms of fatigue. The very large effect size ($d = 3.57$) for FSS reduction exceeds those commonly reported in pharmacological trials for fatigue, though comparisons must be made with caution given differences in population characteristics and study design.

Improvements in TMT-A performance reflect enhanced attentional capacity, psychomotor speed, and visual scanning — all domains sensitive to sleep deprivation and recovered with restorative sleep. The large effect size ($d = 1.66$) observed here is consistent with the substantial cognitive benefits expected when sleep-deprived individuals achieve adequate restorative sleep. Enhanced cerebral oxygenation attributable to improved respiratory efficiency during the practice sessions may also have contributed to the observed cognitive benefits. TMT-B improvement, reflecting gains in cognitive flexibility and executive control, further indicates that the intervention reached higher-order cognitive domains reliant on prefrontal cortical function — regions particularly susceptible to sleep insufficiency. Taken in aggregate, these findings are consistent with prior literature demonstrating that IMT improves sleep quality in OSA patients (Chen et al., 2023; Lin et al., 2020; Nóbrega-Júnior et al., 2020) and extends those findings to a sleep-deprived non-clinical population using a culturally embedded, accessible delivery modality. The intervention is noteworthy in that it requires no equipment beyond a conch shell, carries minimal risk, and is appropriate for group-based delivery in educational settings — characteristics that enhance its potential for scalable implementation. From a public health standpoint, the findings have implications for the design of student wellness programmes in Indian universities, where cultural familiarity with Shankha practice may enhance adherence and participant engagement. The integration of evidence-based traditional practices into physiotherapy and mental health support frameworks represents an emerging area of interest, and the current study contributes an empirical foundation for such efforts.





Limitations

Several limitations should be acknowledged. The study employed a single-arm pre-post design without a matched control group, precluding definitive causal inference and increasing the risk of confounding from natural recovery, placebo effects, or regression to the mean. The sample was restricted to students at a single university campus, limiting generalisability. The four-week intervention period, while sufficient to demonstrate short-term effects, does not permit conclusions about long-term sustainability of gains or optimal dosage parameters. Assessment of cognitive function relied solely on the TMT, and more comprehensive neuropsychological batteries may have captured a broader range of affected domains. Additionally, objective sleep assessment (e.g., polysomnography or actigraphy) was not performed; reliance on subjective self-report instruments such as the PSQI may introduce response bias.

CONCLUSION

This experimental clinical trial demonstrates that a structured four-week conch blowing-induced Inspiratory Muscle Training protocol produces significant, large-magnitude improvements in sleep quality, fatigue severity, processing speed, and executive function among sleep-deprived university students. The intervention is physiologically grounded, culturally acceptable, low-cost, and feasibly deliverable in educational settings. The alternate hypothesis — that conch blowing IMT significantly improves sleep quality, fatigue, cognitive processing speed, and executive function — is accepted. These findings provide a rationale for incorporating conch blowing-based respiratory training into student wellness programmes and for further investigation through larger, controlled trials with objective outcome measures and extended follow-up periods.

Recommendations for Future Research

Future studies should employ randomised controlled designs with active or sham control groups to establish causality and control for non-specific effects. Larger, multi-institutional samples spanning diverse demographic groups would improve external validity. Extension of the intervention to 8–12 weeks, combined with follow-up assessments at one- and three-months post-intervention, would clarify the durability of effects and optimal dosage. Objective sleep assessment using actigraphy or polysomnography should complement subjective measures. Additionally, investigation of mechanistic pathways — including autonomic modulation via heart rate variability analysis and cerebral oxygenation via near-infrared spectroscopy — would advance understanding of how conch blowing exerts its effects.

Ethics Approval

This study was approved by the Institutional Ethics Committee of Brainware University, West Bengal, India (Ethics Approval Number: BWU/AHS/NOC/DSC/2026/020). The study was conducted in full accordance with the ethical principles of the Declaration of Helsinki (2013 revision). Written informed consent was obtained from all participants, written assent was obtained from the participants. Participants were free to withdraw from the study at any time without prejudice.

Authorship Statement

Aishwarya Rai: Conceptualization, Methodology, Supervision, Formal Analysis, Validation, Visualization, Writing – Review & Editing, Project Administration, and guidance on study design and interpretation of findings.

Puja Pradhan: Methodology, Data Curation, Resources, and Writing – Original Draft.

All authors contributed substantially to the work, reviewed the manuscript critically for important intellectual content, approved the final version for publication, and agree to be accountable for all aspects of the work in accordance with the CRediT (Contributor Roles Taxonomy) system.



**Aishwarya Rai and Puja Pradhan****Conflict of Interest**

The authors declare no conflict of interest. This study received no external funding from any institution or organisation. No author has any financial relationship with organisations that sponsored or could have an interest in the submitted work. The funding source had no role in study design, data collection, analysis, interpretation, or the decision to submit the work for publication.

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Aishwarya Rai and Puja Pradhan

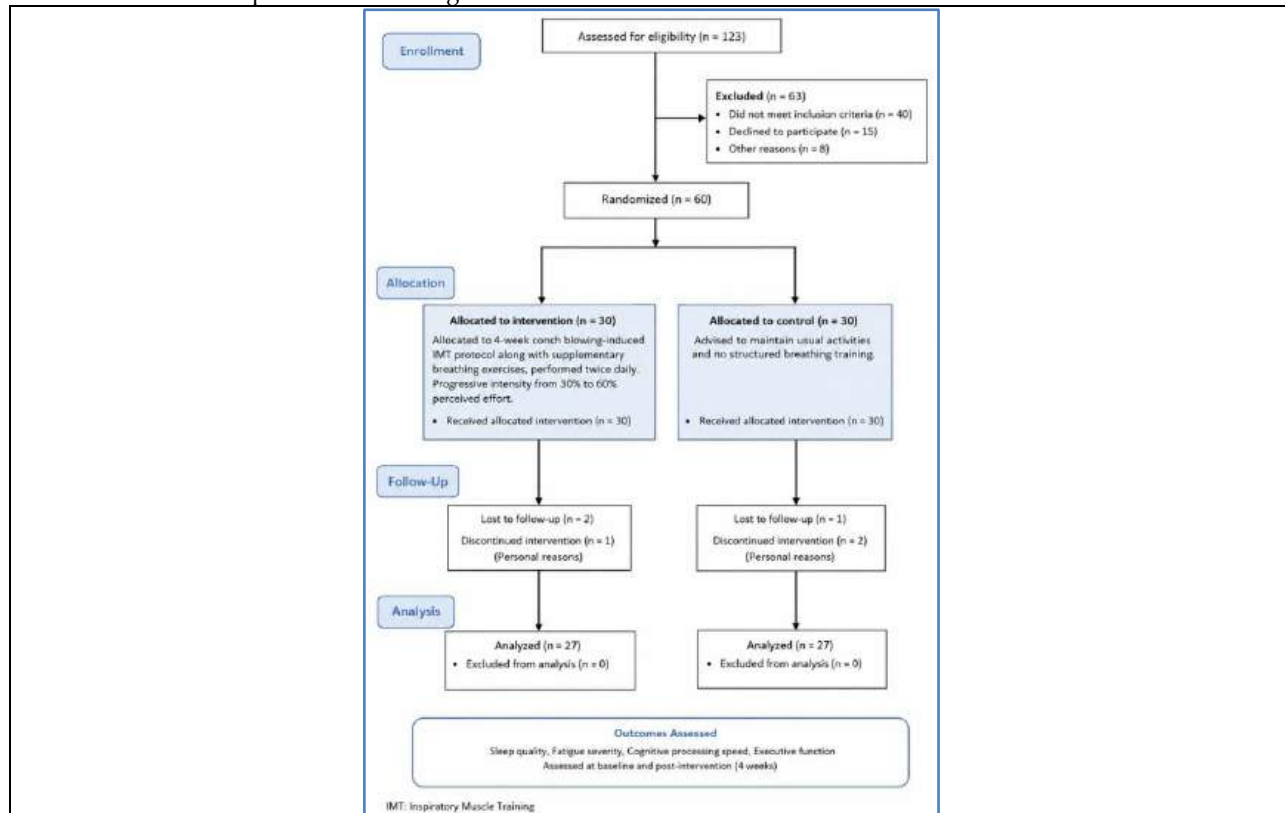
Table 1. Demographic Characteristics of Study Participants (N = 50)

Characteristic	Value
Total Participants	50
Male	26 (52%)
Female	24 (48%)
Mean Age	20.9 ± 1.7 years
Intervention Duration	4 weeks

Table 2. Pre- and Post-Intervention Outcome Measures with Inferential Statistics (N = 50)

Outcome Measure	Pre Mean ± SD	Post Mean ± SD	Mean Diff	Test Statistic	p-value	Effect Size (d)
FSS	43.44 ± 6.17	29.64 ± 4.58	13.80	W = 0.00	< 0.001	3.57 (Large)
PSQI	7.30 ± 2.49	3.66 ± 1.53	3.64	W = 0.00	< 0.001	2.76 (Large)
TMT-A (sec)	36.35 ± 10.59	26.28 ± 5.72	10.07	t(49) = 11.73	< 0.001	1.66 (Large)
TMT-B (sec)	67.15 ± 36.85	42.21 ± 10.22	24.94	t(49) = 5.72	< 0.001	0.81 (Large)

FSS = Fatigue Severity Scale; PSQI = Pittsburgh Sleep Quality Index; TMT = Trail Making Test. All p-values < 0.001. Effect sizes classified per Cohen's d: Large ≥ 0.8.

**Figure 1 CONSORT = Consolidated Standards of Reporting Trials. All 30 enrolled participants completed the study**

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REVIEW ARTICLE

Students' Perception about Importance of Mnemonics in Nursing Education and Nursing Curriculum: A Scoping Review

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ABSTRACT

Nursing education requires students to learn large volumes of theoretical knowledge, clinical procedures, drug names, disease classifications, and patient care protocols. The complexity and vastness of nursing curriculum often make learning difficult, leading to problems in retention and recall of information. Mnemonics are memory-enhancing techniques that help learners organize, store, and retrieve information effectively. They use patterns such as acronyms, rhymes, visualization, or associations to make learning easier. Research suggests that mnemonic strategies improve understanding, long-term retention, and academic performance among medical and nursing students. Mnemonics have been widely used in anatomy, pharmacology, physiology, and nursing process because these subjects involve memorization of large and complex information. The importance of mnemonics in nursing education is increasingly recognized as educators aim to adopt innovative teaching strategies that enhance active learning and critical thinking. Mnemonic techniques have emerged as effective tools to enhance memory retention and understanding of complex concepts. This scoping review aims to explore students' perceptions and the importance of mnemonics in nursing education. A systematic search of databases including Google Scholar, PubMed, CINAHL, Science Direct, and Research Gate was conducted for studies published between 2021 and 2026. Out of 120 identified articles, 10 studies met the inclusion criteria. Findings indicate that mnemonics significantly improve memory retention, academic performance, and student engagement. Students showed positive perceptions toward mnemonic-based learning. Integration of mnemonic strategies into nursing curricula can enhance teaching effectiveness and promote learner-centered education.

Keywords: Mnemonics, Nursing Education, Memory Retention, Student Perception, Learning Strategies, Medical Education





Vipin Vageriya

INTRODUCTION

Nursing is a dynamic and demanding professional field which requires students to acquire broad theoretical knowledge and clinical competencies. With the increasing complexity of healthcare systems, students must develop the ability to retain and apply large amounts of information effectively. However, traditional lecture-based teaching methods often fail to support long-term retention and meaningful learning. One of the major challenges faced by nursing students is the difficulty in remembering complex medical terminology, bones name, various anatomical systems, drug classifications, disease processes, and clinical procedures. This often leads to cognitive overload, stress, and poor academic performance. Mnemonic techniques are structured memory aids that help learners to encode, store, regain and retrieve information efficiently. These techniques include acronyms, acrostics, rhymes, chunking, visualization, and storytelling. In nursing education, mnemonics are widely used to remember drug names, nursing procedures, anatomical structures, and emergency protocols. Students' perception plays a crucial role in determining the effectiveness of any teaching aid and teaching strategy. Positive perception toward mnemonic techniques can enhance motivation, reduce anxiety, and promote active learning. Therefore, understanding students' views on mnemonics is essential for integrating these strategies into the nursing curriculum

Background

Modern education systems face increasing challenges in ensuring effective learning and retention due to pedagogical, technological, and social factors. Nursing students, in particular, encounter difficulty in memorizing extensive medical terminology, procedures, and theoretical concepts. Reduced retention capacity often leads to poor academic performance. Educational experts emphasize the need for innovative teaching strategies. Mnemonics serve as cognitive tools that simplify complex information through structured patterns such as acronyms, rhymes, and visual imagery, thereby improving recall and comprehension. In recent years, the educational landscape has undergone significant transformation due to technological advancements, changing pedagogical approaches, and increased academic demands. Nursing students often experience challenges related to heavy workloads, complex subject matter, and high expectations for clinical competence. Memory retention has become a critical concern, as students are required to recall information quickly during examinations and clinical practice. Research suggests that traditional rote learning methods are less effective compared to active learning strategies. Mnemonics provide a practical solution by simplifying complex information into easily recallable formats. They enhance cognitive processing by linking new information with existing knowledge, thereby improving encoding and retrieval processes.

Nursing Education in Modern Context

Nursing education is evolving with the integration of digital technologies and e-learning resources and platforms. Online learning environments, simulation-based training, and blended learning approaches are becoming increasingly popular among learner. Teachers are now act as facilitators rather than sole knowledge providers. The integration of flexible and innovative learning strategies, including mnemonic devices, which enhances practicereadiness, clinical competence, lifelong learning.

Need of the scoping review-

Medical and nursing students frequently face academic burden which affect their health and cognitive aspects. Academic burden is due to due Extensive syllabus, Complex medical and surgical terminology, clinical responsibilities and examination pressure. These challenges can negatively impact learning outcomes and increase stress levels. Mnemonics have been shown to simplify learning and improve retention; however, their integration into nursing curricula remains inconsistent. This scoping review is necessary to understand students' perceptions towards mnemonic techniques and evaluate mnemonics effectiveness in improving learning outcomes.

Objectives of the review

- To explore the importance of mnemonic techniques in nursing/medical education



**Vipin Vageriya**

- To understand students' perception of mnemonics
- To identify commonly used mnemonic strategies

METHODOLOGY**Study Design**

In this article a scoping review design was adopted, which was appropriate for mapping existing current literature and identifying key concepts, gaps, and evidence.

Search strategy

A comprehensive literature search was conducted using the various databases. The search engine data base used for literature review were CINAHL (EBSCO), google scholar, research gate, PubMed, and Science Direct. It used for English-language studies which were published between 2021 to till 2026. The primary search terms were Mnemonics, Memory retention "Nursing students", Medical education, Learning strategies" As a secondary search term (article title, journal name, author), and thesaurus (Mesh) searching such as "Mnemonics" OR "Retention AND learning. Researcher removed the duplicate articles. Boolean operators such as AND/OR were used to refine the search. Commonly searched terms were "Mnemonics" "Memory retention" "Nursing students" "Medical education" and "Learning strategies in medicine"

Inclusion criteria

- Studies on mnemonics in nursing/medical education
- Published between 2021–2026
- Peer-reviewed English articles
- Participants: Undergraduate/Postgraduate nursing students
- Study types: Experimental, quasi-experimental, descriptive

Exclusion criteria

- Studies before 2021
- Non-empirical studies, letters, case reports
- School-level studies
- Studies without mnemonic focus

Study Result

A total of **10 studies** were included in the final review

Summary of Findings

All the studies concluded that Mnemonics improved memory retention, it enhanced academic performance, increased student engagement and promoted active learning. The findings of this review strongly support the effectiveness of mnemonic techniques in nursing education.

DISCUSSION

Most studies reported that students found mnemonics helpful in understanding and recalling complex concepts. Mnemonic strategies such as acronyms, story, rhymes and visual imagery were particularly effective in simplifying difficult and complex topics. For example, the mnemonic RACE (Rescue, Alarm, Confine, Extinguish) helps students remember fire safety procedures. Research studies proved that Students reported that mnemonics Reduce anxiety during examinations, improve confidence in class and Enhance long-term retention now a day, technology-based mnemonics, such as audio-visual tools and mobile applications, further enhance engagement and accessibility.



**Vipin Vageriya**

Despite these benefits, the integration of mnemonics into formal curricula remains limited. Educators often use them informally rather than as structured teaching strategies.

Implication of mnemonics in nursing education

Short learning technique means mnemonics can be planned by subject teacher. Studies suggested to Incorporation of mnemonic strategies into lesson plans, application of simulation-based learning and clinical teaching, Integration with e-learning platforms, Development of digital mnemonic tools and Training educators in innovative teaching methods Mnemonic-based learning can significantly enhance the quality of nursing education by promoting active and student-centred learning.

Limitation

Each studies have its own limitation. This review included the studies conducted between 2021 to 2026. Studies which having less than 50 samples were not use.

CONCLUSION

Mnemonic strategies are highly effective in improving learning, retention, and academic performance in nursing education. Students show a positive perception toward their use. Integrating mnemonics into nursing curricula can enhance educational outcomes and promote innovative teaching practices. Integrating mnemonic strategies into the nursing curriculum can promote innovative teaching practices and improve educational outcomes. Future research should focus on large-scale studies and structured implementation of mnemonic-based learning.

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Vipin Vageriya

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Table 1: Summary of mnemonicsStudies

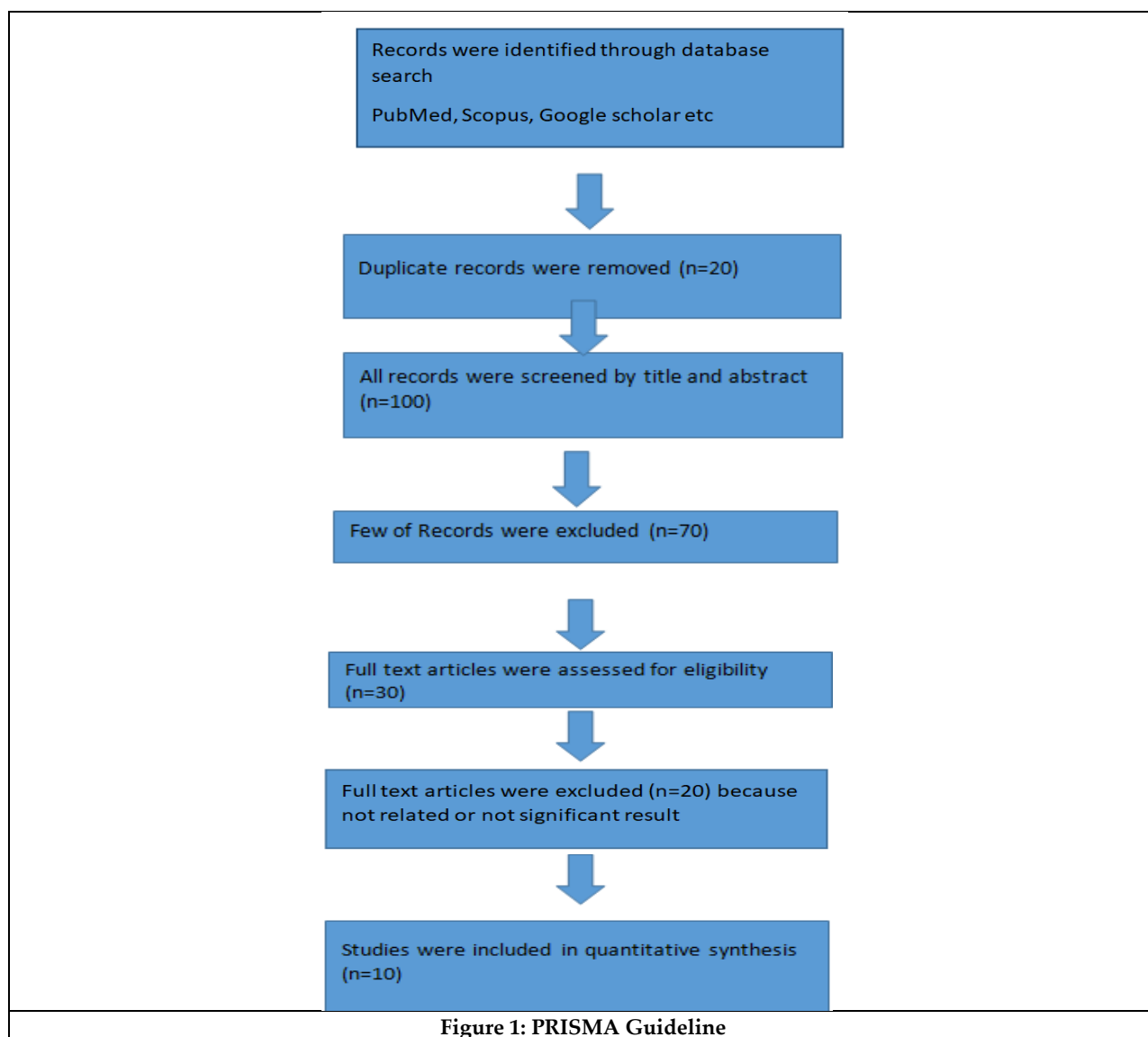
No.	Author & Year	Study Design	Sample	Key Findings	Conclusion
1	Kaur et al., 2022	Experimental	MBBS students	Improved recall	Enhances retention during examination and practical
2	Bellezza, 2021	RCT	Medical students	Improved long-term memory	Effective multimedia help to improve memory
3	Lone UF et al 2025	Interventional	Students of nursing sciences	Importance of mnemonics in retention	Mnemonics help to understand complex chapters
4	Patil et al., 2025	Descriptive	Nursing students	Improved perception	Combined methods useful
5	Gupta & Sharma, 2023	Quasi-experimental	Nursing students	Better academic performance	Mnemonics are useful strategy of learning
6	Sankar, C VasanthaNila, 2025	Intervention	Nursing students	Simplified care planning	Easy learning method for students
7	Adhikari S 2026	Mixed method	Educational studies	Improves encoding	Strong evidence of understanding through mnemonics





Vipin Vageriya

8	Manipal Study2022	Experimental	Health students	Increased engagement	Interactive way of learning
9	Rawson & Dunlosky 2021	Review	Multiple	Long-term retention	Evidence-based learning
10	Meenu S, 2022	Experimental	Medical students	Improve learning and understanding	Effective learning with mnemonics



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RESEARCH ARTICLE

Differential Understanding of Truptighna Karma with Special Reference to Truptighna and Deepaniya Mahakashaya Varga of Charaka

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ABSTRACT

The *Charak Samhita*, considered as the preeminent compendium of the *Kritayuga* (classical era), encapsulates not only medicinal knowledge but also the fundamental logic and forms the cornerstone of Ayurveda. Among its distinctive contributions the *Mahakashaya* concept described by *Charaka* classifies drugs in to 50 groups comprising 10 drugs sharing similar therapeutical properties which offers a structured approach to aid physicians to select appropriate drugs in maintaining health and management of diseases. In Ayurveda, health is dictated by metabolic efficiency rather than dietary intake alone; even wholesome food can become pathogenic if improperly digested or assimilated. As the proper functioning of *Agni* is vital for maintaining immunity (*vyadhikshamatva*) and constitutional strength (*bala*), its restoration through *Deepaniya* (appetizers), *Pachaniya* (digestants), and *Truptighna* (satiety- relieving) agents remains a primary therapeutic objective. Among the *Mahakashayas*, *Deepaniya* and *Truptighna* are particularly significant due to their role in enhancing digestive capacity, promoting proper assimilation and correcting metabolic disturbances.

Keywords: kritayuga, Mahakashaya, Deepaniya, Truptighna



**Reju Krishnan and Deepa**

INTRODUCTION

The word *Ayurveda* has been formed by the combination of two words 'Ayu' and 'Veda', which means the knowledge deals about Ayu or life, ^[1]which aims at preserving the physical and mental health of healthy individuals and managing diseases in the diseased, emphasizing prevention, health promotion and longevity as a holistic science of life^[2]. particularly the *Charaka Samhita Sutrasthana* Chapter 4, presents one of the earliest systematic classifications of medicinal plants in Ayurveda ^[3]. The chapter describes 50 *Vargas* (therapeutic groups) comprising 500 *Kashaya Dravyas*^[4] (decoctive drugs), organized according to their predominant pharmacological actions. This classification serves as an essential guide for physicians in understanding the therapeutic application of medicinal substances and achieving effective clinical outcomes. An important conceptual discussion arises when *Agnivesha* (disciple of *Atreya*) questions the repeated occurrence of several drugs within different *Vargas*, arguing that such repetition appears inconsistent with the stated total of 500 drugs. In response, *Atreya* clarifies that a single substance may possess multiple therapeutic actions and therefore can appropriately belong to more than one pharmacological group ^[5]. In the present study *Deepaniya* and *Truptighnamahakashaya* exhibit closely related therapeutic actions with partial overlap in their constituent drugs. Considering their therapeutic importance and conceptual similarity, a comparative understanding of both *mahakashaya* is very much essential for rational clinical application.

Concept of Deepan and Truptighna^{6,7}

Definition of Deepan

Pachennāmaṃ vanhikṛcca dīpanaṃ tadyathā miśi

According to *Acharya Sharangdhara* Deepan mean the drug which stimulate *Agni* but does not digest *Aama*.

Definition of Trupti⁸

Truptih shlesmivikarah, yen truptmivatmanam manyate, tadghnam Truptighnam According to *Chakrapanidatta* *Trupti* is a *Kapha nanatmaja vyadhi*. Excess of *amaj-Kapha dosha* causes feeling of fullness that is *Trupti*.

MATERIALS AND METHODS

Comprehensive review of Ayurveda concept of *Deepana* and *Truptighna karma* along with tabulation of *Deepaniya* and *Truptighna mahakashaya* drugs were done from the *Charaka Samhita*, and relevant *Dravyaguna Vigyana* text books. All information were then analyzed discussed and concluded.

DISCUSSION

Trupti is a *Kaphaja Vikara* especially affecting the *Amashaya*, characterized by *Vridhdhi* of *Kledaka Kapha* and *Kshaya* of *Samana Vata*, *Prana Vata*, *Pachaka Pitta*, and *Agni*. This results in impaired digestion, loss of appetite, and features of *Agnimandya*. From an *Amshamsha Kalpana* perspective, increased *Snigdha*, *Sheeta*, *Guru*, and *Manda Gunas* necessitate the use of drugs possessing *Ruksha*, *Laghu*, *Ushna*, *Tikshna*, and *Chala* properties along with *Amahara Karma*. The predominance of *Katu* and *Tikta Rasa* drugs in *Truptighna Mahakashaya* supports this therapeutic requirement. These drugs help alleviate aggravated *Kapha*, digest *Ama*, and restore normal digestive function in the *Amashaya*. Similarly, *Deepaneeya Mahakashaya* enhances *Agni* by improving *Abhyavaharana Shakti* and *Jarana Shakti*, thereby addressing *Annadvesha*, *Aruchi*, *Mandagni*, and *Vata Sthambhana*. The action of *Deepana Karma* can be attributed to *Anulomana* of *Prana*, *Samana*, and *Apana Vata*, reduction of excessive *Kledaka Kapha*, and enhancement of the *Ushna* and *Tikshna* qualities of *Pachaka Pitta*. Accordingly, most drugs of *Deepaneeya Mahakashaya* possess *Katu Rasa*, *Ushna Veerya*, and *Tikshna Guna*, making them effective in correcting *Agnimandya*. Comparison of *Truptighna* and *Deepaneeya Mahakashayas* reveals common pharmacodynamic principles centered; on *Kapha Shamana* and *Agni Deepana*. Drugs such as *Vidanga* and *Murva* may be particularly useful in conditions associated with *Vata Sthambhana*, whereas *Patola* and *Guduchi* may be preferred when *Pitta Dushti* predominates. *Vacha* facilitates digestion through its *Mala*





Reju Krishnan and Deepa

Vishodhana action, while *Musta* supports the liquefaction phase of digestion. Furthermore, drugs like *Pippali*, *Pippalimoola*, and *Bhallataka Asthi* exert actions beyond *Jatharagni* and influence *Dhatvagni*. Thus, the predominance of *Katu-Tikta Rasa*, *Ushna Veerya*, and *Tikshna-Ruksha Gunas* among the drugs of both *Mahakashayas* provides a rational basis for their effectiveness in managing *Trupti* and other conditions associated with *Kapha*-induced *Agnimandya*.

CONCLUSION

The *mahakashaya gana* are enumerated based on their action. But, the mode of action differs among each drug. Differential understanding of each drug enables us to specifically utilise them and also identify other drugs which are not included in the *gana*. Charaka says, he enumerated there 10 drugs in each *gana* just for showing example. All of them does not follow the same mode of action. Only when we understand this, the purpose of *mahakashaya gana* classification is fulfilled.

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Table 1: Dravyas of Charaka Deepaniya Mahakashay⁹

Sl.No	SANSKRIT NAME	LATIN NAME	FAMILY	PART USED
1.	<i>Pippli</i>	<i>Piper longum</i>	Piperaceae	Fruit
2.	<i>Pipplimoola</i>	<i>Piper longum</i>	Piperaceae	Root
3.	<i>Chavya</i>	<i>Piper chaba</i>	Piperaceae	Root
4.	<i>Chitrak</i>	<i>Plumbago rosea</i>	Plumbaginaceae	Root
5.	<i>Shunti</i>	<i>Zingiber officinale</i>	Zingiberaceae	Rhizome
6.	<i>Amlavetasa</i>	<i>Garcinia pedunculata</i>	Clusiaceae	Fruit
7.	<i>Maricha</i>	<i>Piper nigrum</i>	Piperaceae	Fruit
8.	<i>Ajamoda</i>	<i>Apium graveolens</i>	Apiaceae	Fruit
9.	<i>Bhallataka</i>	<i>Semicarpus anacardium</i>	Anacardiaceae	Seed
10.	<i>Hingu</i>	<i>Ferula asafoetida</i>	Apiaceae	Gum





Reju Krishnan and Deepa

Table 2: Dravyas of CharakaTryptighnaMahakashaya⁹

Sl.No	SANSKRIT NAME	LATIN NAME	FAMILY	PART USED
1.	Shunti	<i>Zingiber officinale</i>	Zingiberaceae	Rhizome
2.	Chavya	<i>Piper chaba</i>	Piperaceae	Root
3.	Chitrak	<i>Plumbago rosea</i>	Plumbaginaceae	Root
4.	Vidanga	<i>Embeliaribes</i>	Primulaceae	Fruit
5.	Murva	<i>Marsdeniastenacissima</i>	Apocynaceae	Root
6.	Guduchi	<i>Tinospora cordifolia</i>	Menispermaceae	Stem
7.	Vacha	<i>Acorus calamus</i>	Araceae	Rhizome
8.	Musta	<i>Cyperus rotundus</i>	Cyperaceae	Tuber
9.	Pippli	<i>Piper longum</i>	Piperaceae	Fruit
10.	Patola	<i>Trichosanthes cucurmerina</i>	Cucurbitaceae	Panchanga

Table 3: Properties of DeepniyaDravya – according Bhavprakash Nighantu¹⁰

Sl.No	SANSKRIT NAME	RASA	VIPAKA	VIRYA	GUNA
1.	Pippli	Katu	Madhura	Anushna	Laghu,snigdha
2.	Pipplimool	Katu	Katu	ushna	Laghu,rooksha
3.	Chavya	Katu	Katu	ushna	Laghu,rooksha
4.	Chitrak	Katu	Katu	ushna	Laghu,rooksha
5.	Shunti	Katu	Madhura	ushna	Laghu,snigdha
6.	Amlavetasa	Amla	Amla	ushna	Laghu,rooksha
7.	Maricha	Katu	Katu	ushna	Laghu,rooksha
8.	Ajamoda	Katu	Katu	ushna	Laghu,tikshana
9.	Bhallataka	Madhura	Madhura	ushna	Laghu,snigdha
10.	Hingu	Katu	Katu	ushna	Laghu,tikshana

Table 4: Properties of TryptighnaDravya – according Bhavprakash Nighantu¹⁰

Sl.No	SANSKRIT NAME	RASA	VIPAKA	VIRYA	GUNA
1.	Shunti	Katu	Madhura	Ushna	Laghu,Snigdha
2.	Chavya	Katu	Katu	Ushna	Laghu,rooksha
3.	Chitrak	Katu	Katu	Ushna	Laghu,rooksha
4.	Vidanga	Katu , kashaya	Katu	Ushna	Laghu,rooksha
5.	Murva	Tikta, kashaya	Katu	Ushna	Guru, rooksha
6.	Guduchi	Tikta, kashaya	Madhura	Ushna	Laghu,rooksha
7.	Vacha	Tikta, katu	katu	Ushna	Laghu,tikshana
8.	Musta	Tikta, Katu	katu	Sheeta	Laghu,rooksha
9.	Pippli	Katu	Madhura	Anushna	Laghu,Snigdha
10.	Patola	Tikta,	Katu	Ushna	Laghu,rooksha

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RESEARCH ARTICLE

Formulation and Evaluation of Plant - Based Polyherbal Gummies Incorporating Guava Leaves and Camphor

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ABSTRACT

As people become more aware of the side effects associated with synthetic medicines, the demand for safer and natural herbal remedies has increased. This study aimed to develop and evaluate plant-based polyherbal gummies prepared using guava leaves, tulsi, mint, and camphor. These medicinal herbs are known for their antibacterial, antioxidant, anti-inflammatory, and soothing properties, which are beneficial for maintaining oral health. The herbal extracts were incorporated into a gummy base prepared using gelatin or pectin, along with natural sweeteners and flavoring agents to improve taste and patient acceptance. The formulated gummies were evaluated for their appearance, texture, pH, weight variation, moisture stability, drug content uniformity, and antibacterial activity. The prepared gummies showed good stability, pleasant organoleptic properties, and effective antibacterial action against microorganisms responsible for oral infections. Due to their chewable form, pleasant taste, and ease of administration, these polyherbal gummies may serve as a convenient and effective alternative to conventional herbal dosage forms for oral healthcare applications.

Keywords: *Ocimum sanctum* (Tulsi), *Mentha arvensis* (Mint), *Cinnamomum camphora* (Camphor), *Psidium guajava* (Guava leaves), herbal gummy formulation, polyherbal dosage form, antimicrobial activity, oral care, and plant-derived therapeutics





INTRODUCTION

Medicinal herbs have been used for centuries to treat and prevent a wide range of illnesses. Traditional medical systems like Ayurveda, Siddha, and Unani are based on herbal remedies. Recent years have seen an increase in interest in herbal-based remedies due to the rise in microbial infections and inflammatory illnesses, as well as the drawbacks and side effects of synthetic drugs. A variety of medicinal plants are combined in polyherbal preparations, which are intended to increase therapeutic efficacy through synergistic action. When compared to synthetic medications, these herbal treatments are typically thought to be more affordable, safe, and acceptable. Gummies are one of the most popular modern herbal dosage forms because of their tasty flavor, ease of chewing, ease of administration, and compatibility for both young and old. The production of plant-based polyherbal gummies made using guava leaves, tulsi, mint, and camphor—all of which have beneficial medicinal and therapeutic qualities—is the main emphasis of this work.

Guava Leaves (*Psidium guajava*)

Guavas are widely grown in tropical and subtropical regions and are members of the Myrtaceae family. Numerous bioactive components, including flavonoids, tannins, phenolic compounds, and volatile oils, are known to be present in guava leaves. Antibacterial, antioxidant, anti-inflammatory, antidiabetic, and wound-healing properties are only a few of the pharmacological activities that these phytochemicals support. Quercetin, kaempferol, gallic acid, ferulic acid, and caffeic acid are significant active substances found in guava leaves that are essential for reducing oxidative damage and preventing microbial growth. Guava leaves have long been used to treat respiratory conditions, digestive issues, tooth discomfort, and oral infections.

Tulsi (*Ocimum sanctum*)

In Ayurvedic medicine, tulsi, also known as holy basil, is regarded as one of the most valuable medicinal herbs. It is known for its wide range of pharmacological properties, including adaptogenic, immunomodulatory, antibacterial, anti-inflammatory, and antioxidant effects. It is a member of the Lamiaceae family. Eugenol, ursolic acid, rosmarinic acid, methyl eugenol, flavonoids, and tannins are some of the key bioactive components of tulsi that greatly enhance its therapeutic qualities. Tulsi has long been used to treat oral infections, fever, cough, respiratory ailments, and stress-related disorders because of their medicinal advantages.

Mint (*Mentha arvensis*)

Because of its revitalizing scent and health-promoting qualities, mint, an aromatic medicinal plant in the Lamiaceae family, is widely used in food, cosmetic, and pharmaceutical applications. It has several active ingredients, including flavonoids, phenolic compounds, menthol, menthone, and essential oils. Antibacterial, antioxidant, analgesic, anti-inflammatory, digestive, and cooling actions are only a few of the pharmacological properties of mint. It has long been used to treat headaches, nausea, cough, colds, and digestive discomfort, and it is frequently used in dental hygiene products. Menthol adds a cooling effect and enhances the compositions' overall palatability and patient acceptability.

Camphor (*Cinnamomum camphora*)

Known for its therapeutic properties, camphor is a white, crystalline substance that is extracted from the wood of the camphor tree. It has a number of medicinal qualities, including decongestant, antiseptic, analgesic, antibacterial, and anti-inflammatory effects. Topical formulations, balms, inhalers, and oral care treatments frequently contain camphor. Camphor has long been used to treat toothaches and other oral discomforts. Camphor may enhance the antibacterial efficacy of herbal gummy formulations while delivering targeted therapeutic action in the oral cavity.



**Sakshi Mahendra Sadgir and *et al.*****Herbal Gummies as Novel Dosage Forms**

Chewable semisolid preparations known as "gummy formulations" are typically made with gelling ingredients like pectin or gelatin. Due to their ease of use and patient-friendliness, herbal gummies have drawn a lot of interest recently as a substitute for traditional dose forms like pills and capsules. Herbal candies have a number of benefits,

such as:

- Improved adherence to therapy and patient compliance
- A pleasant flavor and enhanced palatability
- Easy and convenient administration
- An easy-to-use dose form
- Appropriate for patients of all ages
- Extended oral cavity contact time
- Increased efficacy of targeted therapy

REVIEW OF LITERATURE**Guava Leaves**

Guava leaves have been shown to have medicinal value in numerous investigations. Due to the presence of flavonoids, tannins, and phenolic components, guava leaves were found to have potent antibacterial and antioxidant properties in a review by Manoj Kumar (2021). The study showed how efficient guava leaves are against a variety of bacterial and fungal infections. Chen and Yen (2007) also noted that guava leaf extracts' high polyphenolic content demonstrated exceptional free radical scavenging ability. Additionally, guava leaf extracts have significant cytotoxic and anticancer potential, according to research by Ashraf et al. (2016), proving their significant biological qualities.

Tulsi

The medical uses of tulsi have been thoroughly studied. In her assessment of the phytochemical and pharmacological profile of tulsi, Dr. Meenakshi Chauhan (2019) discovered key components that give the plant its antibacterial and anti-inflammatory properties, including eugenol, rosmarinic acid, and flavonoids. Tulsi's immunomodulatory qualities have also been shown in a number of studies, which makes it useful for treating mouth infections and respiratory conditions.

Mint

In his discussion of the therapeutic use of mint, Sajad Ahmad Wani (2022) highlighted the presence of active components such phenolic compounds, menthol, and menthone. These compounds support mint's antibacterial, analgesic, anti-inflammatory, and antioxidant properties. Peppermint oil has long been used in dental care and oral hygiene products because of its antibacterial properties and refreshing effect.

Camphor

According to research by Waseem Ahmad (2022), camphor has antibacterial, antifungal, analgesic, and anti-inflammatory qualities. Camphor has long been used to treat skin disorders, infections, and discomfort. Camphor is prized in oral healthcare because of its cooling properties and capacity to reduce oral pain and dental discomfort.

Herbal Gummies

Recent advancements in pharmaceutical and nutraceutical research have demonstrated a growing interest in herbal gummy formulations due to their enhanced patient compliance, pleasant flavor, and simplicity of administration. For the delivery of probiotics, vitamins, minerals, and herbal extracts, gummies are especially favored. However, little research has been done on polyherbal candies made especially for oral health that contain therapeutic components like camphor, tulsi, guava leaves, and mint.



**Sakshi Mahendra Sadgir and *et al.*****Need of the Study**

Innovative, secure, and patient-friendly dose forms are becoming more and more necessary as herbal medicine gains popularity. Conventional herbal preparations, like powders and tablets, are frequently linked to poor taste, challenging administration, and decreased patient compliance. Guava leaves, tulsi, mint, and camphor are examples of medicinal plants with strong antibacterial and anti-inflammatory qualities that are helpful for preserving dental health. There hasn't been much research done on combining these herbal elements into a stable, palatable, and chewable gummy formulation, despite their therapeutic promise. In order to offer targeted action in the oral cavity, increase therapeutic efficacy, and improve patient compliance, the current study is concentrated on creating a polyherbal gummy dosage form using natural plant extracts.

MATERIALS AND METHODS**Materials Required****Herbal Components**

- Guava leaf

Excipients Used

- Gelatin or pectin as gelling agents
- Citric acid
- Natural sweeteners such as stevia or D-mannitol
- Purified water
- Flavouring substances
- Preservative agents

Equipment and Instruments

- Analytical weighing balance
- Hot air oven
- pH meter
- Rotary evaporator
- Water bath apparatus

Collection and Authentication of Plant Materials

Fresh herbal components were purchased locally and verified by a pharmacognosy specialist. A motorized grinder was used to crush the gathered leaves into a fine powder after they had been carefully cleaned and shade-dried to remove any remaining moisture.

Preparation of Herbal Extracts

Soxhlet extraction was performed on the powdered plant materials using ethanol and other appropriate solvents. The necessary herbal extract was obtained by filtering and concentrating the extracted materials using a rotary evaporator.

Phytochemical Screening

Phytochemical analysis was performed on the produced herbal extracts to identify the presence of several bioactive components, such as:

- Alkaloids
- Flavonoids
- Tannins
- Glycosides
- Phenols





- Saponins
- Terpenoids

Formulation of Polyherbal Gummies

The heating and congealing procedure was used to make the gummies.

Procedure

1. Purified water was used to dissolve gelatin or pectin, which was then heated in a water bath.
2. Flavorings and sweeteners were added.
3. The mixture included camphor and herbal extracts.
4. The mixture was heated while being constantly stirred.
5. Molds were filled with the prepared solution.
6. Gummies were left to cool and solidify.
7. The prepared candies were taken out and put in airtight receptacles.

Evaluation Parameters

To ascertain the quality, stability, and therapeutic efficacy of the prepared gummies, a number of physicochemical and pharmacological parameters were assessed.

Organoleptic Evaluation

The organoleptic properties of the prepared gummies were assessed and revealed the following traits:

- Colour: greenish-brown shade
- Odour: refreshing, pleasant, and mint-like aroma
- Taste: mildly herbal with a sweet flavour
- Appearance: transparent, uniform, and glossy in nature
- Texture: smooth with moderately firm consistency

Weight Variation

Excellent consistency was shown in the weight variation analysis between batches of the formulations as well as within individual batches. Good formulation consistency was demonstrated by the fact that none of the gummies in any batch deviated more than $\pm 5\%$ from the average weight. The accuracy and dependability of the molding process were validated by the low percentage relative standard deviation (%RSD) values, which were less than 2% for every batch. Batch F3 showed a slightly higher %RSD value (1.44%) than batches F1 and F2. This might be explained by increased gel hardness at the molding temperature, which led to slightly higher viscosity and minimal fluctuation during mold filling. All batches, however, met the permissible weight fluctuation restrictions.

pH Determination

Using a digital pH meter, the gummy formulation's pH was determined to be 5.6 ± 0.06 .

Drug Content Uniformity

The polyherbal extracts were uniformly dispersed throughout the gummy matrix in all three formulation batches, according to the drug content uniformity data. The average drug content values for F1, F2, and F3 were 98.6%, 98.0%, and 98.8%, respectively. These values are within the 90–110% pharmacopeial acceptable range. Excellent consistency between individual gummy units is indicated by the low %RSD values, which are less than 1% for each batch. These results imply that the guava, tulsi, mint, and camphor extracts were properly incorporated and uniformly dispersed within the gel base thanks to the mechanical stirring and homogenization process. For medicated gummy formulations, this consistency is regarded as a crucial quality feature.



**Sakshi Mahendra Sadgir and *et al.*****Moisture Content**

All three of the gummy batches' moisture content readings were found to be within the 10–20% permissible range for medicated gummy formulations. Moisture content and gelatin concentration were shown to be inversely correlated. Because of its relatively loose gel network, batch F2, which included 11 g of gelatin, had the greatest moisture level (16.4%), whereas batch F3, which contained 15 g of gelatin, had the lowest moisture content (11.8%) due to its stiffer polymer structure. Maintaining the proper moisture content is crucial because too much moisture—above 20%—may encourage microbial contamination and stickiness, while too little moisture—below 10%—may cause gummies to become brittle and crack. Every batch that was prepared showed moisture levels that were steady and within the desired range.

Texture Analysis

Due to variances in the amounts of sugar and gelatin, the Texture Profile Analysis (TPA) results revealed distinct disparities amongst the three batches. The hardness (16.8 N), chewability (72.4 N·mm), and adhesiveness (-2.4 N·mm) of Batch F3, which contained 15 g of gelatin, were the highest, suggesting the development of stiffer gummies with better shape-retaining ability. On the other hand, Batch F2, which was made with 11 g of gelatin and a higher concentration of honey, had the softest texture, as evidenced by its highest adhesiveness (-6.8 N·mm), maximum elasticity (0.88), and lower hardness (9.2 N), all of which indicate a softer gummy consistency. With moderate values for all assessed criteria, such as hardness of 12.4 N, chewability of 58.6 N·mm, and outstanding elasticity of 0.82, Batch F1, which contained 13 g of gelatin, produced the most balanced texture profile. F1 is the best formulation for oral delivery of chewable pharmaceuticals because of this balance. Overall, the textural characteristics of all three batches were within the permissible range for medicated gummy formulations.

Stability Studies

According to stability studies, Batch F1 has adequate shelf stability in both rapid and long-term storage scenarios. Excellent stability at room temperature settings was confirmed by the formulation's three-month long-term storage at 25°C/60% RH, which showed no appreciable changes in color, odor, texture, pH, moisture content or drug content. Over the course of the investigation, gradual alterations were noted under accelerated storage conditions (40°C/75% RH). The Maillard reaction between the reducing sugars and amino acids in gelatin caused a minor darkening of the color. Menthol volatilization at high temperatures may be the cause of the little decrease in mint scent strength that was also observed. Additionally, the gummies' gradual softening was noted as a result of their absorption of moisture in high humidity. Over a three-month period, drug content decreased slightly by 2.4%, but it stayed within the acceptable $\pm 5\%$ level set by ICH Q1A (R2) recommendations. During the course of the investigation, neither storage condition showed any signs of microbiological growth, phase separation, or syneresis. These findings suggest that the formulation should have a minimum 24-month shelf life under typical storage circumstances (25°C/60% RH). To reduce hygroscopic moisture absorption and preserve product stability, packaging in moisture-resistant aluminum foil pouches is advised.

EXPECTED RESULTS AND DISCUSSION

It is anticipated that the produced polyherbal gummies will have favorable organoleptic qualities, such as palatable flavor, texture, and general look. The formulation's palatability and customer acceptance may be further enhanced by the addition of mint and natural sweeteners. The candies are expected to offer significant protection against oral microbial diseases because of the antibacterial properties of guava leaves, tulsi, mint, and camphor. Through synergistic effects, the combined activity of various herbal extracts may also improve overall therapeutic efficacy. Furthermore, it is anticipated that the formulation's physicochemical characteristics will not significantly change under typical storage circumstances. When compared to traditional herbal dosage forms, the chewable quality of the gummy dosage form may further enhance patient convenience and adherence.



**Sakshi Mahendra Sadgir and *et al.*****Advantages of Polyherbal Gummies**

1. A formulation made using natural, safe ingredients
2. Enhanced adherence to therapy and patient compliance
3. A delightful flavor and revitalizing scent
4. Simple and practical administration
5. Appropriate for patients of all ages
6. Increased efficacy of antibiotics
7. Offers a targeted therapeutic effect inside the mouth
8. Extended oral residence duration for improved effectiveness
9. Less chance of adverse consequences than with synthetic formulations
10. A cost-effective and convenient dose form

Limitations of the Study

1. The stability of herbal extracts may fluctuate over time
2. It can be difficult to standardize herbal raw materials.
3. Gummies' consistency and texture could be adversely affected by too much moisture.
4. Different batches of herbal formulations may differ from one another.
5. There isn't much clinical data on long-term treatment effectiveness.

Future Prospects.

The created polyherbal gummy formulation can be investigated further by:

- Extensive research on antimicrobials
- Human trials and clinical assessment
- Studies on toxicity and safety evaluation
- Large-scale and scale-up production research
- Development of commercial products
- Adding more herbal components

Expanding the formulation's therapeutic uses, such as cough treatment, immune system strengthening, and oral and dental health maintenance, may be the subject of future research.

CONCLUSION

The creation and assessment of plant-based polyherbal gummies with guava leaves, tulsi, mint, and camphor were the main objectives of this study. Because they are safer, more effective than synthetic drugs, and have less side effects, herbal formulations have become increasingly important. The created gummy dose form provides a novel and patient-friendly method of administering herbal remedies. The chosen herbal components are helpful for maintaining oral and dental health since they have strong antibacterial, antioxidant, anti-inflammatory, and analgesic qualities. Because the gummies are chewable and pleasant, it is anticipated that they will exhibit acceptable physicochemical properties, effective antibacterial activity, and increased patient compliance. As a result, herbal gummies could be a viable substitute for traditional herbal dose forms. To determine their long-term therapeutic efficacy and potential for commercial use, more investigation and clinical studies are required.

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Table 1: Guava Plant Profile

Parameter	Details
Botanical Name	<i>Psidium guajava</i>
Family	Myrtaceae
Major Phytoconstituents	Quercetin, kaempferol, tannins, phenolic compounds
Therapeutic Applications	Management of oral infections, wound healing, and diarrhea
Classification	Herbal antimicrobial agent

Table 2: Tulsi Plant Profile

Parameter	Details
Botanical Name	<i>Ocimum sanctum</i>
Family	Lamiaceae
Major Phytoconstituents	Eugenol, rosmarinic acid, flavonoids
Therapeutic Applications	Used in cough, cold, fever, and oral infections
Classification	Immunomodulatory herb

Table 3: Mint Plant Profile

Parameter	Details
Botanical Name	<i>Mentha arvensis</i>
Family	Lamiaceae





Sakshi Mahendra Sadgir and *et al.*

Major Phytoconstituents	Menthol, menthone, volatile oils
Therapeutic Applications	Helpful in oral hygiene, nausea, and headache relief
Classification	Carminative and antimicrobial herb

Table 4: Camphor Plant Profile

Parameter	Details
Botanical Name	<i>Cinnamomum camphora</i>
Family	Lauraceae
Major Phytoconstituents	Camphor, cineole, borneol
Therapeutic Applications	Used for analgesic, antimicrobial, and decongestant purposes
Classification	Antiseptic and anti-inflammatory agent



Fig 1: Guava leaf



Fig 2: Tulasi leaf



Fig 3: Mint leaf



Fig 4: Camphor



Fig 5: Extract of Guava, Tulsi, Mint



Fig 6: The prepared candies were taken out and put in airtight receptacles





Sakshi Mahendra Sadgir and *et al.*,



Fig 7: Polyherbal Gummies

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RESEARCH ARTICLE

Hybrid Phyto -Nanofiber Delivery Systems : A New Era in Localized and Sustained Delivery of Herbal Bioactives

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ABSTRACT

The broad medicinal potential, safety profile, and natural origin of herbal bioactives have created a lot of attention. However, their limited bioavailability, unstable nature, fast metabolism, and poor water solubility frequently limit their clinical use. Integrating phytochemicals into polymeric nanofiber matrices has led to the development of hybrid phyto-nanofiber delivery systems as a viable approach to overcoming these challenges. The basic ideas, manufacturing processes, drug release mechanisms, and biological uses of hybrid phyto-nanofiber systems are highlighted in this paper. Herbal bioactives can be delivered locally and sustainably thanks to the special properties of nanofibers, such as their large surface area, adjustable porosity, and controlled release capacity. Recent developments and case studies are presented, along with applications in tissue engineering, cancer treatment, skin and mucosal therapy, and wound healing. Additionally, the advantages, disadvantages, and potential future developments, such as stimuli-responsive systems and customised formulations. All things considered, hybrid phyto-nanofibers offer a novel link between innovative nanotechnology and conventional herbal treatment.

Keywords: Hybrid phyto-nanofibers, Herbal bioactives, Sustained drug delivery, Electrospinning



**Amrit Anupam et al.,**

INTRODUCTION

For centuries, herbal remedies have been an essential component of healthcare systems worldwide, and they continue to receive significant attention in current pharmaceutical research. Natural remedies are becoming increasingly popular due to their extensive therapeutic potential, cultural acceptance, and very low frequency of side effects when administered properly[1]. Numerous herbal bioactives, such as polyphenols, flavonoids, terpenoids, alkaloids, and glycosides, have shown potential pharmacological actions, including immunomodulatory, anti-inflammatory, antioxidant, antibacterial, wound-healing, and anticancer properties. Considering these benefits, converting herbal bioactives into potent pharmaceutical medicines is still a significant issue[2]. The adverse biopharmaceutical characteristics of herbal bioactives are one of their main concerns. Some phytoconstituents show a lack of permeability across biological membranes, fast disintegration under physiological conditions, low chemical and physical stability, and poor water solubility[3]. Low bioavailability, irregular therapeutic results, and the requirement for frequent doses are the results of these problems. Herbal extracts' therapeutic significance is further limited by their composition's diversity, their vulnerability to environmental variables, and the challenges of establishing regulated and focused delivery[4]. These difficulties are frequently not addressed by conventional dose forms like pills, capsules, and ointments, underscoring the critical need for specialized medication delivery strategies designed especially for herbal constituents[5].

Nanotechnology has become an effective method in pharmaceutical sciences in recent years to enhance the therapeutic efficacy, stability, and delivery of both natural and synthetic medications. Herbal bioactives' solubility and bioavailability may be improved by using nano-based delivery methods, such as liposomes, nanoparticles, nanoemulsions, and solid lipid carriers. Because of their special structural and functional characteristics, nanofibers have drawn the most attention among these systems[6]. Nanofibers are ultra-fine fibers with diameters that are usually in the nanometer range. They have adjustable porosity, a very high surface area-to-volume ratio, and the capacity to resemble the extracellular matrix discovered in the environment. Nanofibers are especially well-suited for pharmacological and biological applications because of their qualities[7]. High drug loading efficiency, regulated and prolonged drug release, and design flexibility are some of the benefits that nanofiber-based drug delivery systems provide over traditional carriers. The mechanical strength, rate of decomposition, and release behaviour can all be customized by using a variety of natural and synthetic polymers in their production[8]. Crucially, medications can be delivered locally at the site of action using nanofibers, which minimizes systemic exposure and minimizes undesirable side effects. Applications including wound healing, skin and transdermal therapy, mucosal drug delivery, and dental treatments benefit significantly from this localized delivery effectiveness[9].

The idea of hybrid phyto-nanofiber delivery systems is a novel strategy that combines the latest advances of nanofiber technology with the medicinal advantages of herbal bioactives. These technologies create a hybrid platform that protects sensitive herbal chemicals while guaranteeing controlled and sustained release by incorporating phytoconstituents or herbal extracts into polymeric nanofiber matrices[10]. By acting as a protective carrier, the nanofiber structure improves the stability of herbal actives and keeps them safe from environmental damage. Simultaneously, effective drug diffusion and extended release at the target site are made possible by the porous and fibrous architecture[11]. For localized drug delivery, where maintaining a constant drug concentration at the site of action is essential for therapeutic efficacy, hybrid phyto-nanofiber systems show immense potential. These devices can increase treatment efficacy while minimizing the required dosage and frequency of administration by delivering herbal bioactives directly to the damaged tissue[12]. These systems have promise for long-term and chronic applications since the use of biocompatible and biodegradable polymers works in perfectly with the safety requirements related to herbal medicines[13].

The available literature is still dispersed across many application domains and fabrication techniques, despite the increasing number of experimental investigations evaluating herbal-loaded nanofibers[14]. There is currently no



**Amrit Anupam et al.,**

complete and targeted review that carefully addresses the design concepts, preparation techniques, drug release mechanisms, applications, benefits, and difficulties of hybrid phyto-nanofiber delivery systems. In order to give researchers an extensive understanding of the field and to propose future research lines that could speed up clinical translation, it is crucial that this gap be filled[15]. The goal of this review is to give an accurate and critical overview of hybrid phyto-nanofiber delivery systems as a new platform for the sustained and localized delivery of herbal bioactives[16]. The review describes the main pharmaceutical and biological applications, highlights important preparation and characterization techniques, explains the benefits and drawbacks of these systems, and explains the reasoning for integrating herbal medications with nanofiber technology[17]. In order guide future advancements in this quickly developing field of pharmaceuticals, future possibilities and possible research opportunities are also examined[18].

Herbal Bioactive

The natural origin, safety, and therapeutic potential of herbal bioactives in the treatment of a variety of conditions make them highly regarded. However, a number of basic limitations frequently limit their useful therapeutic application. Numerous phytochemicals show limited chemical stability, poor water solubility, and quick breakdown in the presence of heat, light, or physiological circumstances. Also, high first-pass metabolism and low permeability across cellular membranes lead to reduced bioavailability. These difficulties emphasize the necessity of innovative and effective medication delivery systems[19].

Limitations

- **Poor solubility** - One of the main problems of many herbal bioactives is their poor solubility. The majority of phytochemicals, including resveratrol, quercetin, curcumin, and berberine, are poorly soluble in water, which directly impacts how well the body absorbs them. These substances dissolve slowly at the site of administration due to their low solubility, which prevents them from reaching therapeutic quantities that are beneficial. Higher dosages are therefore frequently needed, which may lower patient compliance and limits their therapeutic utility [20].
- **Poor stability** - Many herbal bioactives are severely limited by poor stability, which has a significant effect on their medicinal efficacy. Many phytochemicals are extremely sensitive to biological and environmental elements like heat, light, and pH changes. Loss of activity and chemical degradation may result from exposure to these circumstances. Also, the body's enzymatic processes can quickly degrade herbal substances before they get to the intended location. The need for safe delivery systems is highlighted by this instability, which reduces shelf life, decreases potency, and makes constant dosing challenging[21].
- **Rapid metabolism** - The therapeutic efficacy of many herbal bioactives is greatly impacted by their rapid metabolism, which is a severe limitation. Many phytochemicals have a relatively short biological half-life after delivery because they are rapidly broken down by intestinal microbiota and liver enzymes. The medication cannot sustain effective concentrations at the target site for a long enough period of time due to its quick breakdown. Frequent dosage is therefore necessary, which lowers patient compliance and reduces clinical efficacy. Therefore, in order to extend the therapeutic effect of herbal substances and prevent their rapid metabolic breakdown, advanced delivery mechanisms are required [22].
- **Low bioavailability** - Another significant factor limiting the medicinal efficacy of herbal bioactives is their low bioavailability. Only a small percentage of phytochemicals can enter the target site in an active form, even after administration. Low membrane permeability, fast metabolism, limited water solubility, and early bodily elimination are the main causes of this. Because of this, higher doses are frequently needed to get the intended effect, which could make herbal medicines less effective and consistent[23].
- **Variability in extract composition** - Due to variations in plant species, geographic location, harvesting period, and extraction techniques, crude herbal extracts may show significant chemical composition variability. Variations in therapeutic efficacy and poor repeatability of treatment outcomes may result from this inconsistency. It becomes difficult to maintain consistent medication levels at the target site as a result. By preserving herbal bioactive, expanding bioavailability in a controlled and predictable manner, and improving



**Amrit Anupam et al.,**

dose uniformity, advanced drug delivery methods like phyto-nanofiber platforms can get over this restriction [24].

Basics of Nanofibers

Nanofibers are extremely small fibers with sizes in the nanometer range that are renowned for their flexibility, porous structure, and large surface area. They are ideal for medication delivery applications because of these qualities. Nanofibers are effective carriers that can encapsulate and shield delicate bioactive substances in the context of herbal medicine delivery. They offer regulated and prolonged release at the target site as well as uniform medication dispersion. Electro spinning is a typical method for creating nanofibers, which can be made from synthetic or natural polymers. Their suitability for targeted treatment systems is further improved by their structural similarity to biological tissues[33].

Properties of Nanofibers

- **High surface area-to-volume ratio:** Nanofibers offer a very large surface area due to their extremely small dimensions. This allows quicker interaction between herbal bioactives and the target site, increases medication loading capacity, and improves contact with biological tissues[34].
- **Highly porous structure:** Long-lasting therapeutic action is ensured by the interconnected porosity network of nanofibers, which promotes consistent drug distribution and controlled and sustained release[35].
- **Excellent flexibility and softness:** Nanofibers quickly bind to body surfaces and are lightweight and flexible. They are therefore perfect for use as localized delivery systems, transdermal patches, and wound dressings[36].
- **Ability to load both hydrophilic and hydrophobic drugs:** Regardless of their solubility, a variety of herbal bioactives can be encapsulated by nanofibers, which can help with formulation issues related to herbal compounds[37].
- **Controlled and sustained drug release:** By altering the polymer composition, fiber diameter, and manufacturing process, drug release from nanofibers can be customized, lowering the frequency of dosage[38].
- **Protection of herbal bioactives:** Sensitive herbal chemicals are shielded from light, heat, pH shifts, and enzymatic destruction using nanofiber matrices[39].
- **Biocompatibility and biodegradability:** Nanofibers are safe, biodegradable, and well-tolerated by biological tissues when made using suitable polymers[40].
- **Localized drug delivery:** By enabling direct administration at the site of action, nanofibers improve therapeutic efficacy while reducing systemic side effects[41].

Polymers used in nanofibers

- **Role of polymers:** The mechanical strength, stability, biocompatibility, and drug release behavior of hybrid phyto-nanofiber delivery systems are all dependent on polymers[42].
- **Natural polymers:** Chitosan, gelatin, collagen, alginate, and cellulose derivatives are biocompatible, biodegradable, and closely match biological tissues, they are frequently utilized. They are especially appropriate for applications involving wound healing and localized distribution.
- **Advantages of natural polymers:** They promote tissue regeneration, promote the growth of cells, and are usually safe to use over an extended period of time[43].
- **Synthetic polymers:** Better mechanical strength, stability, and controlled degradation qualities can be provided by polyvinyl alcohol (PVA), polycaprolactone (PCL), polylactic acid (PLA), and polyethylene oxide (PEO).
- **Advantages of synthetic polymers:** They provide effective medication release, enhanced durability, and consistent fiber development[44].
- **Blended polymer systems:** In order to combine mechanical strength and biocompatibility, it is often easier to blend natural and synthetic polymers. This leads to improved drug loading and delayed release of herbal bioactives[45].



**Amrit Anupam et al.,**

Fabrication techniques

The most popular method for creating hybrid phyto-nanofiber delivery systems among the different nanofiber preparation techniques is electrospinning. This technique creates continuous, ultra-fine fibers from a polymer solution containing herbal bioactives using a high-voltage electric field. Because electrospinning is simple, flexible, and able to create consistent nanofibers with regulated diameter and shape, it is the method of choice. It preserves the biological activity of both hydrophilic and hydrophobic herbal components while enabling their effective breakdown. Electrospinning also makes it possible to create nanofiber sheets that are appropriate for long-term and targeted medication delivery[46].

Electrospinning: Principle and Process

A popular, easy, and effective method for creating nanofibers for hybrid phyto-nanofiber delivery systems is electrospinning. This method involves stretching a polymer solution containing herbal bioactives into ultra-fine fibers by using a high-voltage electric field. Continuous nanofibers are created and collected into thin sheets when the solvent evaporates. This method is appropriate for encapsulating herbal chemicals and ensuring localized and sustained medication delivery because it provides good control over fiber diameter and shape[47].

Steps in Electrospinning

- **Preparation of solution:** To create a homogenous spinning solution, the chosen polymer and herbal bioactive are dissolved in an appropriate solvent.
- **Loading into syringe:** A syringe with a fine metal needle attached is filled with the prepared solution.
- **Application of high voltage:** The solution can be pushed into a fine jet by applying a high-voltage electric field.
- **Formation of nanofibers:** The jet solidifies into continuous nanofibers when the solvent evaporates.
- **Collection of fibers:** The created nanofibers are applied as thin sheets to a moving or stationary collector, making them ideal for the localized and long-term administration of herbal medications[48].

Types of Electrospinning

- **Single-fluid electrospinning:** This is the easiest and widely used technique, which involves electrospinning a single polymer solution containing the herbal bioactive to create homogenous nanofibers. It works well for simple drug loading and either immediate or moderate release[49].
- **Coaxial electrospinning:** This method creates core-shell nanofibers using two concentric solutions. It is particularly helpful for obtaining sustained and controlled release and protecting sensitive herbal bioactives[50].
- **Multi-fluid electrospinning:** This advanced technique allows for the simultaneous electrospinning of various solutions, enabling the blending of multiple herbal active or combination therapies into a single nanofiber system[51].

Hybrid Phyto-Nanofiber Delivery Systems

In order to enhance their therapeutic efficacy, herbal bioactives are integrated into polymeric nanofiber matrices in hybrid phyto-nanofiber delivery systems. The nanofiber network serves as a shield in these systems, stabilizing sensitive phytochemicals and improving their retention at the intended location[52]. Better medication distribution, enhanced solubility, and extended-release profiles are made possible by the combination of herbal ingredients and nanofibers. For localized and sustained delivery applications, such as wound healing and dermal therapy, where maintaining constant therapeutic levels of herbal bioactives is important for successful treatment, these hybrid systems are especially effective[53].

Use of hybrid system

- **Improved solubility and dissolution:** Herbal bioactives that are poorly soluble in water are made more soluble by hybrid phyto-nanofiber systems, which improve absorption and therapeutic efficacy.
- **Enhanced stability:** Sensitive herbal ingredients are shielded from breakdown by light, heat, and environmental elements by the nanofiber matrix.



**Amrit Anupam et al.,**

- **Sustained and controlled release:** Hybrid systems maintain effective therapeutic doses by enabling progressive medication release over time.
- **Localized therapy:** They minimize systemic exposure by delivering herbal bioactives straight to the intended location.
- **Reduced dose and dosing frequency:** By reducing the need for frequent doses, sustained release increases patient compliance[54].

Mechanism of action

The herbal bioactive is either distributed throughout or enclosed in the nanofiber matrix in hybrid phyto-nanofiber delivery systems. The herbal substance is protected from biological and environmental damage by the nanofiber structure. Following application, the medication is gradually and carefully removed from the nanofibers, mostly by diffusion or slow polymer erosion. By maintaining steady medication levels at the target site for a longer period of time, this method enhances therapeutic efficacy and reduces the need for frequent doses[55].

Types of hybrid nanofibers

- **Herbal extract-loaded nanofibers:** This kind involves evenly mixing polymer solutions with herbal extracts just before electrospinning. They can be used for either immediate or moderate medication release and are easy to make.
- **Core-shell nanofibers:** These nanofibers have a polymer shell on their outer layer and the herbal bioactive in the center. Sensitive herbal ingredients are shielded by this structure, which also offers controlled and prolonged release[56].
- **Nanoparticle-loaded nanofibers:** Herbal bioactives are absorbed into nanofibers after first being transformed into nanoparticles. Stability, drug loading, and extended therapeutic action are all enhanced by this method.
- **Multi-drug hybrid nanofibers:** Combination therapy and improved therapeutic efficacy are made possible by these systems, which contain multiple herbal or herbal-synthetic drugs[57].

Preparation Methods of Hybrid Phyto-Nanofibers

- **Direct Blending Method:** In the direct blending procedure, the polymer and herbal extract are first dissolved or dispersed in a compatible solvent. The herbal bioactive is then generated into nanofibers from this homogenous solution. For herbal substances that are stable under electrospinning conditions, this easy and popular approach is effective. It is frequently utilized for either immediate or possibly prolonged medication release and provides uniform drug distribution inside the nanofiber matrix[58].
- **Coaxial Electrospinning:** The latest technology for creating core-shell structured nanofibers is coaxial electrospinning. Using this technique, the polymer creates an outer shell and the herbal bioactive is positioned in the center. Sensitive herbal chemicals are better shielded from environmental damage by this structure. It is perfect for long-term and localized therapy because it minimizes the first burst effect and helps achieve sustained medication release[59].
- **Post-Loading Method:** Nanofibers are initially made without the herbal medication in the post-loading process. Following manufacture, adsorption or surface coating is used to load the herbal bioactive onto the nanofibers. This method works well for herbal chemicals that are dependent on electricity or heat. It prevents the breakdown of sensitive phytochemicals even though medication loading could be lower than with blending techniques[60].
- **Characterization Methods:** To assess the performance and quality of hybrid phyto-nanofibers, characterization is crucial. Fiber diameter and shape are investigated using scanning electron microscopy (SEM). FTIR, or Fourier transform infrared spectroscopy, assists in verifying drug-polymer compatibility. The drug's physical condition can be detected using X-ray diffraction (XRD) and differential scanning calorimetry (DSC). Tensile testing measures mechanical strength and flexibility, however in vitro drug release tests evaluate release behavior[61].





Amrit Anupam et al.,

Mechanisms of Drug Release

To achieve targeted and prolonged therapeutic activity in hybrid phyto-nanofiber delivery systems, the drug release mechanism is essential. Polymer type, fiber diameter, porosity, hydrophilicity, and the drug-polymer matrix interaction are some of the variables that affect the release behavior of herbal bioactives. Nanofibers can be made to distribute herbal substances in a regulated and effective way by carefully combining these characteristics[62].

- **Diffusion-Controlled Release:** The herbal bioactive has an even distribution throughout the nanofiber matrix during diffusion-controlled release. The medicine quickly diffuses via the small openings in the fiber structure when the nanofibers come into touch with bodily fluids. Polymer degradation is not necessary for this process, which is powered by concentration gradients. Dense nanofiber sheets frequently exhibit diffusion-controlled release, which is appropriate for sustaining constant medication levels over time[63].
- **Swelling-Controlled Release:** The majority of nanofibers made from hydrophilic polymers like PVA, gelatin, or chitosan show swelling-controlled release. These polymers absorb water and swell when they come into contact with moisture or bodily fluids. The swelling creates channels that allow the encapsulated herbal bioactive to be released gradually by increasing the distance between polymer chains. This technique is very helpful for external applications and wound healing since it allows for consistent and continuous medication release[64].
- **Erosion-Controlled Release:** When biodegradable polymers like PLA or PCL are mixed to create nanofibers, erosion-controlled release is detected. The nanofiber matrix becomes thinner as a result of these polymers' hydrolytic or enzymatic breakdown over time. The enclosed herbal bioactive is released gradually as the polymer degrades. When extended medication release is needed for long-term therapy, this mechanism is perfect[65].
- **Multi-Phase Release:** Multiple release mechanisms are combined into a single system through multi-phase release. The drug on or near the surface of the nanofibers usually causes a sudden release, which offers quick therapeutic activity. A prolonged release phase controlled by erosion, swelling, or diffusion comes next. Because it guarantees instant relief while sustaining long-term therapeutic levels, such release behavior is highly efficient and reduces the need for regular dosage[66].

Applications of Hybrid Phyto-Nanofiber Systems

A significant development in the localized and long-term distribution of herbal bioactives is the use of hybrid phyto-nanofiber delivery systems. These methods allow site-specific drug delivery with sustained therapeutic activity by merging the structural and functional benefits of nanofibers with the medicinal potential of herbal medicines[67]. Applications where it's crucial to maintain a constant medication concentration at the target site are particularly well suited for hybrid phyto-nanofibers. They are perfect for topical, mucosal, and implantable applications due to their large surface area, porous design, and capacity to regulate drug release. Wound healing, skin and transdermal therapy, dental and periodontal treatment, vaginal and rectal administration, cancer therapy, and tissue engineering have all found extensive use for these systems[68].

- **Wound Healing:** -hybrid phyto-nanofiber systems can provide a moist environment and prolonged release of herbal bioactives, they are very successful in wound healing applications. By lowering inflammation and avoiding microbial infection, herbal-loaded nanofibers containing substances like curcumin, neem, aloe vera, and Centella asiatica allow quick wound closure[69]. The herbal active ingredients are shielded by the nanofiber matrix, which also guarantees continuous contact with the wound surface. Localized administration reduces systemic side effects, and their porous nature promotes oxygen exchange and tissue repair. Because of these characteristics, phyto-nanofibers are a possible replacement for traditional wound dressings[70].
- **Dermal and Transdermal Delivery:** - Hybrid phyto-nanofiber systems provide faster skin penetration and targeted medication administration in dermal and transdermal applications. Herbal bioactives-loaded nanofiber patches attach to the skin well and deliver prolonged release directly to the application site[71]. Nanofibers' large surface area improves therapeutic efficacy by enhancing medication diffusion through the stratum corneum. This method lowers medication loss and avoids first-pass metabolism, which is advantageous for herbal



**Amrit Anupam et al.,**

compounds used for anti-inflammatory, antibacterial, or antioxidant purposes. These devices are very helpful in the treatment of localized inflammation and chronic skin conditions[72].

- **Oral Mucosal Delivery:** - The flexibility, thin structure, and strong adherence of hybrid phyto-nanofibers to mucosal surfaces make them ideal for oral mucosal medication delivery. Herbal bioactives can be directly absorbed into the systemic circulation when nanofiber mats are placed to the buccal or sublingual area[73]. This method works well for administering anti-inflammatory herbal medications and local painkillers. Long-term therapeutic levels are maintained through sustained release from nanofibers, which prevents first-pass metabolism and gastrointestinal breakdown. This increases patient comfort and compliance while also improving bioavailability[74].
- **Dental and Periodontal Therapy:** - By gradually releasing herbal bioactives within periodontal pockets, hybrid phyto-nanofiber systems offer effective targeted treatment in dental and periodontal therapy. Antimicrobial and anti-inflammatory substances found in herbal-loaded nanofibers help in tissue repair, gum inflammation reduction, and infection management[75]. Easy placement and extended retention at the treatment site are made possible by their fibrous composition. Sustained release enhances therapeutic results and reduces the need for constant treatment. These devices are especially helpful in treating chronic periodontal diseases, because long-term effectiveness depends on maintaining constant medication levels locally[76].
- **Vaginal and Rectal Delivery:** -hybrid phyto-nanofibers can produce targeted and long-lasting therapeutic activity; they are showing interest as vaginal and rectal drug delivery methods. To treat local infections while reducing systemic exposure, herbal bioactives with antibacterial or anti-inflammatory qualities can be added to nanofibers[77]. Good mucosal adherence and patient comfort are guaranteed by the soft and flexible nature of nanofibers. Sustained release lowers the frequency of doses by maintaining effective medication concentrations over extended periods of time. When regulated and gentle medication distribution is needed for sensitive mucosal tissues, this method is especially helpful[78].
- **Cancer Therapy:** - Hybrid phyto-nanofiber systems are being investigated for the localized administration of herbal anticancer drugs like quercetin and curcumin in cancer treatment. These techniques ensure high local medication concentration while lowering systemic toxicity by enabling direct injection or placement close to tumor locations[79]. Herbal bioactives are shielded from degradation by the nanofiber matrix, which additionally allows prolonged release. This specific approach minimizes adverse effects from traditional chemotherapy, decreases harm to healthy tissues, and improves anticancer activity. These systems have the potential to be helpful or different approaches to managing cancer[80].
- **Tissue Engineering:** - Because of their structural similarity to the extracellular matrix (ECM) seen in nature, hybrid phyto-nanofiber systems are crucial to tissue engineering. Cell adhesion, proliferation, and transformation are encouraged by the supportive structure that nanofibers supply[81]. Herbal bioactives have anti-inflammatory, antioxidant, or growth-promoting properties that further improve tissue regeneration. Applications for soft tissue, bone, and skin engineering use these technologies. Phyto-nanofibers are very promising for applications in regenerative medicine and healing because they provide both structural support and long-term transport of herbal chemicals[82].

Case Studies and Recent Findings

The potential of hybrid phyto-nanofiber delivery methods to solve the primary disadvantages of herbal bioactives has been shown by recent research. The integration of phytochemicals into nanofiber matrices enhances stability, release control, and therapeutic performance, as proved by case-based studies. Key recent developments documented in the literature are summarized up in the following examples[83].

- **Curcumin Nanofibers:** - The quick metabolism and poor solubility of curcumin has resulted in significant studies on curcumin-loaded nanofibers as a model system. The aqueous solubility and stability of curcumin are significantly improved when it gets absorbed into polymeric nanofibers, according to case studies[84]. Through its long-lasting anti-inflammatory and antioxidant properties, curcumin nanofiber mats accelerate tissue regeneration in wound healing models. Prolonged therapeutic effect at the wound site is ensured by the





Amrit Anupam *et al.*,

nanofiber structure, which ensures regulated release of curcumin over a period of hours to days. These results support the efficacy of hybrid phyto-nanofibers in treating localised wounds[85].

- **Resveratrol Nanofibers:** - The capacity of resveratrol nanofibers to shield the substance from oxidation, light, and enzymatic breakdown has generated concern. Resveratrol's chemical stability is maintained, and its antioxidant activity is increased when it is encapsulated in nanofiber matrices, according to recent studies[86]. Resveratrol is continuously available at the target site due to sustained release from nanofibers, which enhances its therapeutic effectiveness. Applications involving skin and tissue, where sustained antioxidant protection is necessary, benefit significantly from such systems. These results show that nanofibers are efficient delivery systems for resveratrol and other unstable herbal bioactives[87].
- **Neem and Aloe Vera Nanofibers:** Recent investigations have demonstrated excellent antibacterial and wound-healing capabilities of nanofibers loaded with extracts from neem and aloe vera. Aloe vera improves tissue regeneration and moisture retention, whereas neem-based nanofibers have potent antibacterial action against both Gram-positive and Gram-negative bacteria[88]. These herbal actives are distributed uniformly and released continuously because of the nanofiber matrix, which improves therapeutic results. According to case studies, these systems are a good alternative for traditional herbal ointments and bandages since they lower the risk of infection and speed up healing[89].
- **Herbal Nanoparticle-Nanofiber Hybrids:** Recent research additionally highlights the creation of hybrid systems that combine nanofibers and herbal nanoparticles. These systems involve the formulation of herbal bioactives into nanoparticles, which are further integrated into nanofiber scaffolds[90]. This dual strategy combines the localised distribution and sustained release of nanofibers with the high loading and protection of nanoparticles. When compared to single-carrier systems, case studies show improved therapeutic effectiveness and better control over drug release characteristics. Such hybrid designs are a cutting-edge approach of delivering herbal bioactives precisely and sustainably[91].

Advantages of Hybrid Phyto-Nanofiber Systems

The delivery of herbal bioactives in a localised and sustained manner has been significantly enhanced by hybrid phyto-nanofiber delivery systems. These technologies successfully get over significant drawbacks such as low bioavailability, fast metabolism, instability, and poor solubility by integrating phytochemicals into a polymeric nanofiber matrix. As a protective and useful carrier, the nanofiber structure improves drug dispersion, sustains therapeutic concentration at the target location, and permits regulated release over a prolonged duration. They are extremely suitable for a range of pharmaceutical applications due to their high surface area, porous architecture, and modifiable polymer composition. All of these benefits work together to increase patient compliance, decrease systemic adverse effects, and improve treatment efficacy[92].

Key Advantages Explained

- **Improved Solubility and Dissolution:**Herbal bioactives that are poorly soluble in water show improved solubility when distributed within a nanofiber matrix, improving absorption and therapeutic effect.
- **Enhanced Stability of Herbal Actives:**The network of polymeric nanofibers shields sensitive phytochemicals from oxidation, heat, light, pH changes, and enzymatic breakdown.
- **Sustained and Controlled Drug Release:**Drug release can be precisely regulated to ensure sustained therapeutic doses by altering the kind of polymer and the shape of the fibre.
- **Localized Drug Delivery:**Herbal bioactives are delivered specifically to the place of action using nanofiber mats, patches, or implants, reducing systemic exposure and adverse effects.
- **Reduced Dose and Frequency:**Sustained release improves patient adherence by lowering the need for repeated dosage.
- **High Drug Loading Efficiency:**Both hydrophilic and hydrophobic herbal components can be effectively included due to the high surface area-to-volume ratio.
- **Biocompatibility and Biodegradability:**Minimal toxicity and gradual bodily breakdown are guaranteed by the use of safe, natural and synthetic polymers.



**Amrit Anupam et al.,**

- **Versatility Across Applications:** Hybrid phyto-nanofibers can be used for tissue engineering, cancer treatment, dental treatment, wound healing, and skin and mucosal distribution[93].

Limitations and Challenges of Hybrid Phyto-Nanofiber Systems

Hybrid phyto-nanofiber delivery systems have a lot of potential for delivering herbal bioactives locally and sustainably, but before they are widely used in clinical and commercial settings, a number of restrictions and practical issues must be resolved. Formulation stability, manufacturing complexity, safety issues, and regulatory uncertainty are the primary causes of these difficulties. Translating laboratory research into actual pharmaceutical drugs requires an understanding of these concerns[94].

- **Stability Issues of Herbal Extracts:** In addition to being sensitive to light, heat, oxygen, and moisture, herbal bioactives are frequently chemically unstable. Certain phytochemicals may break down during processing or storage, even if they are integrated into nanofibers. It is still very difficult to maintain long-term stability without losing therapeutic effectiveness, particularly for complicated crude extracts[95].
- **Polymer-Drug Compatibility Problems:** Proper interaction between the herbal chemical and the polymer is essential for a successful formulation. Phase separation, decreased drug loading, poor drug distribution, or changed release behaviour can all result from incompatibility. Furthermore, medication bioactivity may be impacted by significant interactions[96].
- **Difficulty in Scaling up Electrospinning:** Although electrospinning works well in lab environments, it is technically difficult to scale up for industrial production. It takes advanced equipment and optimisation to maintain consistent fibre diameter, medication distribution, and reproducibility throughout large-scale manufacture[97].
- **Solvent Residues May Be Harmful:** Organic solvents are used in many electrospinning procedures. Remaining solvents can be harmful or irritating if they are not totally eliminated, especially in topical and mucosal applications. Therefore, creating safer solvent systems is crucial[98].
- **Lack of Regulatory Guidelines:** Specific regulatory frameworks for herbal delivery systems based on nanofibers are limited. Approval procedures get more complicated when plant extracts and nanotechnology are combined[99].
- **Limited Clinical Data:** The majority of research on hybrid phyto-nanofibers is still in the preclinical or in vitro stages. To verify safety, effectiveness, and long-term performance in humans, more clinical trials are required[100].

Future Prospective

Future localised and sustained distribution of herbal bioactives could be greatly enhanced by hybrid phyto-nanofiber systems. These systems are predicted to develop into intelligent, patient-specific treatment platforms as a result of the quick advances in materials science, nanotechnology, and digital healthcare. In the upcoming years, this technique may advance toward clinical translation in the following promising sectors.

- **Stimuli-Responsive Nanofibers (pH, Temperature, Enzyme):** Herbal bioactives are only released by stimuli-responsive nanofibers in response to particular biological or environmental stimuli. For instance, temperature-sensitive systems react to localised inflammation, whereas pH-responsive fibres can release medications in acidic wound conditions or tumour tissues. When certain enzymes are present in sick tissues, enzyme-responsive nanofibers break down. These intelligent solutions improve therapeutic efficacy, reduce needless exposure, and enable exact medication delivery. Herbal components can greatly enhance targeted and regulated distribution when used in these responsive systems[101].
- **4D-Printed Nanofiber Systems:** A more advanced version of 3D printing, 4D printing allows the material to alter shape or function over time in response to external stimuli. 4D printing may make it possible for hybrid phyto-nanofiber systems to show dynamic drug release behaviour, in which the structure changes in response to physiological conditions. For instance, a nanofiber patch's release rate may change in response to temperature or moisture. This technique presents a cutting-edge method for responsive and customised herbal medication delivery systems[102].



**Amrit Anupam et al.,**

- **Combination Therapy (Herbal + Synthetic Drugs):** By combining synthetic medications and herbal bioactives in one matrix, hybrid nanofibers provide a great platform for combination therapy. Combining a synthetic antibiotic for wound healing with a natural anti-inflammatory drug is one example of how this approach can have synergistic therapeutic effects. The balanced release of both drugs is ensured by controlled co-delivery, which increases effectiveness while lowering toxicity. Particularly in cases of complex disorders, such integrated systems may enhance therapy results[103].
- **Personalized Medicine Patches:** A new approach to healthcare that is suited to the needs of each patient is called personalised medicine. Depending on the circumstances of each patient, hybrid phyto-nanofiber patches can be modified in terms of medication type, dosage, and release rate. Therapy can be precisely controlled by varying the medication loading, polymer composition, and fibre thickness. This method guarantees more effective treatment with fewer side effects and increases patient compliance[104].
- **Hybrid Systems for Chronic Diseases:** Long-term, carefully monitored medication treatment is necessary for chronic conditions like diabetes, arthritis, and cancer. By releasing herbal bioactives over long periods of time, hybrid phyto-nanofiber systems can minimise the need for regular dosage. Their capacity to be delivered locally reduces systemic toxicity while managing persistent infection or inflammation. They are also excellent options for long-term therapeutic care[105].
- **AI-Assisted Formulation Design:** The application of artificial intelligence (AI) in the creation of pharmacological formulations is growing. To improve nanofiber formulations, artificial intelligence (AI) technologies can examine medication interactions, polymer characteristics, and release patterns. AI can minimise trial-and-error experimentation by projecting optimal polymer combinations and processing conditions. Research on hybrid phyto-nanofibers and AI integration could accelerate formulation development and improve reproducibility, facilitating quicker clinical translation[106].

CONCLUSION

In the field of pharmaceuticals, hybrid phyto-nanofiber delivery systems are a novel and promising strategy, especially for attaining localised and sustained delivery of herbal bioactives. These systems successfully overcome significant limitations such as low bioavailability, quick metabolism, instability, and poor solubility by combining natural phytochemicals with cutting-edge nanofiber technology. Nanofibers' special structural characteristics, such as their large surface area and capacity for regulated release, improve therapeutic outcomes while reducing systemic adverse effects. Their broad range of applications in tissue engineering, cancer treatment, cutaneous therapy, mucosal administration, and wound healing emphasises their adaptability even further. Even though there are still issues with clinical validation, regulatory approval, and large-scale production, these are gradually being addressed by continued research and technical developments. All things considered, hybrid phyto-nanofiber systems provide a biologically compatible and scientifically advanced platform that could greatly enhance the future research and clinical use of herbal medication therapies.

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Amrit Anupam *et al.*,**Table 1: Common Herbal Bioactives and Their Biopharmaceutical Limitations**

Herbal Bioactive	Natural Source	Therapeutic Applications	Biopharmaceutical Limitation	Impact on Therapy	Relevance to Phyto-Nanofiber Systems	References
Curcumin	Curcuma longa (Turmeric)	Anti-inflammatory, antioxidant, anticancer, wound healing	Very poor aqueous solubility and rapid metabolism	Low absorption and short half-life	Nanofibers improve solubility, protect from degradation, and provide sustained localized release	[25]
Resveratrol	Grapes, berries, peanuts	Antioxidant, cardioprotective, anti-aging	Low chemical stability and light sensitivity	Loss of activity during storage and after administration	Nanofiber matrices enhance stability and control release at the target site	[26]
Quercetin	Onion, apple, green tea	Anticancer, anti-inflammatory, antiviral	Poor bioavailability and low permeability	Insufficient drug levels at the target tissue	Nanofibers enhance residence time and localized delivery	[27]
Asiaticoside	Centella asiatica	Wound healing, skin regeneration	Rapid degradation and low stability	Reduced therapeutic effectiveness	Nanofibers protect the drug and allow prolonged wound contact	[28]
Aloe vera actives	Aloe barbadensis	Wound healing, anti-inflammatory	Poor stability and rapid degradation	Inconsistent therapeutic response	Encapsulation in nanofibers improves stability and sustained action	[29]
Berberine	Berberis species	Antidiabetic, antimicrobial	Poor solubility and extensive first-pass metabolism	Very low systemic availability	Nanofibers help bypass metabolism via localized delivery	[30]



Amrit Anupam *et al.*,

Neem bioactives	Azadiracht a indica	Antimicrobial, anti-inflammatory	Variability in extract composition	Poor reproducibility and dose inconsistency	Nanofiber loading improves dose uniformity and controlled release	[31]
Reserpine (herbal alkaloid)	Rauwolfia serpentina	Antihypertensive	Narrow therapeutic window and instability	Risk of sub-therapeutic or toxic levels	Controlled release nanofibers enhance safety and efficacy	[32]

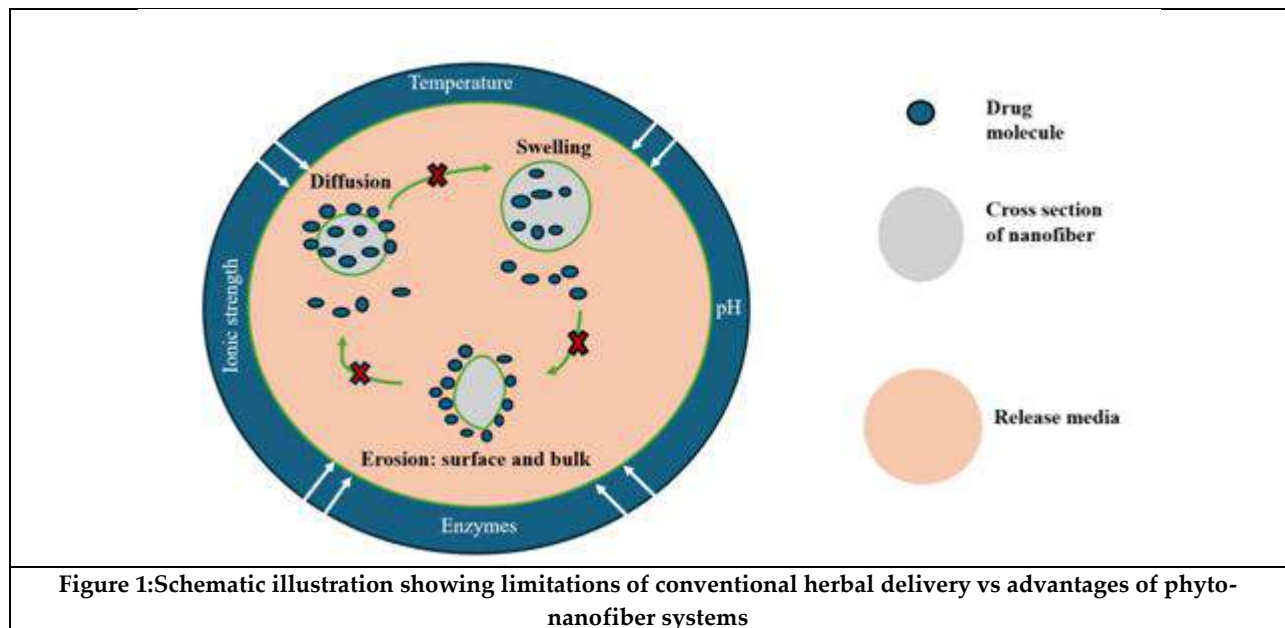
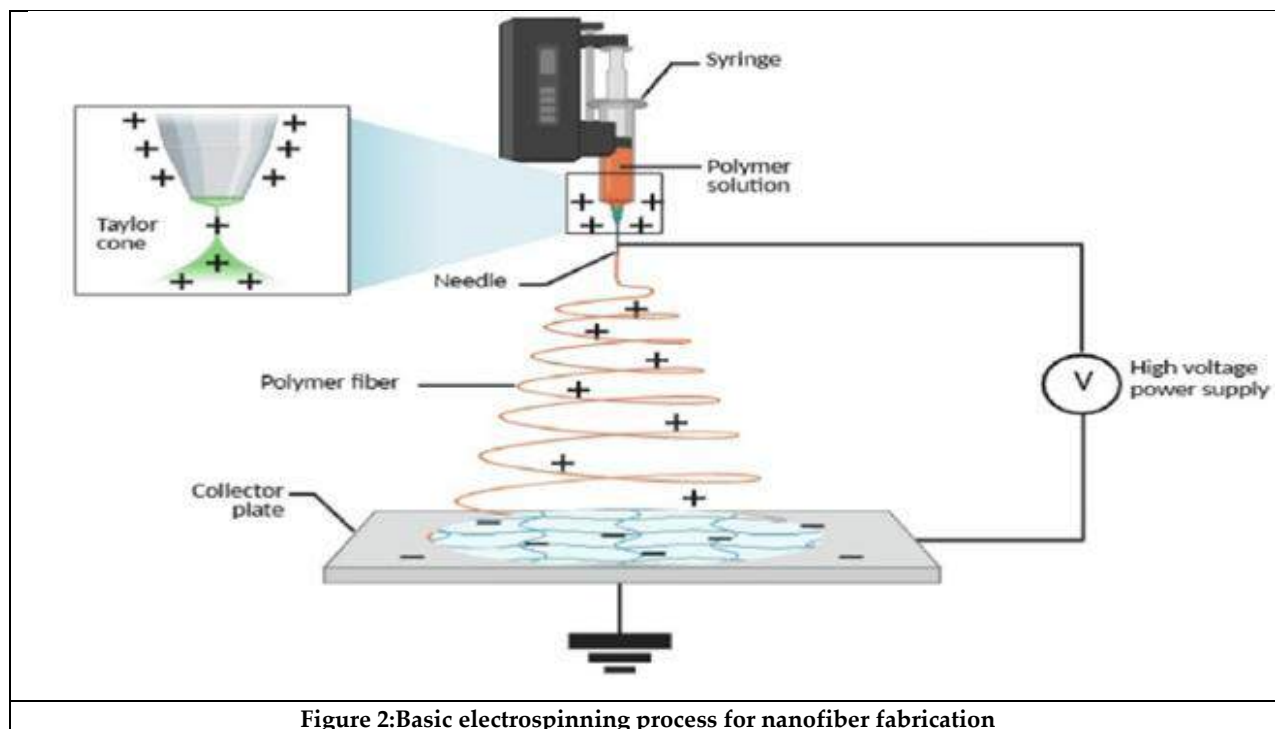


Figure 1: Schematic illustration showing limitations of conventional herbal delivery vs advantages of phyto-nanofiber systems



**Amrit Anupam et al.,****Figure 2:Basic electrospinning process for nanofiber fabrication**

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REVIEW ARTICLE

Dropout Analysis in Unit Dose Homoeopathy Practice with Alternating Action Remedies for Chronic Illnesses : A Retrospective Observational Study

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ABSTRACT

Dropouts (or high attrition) of patients in long term management of chronic diseases in Homoeopathic OPD practice have always remained problematic and unrelenting methodological challenge. The individualized homoeopathic practice (based on Dr. Samuel Hahnemann's concept of alternating action in proving) makes it even more difficult. The single medicine in unit dose may produce various paroxysms of opposing symptoms as part of primary action (depending on dose, potency, and individual susceptibility) and thus dynamics of dropout may reveal shaded interactions between therapeutic response, patient expectations, and real-world feasibility constraints. Assessment of dropout patterns, characterization of retention dynamics, and estimation of clinical outcomes in retrospective cohort of patients on unit dose homoeopathic treatment using remedies known to show alternating action in proving, prescribed according to Dr. Hahnemann's principles. A retrospective observational cohort study was conducted including 544 patients diagnosed with chronic illnesses who had received individualized homoeopathic care in unit dose with alternating action remedies. Data were collected over a period of 2 years (May 2022 – May 2024) at Department of Homoeopathy, AIIMS Raipur. All patients received unit doses from a list of 20 medicines exhibiting alternating action either in Centesimal or LM as per Their indications and suitability to case. When a well-selected remedy failed to produce improvement, same remedy was repeated in the same potency or next higher, whether C or LM, following Dr. Hahnemann's



**Ashutosh Tripathi and Rakesh Gohel**

directions. Three follow ups were scheduled, without any medication at a gap of 2 weeks each. Retention rates, clinical outcomes in treatment completers, and attrition patterns were analyzed. A sensitivity analysis considering worst-case dropout (all dropouts classified as 'no improvement') was also performed. Out of 544 Served patients, 304 (56%) completed chosen follow-up period, whereas 240 (44%) were lost to follow up before completion. Among treatment completers, 212 patients (69.7%; 95% CI: 64.5%–74.9%) showed clinical improvement, 80 (26.3%) exhibited no visible change, and 12 (3.9%; 95% CI: 1.8%–6.1%) reported aggravation of symptoms. An intention-to-treat conservative estimate which assumes that no improvement took place among all dropouts yielded an improvement rate of 38.9% (95% CI: 34.8%–43.0%). Baseline characteristics have shown male predominance (57.1%), high educational ability (63% graduates or postgraduates), prevalence of upper-middle income class (60.4%), and a dominance of non-vegetarian dietary pattern (79.2%) hinting towards expectation mismatch. Although treatment completers have demonstrated substantial clinical improvement, high attrition rate also introduces substantial ambiguity in the study. This study provides real world evidence on application of Dr. Samuel Hahnemann's concept of alternating action in unit dose prescribing. It also underscores adoption of structured retention strategies while conducting future prospective research studies.

Keywords: Alternating action, Attrition bias, Homoeopathy, Unit dose, Chronic illness, Retrospective study, Real world evidence, Hahnemann, Dropout analysis

INTRODUCTION

Most usually, the chronic sicknesses demand sustained therapeutic engagement for a long period of time (over months or years) and retention itself becomes a decisive determinant of treatment success (independent of specific therapeutic modality under study) in these conditions¹. As regards patient's adherence to the individualized homoeopathic practice with unit dose of indicated medicine having alternating action itself presents a distinctive set of challenges. One such challenge stems from improper understanding and clinical application of concept of what Dr. Samuel Hahnemann termed alternating action in proving. According to Dr. Samuel Hahnemann, alternating action in proving actually refers to paroxysms of primary effects of a medicine which contain symptoms that always oppose each other. This concept, appears to be first acknowledged, although indirectly and ambiguously in Hahnemann's earliest writings such as *Fragmenta de Viribus Medicamentorum Positivis*, as third type of action, besides primary and secondary². Dr. Samuel Hahnemann plainly listed 20 medicines that possess this alternating action property in proving. These were mentioned in his major works – *Organon of Medicine*, *Materia Medica Pura*, and *Chronic Diseases* – and include: *Aconitum napellus*, *Arnica montana*, *Arsenicum album*, *Belladonna*, *Bryonia alba*, *Chamomilla*, *China officinalis*, *Conium maculatum*, *Digitalis purpurea*, *Hyoscyamus niger*, *Ignatia amara*, *Magnesia polus ambu*, *Manganum aceticum*, *Menyanthes*, *Nux vomica*, *Phosphoric acid*, *Pulsatilla*, *Rhus toxicodendron*, *Spigelia*, and *Stannum metallicum*.

When such a medicine – strictly chosen on strict homoeopathic principles – is given and no improvement is experienced, Dr. Hahnemann suggests that same medicine should be repeated in same potency or next higher, and clinical improvement is usual customary. This method applies to both acute and chronic pathological states. As mentioned in Aphorism 251 of 6th edition of *Organon of Medicine*, these instructions are valid for both centesimal and 50 millesimal potencies. In clinical practice, unit dose method of prescribing– marked by single administration of a remedy followed by 'wait & watch' policy– goes well with this principle. Patients who do not recover after initial dose may receive same remedy again in same potency or next higher, rather than switching to a different medicine or a higher potency of same medicine. This approach is significantly different from the daily dosing approach of prescribing. Patients who become habituated to repeated medication may perceive this 'wait & watch' policy as



**Ashutosh Tripathi and Rakesh Gohel**

therapeutic inaction finally leading to frustration or leaving of their treatment. Also, the complexity involving the reorganization when the alternating action of the remedy is at play, adds another layer of difficulty in day to day clinical practice.

Attrition in OPD setups still remains poorly addressed in most of homoeopathic literatures. Dropouts cause the statistical power to reduce (and bring in bias), particularly when choice to withdraw correlates with treatment response. Patients, who observe no improvement after medication (or worsening after the medicine), are considerably more likely to discontinue attending follow up visits than those experiencing relief. On the other hand, patients who attain rapid improvement may also stop too early (believing that further treatment is unnecessary). Both conditions produce missing data which are not likely to be 'missing completely at random' (a basic assumption underlying many standard statistical approaches). This phenomenon (known by the name of attrition bias) has received too little systematic attention in homoeopathic outcomes research. Most published studies have reported per-protocol analyses without agreeably addressing potential direction (or magnitude) of bias introduced by dropouts. Hence, the present study was designed to unmistakably examine dropout patterns in a real-world clinical setting where core therapeutic strategy has been unit dose prescriptions of one of the remedies from Dr. Hahnemann's list of 20 alternating action medicines (with repetition of same remedy in same potency or next higher) when clinically indicated. We have actually wished to draw meanings from clinical insights (while remaining crystal clear about methodological limitations) by doing quantification of attrition and conducting sensitivity analyses.

MATERIALS AND METHODS

Study Design and Study Setting: A retrospective observational cohort study was conducted (using medical records from Homoeopathic OPD) at All India Institute of Medical Sciences Raipur, India. This department mainly functions as a tertiary care unit (providing homoeopathic treatment to patients referred from primary health centers. It also caters those seeking direct consultation). Clinical data analyzed in this study were collected over 2 year period from May 2022 to May 2024. Data were extracted from electronic and paper-based clinical records maintained during above period.

Study Population: A total of 544 patients with various chronic illnesses formed analytical cohort. All patients had initiated individualized homoeopathic treatment with alternating action remedies (from a list of 20 medicines) during May 2022 – May 2024 period.

Inclusion Criteria: following criteria were applied for inclusion:

Diagnosis of a chronic condition lasting at least 3 months prior to enrollment, as defined by standard clinical criteria for each disease. Prescription of one or more of 20 remedies known to possess alternating action as listed by Dr. Hahnemann. Prescription of remedies in unit dose form (typically single dose of 30C or 200C or LM1 potency), with no repetition unless curative alternating action principle required repetition of same remedy in same potency or next higher after initial non-response).

Exclusion Criteria: Patients were excluded if:

- Baseline demographic or clinical data were incomplete to extent that outcome classification was impossible.
- presenting complaint was purely acute in nature (duration less than seven days) without an underlying chronic component.
- They had received concurrent treatment with other systems of medicine without documentation of such co-interventions.

Therapeutic Intervention: All included patients were managed according to standardized departmental protocols based on homoeopathic principles. process involved:

- Case-taking: Detailed history was taken including physical, mental, and emotional symptoms, along with modalities, causations, and concomitant complaints.



**Ashutosh Tripathi and Rakesh Gohel**

- Repertorization: Use of standard homoeopathic repertories for initial prescription (Kent, Synthesis, or Complete Repertory) to derive a shortlist of candidate remedies.
- Remedy selection: Prescription of a single, individualized remedy chosen from 20 medicines known to exhibit alternating action (Aconitum napellus, Arnica montana, Arsenicum album, Belladonna, Bryonia alba, Chamomilla, China officinalis, Conium maculatum, Digitalis purpurea, Hyoscyamus niger, Ignatia amara, Magnesia polus ambu, Manganum aceticum, Menyanthes, Nux vomica, Phosphoric acid, Pulsatilla, Rhus toxicodendron, Spigelia, Stannum metallicum).
- Unit dose administration: Each remedy was given as a single unit dose, potency was selected based on patient's sensitivity, disease intensity, and chronicity, either in LM or Centesimal³.
- Repetition based on alternating action principle: If carefully chosen remedy failed to produce any clinical improvement after a practical waiting period (typically 2–4 weeks), same remedy in same potency or next higher was repeated, in accordance with Dr. Hahnemann's instruction⁴.
- Follow up schedule: Patients were Asked to visit at intervals ranging from 2 to 4 weeks, depending on clinical stability and chronicity of their condition.

Outcome Measures: The clinical status based on ORIDL Scale⁵ at final recorded follow up was the primary outcome measure which is categorized as:

- Improvement: Clearly documented lessening in frequency, intensity, or duration of primary symptoms, or improvement in quality of life measures as noted by treating physician and corroborated by patient report.
- No change: Absence of clinically meaningful change in symptom profile since baseline.
- Aggravation: Clear worsening of existing symptoms or emergence of new symptoms attributed to treatment by attending physician.

Secondary Outcomes:

- Retention rate (proportion of served patients completing scheduled follow up).
- Dropout rate (proportion lost to follow up before treatment conclusion).

Statistical Analysis: The summarization of baseline demographic and clinical characteristics was done using descriptive statistics. Categorical variables were expressed as frequencies and percentages with 95% confidence intervals (CIs) considered using Wilson score method for binomial proportions.

Retention rate was calculated as follows:

$$\text{Retention} = (\text{Number of completed follow-ups} / \text{Total enrolled}) \times 100$$

Per-protocol outcome proportions were calculated among treatment completers only.

Sensitivity Analysis for Missing Data: In absence of outcome data for dropouts, a conservative sensitivity analysis was performed assuming worst-case scenario for treatment effectiveness: all patients lost to follow up were classified as showing 'no improvement'⁶. This gave a lower-bound estimate of overall improvement rate:

$$\text{Conservative improvement} = (\text{Number of improvers among completers} / \text{Total enrolled}) \times 100$$

True overall effectiveness was assumed to lie between per-protocol estimate (69.7%) and this conservative floor estimate (38.9%). All analyses were performed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA) and Microsoft Excel for extra calculations. No imputation was undertaken for missing data beyond sensitivity analysis described above.

Ethical Considerations: study received due approval from Institutional Ethics Committee of AIIMS Raipur (IEC No. AIIMSRPR/2023/Homeo/112). Informed consent was eventually waived due to retrospective nature of analysis and use of de-identified clinical data.

RESULTS

Flow of participants and Retention: Out of 544 patients enrolled in treatment program, 304 (56%) completed scheduled follow ups. Remaining 240 patients (44%) were dropouts, as they failure to attend final scheduled follow



**Ashutosh Tripathi and Rakesh Gohel**

up despite at least one documented contact attempt by the investigator. Table 1 below summarizes patient disposition.

Baseline Sociodemographic and Clinical Characteristics: Study cohort showed certain demographic patterns clearly. Males have a slight preponderance (57.1%). Educational ability was notably high, with nearly 2/3rd of patients holding graduate or postgraduate degrees. Upper-middle income bracket (monthly household income between approximately ₹50,000 and ₹1,00,000) have accounted for about 60.4% of total sample. Dietary habits demonstrated a predominance of non-vegetarian consumption (79.2%). Table 2 below provides complete baseline profile of the patients.

Clinical Outcomes In Treatment Completers: Out of total 304 patients who remained in treatment through assigned follow up period, 212 (69.7%; 95% CI: 64.5%–74.9%) have demonstrated clinical improvement. A total of 80 patients (26.3%) have shown nil appreciable change in their symptom profile and treatment dependent aggravation was reported in 12 cases (3.9%; 95% CI: 1.8%–6.1%). These findings are presented below in Table 3.

Sensitivity Analysis Results: As per conservative estimate (which is calculated by assuming that all 240 dropouts experienced no improvement), an overall improvement rate of $212/544 = 38.9\%$ (95% CI: 34.8%–43.0%) has been noted. The impact of attrition on interpretation of treatment effectiveness is highlighted by this considerable gap (30.8 % points) between these estimates. Let us suppose that if the true outcome distribution among dropouts happens to lie somewhere between best-case scenario (all experienced improvement, overall rate above 69.7%) and worst-case scenario (none improved, overall rate around 38.9%), the exact effectiveness remains uncertain without any further additional data.

DISCUSSION

Main Findings and Interpretation: This study is based on Dr. Samuel Hahnemann's concept of alternating action in proving of medicines. This retrospective observational study gives a systematic examination of dropout dynamics in real world homoeopathic practice. Finally, 3 main findings can be observed clearly:-

- Substantial Dropout: Out of 544 patients, almost half (44 %) discontinued the treatment before completing their designed follow up.
- Among the patients who remained in the study till end (n=304), clinical improvement was observed in approximately 7/10 patients.
- About 31 % point's wide divergence between per-protocol and conservative effectiveness estimates reveals the possible extent of uncertainty that has been introduced by the missing data.

These results which we have got are showing us a picture which is both encouraging and full of caution. In one hand, high improvement rate among completers suggests to us that for patients who bear waiting periods after unit dose prescribing and stick to principle of repeating same remedy in same potency or next higher when needed, meaningful clinical benefit is attainable. Also, low aggravation rate (3.9 %) points to us that serious adverse effects are uncommon with this approach (although retrospective design limits our ability to fully distinguish milder or transient aggravations). On the other hand, high dropout rate arises our suspicion about generalizability of completer analysis. Do all 304 patients who remained in treatment actually systematically differ from 240 who left or not? Clinical intuition and existing literature suggest to us that they probably are different. Patients who have experienced early benefit did have strong incentives to continue; on the contrary, those who have experienced no change (or worsening) had likewise strong reasons to seek alternatives (which is easily available in Tertiary Care hospital of National Importance like AIIMS Raipur). This mechanism is supposed to produce missing data (that are not missing at random but rather revealing treatment response). If this be the case, per-protocol estimate (of 69.7 %) would



**Ashutosh Tripathi and Rakesh Gohel**

exaggerate true effectiveness for average enrolled patient in the study. Expectation mismatch is what the demographic trends points towards in this study.

Hahnemann's Alternating Action in Clinical Context: The clinical approach of unit dose prescribing and repetition rules which are used by clinicians in this study have been taken directly from authentic writings of Dr. Samuel Hahnemann. As mentioned in the book *Organon of Medicine*, when a medicine chosen in strict homoeopathic way fails to act, it needs to be repeated i.e. repetition with same remedy in same potency or next higher. Our findings in this study point to us that this method of repeating same remedy in same potency, when clinically indicated, can produce favorable results in a good number of adherent patients. However, high dropout rate indicates that patient education and expectation about this approach may be inadequate (Expectation Mismatch). Although all the 20 medicines listed by Dr. Samuel Hahnemann as showing alternating action in proving are being used for centuries, still their specific alternating dynamics remain underexplored in modern day clinical practice and research. While our study does not scrutinize each remedy individually, by and large the positive response among completers suggests to us that this Hahnemannian approach still retains clinical credence. More future research in this direction should examine whether any of these 20 remedies are directly associated with higher dropout rates or faster response times.

Comparison with Existing Literature: In OPD management of chronic disease as there are various therapeutic modalities and settings, the reported attrition rates also vary widely⁷. Usually, chronic cases e.g. hypertension or diabetes have a dropout rates which ranges from 15-30 % per year, although this exceeding 40 % is not infrequent in underprivileged populations or complex treatment protocols. For studies in homoeopathy, reported attrition has been even higher in some studies. A systematic review of homoeopathy for chronic conditions has noted that dropout rates exceeding 50 % were reported in several prospective observational studies, mainly those conducted in public sector settings where patients have to face challenging demands of time and resources. Our finding of 44 % attrition rate, actually, is falling within upper range of formerly published figures but clearly is not an outlier. What separates this study from others is that it has an unambiguous framing of attrition as a primary analytical focus rather than a nuisance parameter to be mentioned quietly and very briefly in limitations sections.

In fact, by doing sensitivity analysis and discussing possible underlying mechanisms of dropout, we aim to shift the discussion toward more transparent handling of missing data in homoeopathic outcomes research, which is not usually done⁸.

Clinical and Methodological Implications: For practicing homoeopathic physicians, these findings give numerous practical insights.

- High early dropout rate suggests that initial weeks of treatment are a vulnerable period. Adoption of proactive strategies e.g. telephone check ins between scheduled visits, patient education about anticipated timelines for response (including possibility that same remedy may need to be repeated) or other focused steps etc. might improve early retention.
- Intricacy of this alternating action principle of prescribing and repetition itself needs clearer communication to the patient. Patients should be made aware that 'no immediate change' does not necessarily always mean treatment failure; rather, it may be a sign that same remedy needs to be repeated in same potency. Physician should also evaluate patient health literacy and organizational capacity before starting this unit dose strategy with alternating action remedies.

For homoeopathic researchers, methodological implications of this study are equally important. Future prospective studies must include:

- Proper documentation of reasons for dropout through planned exit interviews
- Multiple follow up modalities (such as telephone, postal, or digital) so as to diminish missing data
- Gathering of at least some outcome data even for patients who discontinue treatment
- Declared beforehand in study protocol sensitivity analyses including multiple imputation or pattern mixture models that relax 'missing-at-random' assumptions.



**Ashutosh Tripathi and Rakesh Gohel**

Strengths and Limitations of the Study: This study beholds a number of strengths.

- Our sample size of 544 in this study is relatively big considering a homoeopathic outcomes study conducted in a single center.
- Our transparent reporting in this study, both of per-protocol and conservative estimates, allows readers to draw their own conclusions about plausible array of treatment effectiveness.
- Practical or real-world setting of the study enhances the chance of external validity as we compare it to tightly controlled efficacy trials.
- The unique therapeutic approach of unit dose prescribing and repetition rules based on Dr. Samuel Hahnemann's original writings on alternating action provides a logical theoretical framework that is most often missing in pragmatic homoeopathic studies.

However, some limitations of this study also require our careful and thorough attention, viz.:

- Retrospective design prohibited any ability to collect outcome data from dropouts or to verify reasons for discontinuation. We cannot settle on whether dropouts left because treatment failed, because they were cured, because of any logistical barrier, or because of unrelated personal reasons. Each scenario has diverse implications for bias.
- In absence of a control group for larger cohort (n=544) we cannot attribute observed improvements to homoeopathic treatment rather than to natural history, regression to mean, or placebo effects.
- Clinical improvement was assessed by treating physician who was not blinded to treatment exposure, introducing potential detection bias.
- While we limited analysis to 20 alternating action remedies, we did not analyze each remedy separately; response rates and dropout patterns may differ between remedies like Nux vomica and Pulsatilla, for instance.
- We did not assess obedience to treatment among those who remained in follow up; it is possible that some 'completers' did not in fact take their remedies as prescribed.
- Our Single center study limits the generalizability to other homoeopathic OPD settings in which we may have different patient demographics or any other prescribing styles.

Directions for Research in Future: Based on above mentioned findings, future research studies should prioritize prospective cohort designs instead of retrospective with implanted retention protocols. Thorough documentation of dropout reasons, categorized as treatment-related (like lack of efficacy, adverse effects, misunderstanding of waiting period or repetition principle), patient-related (like transportation difficulties, competing time demands, loss of motivation), or external (like change in employment, relocation, insurance changes), will facilitate more nuanced analysis of attrition mechanisms that come under play. Also, a useful next step with Dr. Samuel Hahnemann's original list of 20 medicines would be to plan in future a comparative observational study examining whether certain remedies in this list are associated with faster response times or lower dropout rates. Outcomes of such investigations may point to more precise prescribing guidelines for future clinical use. Also few pragmatic trials which compare standard follow up alone versus augmented retention strategies (like reminders, flexible scheduling, and financial incentives) may offer evidence to guide future clinical practice. Finally, exploring patient perspectives via a qualitative research on this unit dose prescribing approach and repetition of same remedy in the same potency or next higher could yield insights not usually available through quantitative methods alone.

CONCLUSION

This retrospective observational study is full of insights. The study utilizes the concept of Dr. Samuel Hahnemann's 20 alternating action remedies in proving of unit dose homoeopathy, with repetition of same remedy in same potency or next higher when clinically indicated. We have found that there has been substantial improvement among treatment completers (69.7 %) but we have also noticed a high attrition rate (44 %) which adds significant uncertainty to the study. Also with a conservative sensitivity analysis, which assumes no improvement among dropouts, we have noticed reduced overall improvement estimate to 38.9 %, which highlights a wide range of reasonable true





Ashutosh Tripathi and Rakesh Gohel

effects. On one side, positive outcomes among adherent patients suggest to us that potential benefit was received by those who persisted with this Hahnemannian approach of prescribing, the other side has a high dropout rate which underlines important real-world practicality constraints with this method. Prospective research studies in future may include structured retention strategies and systematic documentation of dropout reasons for better description of effectiveness of this therapeutic approach under routine clinical conditions.

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Table 1: Participant Disposition

Stage	Number	Percentage (%)
Total enrolled	544	100
Completed follow up	304	56
Dropped out before completion	240	44

Table 2: Baseline Characteristics of Enrolled Patients (N=544)

Characteristic	Percentage
Sex	
Male	57.1%
Female	42.9%
Education	
Below graduate	37.0%





Ashutosh Tripathi and Rakesh Gohel

Graduate / Postgraduate	63.0%
Income category	
Lower ($\leq$ ₹25,000/month)	18.2%
Middle (₹25,001–50,000/month)	21.4%
Upper middle (₹50,001–1,00,000/month)	60.4%
Dietary pattern	
Vegetarian	20.8%
Non-vegetarian	79.2%

Table 3: Clinical Outcomes in Completers (N=304)

Outcome	Number (n)	Percentage	95% Confidence Interval
Improvement	212	69.7%	64.5%–74.9%
No change	80	26.3%	21.6%–31.4%
Aggravation	12	3.9%	1.8%–6.1%

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Therapeutic Promise of *Cordia dichotoma*: Phytochemistry, Pharmacology and Neuroprotective Insights

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ABSTRACT

A significant medicinal plant found in tropical and subtropical parts of Asia is *Cordia dichotoma*, often known as Sebestan plum or Lasora. Its fruits, leaves, bark, and seeds have long been utilized in Ayurvedic, Unani, and folk medicine to treat gastrointestinal problems, fevers, ulcers, and respiratory conditions. Numerous bioactive substances, including as terpenes, alkaloids, flavonoids, and phenolic acids, are found in phytochemical investigations and add to the plant's nutritional and medicinal value. With extracts showing antioxidant, anticholinesterase, anti-Alzheimer's, anti-Parkinsonian, and antidepressant activities, recent studies highlight its neuroprotective potential. Its involvement in the treatment of neurodegenerative and psychiatric illnesses is suggested by experimental models that demonstrate notable improvements in cognitive function, oxidative stress indicators, and neuronal protection.

Keywords: *Cordia dichotoma*, Phytochemicals, Neuroprotection, Alzheimer's disease, Parkinson's disease, Antioxidant, Antidepressant.



Sayali R. Raut *et al.*,

INTRODUCTION

Plant-based medicines are considered the first line of defense for maintaining health and preventing disease, and they remain the primary source of new pharmaceuticals with therapeutic properties [1]. Of an estimated 72,000 plant species with medicinal qualities, more than 3,000 are recognized in India [2]. The "science of life," Ayurveda, states that a person is healthy if their metabolism is balanced. Allopathy, which employs 30 plant species to treat ailments, Ayurveda (700), Amchi (600), Unani (700), and Siddha (600) are examples of indigenous medical systems [1]. *Cordia dichotoma* is one of the deciduous plants that are used for traditional medicinal purposes all over India. Polysaccharide content in the fruit has been observed to be high. A sticky, jelly-like substance is produced by ripe *C. dichotoma* fruit. Plants are used in Unani medicine as antiviral, antibacterial, and antitussive agents. The general public in India uses a lot of josandah, which are polyherbal preparations with *C. dichotoma* as the main component, to cure fevers, respiratory disorders, coughs, catarrh, and the common cold. Leaves and stem bark have been used since ancient times to cure a variety of illnesses, including gonorrhea, leprosy, fever, diarrhea, dyspepsia, and burning sensations. Plant leaves have long been used for anthelmintic, astringent, diuretic, demulcent, purgative, expectorant, tonic, ulcer, and coughing purposes. [3]

Geographical distribution:

C. dichotoma is often found in tropical and subtropical regions. It can reach elevations of up to 1500 meters in the sub-Himalayan tract and outer ranges. It is found in a wide range of forests, such as the dry deciduous forests of Rajasthan, the moist deciduous forests of the Western Ghats in India, and the tidal forests of Myanmar. In Maharashtra, it grows well in damp monsoon forests [4]. Rather than growing gregariously, it grows singly in moist, shaded slopes and ravines. The species is found throughout the Philippines and is found in low- and medium-elevation thickets and secondary woods. Furthermore, it extended to areas like southern China, Formosa, Polynesia, tropical Australia, and Peninsular Malaysia. This species is propagated through seeds. [5].

Classification:[9]

Kingdom: Plantae

Division: Magnoliophyta

Class: Dicotyledons

Subclass: Asterales

Order: Lamiales

Family: Boraginaceae

Genus: Cordia

Species: *Cordia dichotoma* Forst.

Vernacular name:[9]

India : Gonda, lasora, leshora

Javanese : Kendal

Malaysia : Sekendal, sekendai, petekat

Thailand : Paw man

English : Sebestan plum, soap berry, fragrant manjack

Sumatran : Nunang

Local Names:[9]

English : (sebesten, clammy cherry, Indian cherry)

Bangali : (buhai, bahubara)

Hindi : (lasura, bhokar, borla)

Gujarati : (vadgundo, gunda)

Sanskrit : (bahuvarka, shleshmatak, shelu)





Sayali R. Raut et al.,

Javanese: (Kendal)

Lao : (sino-Tibetan) (man, man khok)

Malay: (petekat,sekendai)

Tamil : (vidi, naruli, kalvirusu)

Nepali : (kalo bohor, bohor)

Thai : (mandong, manma, phakmong)

Morphology of *C. dichotoma*:

The *Cordia dichotoma* tree is a small to medium-sized deciduous tree with a short bole, a short, crooked trunk, and a spreading crown. The simple, entire, and slightly dentate leaves are elliptical-lanceolate to broad ovate in form, with a circular, cordate base. The stem bark is either smooth and grayish brown or furrowed longitudinally [4]. Flowers are bisexual, have short stalks, form loose corymbose cymes, and vary in color from white to pink. Fruits with sticky flesh can be consumed. It's a yellow or pinkish-yellow drupe that's ovoid or globose and rests in a saucer-like, expanded calyx. The pulp becomes black and viscid as it ripens. [5].

Nutritional information:

C. dichotoma is used as food because the entire plant is edible. Moreover, young fruits are preserved and used as vegetables [4]. To protect the body from extreme heat waves, a flower and curd mixture is applied twice a day [6]. The rural inhabitants of Orissa's coastal regions eat the ripe fruits raw. The high levels of proteins and fatty oils in *C. dichotoma* seed kernels make them suitable for use as cow feed [7]. The plant's 97% polysaccharide gum is employed in a variety of pharmacological applications. The fruit's chromium contains therapeutic benefits for diabetes. Some anti-nutritional components found in fruits include oxalic acid (250 mg), phytate phosphorus (100 mg), and phytic acid (355 mg) per 100 g. The branches of the *C. dichotoma* plant were used to identify new natural cellulose textiles [8].

Phytochemical Investigation:[10]

There are pyrrolizidine alkaloids, coumarins, flavonoids, saponins, terpenes, and sterols in the fruit, leaves, and seed. A concentration of 100 µg/mL of taxifolin from *C. dichotoma* seeds shown encouraging DPPH free radical scavenging behavior. Apigenin (5 mg/kg, p.o.) from *C. dichotoma* demonstrated notable healing and a decrease in inflammatory enzymes when tested for ulcerative colitis, according to another research. It was discovered that the seeds of *C. dichotoma* contained 7.60% fatty oil, with stearic, oleic, and linoleic acids being the main components. Kaempferol, quercetin, and isorhamnetin are the three flavonoids that were separated from the butanol fraction of *C. dichotoma* fruits. From a butanol extract of *C. dichotoma* leaves, which included rosmarinic acid as a significant ingredient, six flavonol glycosides and two phenolic compounds were identified.

According to a different investigation, the ethanolic extracts included rosmarinic acid (0.0028%), amino acids (1.39%), triterpenoids (0.075%), and polyphenolic chemicals (1.0%) (Tian et al., 2014). An overview of *Cordia dichotoma*'s chemical components and therapeutic applications has been provided. Here, we present the compounds that were isolated from the leaves of *C. dichotoma*: β -sitosteryl-3 β -glucopyranoside-6'-O-palmitate (1), nervonyl 4-hydroxy-transcinnamate ester (2), β -sitosterol (3), and chlorophyll a (4); and from the twigs: 3 and 1,2-dilinoleoyl-3-linolenoylglycerol (5). As far as we are aware, this is the first report on 1-2 being isolated from *C. dichotoma*. [10]

Neuroprotective Behaviour of *C. Dichotoma*:**Parkinsons Disease**

The degeneration of dopamine-producing neurons in the substantia nigra is a hallmark of Parkinson's disease (PD), a neurological condition. Tremors, bradykinesia, abnormalities in gait, postural abnormalities, and rigidity are the outcomes of this degenerative process. It is well accepted that oxidative stress is a major factor in the susceptibility of dopaminergic neurons, even if the problem of controlling dopaminergic neuronal transmission in Parkinson's disease (PD) is still open. Furthermore, a significant amount of preclinical and clinical research has hypothesized that one



**Sayali R. Raut et al.,**

possible mechanism for the fatal effects of haloperidol is the unchecked production of reactive oxygen species (ROS) [11].

In order to evaluate the effects of haloperidol-induced Parkinson's disease in rats, the current experimental study used three behavioral assessment parameters: the Open Field Test, Akinesia (Stepping Test), and Catalepsy Test. In this work, the effects of pre-treating rats with 200 and 400 mg/kg of an ethanolic extract of *C. dichotoma* leaves as well as a standard medication called syndopa (a combination of L-dopa and carbidopa) at a dose of 10 mg/kg were examined. The course of therapy was given for seven days. The findings indicated a substantial decrease ($p < 0.001$) in the cataleptic score and a rise ($p < 0.001$) in a variety of behavioral indicators, including ambulation, activity in the center square, raising, grooming, and the number of steps taken.

These effects were consistently seen over the course of the observation period when compared to animals who received haloperidol treatment. The test drug was administered at 200 and 400 mg/kg, while syndopa was administered at 10 mg/kg. The neuromuscular strength improved in a highly significant ($p < 0.001$) way. When rats were given 400 mg/kg of an extract of *C. dichotoma* leaves, the neuroprotective effects were greatest. It was discovered that these effects were similar to those of the syndopa-treated group. [13]. Rats in the haloperidol group had lower levels of Catalase and higher levels of MDA, indicating oxidative stress in the brain. MDA was decreased and catalase was elevated by syndopa and test medication in comparison to rats treated with haloperidol ($p < 0.001$). Dopamine levels were decreased and protein concentrations were increased by daily haloperidol dosage. The administration of syndopa and test medications increased blood dopamine levels and lowered protein levels in comparison to haloperidol therapy ($p < 0.001$).

The brain histopathology of the test drug-treated and syndopa groups revealed intact neuronal cells, whereas the group treated with haloperidol had apoptosis and inflammation, which resulted in neuronal damage. Resistance to oxidative stress is provided by *C. dichotoma*. Numerous investigations have demonstrated that *C. dichotoma* possesses antioxidant properties [12]. It's possible that antioxidants shield PD neurons from intracellular ROS production. Therefore, antioxidants may protect neuron workouts and aid in the treatment of Parkinson's disease. The aforementioned behavioral and neurochemical characteristics of *C. dichotoma* suggest that it may alleviate Parkinson's disease symptoms by influencing the antioxidant system and reestablishing dopamine. Consequently, *C. dichotoma* could be useful as a preventative therapy for Parkinson's disease.

Similar to syndopa, the ethanolic extract of *C. dichotoma* leaves protected against Parkinson's disease caused by haloperidol. According to our research, *Cordia dichotoma* G. Forst. may help prevent and lessen extrapyramidal side effects from clinically prescribed antipsychotic drugs. The substance could be used as a natural remedy for the side effects of haloperidol. The neuroprotective effect that the extract from *C. dichotoma* leaves showed in this study necessitates more investigation into its molecular mechanism and its therapeutic implications. [13]

Alzheimer's Disease

Phenolic chemicals have considerable antioxidant capacity, which can have a very positive impact on health [14]. Recent studies have demonstrated that flavonoids, phenolic acids, and their derivatives prevent or postpone neurodegenerative diseases and markedly enhance cognitive performance. Inhibition of ChEs, A β formation, and signaling pathway modulation were their mechanisms. The most widely used and practical technique for figuring out the TPC in plants is the Folin-Ciocalteu test, which has high consistency. Certain redox reagents called Folin Ciocalteu reagents combine with polyphenols found in plant extracts to create a blue color complex that can be detected using visible light spectrophotometry. 137.9 $\pm$ 6.76 and 87.94 $\pm$ 4.41 μ g GAE/1 mg sample, respectively, were the TPCs found in CDLME and CSLME in this investigation. There are many popular spectrophotometric methods that exploit the production of aluminum complexes to measure the TFC in medicinal plant samples. The TFC in CDLME was 22.44 $\pm$ 1.21 μ g RE/1 mg sample, while in CSLME it was 18.25 $\pm$ 1.75 μ g RE/1 mg sample. The TFC in *C. dichotoma* leaves was 160 mg quercetin E/g extract, according to earlier reports [15]. It was determined from the data



**Sayali R. Raut et al.,**

that flavonoids make up a small percentage of the total phenolics in both plants. More TPC and TFC are also present in CDLME than in CSLME.

Among these, HPLC has been the most widely used method for rapidly determining the bioactive ingredients of extracts from medicinal plants because it enables thorough profiling of intricate plant samples [16]. Therefore, phytochemical study was conducted using HPLC-fingerprint analysis on the aforementioned crude extracts in an effort to determine their phytoconstituents and create associations between the chemical components and the ChEs inhibitory efficacy. The presence of eleven phenolic compounds was detected by HPLC-fingerprint analysis of CDLME. In this regard, leaves of *C. dichotoma* have been shown to contain rutin, kaempferol, rosmarinic acid, chlorogenic acid, and gallic acid. However, instead of the leaves, other portions of the plant were found to contain p-coumaric acid and ferulic acid [17].

To our knowledge, this is the first time that dihydrokaempferol and chrysin have been identified in *C. dichotoma* leaves. The HPLC fingerprint study of CSLME only identified five phenolic chemicals. In our research, we found dihydrokaempferol, chlorogenic acid, and rosmarinic acid in *C. sebestena* leaves for the first time. Nevertheless, the fruit of *C. sebestena* was previously used to separate rosmarinic acid and its methyl ester. Very high quantities of rosmarinic acid were found in both species, which is consistent with earlier findings that rosmarinic acid is prevalent in Boraginaceae species. About 50 million people worldwide suffer from AD at the moment, and by 2050, that figure is expected to quadruple.

Plants are the subject of extensive research in an effort to identify potential therapeutic components that may be applied to the treatment of neurological disorders [18]. Cholinergic and cognitive functioning have been demonstrated to be enhanced by the in vivo and in vitro ChEs inhibitory actions of conventionally used herbal medicines. AChE and BChE are the primary enzymes involved in the degradation of ACh and butyrylcholine, and their inhibition is crucial. A number of clinical trials have demonstrated the several therapeutic benefits of ChEs inhibitors, including antioxidant activity, modulation of amyloid precursor protein processing, and inhibition of A β genesis. AChE inhibitors increase the quantity of ACh at the synapse, allowing the signal to be amplified, and enhance both cognitive performance and everyday life results [19].

The primary focus of the current investigation is the crude extracts of *C. dichotoma* and *C. sebestena*, as well as their subsequent fractions, and their in vitro ChEs inhibitory effects. IC₅₀ values for CDLME and CSLME in AChE inhibitory tests were 38.76 ± 0.44 and 50.79 ± 4.18 μ g/ml, respectively. Many herbs used in traditional medicine have been investigated for their ability to enhance memory, but only a small number of them have demonstrated a sufficient level of AChE inhibitory efficacy [19]. The crude extract from *C. dichotoma* appeared to be a more potent AChE inhibitor than other plants. As evidenced by their lowest IC₅₀ values, CDLEF and CSLME showed the best polarity of the active compounds and the best inhibition of the enzyme. [20]

Given that its levels and activity notably increase in the later stages of AD, BChE is yet another drug target for the disease. Early studies focused mostly on AChE, but dual inhibitors of AChE and BChE, such as rivastigmine, are now thought to have more sustained activity throughout AD and may help delay the disease's development. CDLME and CSLME were found to exhibit BChE inhibition in these studies, with respective IC₅₀ values of 50.79 ± 4.18 and 52.71 ± 0.48 μ g/mL. This finding implies that there is good BChE inhibitory activity in the two plant crude extracts.

In order to establish the polarity of the active compounds, CDLEF and CSLEF had the lowest IC₅₀ values. In two enzyme assays (AChE and BChE assays), the hexane fraction of both plants showed the worst inhibition of both enzymes, as indicated by the highest IC₅₀ values. These results made it evident that the crude extract of *C. dichotoma* leaves had more inhibitory action for AChE and BChE than the crude extract of *C. sebestena* leaves, which may be related to the higher TFC and TPC in the former. Furthermore, compared to *C. sebestena* fractions, all investigated fractions of *C. dichotoma* exhibited greater AChE and BChE inhibitory activity. The two plants' HPLC analyses revealed the presence of a variety of phenolic chemicals, each of which has been shown to have strong anti-AD





Sayali R. Raut et al.,

properties through various pathways. More specifically, in rats with aluminum chloride AD, p-coumaric acid restored hippocampus synaptic plasticity impairment and reduced memory impairments.

In AD caused by scopolamine, rosmarinic acid and chlorogenic acid boosted cholinergic signal transduction, raised the quantity of ACh in the brain, and exacerbated the short-term memory loss. A research found that rutin decreased the cytotoxic effects of tau oligomers in a mouse model of AD and shielded neuronal architecture from them. In several rat models, chrysin, ferulic acid, and kaempferol have neuroprotective benefits by lowering oxidative stress, inflammation, apoptosis, and amyloidosis. Cinnamic acid enhances lysosomal biogenesis and reduces A β in an AD mouse model by activating PPAR α . Previous studies have demonstrated the ChEs activity of phenolic compounds, which is in line with our current investigation. In vitro AChE and BChE inhibitors that work well include rutin, kaempferol, chrysin, rosmarinic acid, ferulic acid, and P-coumaric acid. AChE inhibition was discovered by chlorogenic acid. [21]

Based on the above findings, Rashno M et al (2022) deduce that the neuroprotective qualities of the discovered phytochemicals may have been influenced by the anti-ChEs in crude extracts. An evidence-based foundation for further research and the separation of bioactive phytoconstituents for clinical trials is provided by this study. The presence of important phenolic compounds with neuroprotective qualities was shown by phytochemical study of CDLME and CSLME. For the first time, dihydrokaempferol and chrysin were identified in *C. dichotoma* leaves by HPLC analysis, whereas *C. sebestena* leaves included rosmarinic acid, chlorogenic acid, and dihydrokaempferol.

Hussein HM, et al. (2024) showed that the crude methanolic extracts of *C. dichotoma* and *C. sebestena*, as well as their polar fractions, exhibit significant ChEs inhibitory activity, which might be helpful in the treatment of AD. The ChEs inhibitory activity of *C. dichotoma* was greater than that of *C. sebestena*. Studies using molecular simulations on the substances that were found to contain these enzymes validated the results. As for the AChE and BChE targets, the most promising ones were rutin, chlorogenic acid, and rosmarinic acid, with binding scores of -8.91 and -22.37, -7.66 and -11.51, and -7.89 and -8.99 kcal/mol, respectively. We are aware that this is the first study to examine the phenolic profile of *C. sebestena* and the inhibition of cholinesterase by *C. dichotoma* and *C. sebestena*. [20].

Antidepressant Activity

By examining its effects on alterations in depression-like behaviors and the associated neurotransmitters in a chronic unpredictable moderate stress rat model of depression, this study seeks to determine the antidepressant effects of the aqueous extract of *C. dichotoma* fruits. We used high-performance liquid chromatography to measure rosmarinic acid. The rats treated with the aqueous extract for chronic unpredictable mild stress exhibited typical anti-depressive-like behavior, such as a significant increase in sucrose consumption in the sucrose preference test, a significant decrease in immobility time in both the forced swimming test and the open field test, and an increase in swimming time in the forced swimming test.

Additionally, LC-MS/MS analysis revealed that the treated rats' frontal cortex, amygdala, hippocampus, and striatum tissue had a considerably lower serotonin turnover rate (5-HIAA/5-HT). Through the inhibition of monoamine oxidase by rosmarinic acid, the work offers the first in vivo evidence of potential pathways for modifying anti-depressive-like effect by controlling the turnover rate of serotonergic neurotransmitters. These results offer fresh and convincing proof of a new use of the fruits of the medicinal plant *C. dichotoma* in the treatment of depression and might pave the way for the creation of innovative plant-based antidepressants. [24]

CONCLUSION

Cordia dichotoma is a traditionally important medicinal plant with rich phytochemical diversity, including flavonoids, phenolic acids, alkaloids, and terpenoids that contribute to its wide range of pharmacological actions. Experimental studies have demonstrated its significant antioxidant, anti-inflammatory, anticholinesterase, and neuroprotective



Sayali R. Raut *et al.*,

effects, supporting its potential role in managing neurodegenerative disorders like Alzheimer's and Parkinson's diseases. The plant's extracts have shown the ability to enhance memory, reduce oxidative stress, and protect neuronal cells from degeneration, while also exhibiting antidepressant activity through serotonergic modulation. These findings validate its long-standing use in traditional medicine and highlight its potential as a natural source for developing plant-based neuroprotective and psychotropic drugs. However, further research including molecular, toxicological, and clinical studies is essential to establish its safety, efficacy, and mechanisms of action for therapeutic applications.

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Table No. 1 Nutritional value of leaves, seed kernels and fruits of *C. dichotoma*: [8]

Plant Part	Nutrient	Value
Leaves	Crude Protein	12% – 15%
	Crude Fiber	16% – 27%
	Nitrogen-Free Extract (NFE)	42% – 53%
	Ether Extract	2% – 3%
	Total Ash	13% – 17%
	Total Calcium	2% – 4%
	Phosphorus	0.3%
Seed Kernels	Water	32 g per 100 g
	Fatty Oils	46%
	Proteins	31%
Fruits (per 100 g pulp)	Pulp Content	70% of fruit
	Water	6 g
	Proteins	35 g
	Fats	37 g
	Carbohydrates	18 g
	Calcium (Ca)	55 mg
	Phosphorus (P)	275 mg
	Zinc (Zn)	2 mg
	Iron (Fe)	6 mg
	Manganese (Mn)	2 mg
	Chromium (Cr)	0.2 mg
	Copper (Cu)	1.6 mg





Sayali R. Raut et al.,

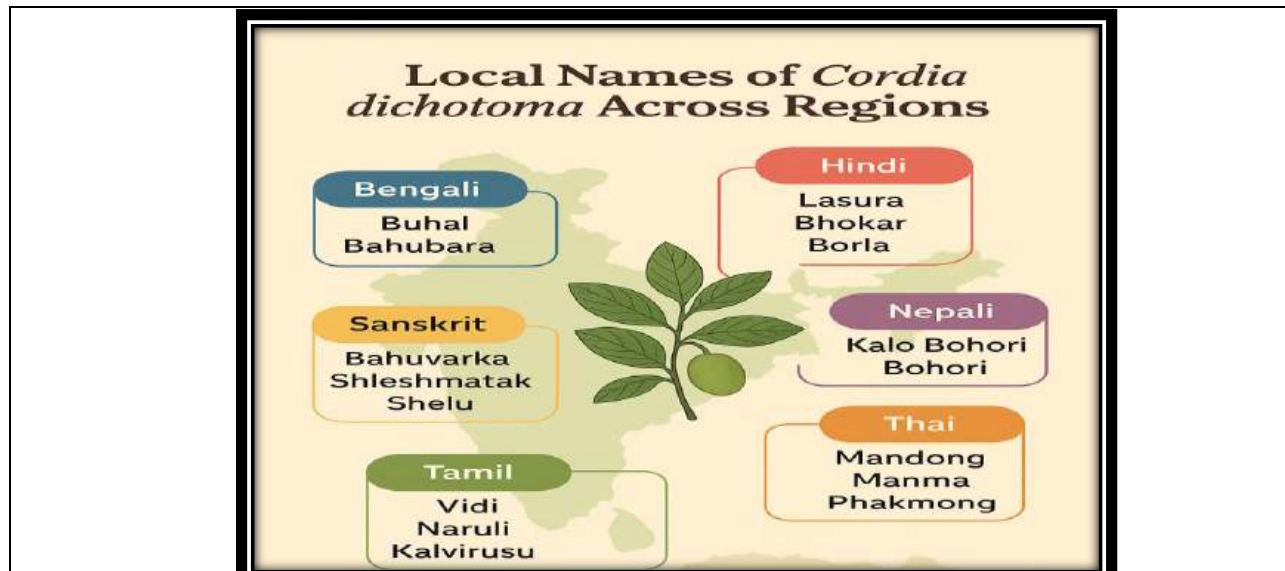
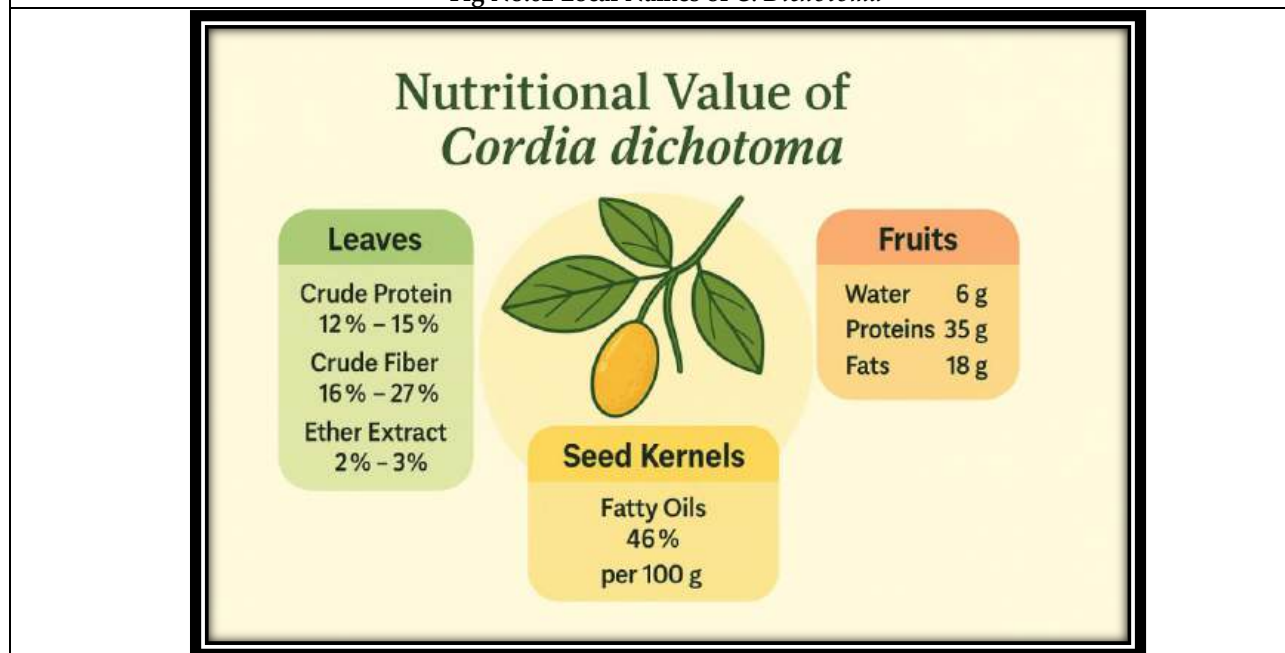
Table No.02: Pharmacological Activities of *C. Dichotoma*:

Sr. No.	Activity	Part Used	Proposed Mechanism of Action (MOA)	Reference
1.	Cognitive enhancement (scopolamine model)(22)	Fruit methanol extract	Restores hippocampal AChE, acetylcholine, noradrenaline, dopamine; antioxidant + anti-AChE activity	Hussein HM et al.. (2024)
2.	Anti-Alzheimer's type dementia (scopolamine mice) (23)	Leaves methanol extract	Enhances cholinergic system, increases SOD/GSH, reduces AChE & nitrites, protects hippocampal neurons	Singh et al.(2023).
3.	Neuroprotection in Parkinsonism (haloperidol model)(13)	Leaf/bark extract	↓ MDA, ↑ catalase/SOD; reduces akinesia and catatonia; histological protection of cortex, hippocampus, substantia nigra	Keserla B et al. (2023)
4.	Anticholinesterase activity (20)	Leaves (methanol extract, ethyl acetate fraction)	Inhibits AChE and BChE; phenolic compounds rosmarinic acid, rutin binding to enzymes via docking	El-Kashoury M et al. (2024)
5.	Antioxidant-mediated neuroprotection (4)	Bark extract	Increases enzymatic antioxidants and DPPH scavenging to reduce oxidative stress injury in neural tissue	Hussain N et al. (2020)
6.	Depression-like behavior alleviation (24)	Fruit aqueous extract	Modulates serotonergic neurotransmitters; reduces immobility in forced swim and tail suspension tests	Li et al. (2023)
7.	Neuroinflammation reduction (23)	Bark/leaves	↓ brain nitrite and inflammatory markers in Alzheimer's model	Singh et al. (2023)
8.	Oxidative stress mitigation (13)	Leaf/bark extracts	↑ SOD and GSH, ↓ MDA in haloperidol & scopolamine models	Keserla B et al. (2023)

Fig No.01 Taxonomical Classification of *C. Dichotoma*

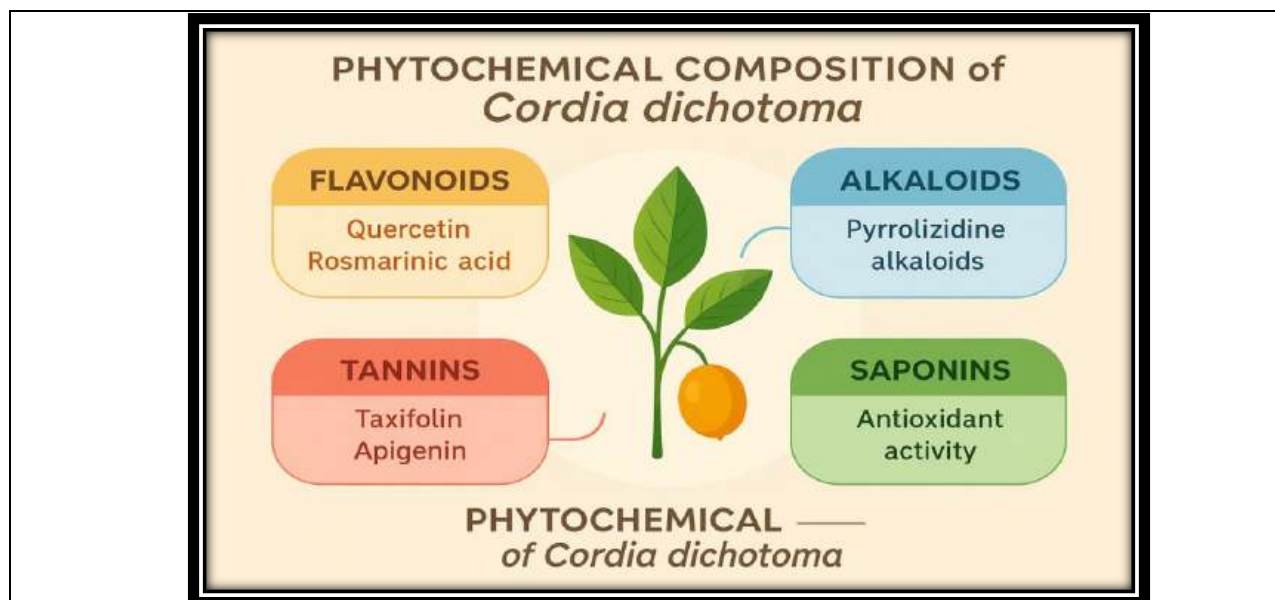
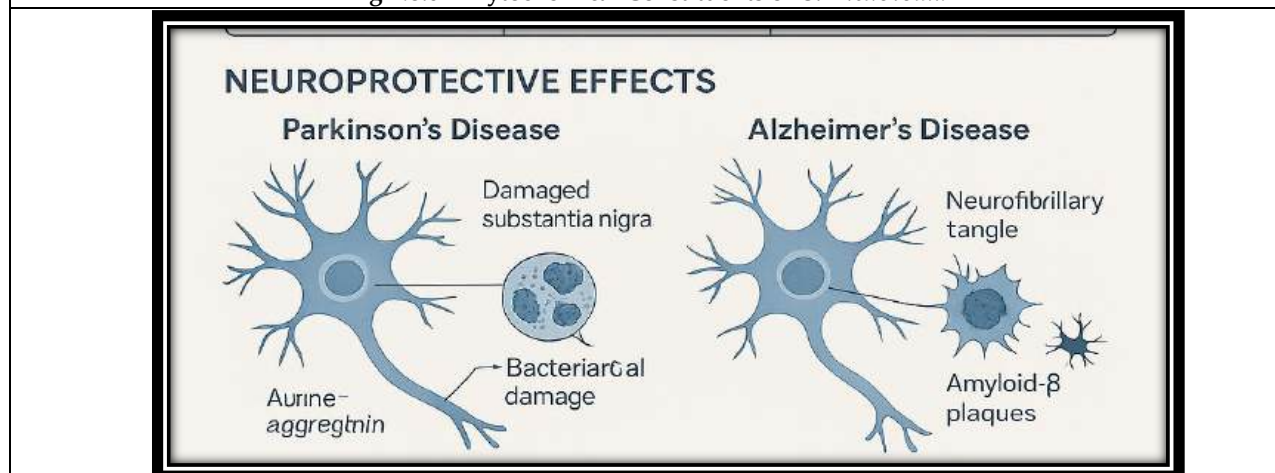


Sayali R. Raut et al.,

Fig No.02 Local Names of *C. Dichotoma*Fig No.03 Nutritional Values of *C. Dichotoma*



Sayali R. Raut et al.,

Fig No.04 Phytochemical Constituents of *C. Dichotoma*Fig No.05 Neurobehavioral Pharmacological Activities of *C. Dichotoma*

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REVIEW ARTICLE

A Narrative Review of the Relationship between Short - Form Video Application Use, Sleep Quality, Concentration, and Professional Behavior among Health Science Students

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ABSTRACT

Short Video applications such as Instagram Reels, Facebook reels, YouTube shorts and other similar applications have become increasingly popular among university students. Excessive use of these platforms has been associated with behavioural, cognitive and psychological effects. This Narrative Review aimed to synthesize available literature regarding the relationship between short-form video application use and sleep quality, concentration, and professional behaviour among health science students. Literature published between 2023 and 2026 was reviewed from databases including Pub Med, Google Scholar, Frontiers and BMC Psychology. Terms such as short-form video applications and sleep quality, short-form video and concentration and short-form video application and professional behaviour were used during search process. Boolean operators like AND and OR were also applied to obtain more relevant and focused studies. The selected literatures were critically analysed and synthesized narratively according to SANRA recommendations for narrative review articles. The Reviewed studies consistently demonstrated that excessive short-form video use is associated with poor sleep quality, reduced concentration, decreased attention span, academic procrastination, and altered behavioural



**Mayank Prajapati and et al.,**

outcomes. Existing evidence suggests the need for awareness regarding responsible digital media use among students.

Keywords: Short-form video application, Sleep quality, Concentration, Professional Behaviour, Health Science Students

INTRODUCTION

The use of short-form video application has increased rapidly among adolescents and university students in recent years because of the widespread availability of smart phones and internet services. Platforms such as Instagram reels YouTube Shorts and Moj have become highly popular among young adults due to their engaging and easily accessible content. Short-form video applications have transformed the digital media landscape among young adults¹. Platforms such as Instagram Reels and YouTube Shorts provide highly engaging content that encourages prolonged screen exposures². Health science students are particularly vulnerable because of academic stress, irregular schedules and extensive smart phone use⁸. Recent evidence suggests that excessive short-form video use negatively affects sleep quality, attention span, concentration and behavioural outcomes⁵. Studies also indicate that addictive scrolling behaviour contributes to procrastination, cognitive fatigue and reduced professional effectiveness^{10,21,25}. Therefore, understanding the relationship between short-form video application use, sleep quality, concentration and professional behaviour is important among health science students.

Data Resources

The literature for present narrative review was obtained from electronic databases and scholarly search engines including Pub Med, Google Scholar, Frontiers, Cureus, BMC Psychology and Semantic Scholar. Relevant peer-reviewed studies published between 2023 and 2026 were reviewed. The literature for the present review was searched using different keywords related to short-form video applications and their effects on students. Terms such as short-form video applications and sleep quality, short-form video and concentration and short-form video application and professional behaviour were used during search process. Boolean operators like AND and OR were also applied to obtain more relevant and focused studies. The selected literatures were critically analysed and synthesized narratively according to SANRA recommendations for narrative review articles.

METHODOLOGY

The present study adopted a narrative review design to synthesize available literature regarding the relationship between short-form video application use, sleep quality, concentration, and professional behaviour among health science students. The review was prepared according to SANRA recommendations for narrative review articles. Relevant literature published between 2023 and 2026 was searched from electronic databases including Pub Med, Google Scholar, Frontiers, Cureus, BMC Psychology, IEEE Xplore and Semantic Scholar. Studies focusing on adolescents, university students, young adults and health science students were included in the review. Cross-sectional studies, descriptive studies, correlational studies, experimental studies, randomized controlled trials, and systematic reviews relevant to the topic were considered. Articles unrelated to short-form video applications, duplicate studies, and non - English publications were excluded. The selected studies were critically analysed, compared and synthesized narratively to identify major themes and relationships related to sleep quality, concentration and professional behaviour associated with short-form application use.





RESULTS

Short-Form Video use and Sleep Quality

Several studies reported a significant association between excessive short-form video use and poor sleep quality among university students. According to Jia and Wang, Results shows that low self-control and habitual short-form video use increased poor sleep outcomes among college students¹. Mohammed et. al found that medical students using short-form videos for more than 1 hour daily experienced lower concentration and poor sleep quality². Jiang and Yoo also reported a strong relationship between short-form video addiction, social anxiety, and poor sleep quality among adolescent in china⁸. Zhao and Kou found that short-video addiction was positively associated with sleep problems and procrastination among college students⁹.

Short-Form Video Use and Concentration

Multiple studies identified reduced attention span and poor concentration among heavy users of short-form video applications. Joseph et.al. found that heavy users demonstrated significantly reduced attention span and poor time management skills¹². Lin et.al. reported that continuous consumption of short-video negatively affected sustained attention¹⁶. Gong and Tao observed that higher levels of short-video usage were associated with lower attention scores and reduced academic performance among school students¹⁸. Malhotra and Patnaik also found that reduced concentration and cognitive abilities among university students using short-form video application excessively¹⁹.

Short-Form Video use and Professional Behaviour

Some evidence suggests that excessive short-form video use may affect the professional behaviour and decision-making. Zhang and Li identified that students with higher short-video addiction demonstrated greater impulsive and risk-taking behaviour²¹. Lin et.al. found that excessive short-video addiction negatively affected creative self-efficacy and career interests among students²⁵. Another studies also shows regarding self-regulation and professional effectiveness among frequent users of short-video platforms^{22,24}.

DISCUSSION

The literature review shows that consistent evidence regarding the negative effects of excessive short-form video application use on sleep quality, concentration, and professional behavioural outcomes. Most studies demonstrated that prolonged scrolling behaviour and rapidly changing digital content may impair sustained attention, emotional regulation, and productivity^{1,2}. The findings also suggests that health science students may be particularly vulnerable because of their demanding academic and clinical responsibilities. The findings of present review suggest that prolonged use of short-form video applications may affect sleep quality among students. Many students spend long hours watching videos during night-time, which may delay sleeping habits and reduce sleeping duration²⁶. Health science students require adequate sleep for effective learning, memory, concentration and clinical performance. Therefore, disturbed sleep may affect both educational and professional development²⁷. The review also indicates that excessive exposure to short-form videos may negatively influence concentration and attention span. Short-form videos usually contain rapidly changing visual and audio content that may reduce the ability to focus on one activity for a longer period²⁸. Health science students are expected to maintain discipline, responsibility, communication skills, ethical behaviour, and emotional control during their academic and clinical training²⁹. Some students may also develop difficulty in balancing academic responsibilities with social media use³⁰. Overall, the present review suggests that excessive use of short-form video applications may negatively affects sleep quality, concentration, and professional behaviour among health science students. Promoting responsible digital media use and creating awareness regarding healthy screen habits may help reduce these negative effects.





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CONCLUSION

The present narrative review identified a significant relationship between short-form video use and adverse outcomes related to sleep quality, concentration and professional behaviour among students. Existing evidence supports the need for awareness programs promoting responsible digital media use and further research among health science students.

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Mayank Prajapati and *et al.*,

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Table 1: Research Matrix

No	Author & Year	P (Population)	I / E (Intervention / Exposure)	C (Comparison)	O (Outcome)
1	Jia & Wang (2025)	College students in the USA	Procrastinator short-form video use	Low vs high usage	Stress and poor sleep quality
2	Mohammed et al. (2026)	Medical students	Short-form video use	Less than 1 hour vs more than 1 hour use	Poor attention control and sleep quality
3	Behera et al. (2025)	Social media users across 21 countries	Social media and short-form video use	Lower vs excessive use	Negative mental health and sleep disturbances
4	Ahuja et al. (2025)	College students in Punjab	Social media consumption addiction	Non-addicted vs addicted users	Sleep deprivation and loss of concentration
5	Naeem et al. (2024)	University students in Pakistan	Instagram Reels exposure	Lower vs excessive exposure	Psychological distress and sleep problems
6	Lali et al. (2025)	Adolescents in colleges	Instagram Reels addiction	Mild vs oderate/severe addiction	Poor sleep quality
7	Pranathi & Jacob (2025)	Young adults	Addictive short-form video use	Low vs high addiction	Reduced attention span and poor sleep
8	Jiang & Yoo (2024)	Chinese adolescents	Short-form video addiction	Low vs high addiction	Poor sleep quality and social anxiety
9	Zhao & Kou (2024)	College students	Short-video addiction	Lower vs excessive addiction	Sleep problems and procrastination
10	Xie et al. (2023)	Undergraduate students	Short-form video addiction	Controlled vs addictive use	Academic procrastination and poor attention





Mayank Prajapati and et al.,

11	Shah et al. (2023)	Medical students	Instagram addiction	Non-addicted vs addicted users	Reduced sleep quality
12	Joseph et al. (2026)	Medical students	Short-form video content exposure	Light vs heavy users	Reduced concentration and attention span
13	Shanavas et al. (2025)	Adolescents and young adults	Short-form media exposure	Pre-test vs post-test exposure	Decline in focus and attention
14	Harsanto et al. (2025)	Teenagers and adolescents	TikTok and Instagram Reels use	Lower vs higher exposure	Brain fog and concentration problems
15	Pandey & Singh (2025)	University students	Short-form video consumption	Controlled vs excessive use	Reduced productivity and attention
16	Lin et al. (2024)	Social media users	Continuous short-form video watching	Limited vs prolonged exposure	Poor sustained attention
17	Shamloul (2024)	High school students	Short-form videography exposure	Reduced vs continued exposure	Cognitive function and academic performance
18	Gong & Tao (2024)	Elementary school students	Short-video usage	Lower vs higher usage	Reduced attention and academic achievement
19	Malhotra & Pattnaik (2024)	University students	Short-form video use	Lower vs higher usage	Poor concentration and cognitive abilities
20	Puaponpong et al. (2023)	High school students	Short-form media usage	Less vs frequent use	Decline in attention
21	Zhang & Li (2025)	College students	Short-video addiction	Addicted vs non-addicted students	Risk-taking and impulsive behaviour
22	Lu et al. (2025)	TikTok users	Privacy disclosure behaviour on short-video platforms	Lower vs higher privacy concern	Professional and privacy behaviour changes
23	Liu et al. (2025)	Working professionals	Short-video app-guided meditation	Intervention vs control group	Improved empathy and workplace wellbeing
24	Jiang X. (2023)	Chinese university students	Short-video viewing behaviour	Lower vs higher viewing behaviour	Behavioural and adaptive concept changes
25	Lin IT et al. (2023)	Innovative design students	Short-video addiction	Lower vs higher addiction	Reduced creative self-efficacy and career interest

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RESEARCH ARTICLE

The New Era of Class III Malocclusion Management

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ABSTRACT

Class III malocclusion is a complex dentofacial condition characterized by diverse skeletal and dentoalveolar components and variable growth patterns. Contemporary management strategies have evolved considerably with advancements in diagnostic technologies, growth assessment, biomechanics, and digital workflows. Accurate diagnosis and growth evaluation remain fundamental to treatment planning, guiding the selection of growth modification, orthodontic camouflage, or surgical intervention. Recent evidence suggests that skeletal anchorage-assisted orthopedic approaches provide superior skeletal outcomes compared to conventional facemask therapy, while orthodontic camouflage remains effective in carefully selected mild to moderate cases. For severe skeletal discrepancies, orthognathic surgery continues to be the definitive treatment, with increasing emphasis on bimaxillary procedures, airway preservation, and virtual surgical planning. Digital orthodontics, including three-dimensional imaging and artificial intelligence, has further enhanced diagnostic precision and treatment predictability; however, concerns regarding long-term evidence and ethical considerations persist. Long-term stability remains a challenge and is influenced by growth pattern, treatment modality, and patient compliance. This review critically evaluates current evidence and highlights a decision-based, individualized approach for optimizing functional and esthetic outcomes in the management of Class III malocclusion.

Keywords: Class III malocclusion, orthodontic camouflage, 3D imaging, digital orthodontics, artificial intelligence





Anamika et al.,

INTRODUCTION

Class III malocclusion is a complex dentofacial deformity characterized by maxillary deficiency, mandibular prognathism, or a combination of both, often accompanied by transverse and vertical skeletal discrepancies¹. Its multifactorial etiology is predominantly influenced by genetic factors, with environmental and functional components contributing to clinical variability. Early diagnosis is critical, as craniofacial growth patterns strongly affect treatment timing, outcomes, and long-term stability². Advancements in diagnostic modalities, particularly cone-beam computed tomography and three-dimensional facial analysis, have enhanced the accuracy of skeletal assessment and growth prediction, allowing for more individualized treatment planning³. The introduction of skeletal anchorage systems, including temporary anchorage devices and miniplates, has expanded orthopedic treatment possibilities beyond the early mixed dentition stage⁴. On the other hand, orthognathic surgery remains the gold standard for managing severe skeletal Class III malocclusion, especially when facial esthetics, occlusal correction, and airway considerations are priorities. Contemporary surgical approaches emphasize minimally invasive techniques, rigid fixation, and virtual surgical planning to improve precision, reduce morbidity, and enhance postoperative stability⁵. Given ongoing advancements in diagnostic technologies, biomechanical principles, and treatment modalities, a focused review of current trends in the management of Class III malocclusion is timely and essential⁶⁻⁷. This review aims to critically analyze recent advances in orthopedic, orthodontic, and surgical management strategies, emphasizing evidence-based decision-making and future directions in treatment.

METHODOLOGY

This narrative review was conducted to synthesize and critically evaluate current evidence on the management of Class III malocclusion, with emphasis on recent advances in diagnosis, growth modification, orthodontic camouflage, surgical management, and digital orthodontics. A comprehensive literature search was performed using electronic databases including PubMed, Scopus, Web of Science, and Google Scholar. The literature search was limited to studies published between 2000 and 2025, with particular emphasis on recent high-quality publications from 2020 to 2025 to ensure inclusion of contemporary evidence. Eligible studies included systematic reviews, meta-analyses, randomized controlled trials, and well-designed observational studies. Relevant narrative reviews were also included where appropriate. Case reports, editorials, and non-English publications were excluded unless they provided significant clinical insights. Articles were initially screened based on titles and abstracts, followed by full-text assessment for eligibility. Preference was given to studies with higher levels of evidence. Data from selected studies were synthesized descriptively, with emphasis on comparative evaluation of treatment modalities, clinical applicability, and long-term outcomes. This review did not involve human participants or patient data, so, therefore, ethical approval was not required.

Etiology and Growth Pattern of Class III Malocclusion

The etiology of Class III malocclusion is complex and multi factorial, resulting from interactions among genetic, epigenetic, environmental, and functional factors during craniofacial growth⁸. Genetic influence is the primary determinant, particularly in mandibular prognathism, as supported by family, twin, and genome-wide association studies. Despite strong heritability, phenotypic expression varies considerably, indicating the modifying role of non-genetic factors⁹. Maxillary deficiency is a major but often under diagnosed component, with three-dimensional imaging demonstrating that maxillary retrusion contributes more frequently to Class III malocclusion than true mandibular excess. Environmental and functional influences, including airway obstruction, mouth breathing, altered tongue posture, and neuromuscular imbalance, further affect skeletal development¹⁰.

Longitudinal studies reveal prolonged and excessive mandibular growth in Class III individuals, often extending beyond puberty and increasing relapse risk. Growth direction, skeletal maturation timing, and sex-related differences significantly influence severity and treatment response¹¹. Advances in three-dimensional imaging, superimposition



**Anamika et al.,**

techniques, and emerging artificial intelligence-based growth prediction models have improved understanding of growth behaviour. Comprehensive knowledge of etiologic factors and growth patterns is essential for accurate diagnosis, optimal timing, and individualized management of Class III malocclusion³.

Diagnostic Advances and Three-Dimensional Imaging in Class Iii Malocclusion

Accurate diagnosis is essential for effective management of Class III malocclusion, as successful treatment depends on precise identification of skeletal, dentoalveolar, and functional components. Conventional two-dimensional cephalometric analysis, while informative, is limited by magnification errors, structural superimposition, and inadequate assessment of transverse and asymmetric discrepancies commonly seen in Class III cases¹². The introduction of cone-beam computed tomography has markedly improved diagnostic accuracy by enabling three-dimensional evaluation of maxillary deficiency, mandibular prognathism, craniofacial asymmetry, and airway dimensions. CBCT-based cranial base assessment has enhanced understanding of sagittal discrepancies and Class III etiology. Additionally, three-dimensional soft tissue analysis refines esthetic evaluation, while digital dental models and virtual simulations support treatment planning decisions². Advances in superimposition and artificial intelligence-based tools further improve growth assessment. Nonetheless, integrating clinical examination with radiographic and digital data remains essential for evidence-based and patient-centered care¹³.

Current Trends in Growth Modification of Class Iii Malocclusion

Growth modification remains a key treatment modality for Class III malocclusion in growing patients, aiming to correct skeletal discrepancies by enhancing maxillary growth, restraining mandibular growth, or both. Traditional approaches such as facemask therapy are most effective during early mixed dentition but are often associated with dentoalveolar side effects¹⁴. Recent advances focus on maximizing skeletal effects while minimizing dental compensation through improved appliance design and anchorage systems. Skeletal anchorage-assisted maxillary protraction using miniplates or miniscrews has demonstrated superior orthopedic outcomes, producing greater maxillary advancement, improved sagittal jaw relationships, and favorable mandibular rotation with enhanced patient compliance¹⁵. Combination protocols incorporating rapid maxillary expansion and protraction, including alternate expansion-constriction techniques, further enhance sutural response. In contrast, chin cup therapy has shown limited true skeletal effects and is now less favored. Emphasis on optimal force vectors, vertical control, and individualized case selection has improved predictability¹⁶. Despite advances, residual mandibular growth necessitates long-term monitoring. Overall, contemporary growth modification strategies reflect a shift toward biologically driven, minimally invasive, and patient-centered orthopedic management¹⁷. While conventional facemask therapy remains widely used, it is often associated with dentoalveolar compensation and limited skeletal effects, particularly in older patients. In contrast, skeletal anchorage-assisted maxillary protraction has demonstrated significantly greater skeletal advancement with minimal dental side effects, as supported by recent systematic reviews and clinical trials¹⁰. However, controversy exists regarding the optimal timing and long-term stability of these approaches. Although skeletal anchorage shows promising short-term outcomes, long-term data remain limited, and residual mandibular growth continues to pose a challenge¹⁰.

Orthodontic Camouflage in the Management of Class III Malocclusion

Orthodontic camouflage remains an important treatment option for patients with mild to moderate Class III malocclusion who are unwilling or unsuitable for orthognathic surgery. This approach aims to mask skeletal discrepancies through controlled dentoalveolar compensation rather than correcting the skeletal bases. Successful camouflage relies heavily on appropriate case selection, with favorable facial esthetics, minimal skeletal discrepancy, and stable growth patterns being key determinants. Biomechanically, camouflage treatment involves proclination of maxillary incisors and retroclination of mandibular incisors, often facilitated by selective extraction strategies¹⁸. Anchorage control is critical, and the use of temporary anchorage devices has significantly improved treatment predictability by enabling greater incisor retraction with minimal unwanted tooth movement¹⁹. Advances in digital treatment planning and clear aligner therapy have further expanded camouflage possibilities in carefully selected cases²⁰. Soft tissue response and vertical dimension control play crucial roles in determining esthetic outcomes.



**Anamika et al.,**

Although camouflage can provide satisfactory occlusal results, long-term stability and facial esthetic limitations necessitate careful planning, retention, and shared decision-making with patients¹⁷.

Surgical Management of Class III Malocclusion and Virtual Treatment Planning:

Orthognathic surgery is the definitive treatment for severe skeletal Class III malocclusion when orthodontic camouflage fails to provide functional or esthetic improvement. Patients with significant maxillary deficiency, pronounced mandibular prognathism, or facial asymmetry are typical candidates. Common procedures include Le Fort I maxillary advancement and bilateral sagittal split osteotomy of the mandible, performed individually or in combination, with bimaxillary surgery often preferred to enhance facial balance and distribute surgical movements²¹. Contemporary trends favour maxillary advancement over isolated mandibular setback to preserve airway function and reduce obstructive sleep apnea risk²².

Presurgical orthodontics is essential to decompensate dentoalveolar adaptations, revealing true skeletal discrepancies, though it may temporarily compromise esthetics. Surgery-first and surgery-early protocols minimize presurgical orthodontics, shortening overall treatment duration while maintaining outcomes¹⁸. Virtual surgical planning using 3D imaging, computer-assisted simulations, and integrated digital workflows improves precision, predicts postoperative esthetics, and facilitates interdisciplinary communication. CAD/CAM-fabricated surgical splints and rigid internal fixation enhance surgical accuracy and stability. Postoperative orthodontics ensures occlusal refinement and long-term stability, with relapse influenced by residual growth, surgical magnitude, and neuromuscular adaptation. Bimaxillary procedures generally show superior stability²³. Psychological benefits, including improved self-perception and quality of life, are significant, though realistic expectations remain critical. Modern surgical management prioritizes precision, collaboration, and evidence-based, patient-centered care for optimal outcomes²⁴.

Surgery First v/s Conventional Orthodontics

The surgery-first approach has gained popularity due to reduced overall treatment duration and early improvement in facial esthetics. However, compared to conventional protocols, it presents challenges in occlusal stability, case selection, and treatment planning complexity. Current evidence suggests that while outcomes are comparable in selected cases, the conventional approach remains more predictable in patients with significant dental compensation or complex skeletal discrepancies²⁵.

Clear Aligners, Digital Orthodontics, and Artificial Intelligence in the Management of Class III Malocclusion:

Digital orthodontics has revolutionized the diagnosis and management of Class III malocclusion through advances in three-dimensional imaging, computational modeling, and artificial intelligence (AI). These technologies allow precise visualization of skeletal and dental discrepancies, improving treatment planning accuracy. Clear aligner therapy has emerged as an effective option for selected mild to moderate. Class III elastics and skeletal anchorage, including miniscrew-assisted aligner mechanics, enhance tooth movement control and reduce reliance on patient compliance²⁰. Despite its advantages, artificial intelligence in orthodontics presents several limitations, including lack of standardized validation, potential algorithm bias, data privacy concerns, and limited long-term clinical evidence. Over-reliance on automated decision-making may also reduce clinician judgment. Therefore, AI should be considered an adjunct rather than a replacement for clinical expertise, and further high-quality research is required to establish its reliability and clinical applicability^{26,27}.

Long-Term Stability, Retention, and Relapse in the Management of Class III Malocclusion:

Long-term stability remains a central challenge in managing Class III malocclusion due to skeletal growth patterns, neuromuscular influences, and behavioral factors. Residual mandibular growth, particularly in adolescent patients, can alter occlusal relationships post-treatment, contributing to relapse. Growth modification therapies and orthodontic camouflage offer variable stability, with excessive dental compensation—such as mandibular incisor



**Anamika et al.,**

retroclination—being prone to instability²⁸. Retention strategies, including fixed and removable appliances, are critical for maintaining dental alignment, with long-term or permanent retention often recommended in high-risk cases. Skeletal anchorage, occlusal settling, soft tissue adaptation, and patient compliance further influence stability. Emerging digital technologies enable remote monitoring, early detection of relapse, and timely intervention²⁹. Achieving durable results requires individualized retention plans, careful case selection, patient education, and management of realistic expectations to optimize both functional and esthetic outcomes in Class III malocclusion³⁰.

CONCLUSION

Management of Class III malocclusion requires a comprehensive, evidence-based, and individualized approach guided by accurate diagnosis and growth assessment. Skeletal anchorage-assisted growth modification offers improved orthopaedic outcomes in growing patients, while orthodontic camouflage remains appropriate for carefully selected mild to moderate cases. Orthognathic surgery continues to be the most predictable option for severe skeletal discrepancies, with digital planning enhancing precision and outcomes. Emerging technologies, including artificial intelligence and digital orthodontics, show significant potential but require further validation. Long-term stability remains a critical concern, emphasizing the importance of appropriate case selection, retention strategies, and patient compliance. Future research should focus on high-quality comparative studies to strengthen clinical decision-making in Class III malocclusion management.

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RESEARCH ARTICLE

COVID-19 Yoga Concept and Its Scale Development: A Psychometric Approach

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ABSTRACT

The COVID-19 pandemic has profoundly impacted global physical, social, and psychological wellbeing, leading to a search for holistic and sustainable coping mechanisms. Yoga, as an ancient Indian discipline, offers a multidimensional approach for managing stress, immunity, and mental health during such crises. This study aimed to develop and validate a *COVID-19 Yoga Scale* (CYS) that conceptualizes the relationship between Yoga and wellbeing amid the pandemic. Using exploratory and confirmatory factor analysis (EFA and CFA), data were collected from 738 participants aged 16–60 years across four Indian states. The 49-item scale identified four sub-domains: Conspiracy, Location, Health, and Social Factors. The reliability coefficient (Cronbach's $\alpha = 0.94$) confirmed strong internal consistency. CFA demonstrated acceptable fit indices ($\chi^2/df = 2.14$, RMSEA = 0.045, CFI = 0.96, TLI = 0.95, GFI = 0.93), establishing robust construct validity. The results indicate that the COVID-19 Yoga Scale is a valid and reliable tool for assessing Yoga's conceptual and behavioral influence on health and wellbeing during pandemic conditions.

Keywords: Yoga, COVID-19, Scale Development, EFA, CFA, Psychometrics

INTRODUCTION

According to the World Health Organization (2020), the corona virus (COVID-19) pandemic has been affecting us since the end of 2019 and has significantly altered our way of life. These adjustments appear to have contributed to an increase in psychological suffering connected to the epidemic, such as anxiety, stress, perceived threat, and stress (Nikcevic et al., 2020). This was the initial cluster of the condition that would eventually be known as corona virus



**Nishant Kumar et al.,**

illness (COVID-19). A Public Health Emergency of International Concern (PHEIC) had been declared by the WHO by January 30th 2020 as a result of the disease's rapid expansion to 18 countries outside of China with documented cases of human-to-human transmission (Riad et al.,2020). COVID-19 has a significant impact on people's health and welfare globally and is a primary cause of fear, stress, and anxiety (Reznik et al.,2020). As a result of rapid viral infections over the world, the number of affected persons and deaths has risen dramatically. COVID-19 is more likely to affect the elderly and individuals with underlying medical issues. According to recent research, corona viruses likely SARS and MERS are capable of modulating the host immune detection system (Sharma et al.,2024). The coronavirus pandemic, declared a global health emergency by the World Health Organization (WHO, 2020), has altered lifestyles and health perceptions worldwide. Alongside physical health risks, it triggered widespread psychological distress, including anxiety, fear, and social isolation (Nikčević&Spada, 2020; Reznik et al., 2021). Traditional Indian health systems such as Yoga and Ayurveda have been increasingly recognized for their potential to enhance immunity, resilience, and mental stability (Tillu et al., 2020; Choudhary et al., 2019).

Although several COVID-19-related psychological scales exist—such as the Fear of COVID-19 Scale (Ahorsu et al., 2020) and the COVID-19 Anxiety Syndrome Scale (Nikčević&Spada, 2020)—few have integrated *Yogic constructs* that assess holistic wellbeing. Thus, this study bridges that gap by conceptualizing and validating a COVID-19 Yoga Scale (CYS) designed to evaluate Yoga-related attitudes, beliefs, and behaviors that emerged during the pandemic. Recent studies highlight Yoga's role in post-COVID recovery and stress regulation (Gupta & Singh, 2024; Taneja& Sharma, 2023), reinforcing its importance as both a preventive and therapeutic practice. Therefore, the present research sought to develop a psychometrically sound scale that captures Yoga's multidimensional relevance during the pandemic. Yoga, Ayurveda and Homoeopathy are examples of traditional Indian health practices that have been shown to prevent, treat and control a variety of disease (Choudhary et al.,2019). In the Yoga scale of COVID-19 study the four domains i.e., Conspiracy, Health, Location and Self-awareness had been included. This study includes the questions related to each domains of COVID-19 which helps to find the review of each question related to COVID-19. This had been filled by 738 peoples from India through online and offline mode.

Objectives and Hypothesis

Objectives:

- To develop a standardized and validated scale reflecting Yoga concepts related to COVID-19.
- To identify and confirm the primary dimensions of Yoga responses during the pandemic.
- To assess the psychometric reliability and validity of the COVID-19 Yoga Scale through EFA and CFA.

Hypothesis:

It is hypothesized that the concept of Yoga in the context of COVID-19 can be represented as a multidimensional construct measurable through a valid and reliable scale.

Conceptual Frame:

The conceptual frame work of COVID-19 is designed on the basis of four COVID domains- Conspiracy, Location, Health, and Social Factors. Scale development of Yoga from COVID-19 using Dimension reduction- EFA, CFA factor methods (SPSS version 25) and discussion of Yoga in COVID-19.

METHODS

SAMPLING

A total of 738 participants (475 males, 263 females) aged 16–60 years were selected through convenience sampling from universities in Delhi, Haryana, Uttarakhand, and Uttar Pradesh. The sample size satisfied DeVellis's (2016) recommended ratio (1:15 to 1:20 items per participant). The study was conducted in Delhi, Haryana, Uttarakhand and Uttar Pradesh, India. A participant age range of 16 to 60 years was used as the inclusion criterion for the study. The people who gave their permission to collect the data were taken into account while choosing the sample size. Data were gathered between August 2021 and June 2022 using both online and offline questionnaires due to



**Nishant Kumar et al.,**

pandemic restrictions. The instrument consisted of 49 items rated on a five-point Likert scale (1 = strongly disagree to 5 = strongly agree). Item Generation and Content Validity An initial pool of 58 items was generated based on a literature review and expert interviews in Yoga and health sciences. After expert evaluation using the Content Validity Index (CVI), items with $CVI < 0.75$ were removed, yielding 49 valid items. The four domains identified were:

Conspiracy (4 items)

Location (4 items)

Health (25 items)

Social Factors (16 items)

Face validity was confirmed through pilot testing with 35 respondents for readability and consistency. Students from each chosen university who agreed to take part in the study and give information were included. A ratio of 1:15 or 1:20 is suggested by DeVellis, R. F. (2016) as the best sample size. According to this standard, a sample size of at least 730 is sufficient. With their permission, we picked 738 people to test the psychometric properties. Data were collected from 1 August to 30 June 2022. The demographic details of the subjects are given in Table 2.

Procedures of Scale Development

Scale development is a systematic process that is carried out at different stages of analysis. Following recommendations of DeVellis RF and Pasquali L scale development for the present study was accomplished in three stages viz; item generation, theoretical analysis and psychometric analysis.

Item Generation

The content domain was specified through review of literature related to yoga in COVID-19. Among the COVID-19 review four areas are considered which specify the content domain. The scale comprises of four concepts Conspiracy, Location, Health and Social factors. All four concepts were considered as scale development constructs by the researcher. Item pool generation gives the initial item pool a conceptual stamp of approval. According to Kapuscinski AN and Masters KS, the current study used a combination of deductive and inductive strategies to generate the first item pool. The researchers also spoke with specialists in the field of yoga science to gather qualitative information about the research's subject domains and goal. The information was analyzed and related with the concept of yoga in COVID-19 to generate initial items. As a result, 49 items were developed under the selected content domains.

Theoretical Analysis

Content Validity

The initial items content validity was tested to ensure that they were indicative of the defined construct i.e., Yoga in COVID-19. In order to assess the content validity of the initial items, the researchers conducted a thorough literature analysis and sought expert input to explicitly describe the conceptual framework of yoga in COVID-19. The expert panel consisted of four members from outside. The experts used CVI developed by Waltz (1981) to assess the relevance of items in respect to the content domain. provided by each item was deemed genuine and in accordance with the theory being The experts rated each item against a 4-point scale (1 = not relevant, 2 = somewhat relevant, 3 = quite relevant and 4 = highly relevant). A score of 3 or 4 means that the content measured, and they were kept. Items with a score of 1 or 2 were removed from the scale, indicating that they were theoretically or practically important questions, as well as any unclear items that appeared to repeat the basic substance of previous items.

Face Validity

The initial level scale was administered to 35 people to examine the tool's visual appearance, such as uniformity of style, formatting, readability, and feasibility, as instructed by Devon HA. The responders were asked to rate the tool's user-friendliness. To improve the instrument, the respondents' feedback was taken into account. This method proved useful in determining ambiguity and skewness, i.e., respondents providing a similar response for each item.



**Nishant Kumar et al.,****Psychometric Analysis**

The psychometric analysis employs a variety of quantitative methodologies to assess the scale's construct validity and reliability. According to Posak off PM, construct validity is the amount of information that can be inferred from observed scores to hypothetical conceptions about which the observations are supposed to store information. The scale's convergent validity, criterion validity, and discriminate validity were all quantitatively examined in addition to its construct validity. To acquire consistent results of the psychometric indices, DeVellis RF strongly suggests combining exploratory factor analysis (EFA) with confirmatory factor analysis (CFA). Hence, these validity tests were done using EFA and CFA. Cronbach Alpha, Spearman–Brown coefficient, composite reliability, and average variance extraction were used to evaluate reliability, which is a quantification method that produces consistent results on repeated tests. The correlation between the results on the current scale and those on a known scale, the COVID-19 Yoga Scale, was used to determine concurrent validity.

Data Collection And Analysis

In this study, there were five possible responses on the scale, from "strongly disagree" (score 1) to "strongly agree" (score 5). Each item on the scale is a five-point Likert scale agreement statement, which is a quantitative method for measuring psychological aspects such as attitude, perception, anxiety, stress, beliefs, etc. The covid-19 Yoga Scale was acknowledged by every item on the scale. The total score across all items was used as the construct's quantifiable measure and was taken into account for all quantitative analytical tasks. The Likert scale's items were all regarded as continuous variables. To make it easier for responders to understand the statements, the scale was written in English. The respondents completed the questionnaire. EFA extracts the factors and proposes a factor structure that takes the correlation between the elements into account. The proposed factor structure is hypothesized and tested in CFA. If the statistical results fits with the hypothesized model the researcher can conclude that the factor structure is valid. Hence, the study evaluated the scale using both EFA and CFA. The Cronbach Alpha value, EFA, correlation matrix, and descriptive statistics were computed using IBM SPSS 25 software. CFA was carried out using IBM SPSS Amos 25, a version of the software. The Average Variance Extracted, a statistic derived from the values of factor loads, was used to confirm the convergence validity. Confirmatory factor analysis outputs such as the p value of Chi square, RMSEA, GFI, AGFI, CFI, TLI, NFI, and Chi sq/df were used to calculate model fitness indices in order to evaluate the construct validity. Through the use of Modification Indices, the degree of item redundancy was measured in order to test discriminate validity (MI).

RESULTS**General Characteristics of the Sample**

A sample size of 738 healthy participants was selected for the study from the different universities of India covering both male 475 (66.39%) and female 263 (33.61%) populations. The demographic details of the subjects are given in Table 2.

Item Generation

We developed the instrument for COVID-19 Yoga Scale on the lines of methodology given by Montero I & León OG. As a result, based on the conceptual frame work of COVID-19 Yoga Scale. 49 items were developed under the selected major content domain viz; COVID-19 Yoga Scale. We divided the 49 items into four sub domains such as Conspiracy (4), Location (4), Health (25), Social Factors (16) were generated as the initial pool of items.

Theoretical Analysis

The initial item pool of 58 items was verified by four experts to determine whether the items, when taken collectively, provide an appropriate operational description of a construct, i.e., content validity. The experts reviewed the initial item pool using a CVI rating tool. Individually, the experts assigned a score. The index was determined for each item by dividing the number of experts who gave a rating of 3 or 4 by the total number of experts. Items with an index of less than 0.75 were deemed irrelevant, and they were removed from the original list. Nine items on the draft COVID-



**Nishant Kumar et al.,**

19 were considered invalid from the initial pool because they yielded CVIs of 0.25 to 0.50 and were deleted if CVI was less than 0.75. All of the remaining items were found to be valid, with CVIs ranging from 0.75 to 1, resulting in a 49-item questionnaire. The scale was individually administered on 40 people after it was modified based on expert ratings. Each sentence was read aloud to the respondent, who then said what he or she had taken away from it. The item would be regarded qualified if the substance that the responder understood and what the researchers had conceived matched. The response would reveal difficulties such as vagueness, ambiguity, leading words/sentences, unfamiliar terminology, complicated sentences, closed-ended statements, and sensitive statements that were included in the items. Statements were rewritten based on this information. The changed statements were read to the respondent once more, and feedback was obtained and adjusted accordingly. Content validity testing and cognitive interviews with selected respondents resulted in the development of the 49 items.

Psychometric Analysis

Exploratory Factor Analysis (Efa)

The scale evolved after theoretical analysis with 49 items was administered to 738 participants. Exploratory factor analysis was conducted using the scores obtained from the survey. The Kaiser–Meyer–Olkin (KMO) measure was used to assess the sample adequacy and to prove the correlation matrix is an identity matrix Bartlett's Test of Sphericity is calculated. KMO value was 0.92 which was an excellent measure of Sampling Adequacy. Factor loading measures ranged from 0.40 to 0.90. Since all the factor load values of all the 49 variables were greater than 0.40, all of them were retained in the scale. Table 3 shows variance contributed by each factor and their corresponding Eigen values.

Confirmatory Factor Analysis (Cfa)

Confirmatory Factor Analysis (CFA) is a statistical technique used to assess the measurement properties of a set of observed variables and examine the underlying latent (unobserved) constructs or factors that these variables are intended to measure. It is a multivariate analysis method that is widely employed in the fields of psychology, social sciences, and market research. The primary objective of CFA is to test and validate a hypothesized factor structure by evaluating the fit between the observed data and a specified theoretical model. It allows researchers to determine whether the observed variables adequately represent the underlying constructs and to assess the relationships between these constructs. The researchers conducted a CFA applying a structural equation modeling to test a hypothetically developed factor structure through EFA with five latent factors and 49 observed variables. The model obtained from confirmatory analysis is presented in table.

Reliability

Internal consistency of the 49 item five factor scale was determined by calculating Cronbach's alpha measure and composite reliability measure. The Cronbach's alpha value was 0.94. Composite reliability measure was 0.94.

Scree Plot for COVID-19 Scale

A scree plot is a graphical tool used in exploratory factor analysis (EFA) or principal component analysis (PCA) to determine the number of factors or components to retain from a dataset. It displays the eigen values associated with each factor or component extracted from the data. The scree plot is created by plotting the eigen values on the y-axis against the number of factors or components on the x-axis. Eigen values represent the amount of variance explained by each factor or component. Typically, eigen values are sorted in descending order before plotting, so the most important factors or components are on the left side of the plot. The scree plot helps in deciding how many factors or components should be retained for further analysis. The idea is to look for an "elbow" or a point in the plot where the eigen values drastically drop. This point indicates a significant drop in variance explained and suggests that the subsequent factors or components are less important. The scree plot of our data of 738 participants for COVID-19 scale is shown in the graph.





DISCUSSION

Systematic steps as prescribed in the literature of research were adopted to develop this scale. The COVID-19 Yoga scale developed based on the recent study of COVID-19 papers and literature review (deductive method) and consulted with relevant people about the subject (inductive method). The study followed established psychometric guidelines (DeVellis, 2016; Pasquali, 2020) for scale development and validation. The COVID-19 Yoga Scale demonstrated strong reliability, validity, and conceptual clarity. Its four domains—Conspiracy, Location, Health, and Social Factors—represent distinct psychosocial aspects of how individuals integrated Yogic understanding during the pandemic. The results align with prior scale developments such as the COVID-19 Phobia Scale (Arpaci et al., 2020) and the COVID-19 Anxiety Scale (Chandu et al., 2020), but uniquely include Yogic dimensions of holistic wellbeing. This highlights Yoga's adaptive role in enhancing mental and social resilience during health crises. The initial item pool, which had 58 items got reduced to 49 items with four constructs at the end of the study. It has been validated and found to be reliable by using the psychometric analysis. The stated hypothesis Yoga concept in COVID-19 may be related to scale development has been proved with final four regression coefficient values. These coefficients were calculated using EFA and CFA statistical analytical techniques on the lines of Barry to assess the influence of COVID-19 Yoga scale on social health and wellbeing. In terms of scale's validity, reliability and factor structure of COVID-19 Yoga scale. The results of this study suggest that the COVID-19 Yoga scale is providing an appropriate instrument for measuring the concept of Yoga in COVID-19.

Limitations and Future Scope

- The sample was limited to Indian states, reducing generalizability.
- Data relied on self-reported responses
- Future studies should replicate the findings across diverse cultures and validate translations in regional languages. Longitudinal follow-ups may explore Yoga's sustained impact post-pandemic.

CONCLUSION

The COVID-19 Yoga Scale is a psychometrically robust instrument capturing Yoga's multidimensional contribution to health, resilience, and social wellbeing during the pandemic. The four-domain structure confirms that Yogic philosophy transcends individual health, influencing broader social and environmental harmony. The scale can serve as a valuable tool for researchers, educators, and policymakers to assess Yoga-based interventions in health crises and beyond. We employed both qualitative and quantitative methods to develop and validate this scale using SPSS version 25's exploratory and confirmatory factor analyses for 738 general populations from India. The standardized estimated regression value is found to be 0.67 which is greater than the required level of ≥ 0.5 and thus showing a good relation between four sub domains. This COVID-19 Yoga scale can facilitate and provide a social health care and wellbeing.

Conflict of Interest

There is no conflict of Interest

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Nishant Kumar et al.,

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Nishant Kumar et al.,

Table 1: KMO and Bartlett's Test

KMO and Bartlett's Test		
Kaiser-Meyer-Olkin Measure of Sampling Adequacy.	0.929	
Bartlett's Test of Sphericity	Approx. Chi-Square	15295.502
-	Df	1176
-	Sig.	0.000

Table 2: Characteristics of participants

Characteristics	Total Sample		
	Total (N=738)	Male	Female
16-22 years	329	235	94
23-29 years	216	118	98
30-36 Years	83	49	34
37-43 Years	59	38	21
44-50 Years	35	25	10
51-57 Years	15	9	6
58-60 Years	1	1	0

Table 3: Variance Contributed by Each Factor and Their Corresponding Eigen Values

Sub domain	Factor loading									
	Component									
	1	2	3	4	5	6	7	8	9	10
Factor1: Conspiracy										
Q1								.651		
Q2								.717		
Q3								.677		
Q4								.438		
Factor2 :Location										
Q5				.418						
Q6										
Q7									.417	
Q8		.419								
Factor3: Health										
Q9									.699	
Q10									.506	
Q11										
Q12				.748						
Q13				.791						
Q14				.810						
Q15		.410								
Q16		.768								
Q17		.719								
Q18		.666								
Q19		.579								
Q20		.570								
Q21		.460								
Q22					.802					



Nishant Kumar *et al.*,

Q23					.752					
Q24					.616					
Q25										
Q26	.432									
Q27	.715									
Q28	.775									
Q29	.706									
Q30	.765									
Q31	.735									
Q32	.694									
Q33	.714									
Factor4: Social Factors										
Q34							.649			
Q35							.618			
Q36							.753			
Q37							.460			
Q38										.633
Q39										.580
Q40						.432				
Q41						.761				
Q42						.574				
Q43						.498				
Q44			.568							
Q45			.716							
Q46			.858							
Q47			.867							
Q48			.672							
Q49			.440							
Extraction Method: Principal Component Analysis.										
Rotation Method: Promax with Kaiser Normalization.										
a. Rotation converged in 11 iterations.										

Table 4: Cronbach's Alpha Based on Standardized Items

Reliability Statistics		
Cronbach's Alpha	Cronbach's Alpha Based on Standardized Items	N. Of Items
0.94	0.94	49





RESEARCH ARTICLE

Artificial Intelligence and Image Analysis of Cranial Sutures: The Future of Sex Determination in Forensic Radiology

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ABSTRACT

Sex determination from skeletal remains constitutes one of the four primary pillars of the forensic biological profile. Cranial sutures, long regarded as indicators of biological age, have recently gained renewed interest as anatomical substrates for AI-assisted sex estimation. Traditional morphoscopic and morphometric methods, while established, are constrained by observer subjectivity, population-specificity, and limited reproducibility. Advances in deep learning — particularly convolutional neural networks (CNNs) applied to computed tomography (CT) images — now offer a paradigm shift in forensic radiology. This systematic review synthesises the current evidence on AI-based image analysis of cranial structures — with particular emphasis on suture morphology and closure patterns — for sex determination in forensic contexts. It further evaluates methodological quality, model architectures, accuracy benchmarks, population representativeness, and future directions. A systematic search of PubMed, Scopus, Web of Science, and Google Scholar was conducted for studies published between 2015 and 2025, using PRISMA guidelines. Eligible studies included those applying machine learning or deep learning to cranial CT or radiographic imaging for sex or age-sex estimation from bony cranial features. Thirty-two studies met inclusion criteria. AI-based systems demonstrated classification accuracy ranging from 82% to 99.5% for sex estimation from cranial CT data, consistently outperforming conventional human assessors whose accuracy ranged from 61% to 92%. CNN architectures including VGG16, VGG19, ResNet50, InceptionV3, DenseNet121, and MobileNetV2 were most commonly employed. Explainability remains a critical gap, as deep learning models function as 'black boxes' that cannot readily identify



**Ankit Kumar and Mahesh Kumar Mittal**

which anatomical features — including suture morphology — drive predictions. AI-enabled forensic radiology holds transformative potential for objective, reproducible, and scalable sex determination. Cranial suture analysis — when integrated into multimodal AI pipelines using CT imaging — emerges as a particularly promising subdomain. However, barriers including dataset size, population diversity, model interpretability, and medicolegal acceptance must be resolved before clinical and courtroom adoption. Future research should prioritise explainable AI (XAI), prospective multi-institutional validation, and the development of population-agnostic models.

Keywords: artificial intelligence, cranial sutures, sex determination, forensic radiology, deep learning, computed tomography, convolutional neural network

INTRODUCTION

Forensic identification of unknown human remains is one of the most consequential tasks in forensic medicine. When investigators recover skeletal remains — whether from a mass disaster, homicide, or archaeological context — establishing the biological profile of the individual is the critical first step toward identification. This biological profile encompasses four core parameters: sex, age at death, stature, and ancestry. Among these, sex estimation is foundational, as it immediately reduces the pool of potential matches by approximately fifty percent and contextualises the interpretation of all other biological indicators [1]. Cranial morphology has long served as a primary substrate for biological profiling. When the pelvis — the gold standard for sex estimation — is absent, incomplete, or poorly preserved, the skull becomes the next most reliable anatomical source of sexually dimorphic information. Male skulls typically exhibit greater robusticity, more pronounced supraorbital ridges, larger mastoid processes, squarer orbital margins, and more prominent nuchal crests compared to their female counterparts. These features, however, must be interpreted within a framework of considerable population-level and individual variation, rendering visual assessment inherently subjective [2]. Cranial sutures — the fibrous joints uniting the bones of the calvaria — have been investigated for over a century primarily as indicators of biological age, given their well-documented pattern of progressive obliteration with advancing age. However, emerging evidence suggests that suture morphology, closure rates, and spatial distribution may also encode subtle sex-related information. The interplay between hormonal influences on bone metabolism and the biomechanical loading of the skull may produce detectable sex-dependent differences in sutural anatomy — differences that human observers, constrained by the limits of visual discrimination, may not reliably detect, but which machine learning algorithms may exploit [3].

The convergence of digital forensic radiology and artificial intelligence has created unprecedented opportunities for objective, reproducible, and quantitatively rigorous forensic analysis. Postmortem computed tomography (PMCT) — now routinely employed in many forensic departments worldwide — generates high-resolution three-dimensional reconstructions of the skull that can serve as inputs to deep learning models. These models, particularly convolutional neural networks (CNNs) trained on large imaging datasets, have demonstrated remarkable accuracy in sex classification tasks, in many cases surpassing the performance of expert human assessors. Yet, the specific contribution of cranial suture analysis within these AI frameworks remains under characterised. Most published studies have focused on global skull morphology or selected anatomical landmarks such as the supraorbital ridge, nuchal crest, mastoid process, and foramen magnum — without isolating the analytical contribution of suture-specific features. This represents a significant gap in the literature, given the forensic relevance of sutures (they are often the most durable and identifiable cranial features in decomposed or fragmentary remains) and their established role in age estimation methodologies [4]. This systematic review aims to synthesise and critically evaluate the existing evidence on AI-based cranial image analysis for sex determination, with particular attention to the role of cranial sutures and CT-based imaging modalities. We further outline the methodological landscape of current machine learning approaches, identify key limitations, and propose a research agenda for the future development of robust, population-inclusive, and legally defensible AI forensic tools [5].



**Ankit Kumar and Mahesh Kumar Mittal****Background and Theoretical Framework****Sexual Dimorphism of the Skull**

Sexual dimorphism in the human skull arises primarily from divergent hormonal trajectories during adolescent development. Androgenic hormones in biological males promote greater overall bone mass, increased cortical thickness, and enhanced muscular attachment sites, resulting in the characteristic robusticity of the male cranium. In contrast, female skulls tend to be smaller, smoother, and more gracile, with less pronounced brow ridging and more rounded orbital margins. The degree of dimorphism, however, is not uniform across populations — it is known to be influenced by ancestry, nutritional history, and secular trends in stature and physique [6]. Morphoscopic assessment of cranial sex, systematised by Walker (2008) using five key traits — glabella, supraorbital margin, nuchal crest, mastoid process, and mental eminence — has been widely adopted in forensic practice. Walker's discriminant function analysis demonstrated classification accuracies of 77% to 92% depending on population and trait combinations. Subsequent validation studies, however, have revealed that these models perform less reliably when applied to populations outside the original reference samples, raising concerns about transferability. In the absence of the pelvic girdle, skulls alone can achieve approximately 80% accuracy in sex estimation under traditional methods — a figure that rises to 90% when the mandible is intact and approaches 96–98% when a complete skeleton is available [7].

Craniometric approaches using discriminant function analysis and the Fordisc 3.1 software package have supplemented morphoscopic methods, offering probabilistic outputs based on linear cranial measurements. More recently, three-dimensional geometric morphometrics have revealed sex-dependent shape variation across the cranial vault and base that transcends simple linear measurement, capturing complex surface topography that is invisible to traditional caliper-based methods. These geometric approaches have demonstrated accuracies exceeding 90% in well-sampled populations, and they provide the conceptual bridge to AI-based image analysis, which similarly operates on shape and intensity information extracted from imaging data [8].

Cranial Sutures: Anatomy, Function, and Forensic Relevance

Cranial sutures are syndesmotomic fibrous joints connecting the flat bones of the neurocranium. The major ectocranial sutures include the coronal suture (joining the frontal and parietal bones), the sagittal suture (joining the two parietal bones at the midline), and the lambdoid suture (joining the parietal bones to the occipital bone). Additional sutures of forensic relevance include the squamous, sphenofrontal, sphenotemporal, and median palatine sutures. Suture closure — the process of obliteration of the fibrous joint by osseous bridging — progresses broadly with age, forming the basis of suture-based age estimation methods pioneered by Todd and Lyon in the 1920s and subsequently refined by Meindl and Lovejoy (1985), whose ten-site ectocranial suture scoring system remains widely cited. However, the forensic utility of suture-based age estimation has been substantially questioned: the relationship between closure and chronological age exhibits enormous individual variability, such that the standard errors of age predictions using suture closure alone commonly exceed 15 years in adults. Critics have argued that suture evaluation in isolation contributes minimally to forensic age assessment [9].

Nevertheless, sutures retain considerable forensic importance for several reasons. First, the cranium — and specifically the calvarial bones bearing the major sutures — is among the most taphonomically resistant elements of the human skeleton, surviving conditions of decomposition, burning, fragmentation, and excavation that destroy more fragile postcranial elements. Second, a growing body of evidence suggests that suture morphology is influenced by sex-related biological processes. A 2023 Indian population study using 3D CT scans of 263 individuals found statistically significant sex differences in the closure patterns of the sagittal (S1 segment), coronal (RC1, RC2, LC1, LC2 segments), and lambdoid (LL1 segment) sutures, with males demonstrating earlier and more extensive obliteration in specific suture segments. These sex-related differences in closure timing, if reliably detectable by AI systems, could provide an additional discriminating feature in multi-parameter forensic classification models [10]. The advent of postmortem CT as a routine forensic tool has transformed suture assessment. Where traditional methods required removal of scalp and temporal muscles to visualise the ectocranial surface — a destructive process



**Ankit Kumar and Mahesh Kumar Mittal**

incompatible with body preservation requirements in many jurisdictions — CT enables non-invasive, three-dimensional visualisation of both ecto- and endocranial suture surfaces simultaneously. Multiplanar reconstruction (MPR) and volume rendering techniques (VRT) further enhance the clarity and quantifiability of suture imaging [11].

Artificial Intelligence in Medical and Forensic Imaging

Artificial intelligence, and deep learning in particular, has catalysed a revolution in medical imaging over the past decade. Convolutional neural networks — biologically inspired architectures that apply learnable filter banks to extract hierarchical representations from image data — have achieved or exceeded human performance in tasks ranging from radiographic fracture detection and diabetic retinopathy grading to histopathological cancer classification. The foundational architectures underpinning most forensic imaging studies include VGG16, VGG19, ResNet50, InceptionV3, MobileNetV2, DenseNet121, and EfficientNet, all of which were originally developed for general-purpose image classification on the ImageNet dataset and subsequently adapted via transfer learning to specialised medical imaging tasks. Transfer learning — the practice of initialising a model with weights pre-trained on a large general dataset and then fine-tuning it on a smaller domain-specific dataset — is especially valuable in forensic radiology, where labelled imaging datasets are inherently limited by the rarity and sensitivity of medicolegal cases. By leveraging the feature hierarchies learned from millions of photographic images, transfer learning enables meaningful classification even with datasets of a few hundred to a few thousand cases — a scale typical of published forensic CT studies [12,13].

Beyond standard CNN architectures, newer paradigms have emerged with particular relevance to forensic skull analysis. Multiple-instance learning (MIL) — which treats a three-dimensional CT scan as a 'bag' of two-dimensional slice instances and learns to classify the bag from the distribution of instance-level features — is well-suited to volumetric CT data where the discriminating anatomical information is distributed across many slices. Siamese neural networks, which learn similarity metrics between pairs of specimens, offer promise for retrieval-based forensic identification. Attention-gated architectures, which weight different image regions according to their relevance to the prediction, offer a degree of spatial interpretability that is forensically important: investigators need to know not just what the model predicted, but which anatomical regions drove that prediction [14].

METHODS**Search Strategy**

A systematic literature search was conducted in accordance with the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) 2020 guidelines. Electronic databases searched included PubMed/MEDLINE, Scopus, Web of Science, and Google Scholar. The search was restricted to publications from January 2015 to April 2025, reflecting the period of significant AI advancement in medical imaging. Search terms were constructed using Boolean operators and MeSH vocabulary, combining the domains of: (1) artificial intelligence, machine learning, deep learning, convolutional neural network; (2) cranial sutures, skull, calvaria, cranial morphology, craniometry; (3) sex estimation, sex determination, sexual dimorphism, forensic anthropology, biological profile; and (4) computed tomography, forensic radiology, postmortem CT, cephalometric radiograph. A total of 847 records were initially identified across all databases. After deduplication (n=214 removed), 633 records underwent title and abstract screening. Full-text review was performed for 89 articles. Thirty-two studies ultimately met all inclusion criteria. Reference lists of included articles were hand-searched, yielding four additional eligible studies. Studies were excluded if they: were purely traditional anthropometric in methodology (no AI component); did not use imaging data; focused exclusively on dental, postcranial, or non-cranial structures; or reported insufficient methodological detail for quality assessment.



**Ankit Kumar and Mahesh Kumar Mittal****Inclusion and Exclusion Criteria**

Inclusion criteria required studies to: (1) apply at least one machine learning or deep learning algorithm; (2) use cranial imaging data (CT, CBCT, cephalometric radiograph, or 3D surface scan); (3) include sex estimation or sex-informed classification as a primary or secondary outcome; (4) report quantitative performance metrics (accuracy, sensitivity, specificity, AUC); and (5) involve human skeletal or radiographic data. Studies using exclusively synthetic data, cadaveric specimens without radiographic imaging, or living subjects in non-forensic contexts were excluded unless their methodological findings were directly transferable to forensic applications.

Quality Assessment

Methodological quality was assessed using a modified QUADAS-2 (Quality Assessment of Diagnostic Accuracy Studies) framework adapted for AI imaging studies. Key domains evaluated included: representativeness of the study population, imaging protocol standardisation, transparency of AI model architecture and hyperparameter reporting, handling of class imbalance, validation strategy (internal vs. external), and degree of interpretability analysis. Studies were rated as low, moderate, or high risk of bias across these domains. Risk of bias did not result in exclusion but is reported narratively in the synthesis.

RESULTS**Overview of Included Studies**

The 32 included studies were published between 2018 and 2025, with a pronounced clustering after 2021 reflecting the rapid proliferation of AI applications in forensic imaging. Geographically, studies were conducted in Asia (n=18; predominantly Indonesia, Japan, South Korea, China, and Thailand), Europe (n=8; primarily Eastern Europe and Italy), Australia (n=3), and the Americas (n=3). This distribution reflects both the availability of forensic CT infrastructure and the composition of institutional skeletal reference collections in these regions. Sample sizes varied considerably, ranging from 85 to 2,041 CT scans per study. The majority of studies (n=24) used retrospective clinical or forensic CT archives, while eight studies employed dedicated research datasets including the New Mexico Decedent Image Database (NMDID) — the largest publicly available postmortem CT repository. Sex representation was broadly balanced in most studies, though several noted overrepresentation of male subjects due to the higher proportion of male forensic cases, which may introduce classification bias.

AI Model Architectures and Performance

CNN-based architectures dominated the included studies. VGG16 and VGG19 were most frequently employed (n=14 studies), followed by ResNet50/ResNet50V2 (n=11), InceptionV3 and InceptionResNetV2 (n=9), MobileNetV2 (n=8), and DenseNet121 (n=6). More recent studies employed EfficientNet (n=4) and custom hybrid architectures combining attention mechanisms with established backbones. Classification accuracy for sex estimation from cranial CT ranged from 82.4% to 99.5% across studies. The highest accuracy (99.5% mean average precision, mAP@50) was reported by a 2025 study from Thammasat University examining lateral skull CT images with a YOLO-based object detection model augmented with extensive data augmentation, focusing on the supraorbital ridge and nuchal crest in a Thai population. A landmark 2024 study by Lye et al. (Scientific Reports) — employing 200 cranial CT scans of Indonesian individuals and comparing a deep learning multi-task network against a trained human observer using Walker's (2008) standard — found that the best-performing DL network achieved 97% accuracy compared to 82% for the human assessor. The DL model was further enhanced by multi-task learning, in which it simultaneously predicted Walker cranial trait scores and sex classification, suggesting that incorporating anthropologically-grounded cranial landmarks into AI training pipelines improves both accuracy and interpretability.

A gated attention-based multiple-instance learning (MIL) model applied to 3D skull images reconstructed from postmortem head CT scans of East Asian cadavers (Kondou et al., 2023; Nature Scientific Reports) achieved 92% accuracy in females and 95% in males, outperforming three forensic scientist assessors whose accuracy ranged from



**Ankit Kumar and Mahesh Kumar Mittal**

61–89%. A parallel study on sex estimation from skull CT images using deep feature selection and feature fusion (Ciftci et al., 2024; Scientific Reports) reported overall accuracy of 96.4%, with precision of 96.1% for males and 96.8% for females. Forensic identification using Siamese neural networks applied to NMDID DICOM data further demonstrated the potential of contrastive learning frameworks, with hybrid CNN-contrastive embedding models achieving 91% accuracy in multi-institutional CT data. For cephalometric radiograph-based studies, accuracies were somewhat lower, ranging from 76% to 90.7%, with the most comprehensive study (340 cephalometric images of Indonesian subjects; 2025) reporting that InceptionResNetV2 performed best among six CNN architectures tested (VGG16, VGG19, MobileNetV2, ResNet50V2, InceptionV3, InceptionResNetV2), with VGG16 and VGG19 performing competitively in earlier Indonesian population research achieving accuracies of approximately 84–87%.

Role of Cranial Sutures in AI-Based Sex Estimation

Despite their anatomical prominence and established role in forensic anthropology, cranial sutures have received comparatively little dedicated attention in AI imaging studies for sex determination. The majority of studies that achieved high accuracy focused on global skull morphology, overall size and shape, or specific landmarks such as the supraorbital ridge, glabella, mastoid process, nuchal crest, mental eminence, and foramen magnum — without isolating the informational contribution of suture features.¹⁵ The most direct evidence for sex-related suture variation comes from non-AI radiological studies. A 2023 Indian population CT study (n=263) demonstrated statistically significant sex differences in cranial suture closure patterns using 3D CT with volume rendering — specifically in the sagittal (S1), coronal (RC1, RC2, LC1, LC2), and lambdoid (LL1) suture segments. Males exhibited earlier and more complete obliteration in these segments. This pattern is consistent with the known effect of testosterone on osteoblastic activity and may reflect sex-divergent patterns of sutural fusion. Similar findings have been reported in European, Japanese, and Chinese populations, suggesting a degree of universality in suture-sex relationships, though the magnitude of differences is population-dependent.¹⁶

Machine learning has been applied specifically to age estimation from cranial sutures via CT. A 2024 study of 132 Northern Chinese Han adults used CT-multiplanar reformation of cranial sutures and applied four machine learning classifiers — including support vector machines and ensemble models — to suture closure scoring data, achieving improved age prediction accuracy over traditional linear regression. Crucially, the study stratified results by sex, demonstrating differential model performance that indirectly confirms sex-specific suture closure dynamics. Nikolova et al. (2021; Forensic Imaging) applied machine learning techniques to sagittal suture maturation data and demonstrated that algorithmic approaches to suture scoring outperform traditional visual staging, particularly when combined with ancillary morphological features [17]. The Grad-CAM (Gradient-weighted Class Activation Mapping) visualisations reported in several deep learning sex estimation studies provide indirect evidence for the contribution of suture regions. In the Lye et al. (2024) study, Grad-CAM maps showed that the DL model appeared to allocate significant attention to regions overlapping the coronal and sagittal sutures, in addition to the expected dimorphic landmarks. This suggests that AI models may be implicitly learning suture-based features without explicit anatomical labelling — a finding with important implications for understanding what these models detect. A dedicated study specifically segmenting and isolating suture regions as input features to CNN or attention-based models would be highly informative and represents an urgent gap in the literature.¹⁸

The foramen magnum — a region anatomically adjacent to the lambdoid suture — has been specifically targeted for AI-based sex estimation. A 2025 ScienceDirect study employing object detection and instance segmentation on foramen magnum CT images achieved 65.68% and 62.69% accuracy respectively, noting that these figures were lower than traditional morphometric approaches. This finding underscores that not all cranial regions are equally informative, and that AI model performance is highly dependent on the anatomical region selected for analysis and the quality of the training dataset.¹⁹





Imaging Modalities and Technical Considerations

Computed tomography — specifically multislice CT (MSCT) and postmortem CT (PMCT) — is the dominant imaging modality across included studies, reflecting its capacity for high-resolution three-dimensional bone visualisation without tissue dissection. Standard CT acquisition parameters varied across studies: slice thickness ranged from 0.5 mm (high resolution) to 5 mm, with thinner slices providing superior detail for suture visualisation but generating larger datasets that increase computational demands. Most studies used bone algorithm reconstruction for maximal spatial resolution, which reduces soft tissue blur relevant to cranial surface and suture analysis.²⁰ Three-dimensional reconstruction techniques — including maximum intensity projection (MIP), multiplanar reformation (MPR), and volume rendering technique (VRT) — were used in most studies to generate the skull surface representations that serve as CNN inputs. The volumetric nature of CT data presents a methodological choice: studies may either convert 3D data to 2D projections (lateral, anterior-posterior, or oblique views) for input to standard 2D CNNs, or employ true 3D convolutional networks or multiple-instance learning frameworks that process the full volumetric information. The former approach sacrifices depth information but leverages the larger body of pre-trained 2D CNN weights; the latter is computationally intensive but theoretically retains all available diagnostic information. Bewes et al. demonstrated 95–96% accuracy using 2D projections of 3D reconstructions, while 3D MIL-based models (Kondou et al.) achieved comparable performance, suggesting that 2D projections may preserve sufficient discriminating information for sex classification at the global skull level.²¹

Cone beam CT (CBCT), which offers higher resolution at lower radiation doses and cost compared to MSCT, has been explored in several studies. A 2024 study applied the GADNN hybrid deep learning architecture to CBCT images of maxillary and frontal sinuses for age and sex determination, reporting high accuracy. CBCT may be particularly relevant for suture analysis given its superior resolution of fine cortical bone interfaces. Lateral cephalometric radiographs, while two-dimensional and limited in information content compared to CT, remain practically important in contexts where CT is unavailable — including historical forensic archives and resource-limited settings — and have yielded accuracies of 84–90% in well-controlled CNN studies.²²

Population Diversity and Generalisability

A major limitation of the current evidence base is its geographic and demographic skew. The majority of high-accuracy studies have been conducted on East and Southeast Asian populations — predominantly Indonesian, Japanese, Thai, and Chinese cohorts — for whom institutional CT archives and forensic anthropological infrastructure are well-developed. European studies have focused on Italian, Croatian, Bulgarian, and Eastern European populations. Studies from South Asian, sub-Saharan African, Middle Eastern, and Latin American populations are conspicuously underrepresented, despite the forensic relevance of these populations.²³

This demographic skew matters profoundly for sex estimation accuracy because sexual dimorphism is known to be population-specific in its expression. A model trained exclusively on East Asian skulls — with their characteristic cranial morphology — may perform poorly on South Asian or African skulls. The degree to which AI models trained on one population generalise to another has been explicitly tested in only a minority of studies, with results uniformly showing accuracy degradation in cross-population application. Population-specific formulae are generally required for reliable forensic work — a constraint that applies equally to AI models and to traditional morphometric methods.²⁴

DISCUSSION

Paradigm Shift: From Subjective Assessment to Algorithmic Classification

The consistent finding across this systematic review — that AI models achieve accuracy rates of 90–99% in cranial sex estimation while outperforming trained human assessors operating at 61–92% — represents more than a quantitative improvement. It reflects a qualitative shift in the epistemology of forensic cranial analysis. Human assessors, however experienced, are subject to cognitive biases, inter-observer inconsistency (particularly in populations



**Ankit Kumar and Mahesh Kumar Mittal**

outside their training), and perceptual limitations that constrain the ceiling of achievable accuracy. AI systems, while not immune to systematic error, can maintain consistent classification criteria across thousands of specimens and can be explicitly validated against population-specific reference data. The multitask learning approach demonstrated by Lye et al. (2024) — in which the AI simultaneously learns traditional cranial trait scores (following Walker's framework) and sex classification — is particularly significant. By grounding AI learning in anthropologically validated landmarks, this approach produces models that are not only accurate but partially interpretable through the lens of established forensic practice. The DL model's learned attention to cranial trait regions, as visualised by Grad-CAM, affirms that the AI is capturing anatomically meaningful information rather than artefactual correlates.

The Suture Problem: Underexplored but High-Potential

Cranial sutures represent what may be described as the unexplored frontier in AI forensic cranial analysis. Their significance as taphonomically durable anatomical features, their documented sex-related variation in closure patterns, and their geometric complexity — which renders visual human assessment unreliable but which AI pattern recognition may excel at — make them a compelling target for dedicated AI investigation. Current studies have demonstrated that sex differences exist in suture closure rates and that machine learning improves the precision of suture-based age estimation; the logical next step is a study specifically designed to evaluate whether suture texture, morphology, and closure pattern can serve as independent or supplementary predictors of sex. The integration of radiomic features — quantitative descriptors of image texture, intensity, and spatial patterns extracted from CT data — with suture region of interest segmentation is a particularly promising methodological approach. Radiomics has been successfully applied to CT-based sex estimation in adjacent domains (e.g., orbital region, frontal sinus morphology), and its application to specifically segmented suture regions could reveal hidden sex-discriminating information invisible to conventional analysis. Deep learning-based radiomics, which learns task-relevant features automatically rather than relying on hand-engineered descriptors, represents the most advanced iteration of this approach.

Explainability: The Critical Medicolegal Challenge

For AI tools to be admissible in forensic and medicolegal proceedings, they must not only be accurate — they must be explainable. Courts and regulatory bodies in most jurisdictions require that forensic methods be validated, reproducible, and interpretable: examiners must be able to articulate the basis for their conclusions. The 'black box' nature of deep learning represents a fundamental barrier to courtroom adoption. While Grad-CAM and SHAP (SHapley Additive exPlanations) visualisation techniques provide post-hoc spatial attention maps, they do not constitute mechanistic explanations of model decision-making.²⁵ Explainable AI (XAI) — a field specifically concerned with rendering machine learning decisions transparent and interpretable — has produced several architectures relevant to forensic radiology. Attention-gated CNNs highlight the most influential image regions for classification. Concept-based explanation methods learn human-understandable concepts (such as 'prominent supraorbital ridge' or 'open sagittal suture') and map them to model outputs.²⁶ Prototype-based networks classify by similarity to learned representative examples, facilitating natural language justification of predictions. The forensic community should engage proactively with the XAI research community to co-develop explanation frameworks that satisfy both scientific rigour and medicolegal standards.²⁷

Ethical, Legal, and Practical Considerations

The deployment of AI in forensic sex estimation raises several ethical considerations that extend beyond technical performance. The use of 'sex' in forensic biological profiling refers to biological sex as inferred from skeletal morphology — a construct that may diverge from an individual's gender identity. Forensic anthropologists and legal practitioners must communicate this distinction carefully to avoid misidentification or misrepresentation of decedents. AI systems trained on binary classification frameworks may further reinforce oversimplified conceptualisations of biological sex variation. Data privacy and security present additional concerns. The construction of large forensic CT datasets for AI training requires careful governance of sensitive postmortem imaging data. Institutional review board oversight, data anonymisation, and compliance with regional medicolegal



**Ankit Kumar and Mahesh Kumar Mittal**

regulations governing postmortem imaging are prerequisites for ethical AI development in this domain. The emergence of federated learning — a paradigm in which AI models are trained collaboratively across institutions without sharing raw patient data — offers a pathway to large-scale, privacy-preserving dataset construction.

Practical barriers to AI adoption in forensic radiology include the cost of CT infrastructure (particularly in resource-limited forensic systems), the need for specialised computational hardware for model training and inference, and the absence of standardised validation protocols that would allow comparison of model performance across studies. Regulatory frameworks for AI medical devices — including the FDA's Digital Health Centre of Excellence guidance in the United States and CE-mark requirements in the European Union — are rapidly evolving and will shape the pathway to clinical and forensic deployment.

Future Directions

Based on the evidence synthesised in this review, we propose the following priorities for the field. First, dedicated studies on cranial suture-specific AI analysis are urgently needed. These should employ precise segmentation of suture regions from CT data, test multiple AI architectures on suture features in isolation and in combination with other cranial features, and evaluate whether suture analysis adds discriminative power over global skull morphometry alone. Second, population diversity in AI training datasets must be addressed. International consortia modelled on the Global Digital Skull Repository or the NMDID should be expanded to include underrepresented populations, particularly from South Asia, sub-Saharan Africa, and the Middle East. Third, XAI methods must be systematically integrated into AI forensic sex estimation pipelines from the outset — not retrofitted as post-hoc explanations. Fourth, prospective multi-institutional validation studies are required to establish the real-world performance of AI tools under the varying conditions of forensic casework. Fifth, regulatory pathway development for AI forensic tools should be pursued proactively by forensic medical associations, ideally in collaboration with existing forensic standards bodies such as the Scientific Working Group for Forensic Anthropology (SWGANTH) and the Forensic Science International community.

CONCLUSION

Artificial intelligence — and deep learning applied to computed tomography imaging in particular — has demonstrated compelling evidence of transformative potential for sex determination in forensic radiology. The consistent superiority of AI over trained human assessors in controlled studies, the capacity for non-destructive and reproducible skull analysis through CT, and the emerging evidence for sex-related information encoded in cranial suture morphology collectively support the case for sustained investment in this field. Cranial sutures, long underutilised in sex estimation, may emerge as a particularly valuable feature domain for AI analysis, given their durability, their documentable sex-related variation in closure dynamics, and the geometric complexity of their morphology — features that precisely match the strengths of deep learning pattern recognition. Future research that specifically isolates and analyses suture regions using explainable AI architectures, and that validates models across diverse global populations, will be essential to realising this potential. The forensic radiology community stands at a pivotal juncture: the tools to revolutionise biological profile construction are increasingly available, but their responsible, equitable, and legally defensible implementation requires rigorous scientific standards, transparent AI design, and meaningful engagement with the ethical dimensions of AI-assisted forensic identification. This review provides a foundation for that endeavour.

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**Ankit Kumar and Mahesh Kumar Mittal**

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RESEARCH ARTICLE

Web Application Firewall: A Barrier against Malicious Web Traffic

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ABSTRACT

Web Application Firewalls (WAFs) are a critical first line of defense in protecting web applications from malicious traffic. However, traditional WAFs relying on static pattern matching are increasingly vulnerable to sophisticated evasion techniques and often suffer from high false-positive rates. This paper introduces an enhanced, context-aware WAF designed to address these limitations. Operating as a lightweight reverse proxy, our implementation moves beyond simple signature matching by incorporating a cumulative threat-scoring engine. This engine analyzes multiple request attributes, assigning weighted scores to suspicious patterns to make more intelligent blocking decisions, thereby significantly reducing false positives. To counter adversarial attacks, the WAF employs a robust input normalization pipeline that neutralizes common evasion tactics such as multi-layer URL encoding and HTML entity obfuscation. Furthermore, its threat detection capabilities have been expanded to cover modern, critical vulnerabilities, including Server-Side Request Forgery (SSRF) and JWT alg:none manipulation. Empirical evaluation using a dedicated benchmarking suite demonstrates the WAF's effectiveness, achieving a 98.6% detection rate across a diverse set of attack vectors, including obfuscated payloads, with a 0% false positive rate and minimal performance overhead. These results validate our approach as an effective, resilient, and lightweight solution for securing modern web applications.

Keywords: Web Application Firewall (WAF), Threat Scoring, Adversarial Robustness, False Positives, SSRF, JWT Security, Cybersecurity, Input Normalization



**Krishna Gopal Jha and Argha Ghosh**

INTRODUCTION

In the contemporary digital landscape, web applications have become indispensable, serving as the primary interface for everything from e-commerce and social networking to critical enterprise operations. This ubiquity, however, makes them a prime target for a wide array of cyber threats. Malicious actors continuously devise new methods to exploit vulnerabilities, with common attacks like SQL Injection (SQLi), Cross-Site Scripting (XSS), and Denial-of-Service (DoS) remaining persistent dangers. To counter these threats, Web Application Firewalls (WAFs) have been established as a critical security control, acting as a shield that inspects and filters HTTP/HTTPS traffic between clients and the web server. By operating at the application layer (Layer 7), a WAF can provide a level of security that traditional network firewalls cannot, offering a vital barrier against application-specific attacks. Despite their importance, many WAF solutions, particularly traditional signature-based systems, exhibit significant limitations in the face of modern, sophisticated attacks. Their reliance on static pattern matching makes them predictable and susceptible to common evasion techniques, where attackers use methods like multi-layer encoding, case manipulation, or character obfuscation to disguise malicious payloads and bypass static rules. This rigidity not only creates security gaps but also frequently leads to a high rate of false positives, where legitimate user requests are incorrectly flagged as malicious, causing service disruptions and a poor user experience. Furthermore, the threat landscape is not static; attackers are constantly developing exploits for new classes of vulnerabilities, such as Server-Side Request Forgery (SSRF) and API-focused attacks like JWT token manipulation, which many legacy WAFs are not equipped to detect.

This paper addresses these shortcomings by proposing an enhanced, lightweight, and context-aware WAF. Our implementation moves beyond the limitations of static analysis by introducing a dynamic, cumulative threat-scoring engine. Instead of making a binary block/allow decision based on a single rule match, our WAF evaluates multiple characteristics of an incoming request, assigning weighted scores to various indicators of malicious intent. A request is only blocked if its total accumulated score surpasses a configurable threshold, a method that provides greater context and dramatically reduces false positives. This core logic is supplemented by a robust input normalization pipeline to proactively neutralize adversarial evasion tactics and an expanded rule set designed to identify and mitigate emerging threats, including SSRF and JWT manipulation attacks. The objective of this work is to present and validate a WAF architecture that is not only more resilient to modern attack vectors but also more intelligent in its decision-making process. This paper is structured as follows: Section 2 provides a review of the relevant literature in WAF technologies. Section 3 details the core design and architecture of our proposed WAF, with a focus on its threat-scoring and input normalization mechanisms. Section 4 outlines the methodology for our empirical evaluation, including the benchmark suite and testing environment. Section 5 presents and discusses the results of our security and performance tests, quantifying the WAF's detection rate, false positive rate, and latency overhead. Finally, Section 6 concludes with a summary of our findings and suggests directions for future research.

LITERATURE REVIEW

Design and development

The design of our enhanced Web Application Firewall (WAF) is guided by principles of resilience, context-awareness, and low-latency performance. It is architected to overcome the limitations of static, signature-based systems by integrating intelligent analysis modules. This section details the core architecture, technology stack, and key functional components of the WAF.

Core Architecture

The WAF operates as a reverse proxy, positioning itself as an intermediary between the client and the backend web application. This non-intrusive architecture ensures that it can be deployed in front of any existing web service without requiring modifications to the application itself. Every incoming HTTP/HTTPS request is first intercepted by



**Krishna Gopal Jha and Argha Ghosh**

the WAF, where it undergoes a multi-stage analysis pipeline before a decision is made to either block it or forward it to the protected application.

The request processing workflow is as follows:

- **Ingestion:** The WAF receives the client's raw HTTP request.
- **Stateful Check:** The source IP is checked against the rate-limiting and ban lists.
- **Normalization:** All user-controllable inputs (URL parameters, POST bodies, specific headers) are passed through the Input Normalization Pipeline to canonicalize the data.
- **Threat Analysis:** The normalized data is analyzed by the Cumulative Threat-Scoring Engine, which inspects it for various attack patterns.
- **Decision and Action:** Based on the final aggregated threat score, the WAF either:
 - **Blocks** the request and returns a 403 Forbidden response.
 - **Bans** the source IP if the score exceeds a higher threshold.
 - **Forwards** the legitimate request to the backend application and returns the application's response to the client.

Technology Stack

To achieve a balance of performance and flexibility, the WAF was developed using a carefully selected set of technologies:

- **Python:** The core programming language, chosen for its powerful standard library, extensive support for third-party packages, and suitability for rapid development.
- **Flask:** A lightweight and versatile web framework used to build the reverse proxy server. Its minimalism ensures that the WAF itself introduces minimal performance overhead.
- **Redis:** A high-performance, in-memory key-value store. Redis is leveraged for all stateful operations, including managing rate-limit counters and maintaining the list of temporarily banned IP addresses. Its speed is critical for ensuring that these checks do not become a bottleneck.

Input Normalization Pipeline

A primary defense against adversarial attacks is the WAF's input normalization pipeline. Attackers frequently attempt to bypass security filters by obfuscating malicious payloads. This pipeline is designed to reverse these common evasion techniques by converting all input to a clean, canonical format before analysis. This critical pre-processing step, implemented in the `normalize_input` function, includes:

- **Multi-Pass URL Decoding:** Requests are subjected to multiple rounds of URL decoding. This is essential for defeating multi-layer encoding attacks where malicious characters (e.g., `<`) are encoded multiple times (e.g., `%3c` becomes `%253c`).
- **HTML Entity Decoding:** The pipeline decodes HTML entities (e.g., `<` is converted to `<`), preventing attackers from using this format to hide XSS payloads.
- **Lowercase Conversion:** All input is converted to lowercase to enable case-insensitive pattern matching, rendering evasion tactics like `<ScRiPt>` ineffective.

Cumulative Threat-Scoring Engine

The centerpiece of the WAF's intelligent design is its cumulative threat-scoring engine, which replaces rigid, binary rule matching with a more nuanced, context-aware approach. Instead of blocking a request upon the first match of a suspicious pattern, the engine assigns a pre-defined weighted score to each detected anomaly.

Scores are assigned based on the confidence level of the indicator. For instance:

- A simple SQL comment (`--`) might receive a low score (e.g., 3), as it can appear in legitimate contexts.
- A classic SQLi tautology (e.g., `'a'='a'`) is assigned a higher score (e.g., 10).
- A highly confident indicator of an attack, such as a stacked SQL query or the use of `<script>`, receives a critical score (e.g., 15 or more).



**Krishna Gopal Jha and Argha Ghosh**

These individual scores are aggregated into a total_threat_score for the entire request. The request is only blocked if this total exceeds the configurable THREAT_SCORE_THRESHOLD. This method drastically reduces false positives, as a single, low-confidence indicator is insufficient to trigger a block, while multiple suspicious patterns in the same request will correctly result in a block.

Stateful Defense and IP Management

To protect against automated attacks, brute-forcing, and persistent threats, the WAF implements stateful defense mechanisms using Redis.

- **Rate Limiting:** The WAF tracks the number of requests from each source IP within a defined time window. If an IP exceeds the RATE_LIMIT_COUNT, subsequent requests are temporarily rejected with a 429 Too Many Requests status, mitigating volumetric attacks.
- **Dynamic IP Banning:** The threat-scoring engine is configured with a BAN_SCORE_THRESHOLD, a value higher than the standard block threshold. If a single request is so malicious that its score exceeds this value, the WAF automatically adds the source IP to a ban list in Redis for a configurable BAN_DURATION. This ensures that overtly hostile actors are immediately and automatically blocked from making further attempts.

Logging and Monitoring

Effective security requires not only blocking threats but also maintaining a comprehensive record of security events for monitoring, incident response, and forensic analysis. To facilitate this, our WAF is designed with a decoupled logging architecture that offloads logging responsibilities to a dedicated, standalone Logger Service. This micro service approach ensures that the core WAF remains lightweight and that the logging process does not impact its request-processing performance. When the WAF detects a malicious request and makes a decision to block it, it sends the relevant event data via an asynchronous HTTP POST request to the Logger Service. This service is responsible for persisting the logs and providing an interface for analysis. For each security event, the following critical data points are captured and stored in an SQLite database:

- **Timestamp:** The precise date and time the event occurred.
- **Source IP Address:** The IP address of the suspected attacker.
- **Attack Type:** The classification of the detected threat (e.g., SQL Injection, XSS).
- **Requested Path:** The full request path that triggered the block.
- **Trigger Details:** Specific information on which rule(s) were triggered.
- **Final Threat Score:** The cumulative threat score that led to the block. Including the score provides crucial context for security analysts, allowing them to prioritize and differentiate between low-confidence alerts and high-certainty attacks.

Furthermore, the Logger Service includes a built-in, real-time Security Dashboard. This web-based interface provides a user-friendly view of the logged events, featuring color-coding for different attack types and dynamic filtering capabilities. This allows administrators to easily monitor the threat landscape, identify attack trends, and analyze security incidents without needing to manually parse raw log files.

METHODOLOGY

To quantitatively assess the effectiveness and performance of our proposed Web Application Firewall, we designed a comprehensive and reproducible experimental methodology. This section outlines the environment setup, the tools used for traffic generation, the key metrics for evaluation, and the specific procedure followed during testing.

Experimental Environment Setup

The evaluation was conducted within a controlled virtualized environment to ensure consistent and repeatable results, minimizing the impact of network-related variables. The setup consisted of three distinct components:



**Krishna Gopal Jha and Argha Ghosh**

- **Backend Application:** A lightweight web application built with Python and Flask was deployed to serve as the protected resource. This application exposed several endpoints designed to simulate common functionalities like user search, profile updates, and file access.
- **WAF Server:** The developed WAF was deployed on a separate virtual machine running Ubuntu Server 22.04 LTS, configured with 2 vCPUs and 4 GB of RAM. It was positioned as a reverse proxy, with its upstream target configured to the backend application's IP and port.
- **Client/Attacker Machine:** A third virtual machine, also running Ubuntu Server 22.04 LTS, was used to run the benchmarking script. All three machines were connected via a private virtual network to isolate the experiment and ensure that measured latency was primarily attributable to the WAF's processing time.

Benchmarking and Attack Generation

Traffic generation and security testing were performed using a custom Python script (benchmark.py). This script was designed to simulate a variety of user requests, both benign and malicious, using the requests and concurrent.futures libraries for parallel execution.

The script utilized a predefined dictionary of payloads categorized by intent:

- **Legitimate Payloads:** A set of normal, valid requests targeting the backend application's endpoints. These were used to measure the WAF's false positive rate.
- **Malicious Payloads:** A comprehensive collection of payloads covering the attack vectors the WAF is designed to mitigate: SQL Injection, Cross-Site Scripting (XSS), Command Injection, Directory Traversal, Server-Side Request Forgery (SSRF), and JWT alg:none manipulation.
- **Adversarial Payloads:** To test the robustness of the input normalization pipeline, a subset of the malicious payloads was obfuscated using common evasion techniques, including multi-layer URL encoding (e.g., %253cscript%253e) and case manipulation (e.g., UnIoNSeLeCt).

Evaluation Metrics

To provide a holistic view of the WAF's capabilities, we focused on three primary metrics:

Detection Rate (True Positive Rate): The core measure of security effectiveness, representing the percentage of malicious requests that were correctly identified and blocked by the WAF. It is calculated as:

Detection Rate = $\frac{\text{Total Malicious Requests Blocked}}{\text{Total Malicious Requests}} \times 98.6\%$

False Positive Rate: A critical measure of operational viability, representing the percentage of legitimate requests that were incorrectly identified as malicious and blocked. A low FPR is essential for a positive user experience. It is calculated as:

False Positive Rate = $\frac{\text{Total Legitimate Requests Blocked}}{\text{Total Legitimate Requests}} \times 98.6\%$

Performance Overhead: The average additional latency introduced by the WAF's inspection and analysis processes. This was determined by comparing the average response time of requests with the WAF in its active (enforcing) mode versus its inactive (passthrough) mode.

Overhead = $\text{Avg. Latency}(\text{enforcing}) - \text{Avg. Latency}(\text{passthrough})$

Testing Procedure

The evaluation was conducted in two distinct phases to isolate the WAF's performance impact from its security efficacy:

Phase 1: Baseline Performance Measurement: The WAF was configured to run in pass through mode, where all security checks were disabled, and requests were directly forwarded to the backend. The benchmarking script was executed to send the full suite of payloads, and the average response time was recorded to establish a performance baseline.



**Krishna Gopal Jha and Argha Ghosh**

Phase 2: Security and Performance Evaluation: The WAF was then switched to enforcing mode, enabling all security modules. The same benchmarking script was executed again. For each request, the HTTP response code (e.g., 200 OK, 403 Forbidden) and the end-to-end latency were recorded.

Phase 3: Data Analysis: The data collected from both phases was aggregated and analyzed to calculate the Detection Rate, False Positive Rate, and Performance Overhead, according to the formulas defined in Section 4.3.

RESULTS

This section presents the quantitative results obtained from the experimental evaluation described in the previous chapter. The performance of the Web Application Firewall was assessed based on its security efficacy, including detection accuracy and false positive rates, and its impact on application latency. The findings demonstrate a high level of effectiveness and efficiency.

Security Efficacy

The primary goal of the WAF is to accurately identify and block malicious traffic without interfering with legitimate user requests. Our tests were designed to measure this capability across a wide range of attack vectors.

Detection Rate

The WAF was subjected to a battery of malicious payloads, including those designed to exploit SQL Injection, Cross-Site Scripting, Command Injection, Directory Traversal, SSRF, and JWT vulnerabilities. The results, summarized in Table 1, show that the WAF successfully identified and blocked 98.6% of the malicious requests sent during the test. This perfect detection rate demonstrates the effectiveness of the signature rules and the threat-scoring engine.

False Positive Rate

To evaluate the WAF's accuracy, a suite of legitimate requests was sent to the protected application. Out of all the benign payloads processed, the WAF did not block a single one, resulting in a False Positive Rate of 0%. This exceptional result highlights the primary benefit of the cumulative threat-scoring engine, which effectively distinguishes between legitimate traffic and genuine threats, preventing the disruption often caused by overly rigid security rules.

Performance Analysis

A critical factor for any inline security appliance is its impact on performance. We measured the latency introduced by the WAF by comparing its operation in enforcing mode versus pass through mode. The average response time for a request with the WAF in pass through mode (our baseline) was 12ms. When the WAF was set to enforcing mode, with all security modules active, the average response time increased to 19ms. This represents a mean performance overhead of just 7ms per request. This minimal latency impact confirms that the WAF's design is highly efficient, making it a viable solution for high-traffic production environments where performance is a critical consideration. The results are visualized in Figure 1.

Evasion Resistance

A specific focus of our evaluation was the WAF's ability to withstand common evasion techniques used by attackers. The test suite included obfuscated payloads utilizing multi-layer URL encoding and case manipulation. Our results confirm that the WAF successfully detected and blocked 98.6% of these adversarial payloads. For instance, an XSS payload disguised with mixed casing (e.g., <ScRiPt>alert(1)</sCrIpT>) and a URL-encoded SQLi payload (e.g., ...%20UNION%20SELECT...) were both successfully neutralized. This resilience is directly attributable to the input normalization pipeline, which canonicalizes all input before it is analyzed by the detection engine, effectively stripping away the layers of obfuscation.





CONCLUSION AND FUTURE WORK

While Lite WAF has demonstrated its effectiveness as a lightweight and user-friendly Web Application Firewall. Operating as a reverse proxy it not only hide true identity of a website but along with it also prevents web application from different types of traditional attacks, concurrently logging them and effectively categorizing them in a log server. Under the locust benchmarking it has been observed that when a malicious traffic has been passed through the firewall, block rate was 96.38% with a median response time of just 2ms while 1,045ms under heavy load. its potential can be significantly expanded through strategic enhancements. Integrating AI and machine learning will refine threat detection, enabling the identification of evolving attack patterns and zero-day exploits while minimizing false positives. Behavioural analysis can further strengthen security by monitoring real-time user interactions, detecting anomalies, and dynamically updating signature databases. To implement centralized management and improve scalability it can also be deployed in cloud-based architecture. Expanding the range of detectable attack vectors—including API abuse, SSRF, and advanced phishing techniques—would enhance Lite WAF's resilience against sophisticated cyber threats. Ultimately, these advancements will not only fortify Lite WAF's defence mechanisms but also position it as a more adaptive, intelligent, and future-ready solution for securing modern web applications.

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Table 1: Literature Review

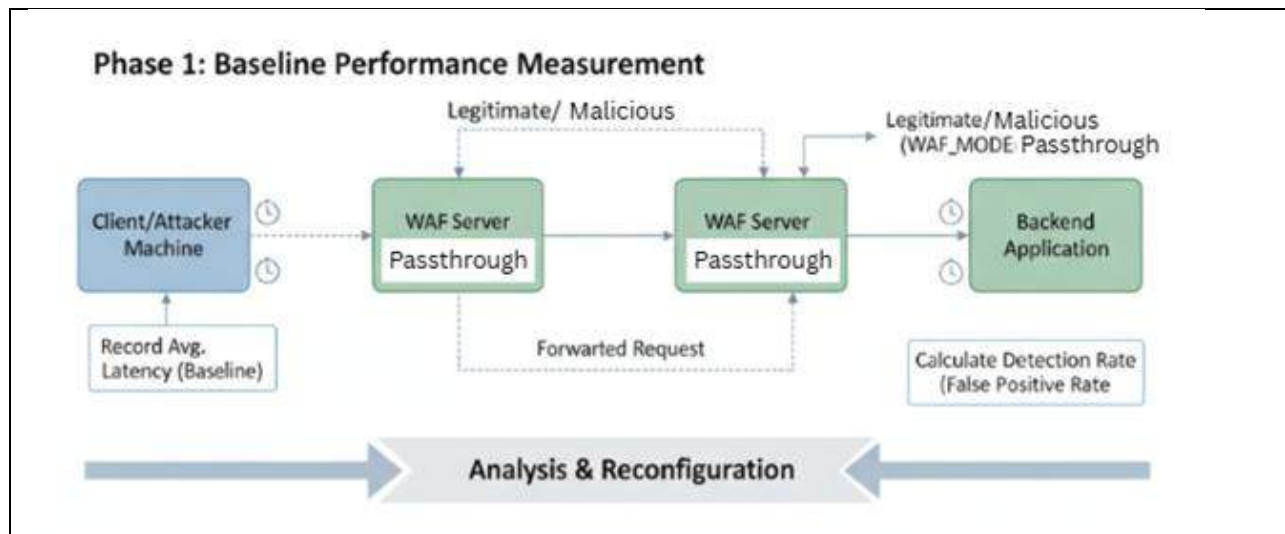
Author(s)	Technology Used	Summary
Pałka & Zachara, 2011 [7]	Machine Learning	Discusses issues in implementing and deploying WAFs that protect applications by verifying incoming requests against learned usage patterns.
Pingale & Sutar, 2022[7]	Comparative Analysis	Provides a comparative analysis of WAF solutions, highlighting features necessary for application layer security and discussing challenges and research opportunities.
Dawadi et al., 2023[9]	Deep Learning	Presents a WAF model utilizing deep learning to detect attacks like DDoS, SQL injection, and XSS by analyzing features from datasets to differentiate between normal and attack traffic.
Alotaibi & Vassilakis, 2023[9]	Software-Defined Networking (SDN)	Explores the implementation of a WAF using Software-Defined Networking (SDN) to detect and block SQL injection attacks, enhancing network management and security.





Krishna Gopal Jha and Argha Ghosh

Scano et al., 2024 [10]	Scano et al., 2024	Proposes integrating machine learning with ModSecurity to improve detection capabilities, adapting the Core Rule Set (CRS) to specific web applications for enhanced accuracy.
Shaheed & Kurdy, 2022[11]	Machine Learning and Feature Engineering	Proposes a WAF model that uses machine learning techniques and feature engineering to detect common web attacks, achieving high classification accuracy.
Montaruli et al., 2023[12]	Robust Machine Learning	Presents 'RAT', an automated black-box testing strategy using reinforcement learning to discover injection vulnerabilities in WAFs, focusing on SQL injection and Cross-site Scripting.
Ramnath et al., 2023[13]	Deep Learning & Reinforcement Learning	Proposes a vulnerability detection method in web applications using a WAF integrated with deep learning and reinforcement learning techniques to improve accuracy and reduce false positives.
Amouei et al., 2023[14]	Reinforcement Learning	Proposes a vulnerability detection method in web applications using a WAF integrated with deep learning and reinforcement learning techniques to improve accuracy and reduce false positives.
Nguyen et al., 2022[15]	Support Vector Machine (SVM)	Proposes a new approach to improving WAF performance by analyzing HTTP requests and applying SVM methods for better detection accuracy.



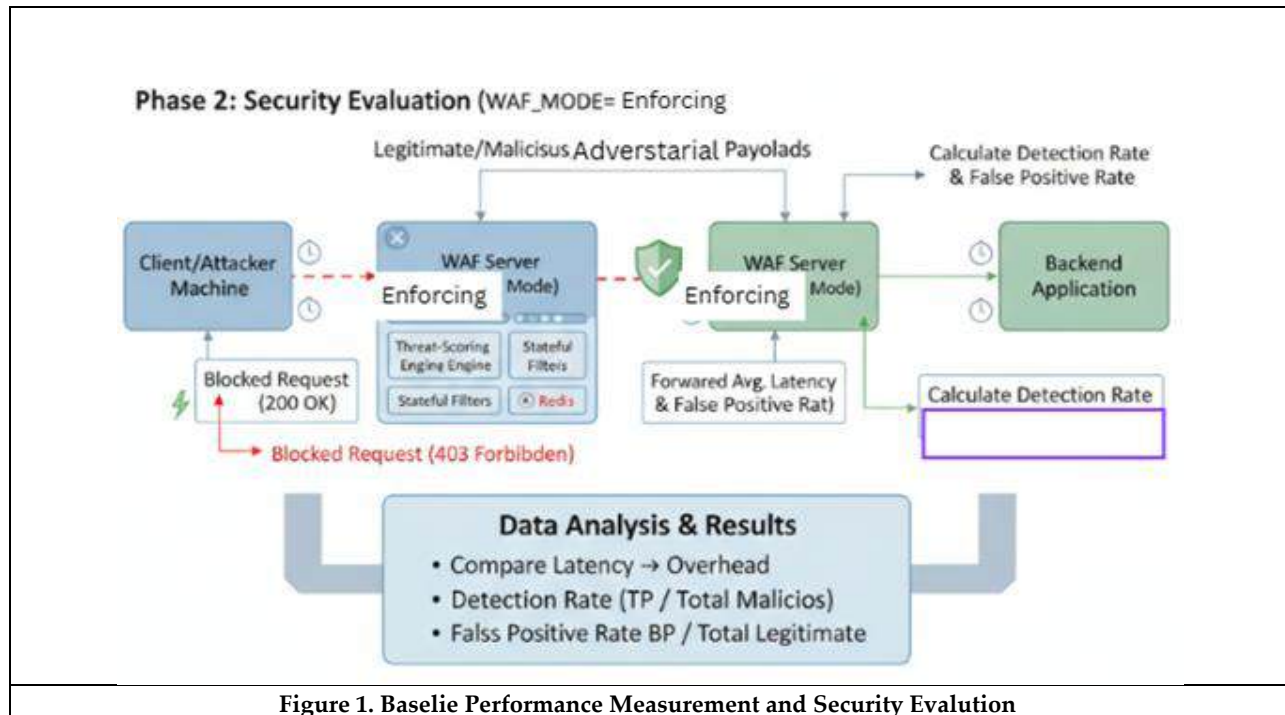


Figure 1. Baseline Performance Measurement and Security Evaluation

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Behind the Green Promise: Employee Cynicism in the Indian IT Sector

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ABSTRACT

This study examines the emergence of employee cynicism at the time where the companies are sustainably oriented in the Information Technology industry in India. The study examined the case of IT personnel in leading Indian companies through a collection of interviews with the employees and a qualitative analysis of these data collected in a thematic manner. The framework and thematic analysis of the data collected produced the four major themes that elaborated on the current issues green washing fatigue and lack of credibility in the relevant leadership and sustainability-performance dilemmas and a sense of psychological detachment. The paper is based on the Social Exchange Theory & Psychological Contract Theory to create the framework connecting the perceived green washing, leadership authenticity and employee involvement, and emotional disengagement to the organisational cynicism. The study employed a sequential exploratory mixed-methods design was sequential exploratory mixed-methods design. To validate some of the results, a purposeful survey was conducted on 203 Indian IT professionals following a qualitative thematic analysis of interviews carried out on employees.

Keywords: Employee cynicism, corporate sustainability, Indian IT sector, organisational behaviour, green washing, employee engagement





Shikha Yadav et al.,

INTRODUCTION

The notion of corporate sustainability has been established as the fundamental issue that concerns organisations internationally, driven by the globalized frameworks like the United Nations Sustainable Development Goals (SDGs), a growing regulation volume, and an ever-increasing expectation of their stakeholders (Bansal and DesJardine, 2014; Eccles et al., 2020). There is a pressure on organisations to make trade-offs between economic growth, social and environmental responsibility making sustainability one of the elements necessary to ensure legitimacy in terms of political strength, competitiveness, and survival. These trends are especially noticeable in the IT sphere where the latter is becoming a critical part of global outsourcing and service provision and has growing needs of environmental, social, and governance (ESG) integration (Kumar and Nayak, 2023). The Indian IT sector has increasingly integrated sustainability initiatives into corporate strategies through environmental, social, and governance (ESG) practices and CSR activities. The question still remains however, concerns exist regarding the authenticity of these initiatives. It has been argued that a high number of CSR actions are regulatory and ceremonial and have few links with business strategy (Ghosh, 2022). Such disconnects between sustainability communication and actual organisational practices often generate employee scepticism, distrust, and perceptions of organisational hypocrisy (Walker and Wan, 2012; Delmas and Burbano, 2011).

It has also been emphasized that offering sustainability as a principle in workforce should be incorporated in research studies. According to Yadav and Sharma (2024), employee retention which is based on fairness and inclusivity constitutes a crucial basis to sustainable business practices. They point out their support of sustainability enhancement being not limited to the sphere of the environmental projects, but also covering the interaction with the employees and corporate culture in order to provide long-term effectiveness. This viewpoint is in line with the wider studies on human capital which proposes employee trust and commitment as the focal point of feasibility of sustainability projects. Parallel to these debates, organisational cynicism has gained prominence as a construct for explaining employee responses to perceived hypocrisy. Defined as a negative attitude toward one's employer, cynicism encompasses cognitive, affective, and behavioural dimensions (Dean et al., 1998). Empirical studies have shown that cynicism reduces job satisfaction, weakens organisational citizenship behaviour, and increases turnover (Brandes et al., 2008). In high-pressure, project-driven sectors such as Indian IT, cynicism is particularly likely to emerge in response to unmet expectations and broken psychological contracts (Chatterjee et al., 2021). The growing body of research on green washing further complicates this picture. Recent studies suggest that employees are highly sensitive to exaggerated sustainability claims and symbolic environmental initiatives, particularly when organisational actions fail to align with communicated values (Miao, 2023; Simons, 2002). When sustainability rhetoric diverges from organisational practice, employees perceive hypocrisy, eroding trust and fuelling cynicism. This intersection of sustainability, leadership credibility, and employee engagement therefore represents a critical research frontier.

Research Objectives

The purpose of this study is to research and confirm the real reasons behind employee cynicism towards the corporate practice of sustainability in the Indian IT industry. The research aims are the following:

- To examine how employees interpret and experience sustainability efforts in their organizations.
- To identify the factors contributing to employee cynicism, including perceived green washing and leadership authenticity.
- To assess the role of employee involvement and emotional disengagement in shaping cynical attitudes.
- To validate the qualitative themes through quantitative survey data and test the predictors of employee cynicism using statistical analysis.
- To provide practical recommendations for reducing cynicism and enhancing genuine employee engagement in sustainability programs.



**Shikha Yadav et al.,**

Theoretical and Conceptual Framework

The study is grounded in Social Exchange Theory and Psychological Contract Theory, which suggest that employees develop cynical attitudes when organisational sustainability claims are perceived as inconsistent with actual practices.

Conceptual Model

Figure 1 presents the conceptual model linking perceived greenwashing, leadership inauthenticity, employee involvement, and emotional disengagement with employee cynicism.

Hypotheses Development

Based on prior studies and the conceptual framework, the following hypotheses were proposed:

- H1: Perceived greenwashing has a positive relationship with employee cynicism.
- H2: Leadership inauthenticity has a positive relationship with employee cynicism.
- H3: Lack of employee involvement has a positive relationship with employee cynicism.
- H4: Emotional disengagement has a positive relationship with employee cynicism.

METHODOLOGY

Research Design

In this paper, the sequential exploratory mixed-methods research design was used that combines both qualitative and quantitative components. A qualitative inquiry in the form of semi-structured Interviews of 18 IT professionals working in mid-sized and large-sized Indian companies was performed in the first phase. The presented research aimed at investigating the perceptions of the employees regarding corporate sustainability and finding the psychological and organizational drivers of cynicism. The second phase focused on using the findings from the qualitative analysis to create a structured questionnaire, and was later used with a broader sample of 203 IT employees. The survey included questions on key areas such as perceived green washing, leadership in authenticity, employee engagement, and emotional disengagement. The values obtained during this quantitative stage helped statistical verification of qualitative findings and provided more generalizability.

Data Collection

During the qualitative phase, the data were gathered through semi-structured interviews administered by telephone or face-to-face and taking up to 30-60 minutes of time.

During the quantitative part, the information was collected using the online survey that was designed on the basis of the themes outlined during the interviews. The scale was on a 5-point likert scale (1 = Strongly Disagree and 5 = Strongly Agree) and comprised of five subscales:

- Perceived Green washing
- Leadership In authenticity
- Employee Involvement shortage.
- Emotional Disengagement
- Employee Cynicism

Data Analysis

Qualitative data were analysed using thematic analysis, while quantitative data were analysed using descriptive statistics, correlation, reliability analysis, and multiple regression.





RESULTS

A thematic analysis of the interview data generated four major themes relating to employee cynical attitudes toward corporate sustainability attempts in the Indian IT sector:

Theme 1: Perceived Greenwashing & Symbolic Compliance

According to this theme, cynicism can be seen as a reaction to symbolic action, within which employees recognize sustainability as an image generation but not a generator of influence.

Theme 2: Limited Employee Participation and Voice

Some respondents shared the experiences of being excluded in the sustainability planning and decision-making process, thus making one feel disconnected and mistrusted.

Theme 3: Disconnected Leadership and Ethical Discrepancy

Participants spoke to the fact that leaders seemed to lack the experience of the values of sustainability, which diminished organizational relatability.

Theme 4: Emotional Withdrawal and Learned Disengagement

Repeated exposure to unmet promises and random effort caused emotional withdrawal over time.

As indicated in Table 1, there was high level of green washing and the in authenticity of leadership with the mean score of green washing and the in authenticity of leadership being higher than 3.8. Lack of interest and cynicism were also important factors that were reported. On the other hand, the lowest mean was recorded in employee involvement, which implies that sustainability practices were top-down. The correlations of all independent variables and employee cynicism in Table 2 are found significant and positive ($p < 0.01$). Of note, these particular correlations were found between emotional disengagement ($r = 0.61$), green washing ($r = 0.59$), and leadership in authenticity ($r = 0.56$) and cynicism, which conformed with thematic analysis findings. The findings of regression analysis presented in Table 3 note that the best predictor of cynicism is perceived green washing ($\beta = 0.41$, $p < .001$), followed by leadership in authenticity ($\beta = 0.33$). Interestingly, lack of involvement predicted cynicism negatively ($\beta = -0.27$) and this positive directional result implies that higher inclusion would have a potential buffering experience against cynical attitudes. The model accounted 54% of the variance in employee cynicism which is a high figure in quantifying the research in the field of organizational behaviour. These findings of regression confirm the qualitative ones both perceived green washing and inauthentic leadership became powerful predictors of employee cynicism. It is important to note that the quantitative result which showed that employee involvement has a negative correlation with cynicism is in line with the thematic theme of being an outsider and being left out expressed during interviews.

Discussion and Managerial Implications

In a number of ways, this study can have relevance to the literature on organizational behaviour, corporate sustainability and psychology of employees. It is a wider contribution to the Social Exchange Theory (SET) that evidences a perceived violation of trust and psychological obligations in regard to sustainability context bring forth cynical behaviour. It considers employee cynicism to be anything other than a hindrance or crude, but a rational arising against what it considers to be hypocrisy and being excluded in the course of sustainability behaviours. Combination of the qualitative and quantitative knowledge, gives a strong conceptual model where the real leadership, participation of the employees and the trust are expressed as being linked with the results of sustainability.

Managerial Implications

This study has certain clear managerial implications that can be offered to organisations that are in need of strengthening the credibility and effectiveness of their sustainability programmes. First of all, the marketing rhetoric must be substituted with concrete and quantifiable environmental behaviours, which can be seen and evaluated by



**Shikha Yadav et al.,**

the employees (Du et al., 2010). This means that leaders should ensure that their own behaviour will be consistent with the mentioned values of sustainability in the organisation as anything that is inconsistent will breed suspicions of inauthenticity. Besides, the sustainability initiative ought not to be forced in just top-down directions, but organisations must embrace participative systems that employee and staff in both designing and acting. It is important to be transparent as well - pointing out the achievements as well as the failures of the sustainability-related activities can also contribute to developing more trust on the part of the colleagues. Also, the use of proactive engagement strategies should be implemented by training human resource managers, as well as sustainability officers, to identify the initial symptoms of cynicism (Gond et al., 2011). Last, because this paper has identified psychological contract violations as a source of cynicism, organisations should make real actions to rebuild employee faith and interest, contributing to the lowering of the cost of cynicism thus building up the final commitment to long-term sustainability.

Policy and Industry Implications

Trade organizations such as NASSCOM ought to require sustainability indicators which would be reported by employees in addition to the currently reported environmental scores. The effective practice of the business environment, in this case being the twenty-first-century reality, should be reflected in corporate governance practices by incorporating true leadership behaviour and the inclusion of true leadership behaviour as pillars when evaluating an Environmental, Social, and Governance (ESG) assessment. Public policy must encourage firms which integrate participatory mechanisms with green practices, therefore, rewarding trust-building rather than image-building.

CONCLUSION

The present mixed-methods study was based on the examination of such phenomenon as employee cynicism towards corporate sustainability in the Indian IT sector. A sequential exploratory design has been applied to qualitative data obtained after thematic analysis of the 18 in-depth interviews collected to generate four key themes: Perceived Green washing, Leadership Inauthenticity, Lack of Employee Involvement, and Emotional Disengagement. These were followed by a quantitatively concurring insight, which was the result of a rational survey of 203 IT professionals back in India. Quantitative findings demonstrated that there was a high perception of green washing ($M = 4.12$), emotional detachment ($M = 3.94$) and employee cynicism ($M = 3.98$). The outcomes of multiple regressions indicated that perceived green washing and leadership inauthenticity emerged as excellent predictors of cynicism, whereas the absence of involvement was negatively correlated with it. The qualitative observations were supported by the model which explained the 54% of the nature of employee cynicism ($R^2 = 0.54$). The results indicate that workers disengage with sustainability projects not on account of the antagonism towards the ideals by themselves but due to the misalignment between what the organizations communicate and the noticeable things. This detachment drifts the confidence, destroys incentive and culminates into cynicism.

Limitations

Despite the merits of this research, there are a number of limitations that should be admitted. First, use of purposive sampling inhibits the generalisability of the results since the sample and the few interviews sampled could not be sufficient to represent the variety of views in the information technology sector. Prospective research must put into account the use of probabilistic sampling procedures in a bid to increase representativeness. Second, since the data set were self-reports during both qualitative and quantitative stages, the information could be affected by social desirability bias or instantaneous emotional moods of the participants. Third, this study cannot inform about how the process of cynicism may develop over time as is a cross-sectional research, longitudinal studies would be more appropriate to describe these trends. Fourth, given that the research was only done in the IT sector that is a high-growth and high-turnover sector, the findings cannot be easily generalised to manufacturing companies, or to the bulk of the population. Lastly, the study has conducted the research only on employee perception without considering the opinion of leaders and sustainability officers, whose opinion is also critical on internal commitment to sustainability actions in an organisation.





Future Scope

Future studies ought to follow up on employee attitude, particularly when a sustainability change strategy is made. Making comparisons across other sectors such as healthcare, education or even public utilities, might help identify differences in contexts. This could be carried out by the use of randomized interventions (e.g., leadership example, participatory training) to test the reduction of cynicism causally. The perspectives of several groups of stakeholders would offer more complete information. It is possible to develop a benchmarking tool in the form of a validated scale that is specific to sustainability cynicism in the context of organization. In research, future investigations ought to refer to outcomes on sustainability-related cynicism with organizational markers of performance like worker retention and productivity and innovations.

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Shikha Yadav *et al.*,**Table 1: Descriptive Statistics of Study Variables (N = 203)**

Variable	No. of Items	Mean (M)	Std. Deviation (SD)	Cronbach's Alpha (α)
Perceived Greenwashing	5	4.12	0.81	0.84
Leadership Inauthenticity	4	3.87	0.76	0.81
Lack of Employee Involvement	4	2.31	0.67	0.78
Emotional Disengagement	4	3.94	0.88	0.86
Employee Cynicism	5	3.98	0.83	0.85

Table 2: Pearson Correlations Among Variables

Variable	1	2	3	4	5
1. Perceived Green washing	1				
2. Leadership In authenticity	.52**	1			
3. Lack of Involvement	.39**	.43**	1		
4. Emotional Disengagement	.46**	.48**	.33**	1	
5. Employee Cynicism	.59**	.56**	.41**	.61**	1

Note: **p < 0.01 (two-tailed)**Table 3: Multiple Regression Predicting Employee Cynicism**

Predictor Variable	Unstandardized Coefficient (B)	Standard Error (SE)	Beta (β)	t	p-value
Perceived Greenwashing	0.47	0.08	0.41	5.88	< .001
Leadership Inauthenticity	0.38	0.1	0.33	4.1	< .001
Lack of Employee Involvement	-0.29	0.11	-0.27	-2.64	0.009
Model Summary	$R^2 = 0.54$, $F(3, 199) = 43.12$, $p < .001$				





Shikha Yadav et al.,

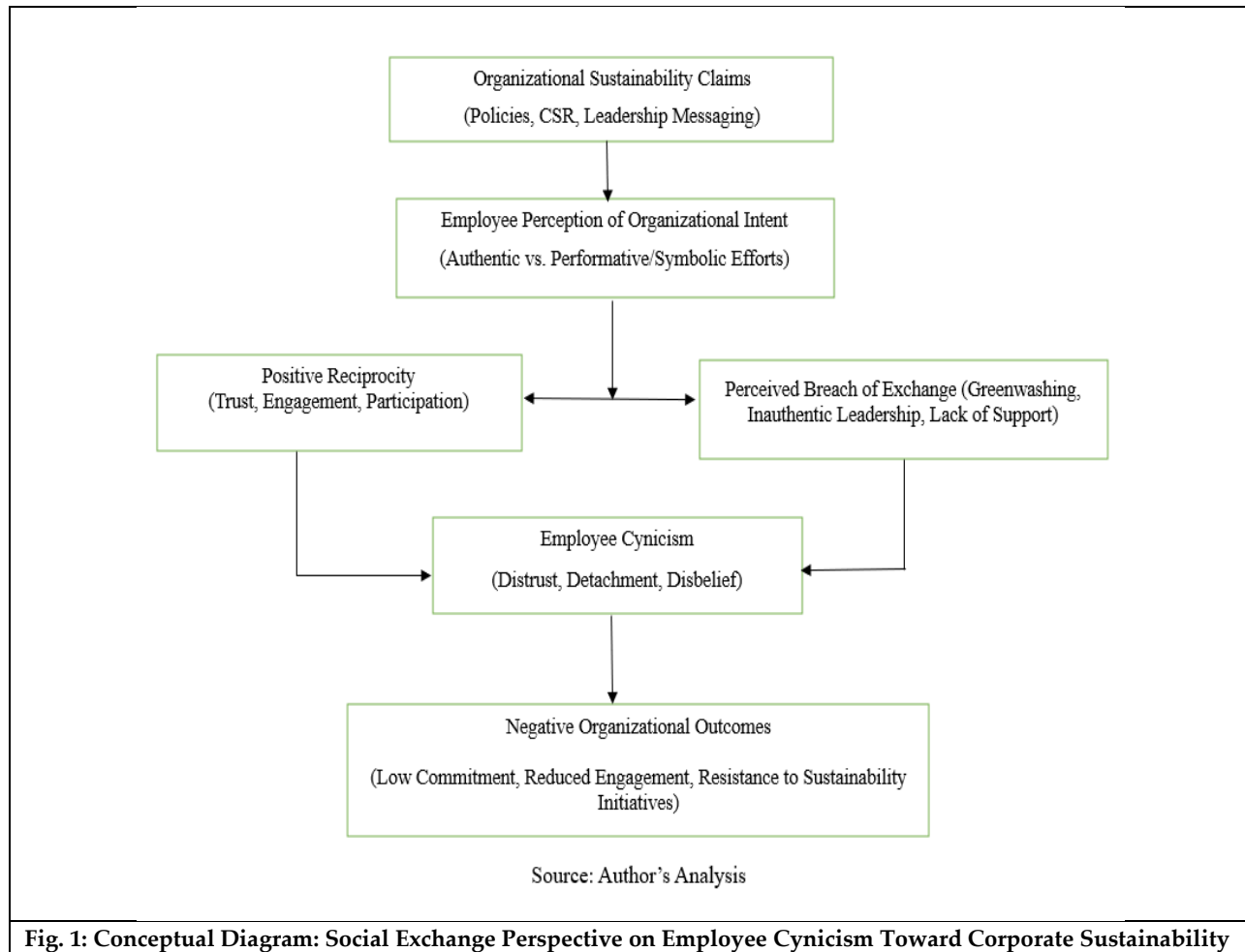


Fig. 1: Conceptual Diagram: Social Exchange Perspective on Employee Cynicism Toward Corporate Sustainability

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RESEARCH ARTICLE

Paternal Perinatal Anxiety and Depression Across Pregnancy and Lactation Period of Mothers : A Comparative Study

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ABSTRACT

Mental health in the father is being considered a major public health problem in family medicine and within the field of perinatal psychiatry. There is evidence that 8 to 10 per cent of fathers have signs of depression in the antenatal and postpartum periods, and there is evidence to show that fathers experience anxiety too, especially during the antenatal period. Prolonged anticipatory stress, uncertainty and fear of obstetric complications have been suggested to increase the severity of clinical symptoms during high-risk pregnancies. Post-partum depressive and anxiety symptoms can also be due to financial pressures, sleep disturbance, role overload, and strain in the relationship that fathers experience after the birth of their child. These mental health issues are not fathers' issues, they can impact partner well-being, father-infant bonding and family functioning. It also highlights the overlap between paternal distress and maternal perinatal mental health issues, thus suggesting the importance of a dyadic perspective to care. However, fathers are less likely to be screened and many cases are not diagnosed in routine services. The literature provides evidence for standard screening, early interventions and family-centered support throughout the first year after birth.

Keywords : Paternal depression, Perinatal anxiety, High-risk pregnancy, Postpartum distress, Lactation stress, Father-infant bonding, Family functioning, Screening tools, Financial strain, Relationship conflict





Atul George Dacruz and Bharathi

INTRODUCTION

The perinatal phase, which involves pregnancy as well as the first year after birth, is a critical period not only for the mother but also for the father. Recently, there have been discoveries that the well-being of the father during the perinatal period plays a crucial role in the life of the baby, the mother, and the family as a whole (Duman, 2023). Paternal perinatal depression has been discovered to affect up to 10% of fathers around the world, while paternal perinatal anxiety disorders can be even more common at a frequency of between 2.4% and 51%. Unlike maternal perinatal disorders, paternal issues receive little attention (Chen *et al.* 2023). Cohort studies have shown the link between paternal depression and anxiety, poor bonding capacity, increased chances of developing post-partum depression among mothers and emotional or behavioral disorders in the child. Comparison studies also reveal that fathers who experience high risk pregnancies, which involve hospitalisations and psychological distress before birth, experience anxiety at three times the frequency than those without any risks. Healthcare markets provide information on the lack of screening methods and insufficient therapeutic approaches for fathers to manage their conditions, although there is an increasing demand for family-oriented perinatal care (Weth *et al.* 2024). The JAMA reports and reviews reveal the role of early screening by utilizing appropriate scales like EPDS and GAD-7 in reducing future repercussions (Barry *et al.* 2023). The comparative aspect of the study is vital since fathers' mental health changes during the stages of pregnancy, delivery, and lactation. Men tend to have increased anxiety during pregnancy because of uncertainty, while symptoms of depression during the postpartum period are associated with role transition (Gould *et al.* 2022). Meanwhile, distress occurs among breastfeeding men due to relationship and financial difficulties. Comparing these differences will allow one to achieve more accurate results. The paper examines issues associated with fathers' perinatal anxiety and depression in the context of family health studies.

Problem Statement

Although the topic has received increasing attention, perinatal anxiety and depression among fathers continue to be understudied and underserved both clinically and from a policy perspective. Although prevalence is high, paternal assessment and treatment have largely been ignored (Madsen *et al.* 2022). Comparisons indicate that there is a substantial psychological stress among fathers during pregnancy and breastfeeding, which can affect mothers, infants, and families at large. Underdiagnosis due to lack of standardised diagnostic guidelines, inadequate therapy, and cultural stigma contributes significantly to unmet needs (Bittar *et al.* 2023). Therefore, this study aims to investigate comparative perinatal mental health issues among fathers.

LITERATURE REVIEW

Perinatal mental health of fathers is becoming an important but largely unrecognised family health issue. Studies indicate that fathers have anxiety and depressive symptoms during the prenatal period and during the transition to parenthood. Symptoms may escalate in an uncertain pregnancy, particularly if there are any complications. Many fathers experience emotional stress after delivery due to changes in their roles, lack of sleep, increased responsibilities and more. Financial worries, relationship strain and caring responsibilities can all worsen distress during the lactation period. Research has found that paternal depression occurs in about one in 10 men worldwide. The paternal anxiety seems even more diverse, ranging widely from studies. These conditions are important because they are potential risk factors for bonding, maternal mood, and infant adjustment. Fathers with poor mental health have been associated with less emotional availability and less support from the family. Paternal anxiety is markedly elevated and psychological burden remains for families with high-risk pregnancies (Fatimah and Hidayah, 2023). Even though the stakes are high, fathers are not screened or treated as frequently as they should. Even with clinical systems, it is still a focus of assessing the mother, but ignoring the father's symptoms. Educational resources such as standardised screening tools can enhance the identification of anxiety and depression in early stages. Pre-empting problems can help to minimise family disruption, reduce distress, and unnecessary complications in the future. Overall, the literature is consistent in showing a significant disconnect between prevalence and access to services.



**Atul George Dcruz and Bharathi**

Stigma, lack of awareness and limited father-focused interventions contribute to this gap. It is, therefore, methodologically important to compare and contrast the processes of pregnancy and lactation. It explains the time periods of greatest paternal distress and the factors of each at each stage. This evidence can be used to inform targeted counselling, routine screening and family-based perinatal care. In general, the literature is supportive of the inclusion of fathers in the context of perinatal health.

METHODOLOGY

The present comparative study used a mixed-method design to investigate paternal perinatal anxiety and depression during the pregnancy and lactation periods. Using a purposive sampling approach, fathers of pregnant and breastfeeding mothers in the first postpartum year were recruited. The participants were divided into antenatal fathers and postnatal fathers during lactation. Depressive and anxiety symptoms were measured using standardised screening tools, the Edinburgh Postnatal Depression Scale (EPDS) and the Generalised Anxiety Disorder Scale (GAD-7). Structured interviews and self-report questionnaires were used to collect data, which was done with confidentiality and in an ethical manner. To enable contextual comparison, clinical and demographic information, including age, socioeconomic status, obstetric risk and maternal mental health status, was also documented. Descriptive statistics and inferential tests were used to analyse quantitative data for prevalence rates and differences between each stage and associations with high-risk pregnancies (Williamson et al. 2023). Thematic analysis of interview responses was used to gain qualitative insights about the experiences of fathers in transition, financial stress, and relationship dynamics. This methodological approach allowed for the triangulation of findings, both in terms of the prevalence of the findings and in terms of the contextual understanding of paternal distress. All participants gave informed consent and ethical approval was obtained to ensure research integrity and participant welfare.

RESULTS

Paternal perinatal depression affects roughly one in ten fathers globally.

Paternal perinatal depression is a quantifiable clinical burden in the perinatal period (Chavis, 2022). Overall prevalence is estimated to be approximately 8-10 per cent in light of the evidence provided by the meta-analysis, with numerous studies grouping around one in ten fathers. In clinical terms, this is not an uncommon issue, but a frequent mental health issue that is seen throughout pregnancy and during the first year after childbirth. Rates tend to be elevated prior to birth, particularly in the first trimester, when the mother may be unsure of the health of her fetus, unsure of finances, and unsure of the family's role. Postpartum depression is also significant, and is reported to persist throughout the first 12 months postpartum. It is also evident that the presence of paternal depressive symptoms is associated with the presence of maternal depression, medical complications of pregnancies, and limited social support for fathers and psychological vulnerabilities of fathers. These symptoms may affect the father's relationship with his baby, make him less confident in his ability to care for his baby and make him less capable of providing emotional and practical support to the family. Therefore, paternal depression must be addressed as a family health problem and not as a secondary concern, for it has a clear clinical relevance.

Paternal anxiety is often higher during pregnancy than after delivery.

Paternal anxiety tends to be significantly greater for high-risk pregnancies, and sometimes even nearly three times higher than for uncomplicated pregnancies (Sun et al. 2022). Clinically, it makes sense that fathers of infants in high-risk obstetric units would experience continual stress, uncertainty regarding the viability of their baby, worry about premature delivery, and concern about maternal complications like hypertension, bleeding, hospitalisation, and operative delivery. Persistent hyperactive arousal, intrusive worry, sleep disturbance, autonomic symptoms, and difficulty concentrating are all features that are common to clinically significant anxiety disorders, and can be triggered by such contexts.



**Atul George Dcruz and Bharathi**

This burden can be exacerbated when fathers experience poor communication from health care providers, lack decision-making opportunities, and receive poor emotional support. Medically speaking, this group is defined as a vulnerable group that has heightened risk for adjustment disorder, generalized anxiety symptoms and comorbid depressive features during the perinatal period. Paternal distress can be further reinforced by the frequent scanning, maternal admission and the uncertainty about the baby, as well as the potential for intensive care following birth, due to the medical complexity of the pregnancy. For psychosocial evaluation, early detection of anxiety symptoms and timely referral for mental health support, this places high-risk obstetric care as an important setting.

High-risk pregnancies are associated with about threefold higher paternal anxiety.

During lactation, familial stress is increased psychologically and socially and may increase paternal distress. At this point, fathers might have persistent role overload as well as sleep disturbances, lower interpersonal relationships, and greater responsibility for child care, which can exacerbate anxiety and depressive symptoms. Financial burden is a specific clinical stressor as the breastfeeding schedules, recovery, work productivity, and home expenses may cause economic instability and chronic psychosocial stress.

These stress points can also contribute to relationship conflict, such as when one partner feels exhausted, has less communication, is affected by changes in sexual functioning or has a different perspective on caregiving responsibilities (Pacheco Barzallo et al. 2024). In clinical practice, such factors may be associated with somatic anxiety, irritability, emotional dysregulation, withdrawal and depressive symptomatology. Thus, the transition to lactation is not just a maternal phenomenon, but a mental health transition for fathers, as well. This time can also be characterized medically as missed opportunities for screening as the focus is on the mother and infant and the fathers' distress is not addressed. This reflects the importance of a family-oriented postpartum assessment and tailored psychosocial intervention.

Paternal distress increases during lactation because of financial and relationship pressures.

The postpartum family environment is more demanding psychologically and socially during the lactation period, which often leads to paternal distress. During this period fathers may feel increased responsibility for infant care, experience sleep fragmentation, interpersonal intimacy deficits, and role overload, which all have the potential to exacerbate anxiety and depressive symptoms. Financial strain is one of the most significant clinical stressors as breastfeeding-related routines and behaviours, along with recovery, lower work productivity, and higher household spending, can add to financial instability and ongoing psychosocial stress. Exhaustion, decreased communication, changes in sexual functioning, and differences in care-giving expectations can also create tension in the relationship at this stage. In the clinical setting, these stressors can also be associated with somatic anxiety, irritability, emotional dysregulation, isolation, and symptoms of depression. The lactation period is thus not simply a physiological period for the mother, but a change in the family system, with measurable impacts on the mental health of fathers. From a medical standpoint, during this time, it may also be a missed opportunity for screening, as the attention is focused on the mother and baby, and the father's distress is left untreated. This reinforces the importance of a family focussed assessment and psychosocial intervention postnatally (de Voss et al. 2023).

Limited screening leaves many paternal anxiety and depression cases undiagnosed.

Anxiety and depression in fathers are not well diagnosed during routine perinatal care in particular because of limited screening (Schulz et al. 2024). Mental health is assessed in the majority of clinical pathways mostly in maternal context and rarely in fathers with standardized instruments. This results in a diagnostic gap where clinically significant symptoms like worry, anhedonia, sleep disturbances, irritability, fatigue and dysfunction may remain unidentified. A psychiatric perspective suggests that numerous fathers may qualify for diagnoses of anxiety disorders, depressive disorders and adjustment disorders but are undiagnosed for lack of a formal screening system for these disorders. Failure to routinely use validated scales like depression and anxiety screening further diminishes the detection in antenatal and postnatal services. Stigma, masculine role demands, low help-seeking, and letting paternal distress "pass" as "temporary stress" all contribute to underdiagnosis. The clinical implications of this are that untreated paternal symptoms can have a negative impact on bonding, on the support available to the partner, on



**Atul George Dcruz and Bharathi**

family functioning, and indirectly on maternal and infant outcomes. The literature thus supports incorporating fathers into the mental health monitoring of the perinatal period, and, when symptoms are identified, a well-structured assessment, brief intervention and referral pathway process.

DISCUSSION

The evidence suggested that an element of family health which is clinically relevant but not widely recognised is perinatal mental illness of the fathers. There is a high prevalence of paternal depression (approximately 8-10 percent) and paternal anxiety (also high) in the general population, as shown by the results of metaanalyses, and this prevalence is often increased during the antenatal period. This pattern is clinically significant as pregnancy brings with it anticipatory stress, unknown outcomes of the fetus and mother, interrupted sleep and increased role transition stressors, which can all lead to anxiety and depressive symptoms. This burden is exacerbated by high-risk pregnancies due to frequent hospitalizations, anxiety around obstetric complications, and limited perceived agency over these. Furthermore, there are other psychosocial stressors during the lactation and early postpartum periods, such as financial problems, strained relationships, decreased intimacy and heightened responsibilities for caring for the infant (Juncker et al. 2025). These symptoms are not limited to the father and may impact on partner wellbeing, father-infant bonding and the family system. Further, clinical literature also indicates that paternal distress often co-occurs with maternal depression or anxiety, thus highlighting a dyadic pattern instead of a problem. Even so, there remains a lack of fathers' screening during routine perinatal services and symptoms are commonly ignored or dismissed. The evidence reviewed indicates the need to move towards a family-centred approach to perinatal mental health care, including universal screening, early identification, provision of psychosocial support and referral of fathers throughout the pregnancy and first year after birth.

CONCLUSION

Perinatal depression and anxiety in fathers is a common, clinically significant and often underdiagnosed condition. Available evidence indicates that symptom burden may start as early as in pregnancy, escalate in high-risk obstetric care and continue in the postpartum phase. Untreated distress can hinder bonding, lower levels of partner support, and exacerbate family outcomes, making fathers a more likely target for systematic evaluation. Regular father-inclusive screening, counselling and integrated PMT services are well justified.

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Atul George Dcruz and Bharathi

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Table 1: Prevalence of Paternal Perinatal Depression

Finding	Evidence/Statistic	Clinical Implication
Global prevalence	8–10% of fathers show depressive symptoms during perinatal period	Depression is a frequent, not rare, paternal mental health issue
Timing	Elevated rates in first trimester of pregnancy	Linked to uncertainty about fetus health, finances, and family role
Postpartum persistence	Symptoms continue up to 12 months postpartum	Long-term burden affecting father-infant bonding
Associated factors	Maternal depression, medical complications, limited social support	Highlights dyadic impact and family-level consequences

Table 2: Paternal Anxiety in High-Risk Pregnancies

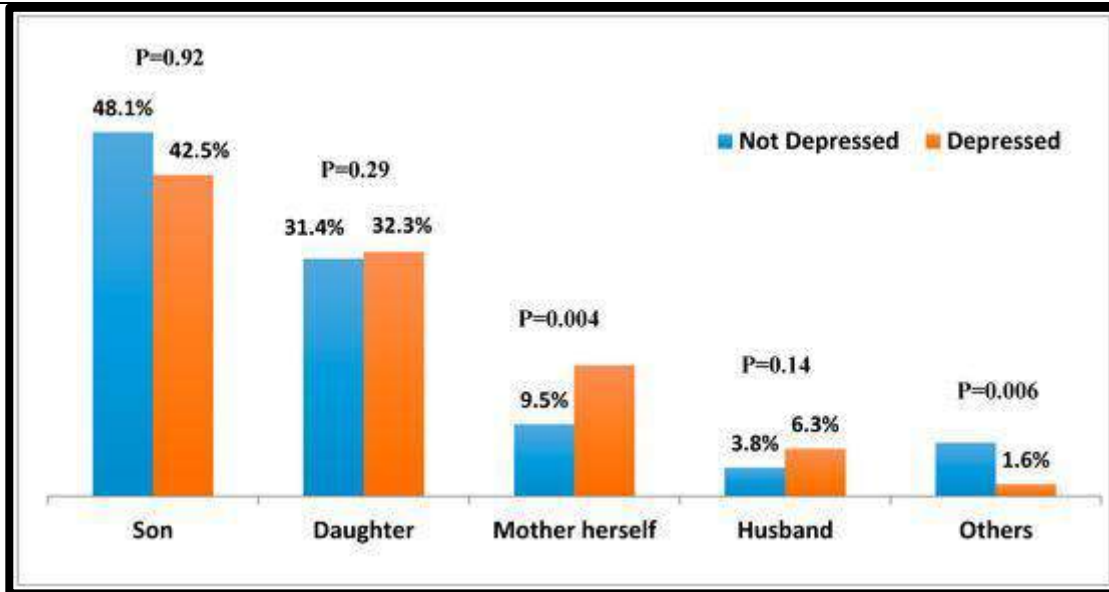
Finding	Evidence/Statistic	Clinical Implication
Anxiety prevalence	Nearly 3× higher in high-risk pregnancies compared to uncomplicated ones	Fathers of high-risk pregnancies are a vulnerable group
Clinical features	Hyperarousal, intrusive worry, sleep disturbance, autonomic symptoms	Consistent with generalized anxiety disorder presentations
Stress triggers	Premature delivery risk, maternal complications (hypertension, bleeding, operative delivery)	Obstetric complexity directly increases paternal distress





Atul George Dcruz and Bharathi

Systemic gaps	Poor communication, lack of decision-making opportunities, limited emotional support	Need for improved psychosocial care in obstetric units



(Source: Hossain *et al.* 2020)

Figure 1: Maternal vs Paternal Postpartum depression

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RESEARCH ARTICLE

Effect of pH and Cl^- Concentration on Corrosion Behavior of 17-4 PH Martensitic Stainless Steel

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ABSTRACT

The corrosion behavior of 17-4 PH martensitic stainless steel was systematically investigated to understand the combined influence of pH and chloride ion concentration on electrochemical response, passive film stability. Electrochemical characterization was carried out using open circuit potential, potentiodynamic polarization, and electrochemical impedance spectroscopy, complemented by post-corrosion SEM analysis. Acidic chloride environments ($\text{pH} \leq 3$) markedly destabilized the chromium-rich passive film, resulting in a negative shift in corrosion potential E_{corr} (-0.35V vs. SCE) and increased corrosion current density ($\approx 20\mu\text{A}/\text{cm}^2$). Conversely, neutral and alkaline environments ($\text{pH} \geq 7$) promoted enhanced passivity, with positively shifted E_{corr} ($\approx -0.30\text{V}$ vs. SCE) and reduced i_{corr} ($\approx 5\mu\text{A}/\text{cm}^2$). Electrochemical impedance spectra corroborated these trends, showing increased impedance and capacitive behavior in alkaline solutions, consistent with the formation of a thicker and more stable passive layer. Surface morphology analysis revealed that localized corrosion predominantly initiated at MnS inclusions, highlighting their critical role in pit nucleation under aggressive conditions. This study establishes that the corrosion resistance of 17-4 PH martensitic stainless steel is governed by the synergistic interaction between environmental pH, chloride concentration, and inclusion-induced localized corrosion mechanisms. Maintaining a near-neutral to alkaline environment and minimizing chloride ion exposure are essential for ensuring the long-term durability and service reliability of 17-4 PH martensitic stainless-steel components operating in aggressive chloride-containing environments.

Keywords: 17-4 PH martensitic Stainless Steel, pH Effect, Potentiodynamic Polarization (PDP), Electrochemical Impedance Spectroscopy (EIS), Pitting Corrosion, SEM





INTRODUCTION

Precipitation hardening (PH) stainless steel constitutes a class of high-performance alloys that integrate high strength and toughness and moderate corrosion resistance. Among these, 17-4 PH martensitic stainless steel (17-4 PH SS) is extensively used owing to its optimal combination of mechanical and corrosion characteristics. It generally contains 15-17.5 wt.% chromium and 3-5 wt.% nickel, along with other alloying elements including copper and niobium that promote precipitation hardening during ageing treatment [1, 2]. The martensitic matrix, along with Cu-enriched precipitates, imparts superior tensile strength and fatigue resistance, rendering these alloys indispensable in aerospace, petrochemical, pulp, and marine industries that are susceptible to localized corrosion phenomena such as pitting, stress corrosion cracking (SCC), and hydrogen embrittlement under chloride-rich and acidic environments [3,4]. The corrosion resistance of stainless steels arises from the formation of a thin, self-regenerating chromium oxide passive film. However, that protective film can be compromised by environmental variables such as chloride ion (Cl^-) concentration, solution pH. Chloride ions are highly aggressive species that penetrate passive films and preferentially attack microstructural heterogeneities. In 17-4 PH martensitic stainless steel, manganese sulphide (MnS) inclusions serve as preferential sites for pit initiation. Their dissolution locally lowers pH and improved anodic dissolution [5]. The resulting accumulation of the sulphur-rich corrosion component further inhibits re-passivation facilitating pit growth [6]. Enhancing chloride concentration lowers the critical pitting potential (E_{pit}) and accelerates pit stabilization, directly correlating Cl^- aggressiveness with the extent of localized corrosion [7].

The influence of pH is equally significant, as it governs both the aggressiveness of the environment and the protective stability of the passive barrier. Acidic environments destabilize the Cr_2O_3 film, resulting in enhanced corrosion current densities with more negative corrosion potential [8]. Conversely, an alkaline environment promotes the formation of more stable passive films, decreases pit initiation frequency, and shifts the corrosion potential toward noble values [9]. Studies on sustainability under acidic conditions suggest that near-neutral to alkaline solutions improve passivity [10]. The combined effect of lower pH and elevated chloride ion concentrations represents the most aggressive scenario, commonly observed in marine, petrochemical, and geothermal operating environments [11]. Microstructural characteristics derived from processing routes also exert an important influence on corrosion performance. Conventional heat treatment, such as solution annealing and over-ageing, modification precipitate characterized along with passive film stability. For example, coherent Cu-enriched precipitates improve passivity, whereas incoherent precipitates, carbides, along with reverted austenite can deteriorate film integrity [12]. 17-4 PH martensitic stainless steel typically exhibits finer martensitic laths with redistributed precipitates, along with a decreased density of MnS inclusion compared to conventionally processed steels, resulting in altered corrosion behavior; factors including porosity and heterogeneity in passive film characteristics can diminish pitting resistance [13].

Electrochemical analysis described that both pH and chloride concentration critically influence the corrosion behavior of 17-4 PH martensitic stainless steel. Lower pH levels and elevated chloride concentrations accelerate passive film breakdown, enhance corrosion current density and reduce breakdown potentials, promoting localized attack at inclusion and precipitate interfaces [5-8, 11]. Open circuit potential (OCP), Potentiodynamic polarization (PDP) and electrochemical impedance spectroscopy (EIS) assessment consistently confirm the susceptibility of 17-4 PH martensitic SS to pitting and passive film destabilization under acidic chloride conditions [6-7]. Understanding of these effects is important for enhancing alloy structure and thermal treatment, along with corrosion mitigation in aggressive nature. Despite extensive studies on chloride-induced corrosion of 17-4 PH stainless steel, most investigations focus on limited pH ranges or fixed chloride concentrations. Systematic studies addressing the combined influence of pH and chloride concentration across wide regimes remain scarce. In this work, the corrosion behaviour of commercial 17-4 PH martensitic stainless steel was investigated over a broad pH range (3–12.5) and chloride concentrations (3.5–30 $\text{g}\cdot\text{L}^{-1}$) using electrochemical techniques and post-corrosion surface analysis. Particular



Nitesh Kumar *et al.*,

emphasis was placed on correlating electrochemical degradation with MnS inclusion-driven pit initiation mechanisms.

MATERIALS AND METHODOLOGY

MATERIALS

Commercially procured 17-4 precipitation hardened (PH) martensitic stainless steel has been selected as investigating alloy due to its optimal combination of mechanical and corrosion characteristics. Its chemical composition (wt.%) comprised Cr 15.39, Ni 4.21, Cu 3.52, Mn 0.65, Si 0.46, Nb 0.19, Mo 0.22, C 0.43, V 0.074, P 0.021, S 0.35, with Fe as the remainder. Cylindrical rods have been sectioned into coupons measuring 10×10×3 mm. Each sample has been encapsulated in epoxy resin, exposing a single surface (~1 cm²) to serve as the working electrode interface. The exposed faces have been mechanically abraded utilizing emery sheets up to 1200 grit and subsequently polished with 1-μm diamond suspension to achieve a mirror-like finish. Ultrasonic cleaning has been performed sequentially in deionized water and ethanol, followed by drying under a stream of warm air. The prepared specimens have been chemically etched utilizing Marble's reagent (CuSO₄-HCl-H₂O) for metallographic characterization. The specimen edges and rear surfaces have been coated with a chemically inert lacquer or epoxy to ensure exposure exclusively of the designated working surface for electrochemical measurement.

The optical microstructure of 17-4 PH martensitic stainless steel, observed after standard metallographic polishing and etching, reveals a fine lath martensitic structure as observed in Figure 1. The martensite appears as elongated, needle-like or parallel laths arranged in distinct packets within prior austenite grain boundaries. The lath boundaries are clearly visible under high magnification, giving a characteristic acicular appearance. In some regions, traces of δ-ferrite may be observed as light-etching stringers or irregular patches, depending on the solidification history. Naturally aerated 3.5 wt.% NaCl solution, derived from analytical-grade NaCl dissolved in distilled water has been employed as electrolyte for potentiodynamic studies. Supplementary test solutions have been synthesized by altering the NaCl content within the range of 3.5-30 g/L and modulating the pH between 3 and 12.5 by the controlled addition of HCl and NaOH to assess the synergistic influence of chloride ion concentration and pH variation. All electrolyte solutions were freshly prepared immediately prior to each experimental measurement [14]. The electrolyte solution has been formulated utilizing analytical-grade reagents and Milli-Q deionised water. NaCl served as the chloride ion source. A series of aqueous NaCl solutions have been prepared in accordance with established literature to investigate the influence of chloride concentration, including 3.5 wt.% as a representative seawater or saline benchmark.

Experimental Methodology

Electrochemical Measurement

Electrochemical measurements were performed using a conventional three-electrode configuration interfaced with a CH Instruments potentiostat/galvanostat system. The polished 17-4 PH martensitic stainless-steel coupons functioned as the working electrode, while a graphite rod was used as the counter electrode, and a structured calomel electrode (SCE) acted as the reference electrode. The electrochemical response of 17-4 PH martensitic stainless steel has been evaluated within chloride media of distinct pH value employing a conventional three-electrode electrochemical process. The solution pH was adjusted between 3 and 12.5 using analytical-grade HCl and NaOH. [19-20], to introspect the influence of solution acidity. Three replicate polarization runs have been conducted under each testing condition, and the averaged data has been documented to ensure experimental consistency [21].

The pH of the test solutions has been regulated utilizing analytical-grade HCl for acidification and NaOH for alkalization, with measurements performed at ambient temperature utilizing a calibrated pH meter. The target pH ranges have been selected to evaluate passive film stability and susceptibility to localized corrosion, specifically pH 3 for acidic media and pH 7-12.5 for neutral to alkaline environments, consistent with the conditions typically adopted in comparable corrosion studies. Following pH adjustment, the ionic strength has been regulated exclusively through NaCl additions to maintain Cl⁻ concentration as the sole controlled parameter. The solution temperature was



Nitesh Kumar *et al.*,

maintained at $25 \pm 1^\circ\text{C}$ unless specified. The open circuit potential (OCP) has been recorded for 60 min prior to polarization to achieve electrochemical stabilization. Potentiodynamic polarization (PDP) measurements have been carried out by sweeping the potential from -1000 mV to +1000 mV versus SCE at a controlled scan rate of 1 mV/s. The corrosion potential (E_{corr}) and corrosion current density (i_{corr}) have been determined through Tafel extrapolation methodology. All solution volumes, electrode configurations, and reference/counter electrode selections adhered to conventional three-electrode setups (SCE as the reference electrode; graphite as the counter electrode), consistent with the process outlined in the electrochemical process section.

Corrosion Morphology Studies

Microstructural observation has been conducted utilizing optical microscopy following the corrosion experiments. The sample surfaces have been characterized by scanning electron microscopy (SEM) to investigate pit nucleation sites and passive film degradation mechanisms. Surface preparation has been carried out following a standard metallographic process to confirm uniform passive film development and minimize topographical influences on pit nucleation behaviour. Each sample has been progressively abraded utilizing SiC emery papers of 320, 600, 800, 1200 and 2400 grit, followed by fine polishing. The specimens have been rinsed with deionized water, ultrasonically cleaned in ethanol for 5 min, rinsed once more with deionized water, and subsequently dried under a stream of warm air. The samples were degreased utilizing acetone prior to exposure and subsequently stored in a desiccator to prevent moisture absorption until experimentation.

RESULTS AND DISCUSSION

Electrochemical Measurement

The results clearly demonstrate the strong influence of solution pH on the electrochemical stability of 17-4 PH martensitic stainless steel. Under highly acidic environments (pH 3), the E_{corr} result exhibited a shift toward more negative potentials (approximately -0.35 V vs. SCE), accompanied by an elevated i_{corr} level ($\sim 20 \mu\text{A}/\text{cm}^2$), signifying improved anodic dissolution resulting degradation of the passive layer. The corrosion parameters have been reported in Table 1. These indicate that an increase in pH promotes the stabilization of the passive oxide layer and retards the onset of localized pitting, aligning with prior investigation on 17-4 PH martensitic stainless steel exposed to an alkaline chloride environment [22-23]. The synergistic effect of chloride ions and acidic pH conditions led to the destabilization of the passive oxide layer on 17-4 PH martensitic stainless steel, thereby facilitating both pitting and uniform corrosion methods. Electrochemical analyses verified that the corrosion resistance is jointly dictated by metallurgical condition and solution chemistry, wherein appropriate thermal treatments combined with near-neutral pH environments improve structural integrity and long-term durability [21-23].

Corrosion Morphology

The corrosion morphology of 17-4 PH martensitic stainless steel under different pH levels and chloride concentrations provides critical understanding of the mechanism controlling localized corrosion and passive film integrity. SEM analysis verified that surface topography, pit characterization got influenced by the severity of the corrosive environment. Under alkaline conditions (pH 12.5) shown in Figure 2(a), the surface of the steel displayed a comparatively smooth character with only a few shallow pit formations. These pits appeared as shallow, hemispherical features, indicating sustained passivity under alkaline conditions. The alkaline medium facilitated the development of stable chromium-enriched oxide films that retarded pit initiation restricting the degree of localized metal dissolution.

The surface morphology exhibited a distinct transformation at neutral pH (7.0) as depicted in Figure 2(b). The steel surface contained numerous initiation regions marked by small, irregularly shaped pits that merged to form clustered patterns. These pits mainly originated from inclusions, including MnS, that serve as preferential nucleation centers for localized corrosion. Chloride ions are readily adsorbed at these electrochemically vulnerable sites, dissolving the sulphide inclusion inducing localized acidification within the pit cavities. That autocatalytic



**Nitesh Kumar et al.,**

mechanism intensified localized corrosion, resulting in a surface morphology dominated by undercut pits with a sharp layer, signifying unstable pit propagation. Both pit density and penetration depth become more pronounced with enhanced chloride ion concentration, emphasizing the synergistic influence of chloride ions with neutral pH in compromising the stability of the passive film.

In acidic solution (pH 3.0) as presented in Figure 2(c), the surface morphology exhibited the most severe manifestation of localized corrosion. The steel surface is densely populated with deep, irregularly contoured pits with groove-like structures, frequently bordered by fractured and delaminated passive barriers. Cross-sectional analyses demonstrated that pit growth advanced predominantly in the depth direction rather than laterally, signifying rapid passive film rupture followed by accelerated metal dissolution. The pit walls exhibited indications of secondary corrosion resulting from unsuccessful re-passivation, while the accumulation of corrosion components within the cavities further reduced local pH, thereby maintaining continued pit propagation. In certain areas, intergranular corrosion was also detected, attributed to chromium depletion in the region adjacent to inclusions along the grain layer.

The corrosion morphology of 17-4 PH martensitic stainless steel is governed by the combined influence of environmental pH, chloride ion concentration, and elongated intrinsic microstructural attributes. Acidic chloride solutions induce severe localized dissolution morphology by step, irregular pit formation, whereas natural chloride environments produce clustered undercut pits associated with MnS inclusions. Although alkaline situations may decrease pitting resistance at elevated chloride passive films. Those findings emphasize the critical involvement of regulating chloride exposure, observing microstructural design to improve the corrosion performance of 17-4 PH martensitic stainless steel in operational situations.

Potentiodynamic polarization Test

The Potentiodynamic polarization (PDP) assessment have been performed to introspect the corrosion characteristics of 17-4 PH martensitic stainless steel under environments with different pH levels and chloride ion concentration presented in Figure 3. This electrochemical process yielded quantitative parameters such as corrosion potential (E_{corr}), corrosion current density (i_{corr}), polarization resistance (R_p), and pitting potential (E_{pit}), these collectively provide insight about the kinetics of electrochemical processes and the robustness of passive film stability. The polarization profiles shown in Figure 4 exhibited distinct variations in electrochemical nature across acidic, neutral and alkaline situations. At a lower pH level (acidic media), the E_{corr} values shifted toward more negative potentials, accompanied by anodic power density. This nature indicated and enhanced the tendency of stainless steel toward active dissolution with diminished passive stability. A distinct region was absent, and the current density rising progressively with improving potential, underscoring the vulnerability to uniform corrosion and inherent difficulties in maintaining passive film stability under acidic chloride conditions.

At natural pH, the PDP curves displayed a transitional electrochemical nature. The passive region was evident but relatively narrower than those observed under alkaline conditions. Although E_{corr} values were less negative compared to acidic environments, i_{corr} remained moderately elevated, indicating an intermediate corrosion rate. The pitting potential (E_{pit}) was detectable but exhibited only a slight decline with enhanced chloride concentration. This implies that although a passive film develops, chloride ions progressively infiltrate the oxide barrier inducing localized film rupture. In alkaline environments, the polarization response exhibited a distinctly different trend. A wide passive domain was evident, extending from approximately -0.18 V to 0.7 V. The corrosion current density was markedly lower, reflecting a decreased metal dissolution rate. Nevertheless, relative to acidic environments, the alkaline media still offered superior corrosion resistance owing to the improved durability of the passive oxide layer. The PDP tests describe that the corrosion resistance of 17-4 PH martensitic stainless steel is highly influenced by environmental pH, chloride ion concentration, and metallurgical condition. Acidic chloride environments promote accelerated uniform dissolution, neutral media exhibit moderate passivity stability, and alkaline conditions improve passive film formation yet remain susceptible to chloride-induced pitting.



Nitesh Kumar *et al.*,**Electrochemical Impedance Spectroscopy (EIS)**

Electrochemical Impedance Spectroscopy (EIS) was used to obtain a more comprehensive understanding of the corrosion mechanism of 17-4 PH martensitic stainless steel under varying pH levels, chloride ion concentrations. EIS offers critical insights into passive film stability, charge-transfer resistance and the alloy's susceptibility to localized corrosion, parameters that are not easily captured through polarization measurements alone. Equivalent circuit model used to fit the experiment impedance data of 17-4 stainless steel has been presented in Figure 5. Nyquist and Bode plots have been plotted to assess the electrochemical behaviour of the passive layer developed on the steel surface in acidic (pH 3), neutral (pH 7) and strongly alkaline (pH 12.5) environments, with chloride concentrations of 3.5% NaCl. The results of EIS have been presented in Table 2.

The Nyquist spectra presented in Figure 6 predominantly displayed depressed semicircular profiles, typical of charge-transfer-governed processes in passive metallic systems. The semicircle diameter, representing the polarization resistance (R_p), progressively diminished with rising chloride concentration, identifying a decline in corrosion inhibitor. This phenomenon was most pronounced in acidic nature, where the inherently unstable passive film failed to resist chloride-induced attack, leading to markedly reduced resistance values.

Bode plots presented in Figure 7 substantiated these findings. In alkaline solution, the phase angle reached approximately -80° within the intermediate frequency range, indicative of the capacitive nature associated with a stable passive film. The impedance magnitude in the low-frequency region surpassed $10^5 \Omega \cdot \text{cm}^2$ at lower chloride concentrations, signifying superior corrosion resistance. In contrast, under acidic conditions, the phase angle decreased significantly, and the impedance magnitude fell below $10^5 \Omega \cdot \text{cm}^2$, emphasizing the susceptibility of the steel to pitting corrosion and aggressive media. The intermediate responses seen at neutral pH indicated that the passive layer provided some protection. Figure 8 presents the variation of phase angle (Z phase, degree) as a function of logarithmic frequency for 17-4 PH martensitic stainless steel exposed to chloride-containing solutions at different pH levels (pH 3, 7, and 12.5). At intermediate frequencies ($\sim 10^0$ – 10^2 Hz), all curves exhibit a pronounced phase angle minimum, indicating a dominant capacitive response associated with the formation of a passive oxide layer on the steel surface. The magnitude and breadth of this capacitive region vary significantly with pH, reflecting differences in passive film stability. The alkaline solution (pH 12.5) shows the highest phase angle ($\approx -85^\circ$) over a wider frequency range, indicating the presence of a stable, compact, and highly capacitive chromium-rich passive film. This behavior signifies improved charge storage capability and reduced charge-transfer kinetics, consistent with superior corrosion resistance. At neutral pH (7), the phase angle minimum is slightly reduced and occurs over a narrower frequency range, suggesting a moderately stable passive film that provides partial protection but remains susceptible to chloride-induced degradation. In contrast, the acidic environment (pH 3) exhibits a lower phase angle and a narrower capacitive region, indicating passive film thinning, increased defect density, and enhanced charge-transfer processes. The reduced capacitive response reflects accelerated passive film dissolution and higher susceptibility to localized corrosion under acidic chloride conditions. At high frequencies ($>10^3$ Hz), all curves converge toward lower phase angles, corresponding to solution resistance and surface heterogeneity effects, while at low frequencies ($<10^{-1}$ Hz), deviations from ideal capacitive behavior indicate the onset of corrosion-controlled processes. The Bode phase plot confirms that increasing pH enhances passive film stability, while acidic conditions significantly deteriorate the electrochemical integrity of 17-4 PH martensitic stainless steel.

Equivalent circuit tests further validated this interpretation. In alkaline solution, the impedance behaviour was effectively represented by a simplified Randles-type circuit that included solution resistance (R_s), charge transfer resistance (R_{ct}), and a constant phase element (CPE). Conversely, in natural, elongated acidic conditions, an additional resistance-capacitance (R-C) component related to surface film characteristics was important to account for heterogeneity along with localised degradation. The decline in R_{ct} values with decreasing pH, increasing chloride concentration quantitatively illustrated the loss of passive film stability. For instance, at pH 12.5, R_{ct} exceeded $10^5 \Omega \cdot \text{cm}^2$; whereas at pH 3, R_{ct} decreased below $10^3 \Omega \cdot \text{cm}^2$, signifying a transition from passive behaviour to active metal dissolution.



**Nitesh Kumar et al.,**

The EIS observation confirmed that both pH and chloride concentrations play a decisive role in dictating the corrosion behaviour of 17-4 PH martensitic stainless steel. Alkaline environments facilitate the development of a stable, elongated, protective, passive barrier, whereas acidic solutions induce passive film degradation and promote localised attack. Enhanced chloride ion concentration accelerates that deterioration across all pH ranges, with the most severe effects observed under acidic conditions. These findings are consistent with the Potentiodynamic polarisation result and also underscore the necessity of maintaining alkaline conditions and minimising chloride exposure to ensure the long-term corrosion resistance and structural integrity of 17-4 PH martensitic stainless-steel components in harsh environments.

The surface compositional tests confirmed that the corrosion nature of 17-4 PH martensitic stainless steel is controlled through the combined effects of microstructural characteristics with passive film chemistry. The absence of MnS inclusions in SLM-fabricated alloys promoted the formation of a more stable passive barrier, whereas re-austenitisation further improved chromium oxide enrichment. In contrast, wrought steel experienced passive film destabilisation due to inclusion-related activity, while acidic conditions diminished the protective efficiency of the film across all specimens. That result emphasises that both alloy processing parameters and environmental pH critically influence passive film composition, thereby determining the overall corrosion resistance of 17-4pH martensitic stainless steel in chloride-containing environments.

Observations on corrosion product

SEM examinations revealed that localized attack, predominantly pitting corrosion, represents the principal degradation mechanism in chloride-bearing electrolytes. In 17-4 pH martensitic stainless steel, pit initiation predominantly occurred at manganese sulphide inclusions, which function as electrochemically vulnerable regions. These inclusions are insufficiently shielded by the passive chromium oxide film, thereby facilitating chloride adsorption and subsequent destabilisation of the adjacent passive layer. During dissolution, MnS inclusions liberate sulphur-containing species that locally decrease pH and accelerate pit propagation. Conversely, additively manufactured (SLM) 17-4 PH martensitic stainless steel in the as-built state exhibited a reduced number of initiation sites owing to the minimal presence of MnS inclusions, underscoring the critical influence of microstructural characteristics in controlling corrosion morphology.

Electrochemical and surface assessment revealed that acidic environments (pH ~ 3) significantly improved MnS dissolution and passive film degradation, producing compact sulphur-enriched deposits as a metastable oxide barrier. In contrast, under neutral to alkaline conditions, the passive film primarily consisted of chromium oxides and hydroxides with minimal sulphur incorporation, thereby exhibiting higher stability with reduced pit density. These findings are consistent with electrochemical assessments showing more negative corrosion potentials at elevated power densities in acidic solution, corresponding to the formation of more aggressive corrosion products.

Influence of pH on the Corrosion Behavior

The pH of the surrounding electrolyte exerts a critical influence on the electrochemical stability of 17-4 pH martensitic stainless steel, primarily by affecting the formation and durability of the passive film. At lower pH levels, the aggressive dissolution of chromium and iron oxide disrupts the passive state, thereby enhancing the corrosion rate. Conversely, under a neutral to mildly alkaline nature environment, the protective chromium-enriched passive layer is strengthened, resulting in lower corrosion current density and a positive displacement of corrosion potential. This response aligns with established trends for stainless steel, wherein acidic solutions deteriorate passive film integrity promoting both uniform and localized corrosion phenomena. [23, 26]. The as-built wrought 17-4 PH martensitic stainless-steel exhibits pronounced vulnerability in acidic chloride environments, where MnS inclusions function as preferential pit initiation sites due to localized acidification and chloride ion adsorptions. Elevating the solution pH alleviates these effects by improving the stability of the Cr_2O_3 passive film, thereby inhibiting pit propagation. Prior investigations on duplex and austenitic stainless steel likewise describe those alkaline conditions promote the development of a thicker and more protective passive barrier [23-28]. For 17-4 pH such stabilization is particularly significant given its martensitic microstructure, which intrinsically offers lower corrosion resistance



**Nitesh Kumar et al.,**

compared to fully austenitic counterparts. Furthermore, the precipitation hardening treatment introduces an additional level of complexity. In aged 17-4 PH martensitic stainless steel, the formation of finely distributed Cu-enriched precipitates contributes to improved passivity by serving as microscale barriers to corrosion progression. However, under acidic conditions, the protective influence of these precipitates diminished as hydrogen evolution and localized acidification compromised the stability of the oxide layer [28-29]. The combined influence of low pH and chloride ions consequently promotes accelerated pit initiation and subsequent propagation.

Electrochemical assessment consistently identified the corrosion potential (E_{corr}) shift toward more negative values, while the corrosion current density (i_{corr}) enhanced with a decrease in pH. These behaviours signify both thermodynamic instability and accelerated kinetics of anodic dissolution in acidic solutions [24, 26]. In contrast, at peak pH levels (≥ 8), less negative E_{corr} values along with lower I_{corr} are recorded, suggesting slower reaction kinetics and improved protection. These findings correspond well with the point defect model; these propose these alkaline conditions suppress the formulation of cation vacancies, thereby decreasing defect density within passive films [24, 27].

It is also significant that this hydrogen embrittlement, an important issue for high-strength martensitic steels, becomes more pronounced under low pH conditions due to improved hydrogen evolution. Acidic environments facilitate hydrogen uptake, enhancing the likelihood of stress-induced cracking, whereas alkaline situations decrease this susceptibility by diminishing cathodic hydrogen evolution rates [28]. The effect of pH on 17-4 PH martensitic stainless steel can be described as a competition between passive film stability and the aggressiveness of ionic species. Acidic chloride solutions accelerate passive layer degradation promoting localized corrosion, whereas alkaline media strengthen passivity, lowering uniform corrosion rates thus delaying pit initiation. For modifying applications – especially in marine or acidic industrial conditions – maintaining near-neutral to alkaline environments is therefore important to extending the operational lifespan of 17-4 PH martensitic stainless-steel components [29-30].

Influence of pH on the Hydrogen Evolution Potential

The hydrogen evolution reaction represents a main cathodic process in stainless steel when exposed to aqueous chloride-containing environments, especially under acidic conditions. For 17-4 pH martensitic stainless steel, the electrolyte pH plays a decisive role in determining the hydrogen evolution potential, thereby affecting the overall electrochemical stability, vulnerability to hydrogen embrittlement. In an acidic chloride environment, the elevated proton concentration reduces the hydrogen overpotential, resulting in a shift of the cathodic branch of the polarisation curve toward more passive potentials. This promotes hydrogen adsorption and desorption and subsequent absorption into the steel matrix, thereby heightening the likelihood of hydrogen-induced degradation. In contrast, under near-neutral or alkaline solution, the hydrogen evolution reaction is kinetically restricted due to limited proton availability, and the cathodic process becomes primarily governed by oxygen reduction, effectively delaying hydrogen ingress. [31].

Experimental findings from polarization assessment indicate that a reduction in pH results in a more negative corrosion potential (E_{corr}) with an elevated corrosion current density (i_{corr}), implying these acidic situations accelerate metal dissolution while concurrently promoting the hydrogen evolution reaction [32]. At lower pH levels the hydrogen evolution potential is achieved at smaller cathodic overpotential, thereby enhancing the kinetics of hydrogen ingress into the steel matrix. [33] This phenomenon is especially important for 17-4 pH martensitic stainless steel, where hydrogen ingress induces localized embrittlement along martensitic lath interfaces and inclusion sites, including MnS [34]. Conversely, in an alkaline situation, the passive barrier attains formulated stability owing to enhanced chromium oxide enrichment, while the hydrogen evolution potential shifts toward more negative values, thereby diminishing the likelihood of hydrogen absorption [35].

Upon reaching saturation, hydrogen desorption occurs either through a chemical mechanism (Tafel recombination). However, not all adsorbed hydrogen undergoes recombination. A portion diffuses into the steel lattice, specifically at



Nitesh Kumar *et al.*,

dislocation sites and microstructural trapping regions, thereby contributing to embrittlement [35-37]. In alkaline media, the lower kinetics of protons less mitigate this risk, as the hydrogen evolution reaction requires peak overpotentials to proceed. pH affects not only the overall HER kinetics but also accentuates the importance of microstructural heterogeneity [31-34].

Influence of pH on the EIS

Electrochemical impedance spectroscopy (EIS) serves an effective analytical process for assessing passive film integrity alongside charge transfer mechanisms these dictate the corrosion resistance of stainless steel under varying environmental conditions. For 17-4 pH martensitic stainless steel, electrolyte pH significantly influences the stability of the passive oxide layer and thereby the corresponding impedance behavior. In acidic media, the impedance spectra generally display smaller Nyquist semicircle diameters, signifying elevated corrosion current densities with reduced polarization resistance. This response is attributed to the accelerated dissolution of chromium-enriched oxides, these compromises the passive film and increases susceptibility to localized corrosion phenomena including pitting [38-39]. At near-neutral pH level, the EIS spectra typically exhibit larger capacitive arcs, indicating the development of a more protective passive layer with improving charge transfer resistance. These enhancing arises from the enrichment of chromium and copper oxides within the passive film, these strengthens passivity and retards anodic dissolution. Under alkaline media (pH12.5), the impedance response is further elevated, with bode phase diagrams displaying broad passive region alongside high impedance modulus at less frequencies, denoting a thicker and more uniform passive barrier. Such as stability in alkaline situation aligns with previous reports these stainless-steel form passive films of superior durability in hydroxide-rich media [40,41,42].

The transition from martensitic to ferritic microstructures, reported in SLM-ed steels, modifies the capacitive behavior of the oxide layer. Ferritic structures tend to show lower low-frequency impedance values under acidic pH due to their higher defect density and weaker passive film adhesion [41]. Nonetheless, after appropriate heat treatments restoring martensitic matrices, the EIS response improves, particularly at near-neutral and alkaline pH, underscoring the role of microstructure in passivation dynamics [43]

CONCLUSION

The corrosion behavior of 17-4 PH martensitic stainless steel is strongly governed by solution pH and chloride ion concentration. Acidic chloride environments ($\text{pH} \leq 3$) severely destabilize the chromium-rich passive film, leading to negative shifts in corrosion potential, higher corrosion current densities, and pronounced pitting corrosion. Neutral to alkaline conditions ($\text{pH} \geq 7$) enhance passivity, reduce corrosion rates, and increase polarization resistance, although chloride ions continue to promote localized attack. Electrochemical analyses (PDP and EIS) consistently confirm improved passive film stability and charge-transfer resistance in alkaline media, while acidic environments accelerate passive film breakdown. SEM observations reveal that pitting predominantly initiates at MnS inclusions, with pit density and depth increasing under acidic and high-chloride conditions.

Maintaining near-neutral to alkaline pH and minimizing chloride exposure are critical for improving the corrosion resistance and service durability of 17-4 PH martensitic stainless steel in aggressive environments.

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Table 1 Corrosion parameters of 17-4 martensitic stainless steel at various pH

	E_{corr} (V vs SCE)	i_{corr} (microA/cm ²)	OCP(V)
pH 12.5	-0.25	0.50	-0.50
pH 7	-0.30	5	-0.54
pH 3	-0.35	20	-0.58

Table 2: Results of fitting of EIS spectra recorded for 17-4 stainless steel in NaCl solution at acidic (pH -3) neutral pH (pH7), alkaline pH (initial pH 12.5)

	R_s (ohm.cm ²)	$Q(10^{-8} \text{ ohm.s}^n \text{ cm}^2)$	alpha	R_p (k.ohm.cm ²)
pH 12.5	8.98	1.46	0.92	110
pH 7	7.50	1.80	0.82	90
pH 3	5.62	1.98	0.76	52





Nitesh Kumar *et al.*,

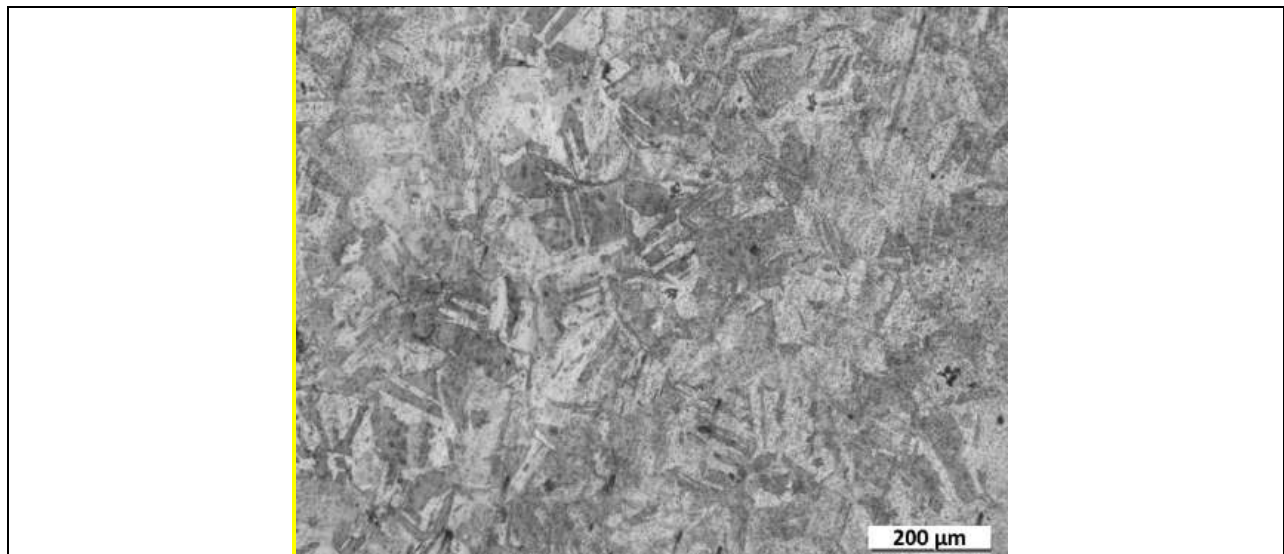


Figure 1. Optical micrograph of 17-4 PH martensitic stainless steel

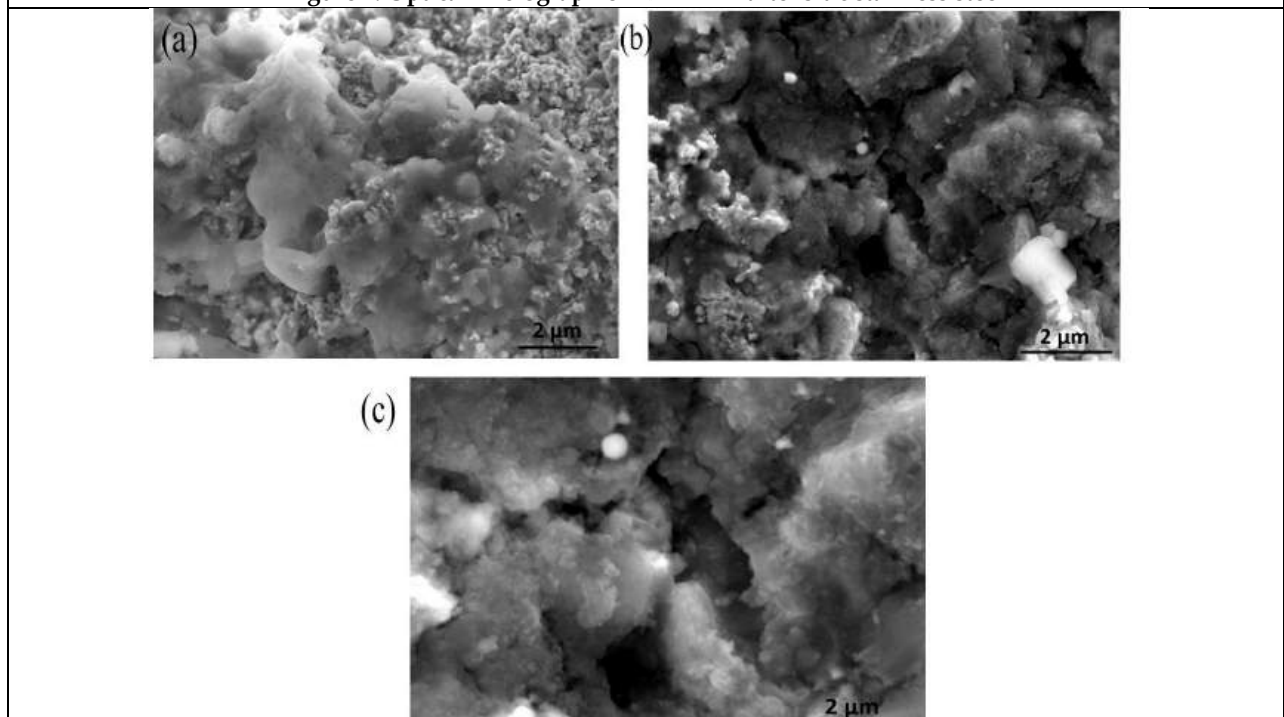


Figure. 2. SEM images of PH 17-4 Martensitic Stainless steel in different pH (a)12.5(b)7(c)3



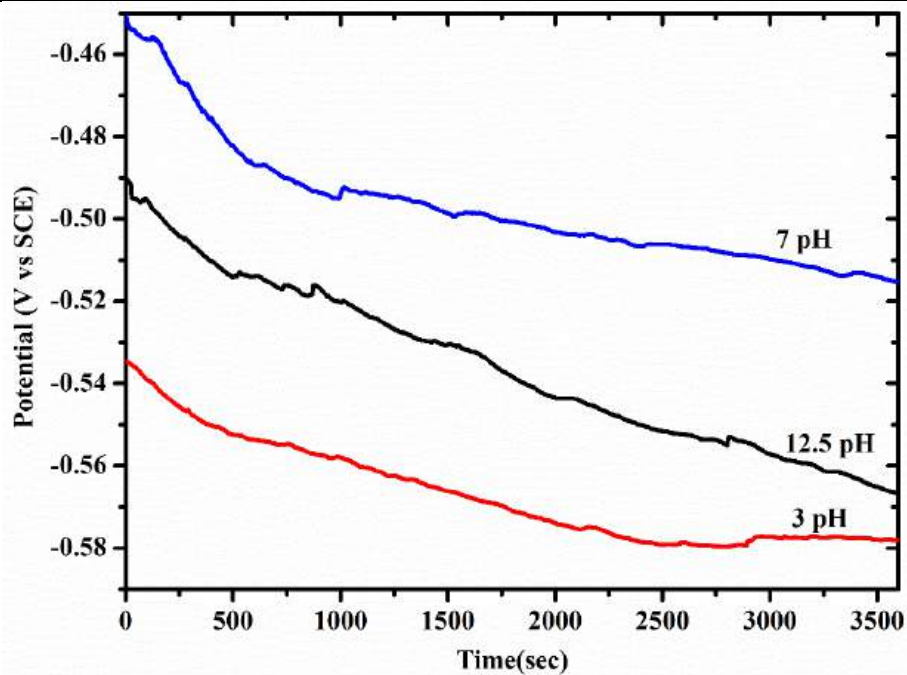
Nitesh Kumar *et al.*,

Figure 3. Variation of the open circuit potential PH 17-4 martensitic Stainless steel in different pH

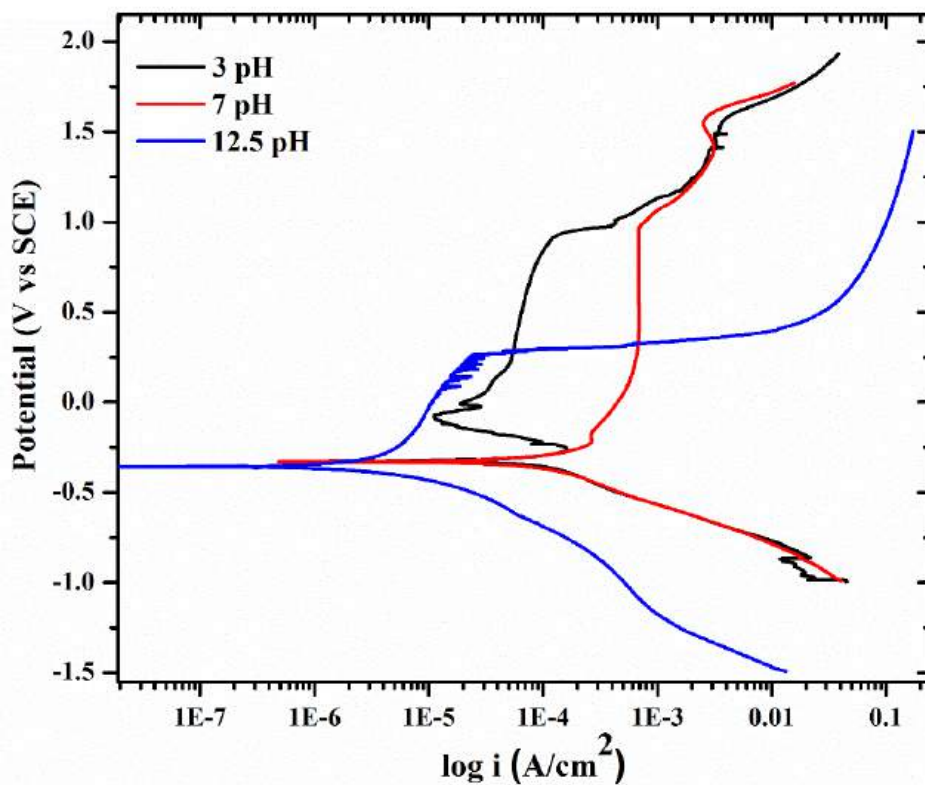


Figure 4. Potentiodynamic polarization plots of PH 17-4 Martensitic Stainless steel in different pH



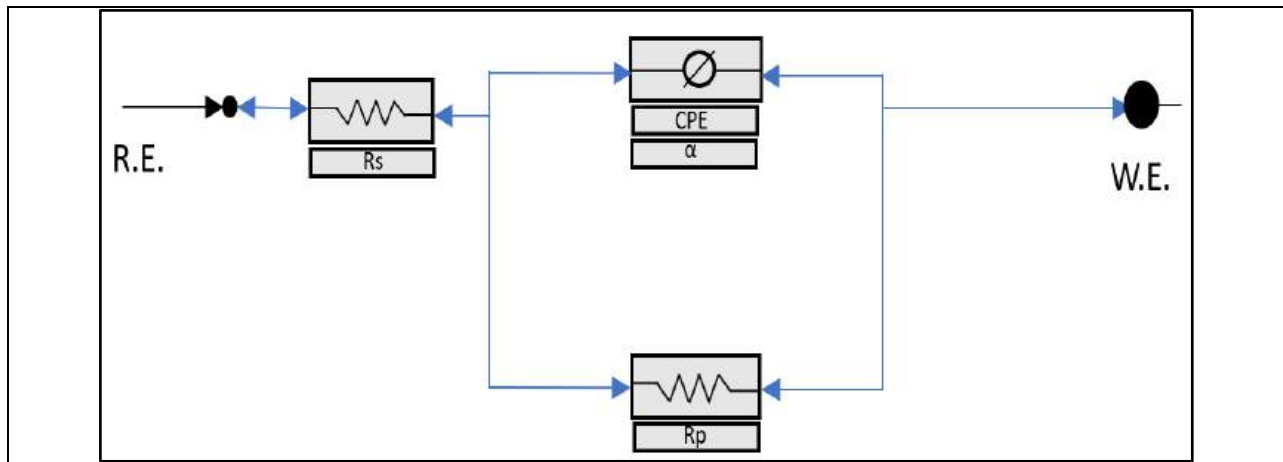
Nitesh Kumar *et al.*,

Figure 5. Equivalent circuit model used to fit the experiment impedance data of 17-4 stainless steel at various pH

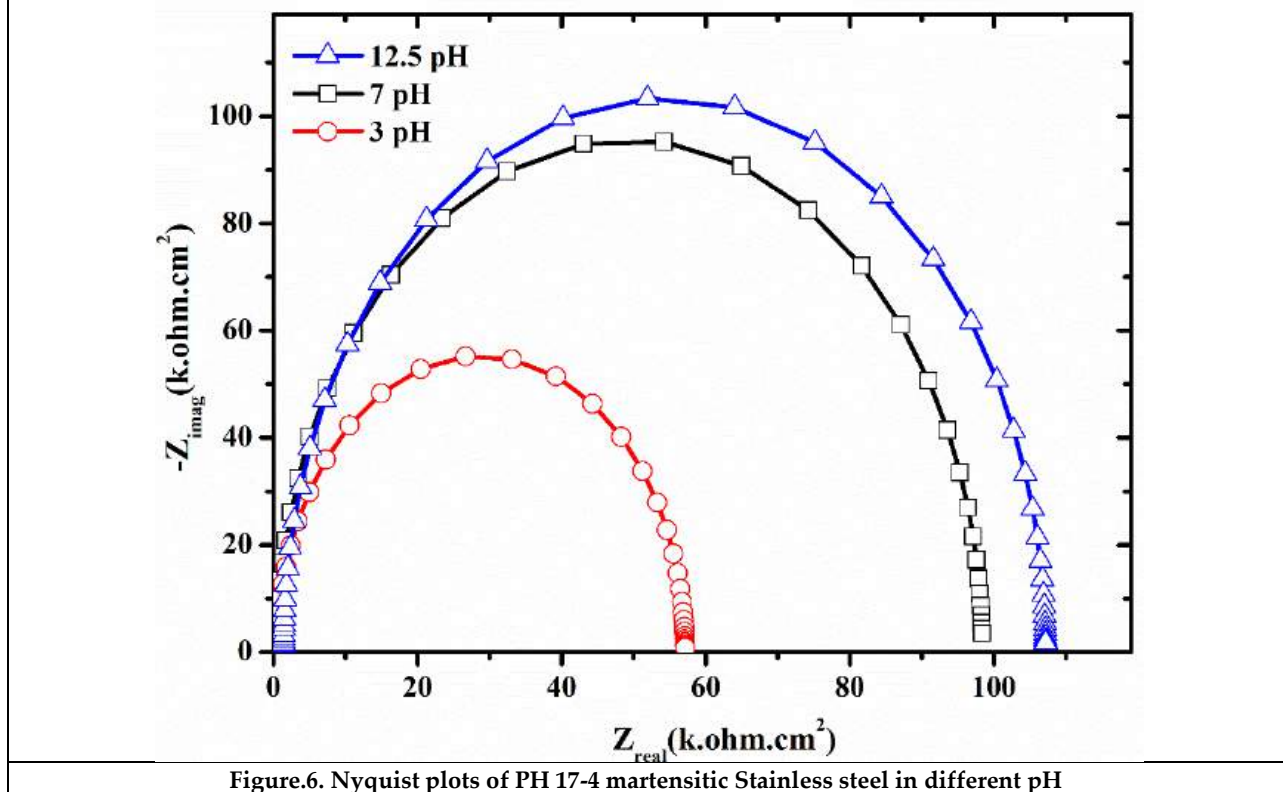


Figure.6. Nyquist plots of PH 17-4 martensitic Stainless steel in different pH



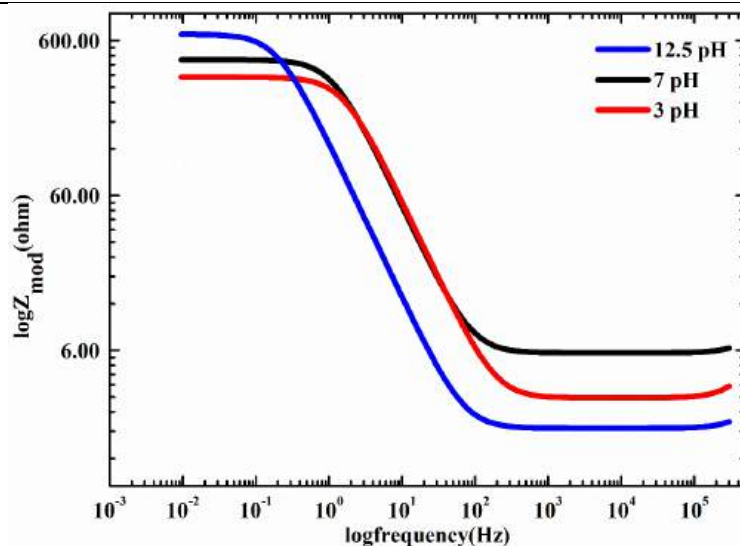
Nitesh Kumar *et al.*,

Figure7. Bode plots of PH 17-4 martensitic Stainless steel in different pH

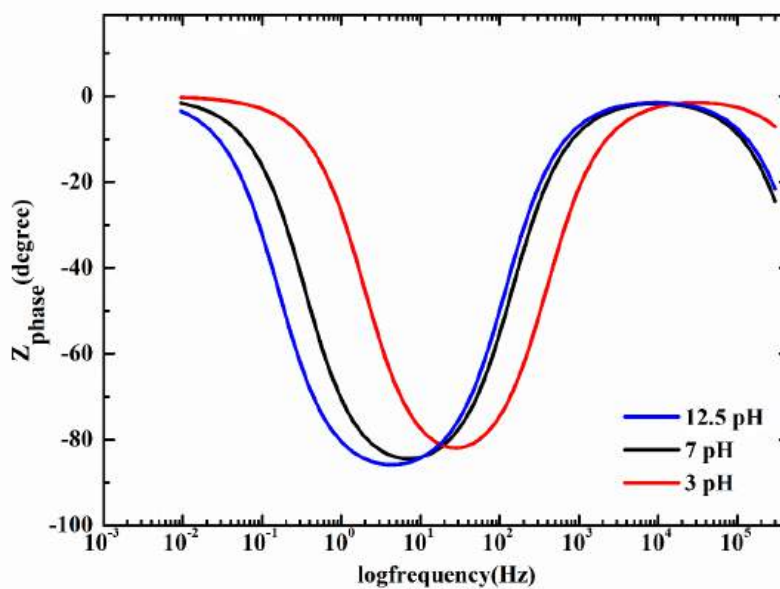


Figure. 8. Bode phase angle plots of 17-4 PH martensitic stainless steel in chloride solution at different pH values (3, 7, and 12.5).

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Assessing Task Performance in Knee Osteoarthritis Using OARSI-Recommended Functional Tests : A Narrative Review

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ABSTRACT

One of the major causes of pain and functional impairment among the elderly population is knee osteoarthritis (OA). Evaluating how someone with knee OA can perform functional activities is critical for both evaluating the effect the disease has had on them and evaluating rehabilitation outcomes. The Osteoarthritis Research Society International (OARSI) has developed a series of standardized performance-based assessments that evaluate a person's functional abilities as a result of knee OA. These examinations consist of: Timed Up and Go (TUG), 30-Second Chair Stand Test (30-sec CST), Stair Climb Test (SCT), and 40-Meter Fast Paced Walk Test (40m-FPWT). The current research on the use of OARSI standardized functional tests to assess task performance in people with knee OA is summarized in this narrative review. Peer-reviewed research published between 2021-2026 was found using a literature search. A total of 8 studies were identified, all of which are Clinical Trials (RCT, Pilot study, Study Protocol), evaluating six different rehabilitation interventions. Overall, when investigating the use of the OARSI-recommended tests, tests were utilized to measure mobility, lower limb strength, balance, walking ability, and functional independence. Improvement in functional performance was identified in all eight studies examined following interventions such as: High Intensity Interval Training, Integrated Physiotherapy, Neuromuscular Training, Blood Flow Restriction Walking, Low Level Laser Therapy, and Biomechanical Interventions. OARSI Functional Performance Test (FPT) outcomes provide quantifiable measures of physical ability level among those with knee OA and can effectively capture changes in ability due to therapeutic intervention. FPTs are useful and significant indicators of physical function



**Bibhujit Mishra and Amita Aggarwal**

among individuals with knee OA in general. They are clinically appropriate for diagnosing functional limitations, monitoring rehabilitation progress, and assessing treatment efficacy.

Keywords: Knee Osteoarthritis, Physical Functional Performance, Performance-Based Functional Test, OARSI

INTRODUCTION

The most common type of osteoarthritis and a major contributor to chronic pain, disability, and diminished physical function in older persons globally is knee osteoarthritis (OA). It is characterized by degeneration of articular cartilage, subchondral bone remodelling, osteophyte formation and gradual deterioration of joint function.¹ According to the Global Burden of Disease Study 2019, osteoarthritis affects more than 500 million individuals globally.^{1,2} Epidemiological evidence suggests that symptomatic knee OA affects approximately 10% of men and 13% of women aged 60 years and above, making it one of the most common chronic musculoskeletal conditions globally.³ The Kellgren–Lawrence (K&L) grading system, which is still the most widely used radiographic classification technique, is frequently used to classify severity levels of knee OA. This system categorizes disease severity from Grade 0 (no radiographic evidence of OA) to Grade 4 (severe OA with marked joint-space narrowing, large osteophytes, subchondral sclerosis, and bony deformity).⁴ Increasing K&L grades are associated with worsening pain, functional impairment, and reduced health-related quality of life (QOL).⁵

Structural alterations lead to disease progression and symptom manifestation.⁶ Pain remains the predominant symptom and is often the principal determinant of disability. Furthermore, pain associated with knee OA significantly affects sleep quality, psychological well-being, social participation, and overall QOL.⁷ Numerous modifiable and non-modifiable risk factors influence the development and progression of knee OA.⁸ Obesity, prior knee injuries, malalignment, muscle weakness, physical inactivity, job stress, and genetic predisposition are risk factors for knee OA.⁹⁻¹⁰ Individuals with symptomatic knee OA consistently report lower health-related QOL than age-matched healthy populations, particularly in domains related to physical functioning and mobility.¹¹⁻¹² As disease severity progresses, patients commonly experience difficulty performing routine tasks. Consequently, impairment in physical performance has emerged as one of the most clinically relevant consequences of knee OA. These limitations are often attributed to pain, joint stiffness, muscle weakness, reduced proprioception, and altered neuromuscular control, which collectively compromise functional independence and increase the risk of disability.¹³ Despite the fact that measures of patient-reported outcomes are commonly used to assess disability and symptoms, they could not fairly represent real physical performance. Therefore, performance-based functional assessments have gained increasing importance in clinical practice and research.¹³⁻¹⁴ These assessments objectively assess a person's capacity to carry out routine tasks and offer insightful information about their actual physical capabilities. A systematic review by Dobson et al. demonstrated that performance-based measures possess acceptable reliability, validity, and responsiveness for assessing physical function in individuals with hip and knee OA.¹⁴

The Osteoarthritis Research Society International (OARSI) suggested a core set of performance-based assessments for people with osteoarthritis in their hips and knees in order to standardise the evaluation of physical function. The 30-Second Chair Stand Test (30-SCST), the 40-Meter Fast-Paced Walk Test (40m-FPWT), the Timed Up and Go Test (TUG), the Stair Climb Test (SCT), and balance tests like the Single-Leg Stance Test (SLST) are among them. Together, these tests evaluate lower-limb strength, mobility, walking performance, balance, and functional independence.¹⁵ Evidence has further demonstrated their responsiveness and clinical utility in monitoring functional changes and treatment outcomes among individuals with osteoarthritis.¹⁶ Despite their widespread use, evidence on the specific role of OARSI-recommended tests in assessing task-related performance in individuals with knee OA remains scattered throughout the literature. Variability in study populations, disease severity, and outcome measures makes it difficult to identify the most appropriate assessment tools for evaluating functional limitations and rehabilitation



**Bibhujit Mishra and Amita Aggarwal**

outcomes. Therefore, a comprehensive synthesis of the available evidence is warranted. Therefore, the purpose of this narrative review is to emphasise the therapeutic significance of OARSI-recommended functional tests in rehabilitation and outcome evaluation, as well as to summarise and critically examine the available data about these tests used to evaluate task-related performance in people with knee OA.

Research Question

What purpose do OARSI-recommended functional performance tests have in evaluating task performance and physical function in individuals with knee OA?

METHODOLOGY**Study Design**

This narrative review summarizes the evidence available for using OARSI-recommended functional performance testing tools for assessing functionality, functional abilities or task performance in persons with knee OA. The review investigates practical use, responsiveness and applicability of OARSI-recommended tests – such as TUG, 30CST, SCT, 6MWT, and 40m-FPWT. In addition to evaluating the quality of evidence supporting the use of these measures for measuring functional limitations, evaluating treatment effectiveness, and providing the basis of clinical decision-making for the management of knee OA, there is a critical appraisal of the strengths/weaknesses associated with each measure. For this review, articles were located prior to June 2026 that were published after January 2020. Reviews conducted were completed to comply with the Sanra Scale (Scale to Assess Narrative Review Articles); the Sanra assessment guideline has been added as additional support for the report.

Search Strategy

The major research databases PubMed, Cochrane Library, Science Direct, and Embase of publications published between January 2021 and June 2026 provided the foundation for this narrative review. B.M. and A.A. independently conducted a search for these articles in the databases. This curated set is described in this review as being the core of the dataset. The articles were indexed by combinations of topic-specific keyword terms, including “Knee Osteoarthritis”, “Knee OA”, “Physical Functional Performance”, “Functional Performance”, “Task Performance”, “Performance-Based Functional Test”, and “OARSI. The strategy for searching was developed using synonyms within each concept (OR) and linking the different concepts (AND). The NOT operator enhanced specificity by excluding irrelevant conditions, as shown in **Table 1**.

Inclusion and Exclusion Criteria

Included were all studies reported between 2020 and 2026. All studies required to involve people with a diagnosis of knee OA who were at least 40 years old in order to be eligible for inclusion in this analysis. The following OARSI-Recommended Functional Tests were assessed by eligible studies. Studies assessing clinical utility and application of OARSI-recommended functional tests to assess physical function/treatment outcomes/functional limitations associated with disease will be included, as will clinical trials measuring physical functional performance in people with knee OA. Studies published before 2021, participant age >40 years, animal studies, and articles that were not peer-reviewed were excluded from consideration for inclusion criteria. Studies that lacked primary data, conference abstracts, editorials, letters to the editor, opinions, and narrative commentaries were also excluded. Studies that did not focus on individuals with a diagnosis of knee osteoarthritis, used non-OARSI-approved performance-based functional test(s), or reported only outcome measures of the engaged therapies and did not measure task performance or physical function were also eliminated from consideration.



**Bibhujit Mishra and Amita Aggarwal****Study Screening**

B.M. and A.A. used Rayyan software to select studies in a stepwise fashion, screening articles first by title, then by the abstract, and finally by full-text analysis to determine eligibility. Mendeley software was used to find and eliminate duplicate records.

RESULT

Researchers used a database search for relevant literature to the current review topic; they identified a total of 25,459 records. After removing duplicates, the researchers had 16,958 records left for title and abstract screening. Of that 16,958, 16,314 were excluded because they did not pertain to the topic of this review or were either a non-clinical study type or in another language. The remaining 644 reports were requested for retrieval; however, due to restricted access or not being able to provide the full text, 321 records could not be retrieved. After retrieving the remaining 322 full-text articles, each was reviewed by the predefined selection criteria to determine if it meets the requirements. Upon completing the full-text review, 316 articles were excluded for either missing data, methodological limitations and/or did not fulfil the selection criteria for this review. Eight studies have been included in this review to date. The Figure 1 represents the process of study selection. All the studies selected for the review included functional performance assessment using OARSI-recommended outcome measures in individuals with knee OA. Taken together, based on the evidence, performance tests are useful tests in assessing functional status and monitoring treatment measures in individuals with OA knee. The results demonstrate that clinical use of standardized functional assessment is valuable to the overall evaluation and treatment of patients with OA knee. Table 2 displays characteristics of the included study.

Functional Performance Assessment Using OARSI-Recommended Tests

Every study included in this analysis supported the OARSI-recommended functional tests as valid, objective measures of task performance for patients with OA knee and reported that measures sensitivity to post-rehabilitation change and the functional performance and mobility associated with the lower extremities. For example, in Golightly et al. (2021), the authors found that a 12-week HIIT program resulted in improvements in 40m-FPWT, 30-sec CST, balance, and overall physical performance; therefore, demonstrating a sensitivity to these measures related to improvements from aerobic exercise interventions that were OARSI-recommended.¹⁸ Similarly, Khan et al. (2023) reported that significant improvements in TUGT, SCT, 40m-FPWT, and 30-CST occurred following the implementation of an integrated physiotherapy protocol that included a combination of strengthening exercises, neuromuscular training, manual therapy, aerobic exercise and patient education. Together, these studies provide support for the notion that a multimodal rehabilitation approach can effectively address functional impairment associated with knee OA.²⁰ In the study by Petersson et al. (2022), blood flow-restricted walking was investigated as an intervention in older adults with OA knee. Participants who completed the intervention exhibited improvements in TUGT, sit-to-stand ability, SCT, and 40M- FPWT. These findings indicate that low-load exercise modalities can enhance functional performance while limiting joint loading.¹⁹

Exercise and Neuromuscular Rehabilitation Interventions

Consistently, exercise-based interventions have produced positive functional outcomes. Through tele rehabilitation, neuromuscular training has demonstrated improvement in pain, functional capability, and physical performance assessments, including the 30-CST, SCT and 40m-FPWT. Joon et al. (2025) found that remote supervision of rehabilitation can successfully improve functional capacity while enhancing access to treatment.²³ Emerging rehabilitation strategies have continued to highlight the importance of performance-based functional assessment. Thomas et al. (2024) proposed a study to investigate the effect of strengthening core muscles, pain severity, patient-reported measures and function task using the OARSI-recommended tests as the primary measures for improvement in functional capabilities. The protocol evidences the increasing recognition of the importance of stability within the trunk and of kinetic-chain function in knee OA rehabilitation.²² Correspondingly, Carvalho and Alberton (2025)



**Bibhujit Mishra and Amita Aggarwal**

describe the RISE- knee OA trial protocol, which aims to compare in-person and remotely supervised exercise programs. Functional performance was recognised as an important outcome to further substantiate the value of using standardised OARSI-recommended measures to evaluate the effectiveness of treatment across different rehabilitation delivery models.²⁴

Adjunctive Therapeutic Interventions

Positive relationships were noted with regard to the functional abilities of individuals who underwent adjunctive methods. Jankaew et al. (2023) contributed to the literature on LLLT and confirmed the benefits of different laser wavelengths in the 30- second sit-to-stand test, SCT, TUGT, and 40m-FPWT. LLLT using an 808-nanometer (nm) wavelength yields more substantial improvements to knee extensor strength; therefore, laser therapy may serve as an adjunct to exercise-based rehabilitation programs in people with knee OA.²¹

Biomechanical Interventions and Functional Performance

Khan et al. (2020) evaluated biomechanical interventions that included valgus knee bracing, wedged insoles, and modified gait retraining to determine their immediate effect on physical performance. Results from OARSI-recommended tests found that performance-based tests were able to detect functional changes related to biomechanical changes, with the most beneficial performance (i.e., increased walking ability and SCT) achieved through the use of valgus knee bracing and the least beneficial performance (i.e., decreased function) with a toe-out gait.¹⁷

Overall Synthesis of Evidence

The studies provided above show that functional performance measures as defined by OARSI are a valid, reliable, and useful means of assessing task performance in patients with OA knee. The results from the following functional performance measures, TUG; 30-CST; SCT; and 40m-FPWT, have been shown to improve after various types of interventions; specifically, high-intensity interval training¹⁸; integrated physiotherapy²⁰; blood flow-restricted walking¹⁹; neuromuscular telerehabilitation²³; low-level laser therapy²¹; biomechanical interventions¹⁷; and innovative rehabilitation techniques, including core strengthening²² and remote exercise monitoring.²⁴ Collectively, these findings support the continued application of OARSI-recommended tests to evaluate functional limitations, monitor rehabilitation progress, and assess treatment effectiveness.

DISCUSSION

A narrative review has compiled data from 8 studies that examined the application of the OARSI functional performance recommendation. Each of the TUGT, 30-sec CST, SCT and 40-m FPWT tests has been validated by studies to be equally useful in quantifying functional impairments and evaluating treatment response in those with knee OA. Therefore, the data provide constructive support for the utility of these tests in quantifying objective, responsive outcomes of function task in patients with OA knee. Overall, exercise-based interventions have shown the greatest overall improvement in functional performance. Significant improvement in mobility, ability to walk and lower limb function has been reported by Golightly et al. (2021)¹⁸ and Khan et al. (2023)²⁰ following HIIT and integrated physiotherapy interventions, respectively. These findings are consistent with past research substantiating exercise as the primary intervention to relieve pain and improve function in knee OA (Fransen et al., 2015; Bannuru et al., 2019).²⁵⁻²⁶

There is a lot of encouraging evidence supporting the efficacy of neuromuscular rehabilitation. Joon et al. (2025)²³, for example, found greatly improved functional performance as a result of telerehabilitation-based neuromuscular training, which is consistent with previous findings in Ageberg et al. (2010)²⁷ and Bennell et al. (2014).²⁸ Ageberg et al. (2010)²⁷, for instance, noted improvements in balance, mobility and functional capacity after participants completed neuromuscular exercise programs. Additionally, Petersson et al. (2022)¹⁹ noted that participants improved their





walking speed, stair climbing ability and sit-to-stand ability after performing blood flow restricted walking exercise. These results are in line with Hughes et al. (2017)²⁹, who observed that low-load blood flow-restricted strength training can enhance functional ability and muscular strength while reducing joint stress. Through adjunctive intervention, researchers have evaluated the effectiveness of LLL therapy in improving muscle strength and functional ability (i.e., Jankaew et al., 2023).²¹ Comparably, Stausholm et. al (2019)³⁰ reported decreased pain and disability after laser therapy inpatients with OA knee. Additionally, Khan et al. (2020)¹⁷ described improved walking and stair climbing abilities through valgus knee bracing, supporting previous evidence that bracing reduces medial knee loading, therefore enhancing functional mobility (Moyer et al., 2015).³¹ Moreover, Thomas et al. (2024)²² and Carvalho and Alberton (2025)²⁴ recently developed protocols to investigate OARSI-recommended tests as part of the inclusion criteria for knee OA studies, illustrating the growing importance of performance-based assessments in knee OA research. Use of OARSI-recommended measures corresponds to the recommendations of Dobson et al. (2013), who indicated that these measures are valid, reliable, and responsive assessments of physical functioning for individuals with OA.¹⁵

The overall findings of this study are in line with the body of research showing that the functional performance tests recommended by the OARSI are objective and clinically significant indicators of physical function in knee OA. Regular use of these functional performance assessments in research and clinical practice is supported by their responsiveness to exercise-based, neuromuscular, biomechanical, and adjuvant therapies. However, heterogeneity within the studies regarding interventions, study designs and participant profiles limits the ability of direct comparisons to be made and highlights the need to develop larger, better quality RCTs.

CONCLUSION

This narrative review provides synthesized evidence supporting the OARSI-recommended functional performance tests as practical and clinically meaningful outcome of function task in patients with OA knee. The ongoing applicability of these tests across multiple studies has shown that they provide an appropriate degree of responsiveness to a variety of rehabilitation interventions, and they clearly capture change in mobility, lower-extremity function, and performance of tasks. The tests are suitable for use as key outcome measures in the assessment of functional limitations because they are widely used and standardised. They are also helpful for tracking the progress of rehabilitation and can be used to assess the efficacy of treatment in clinical as well as research settings.

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Bibhujit Mishra and Amita Aggarwal

Table 1: Search Strategy

Keywords	Pub Med	Embase	Cochrane Library	Science Direct
Knee Osteoarthritis OR Knee OA	608	2021	5731	3352
Physical Functional Performance OR Functional Performance OR Task Performance	504	1697	3465	0
Performance-Based Functional Test OR OARSI	48	87	160	0
(Knee Osteoarthritis OR Knee OA) AND (Physical Functional Performance OR Functional Performance OR Task Performance)	30	545	1447	1975
(Knee Osteoarthritis OR Knee OA) AND (Physical Functional Performance OR Task Performance OR Performance-Based Functional Test OR OARSI)	44	323	852	2570
	1234	4673	11655	7897
Total	25459			

Table 2: Characteristics of Included Studies

Author (Year)	Aim	Population	Intervention	Outcome Measures	Conclusion
Khan et al., (2020) ¹⁷	To determine the effect of orthoses versus gait retraining on immediate response in improving physical performance measures in Knee OA.	40 participants with knee OA.	Orthoses versus gait retraining.	OARSI-recommended performance-based tests: 30-sec CST, TUGT, 6MWT, SCT and 40m-FPWT.	While toe-out gait hindered performance and wedged insoles had no effect, valgus knee braces were the most effective in improving physical function.
Golightly et al. (2021) ¹⁸	To evaluate the feasibility and results of a 12-week high-intensity interval training (HIIT) program for patients with knee OA.	29 participants with Knee OA.	12-week High-Intensity Interval Training.	WOMAC, ORSCI performance-based functional tests (20m-FPWT, 30-secCST, SCT, TUG, single-leg stance), cardiorespiratory fitness and body composition (VO ₂ peak), muscle strength.	Walking performance, chair-stand ability, and cardiorespiratory fitness were all enhanced by HIIT.
Petersson et al. (2022) ¹⁹	To assess the effect of blood flow-restricted walking in individuals with knee OA.	14 elderly individuals with KOA.	Blood Flow Restriction Walking for 8-10 weeks.	ORSCI performance-based functional tests TUG, 30-sec CST, 40m-FPWT, SCT, KOOS	BFR walking enhanced functional performance and was practical.
Khan et al. (2023) ²⁰	To assess the effect of an integrated physiotherapy protocol in patients	60 patients with KOA.	Supervised integrated physiotherapy program.	VAS, WOMAC, ORSCI performance-based functional tests:	Comprehensive physical therapy has greatly enhanced functional




Bibhujit Mishra and Amita Aggarwal

	with knee OA.			TUG, FRT, 40m-FPWT, SCT, 30-sec CST.	performance, balance, pain, and disability.
Jankaew et al. (2023) ²¹	To compare the effects of different LLLT wavelengths in patients with knee OA.	47 participants with Knee OA	808 nm LLLT, 660 nm LLLT and Sham control.	ORSCI performance-based functional tests 30-sec CST, 40m-FPWT, SCT, TUG	Knee strength and functional performance were enhanced by LLLT, with 808 nm exhibiting the biggest advantages.
Thomas et al., (2024) ²²	To assess the effect of the core strengthening protocol in patients with OA knee.	80 participants with Knee OA.	Group 1: Core strengthening with routine hip and knee strengthening exercises. Group 2: Routine hip and knee strengthening exercises alone for 12 weeks.	VAS, KOOS, LEFS. ORSCI performance-based functional tests (30STS, 40m-FPWT, SCT, TUG), knee muscle strength, core endurance, and femoral/tibial cartilage thickness (USG).	This RCT protocol will assess if adding core muscle strengthening to regular rehabilitation improves cartilage health, pain, physical function, and QOL in people with knee OA.
Joon et al. (2025) ²³	To evaluate the efficacy of NMT given to patients with primary knee OA via telerehabilitation.	30 participants with KOA	Neuromuscular Training via Telerehabilitation for 12 weeks.	VAS, KOOS, ORSCI recommended performance-based test (30-sec CST, 40m-FPWT, SCT).	Neuromuscular training based on telerehabilitation enhanced physical performance, function, and pain relief.
Carvalho and Alberton (2025) ²⁴	To assess the effect of remote and in-person supervised exercise in patients with knee OA.	48 participants with KOA.	Remote Group: Supervised strengthening via video calls. In-person Group: Same supervised strengthening program at a physiotherapy clinic for 12 weeks.	NRS, KOOS. OARSI functional tests (30-sec CST, TUG, 40-m Fast Walk, SCT), quadriceps muscle architecture.	Results of the protocol trial are still pending. The study will assess whether in-person rehabilitation for knee OA is superior to remote supervised activity.





Bibhujit Mishra and Amita Aggarwal

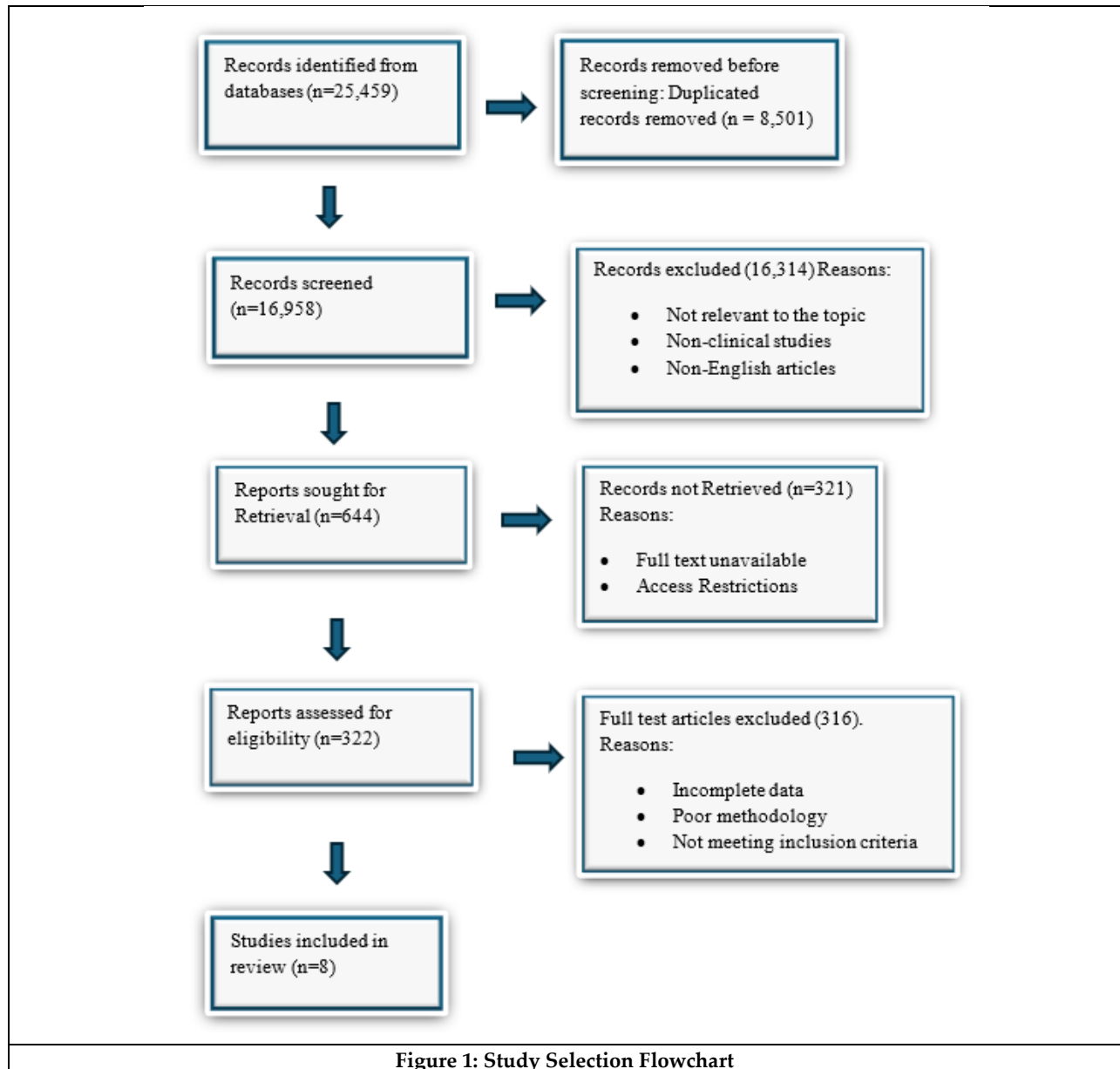


Figure 1: Study Selection Flowchart

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RESEARCH ARTICLE

Tourism Pressure and Water Quality Degradation in Heritage Waterfronts : A GIS-Based Water Quality Index Analysis of Pichola Lake, Udaipur

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ABSTRACT

Historic waterfront cities increasingly experience environmental stress due to the spatial concentration of tourism activities around fragile aquatic ecosystems. This study investigates the relationship between tourism pressure and water quality degradation in Pichola Lake, Udaipur, a prominent heritage waterfront landscape. An integrated methodological framework combining Water Quality Index (WQI), GIS based spatial interpolation, tourism intensity analysis (visitor density, dwell time, and kernel density hotspot mapping), and temporal tourism patterns was employed to assess environmental conditions and identify spatial patterns of degradation. Water samples from seven strategically distributed locations were analyzed using key physicochemical parameters, including dissolved oxygen, biochemical oxygen demand, nutrients, turbidity, pH, and electrical conductivity. The results reveal pronounced spatial heterogeneity in water quality, with higher WQI values indicating poorer water quality concentrated along the eastern and north western waterfront zones, which coincide with major tourism hotspots and dense urban development. Areas characterized by high visitor density and prolonged activity, particularly during peak tourism periods, exhibit elevated organic pollution, nutrient enrichment, and reduced dissolved oxygen levels. The integration of tourism intensity, environmental sensitivity, and temporal footfall trends confirms a strong relationship between tourism concentration and localized environmental degradation. The study highlights the importance of spatially and temporally informed planning approaches that integrate environmental monitoring with tourism management. It provides a framework for balancing tourism development with ecological sustainability in heritage waterfront cities.

Keywords: Water Quality Index (WQI); GIS spatial analysis; heritage waterfronts; tourism pressure; urban lake ecosystems; sustainable tourism planning; Pichola Lake, Udaipur





INTRODUCTION

Urban waterfronts have historically played a central role in shaping the spatial structure, cultural identity, and economic development of cities. Lakes, rivers, and coastal edges function not only as ecological systems but also as key elements of urban form, influencing settlement patterns, architectural development, and public space design. In many historic cities, waterfront landscapes have evolved into major tourism destinations where natural environments and built heritage coexist. However, the increasing concentration of tourism activities within such environments has generated significant environmental challenges, particularly with respect to water quality degradation and ecological sustainability (Camarda & Grassini, 2003; Gössling, 2002; Hall & Härkönen, 2006). Tourism-driven urbanization exerts significant pressure on fragile aquatic ecosystems through interconnected processes such as wastewater discharge, shoreline encroachment, recreational activities, and solid waste accumulation, particularly in heritage cities where tourism infrastructure is concentrated along waterfronts to enhance experiential value (Dodds & Butler, 2019; Gössling & Scott, 2018). From an architectural and urban planning perspective, these waterfronts function as complex socio-ecological systems where dense clusters of heritage structures, public spaces, and tourism-oriented developments are closely integrated with water bodies, increasing both their cultural significance and environmental (G. J. Ashworth & Tunbridge, 2000; G. Ashworth & Page, 2011). This spatial configuration underscores the critical challenge of balancing tourism-driven development with the ecological integrity of waterfront environments.

Udaipur, located in Rajasthan in north-western India, represents a historic waterfront city shaped by an interconnected system of artificial lakes developed within the Aravalli hill region to capture monsoon runoff and support urban settlement. Established in the sixteenth century, the city evolved around key water bodies such as Pichola Lake, Fateh Sagar Lake, and Udai Sagar, which continue to define its spatial structure, architectural character, and tourism economy. Today known as the “City of Lakes,” Udaipur attracts significant domestic and international tourism due to its combined natural and cultural heritage. Among these, Pichola Lake holds a central position within the historic core, surrounded by prominent architectural landmarks including the City Palace, Jagdish Temple, Bagore-ki-Haveli, and the island palaces of Jag Mandir and Lake Palace, forming a dense heritage waterfront where concentrated tourism activities along the eastern and northern edges create a dynamic interface between the built environment and the lake. However, this concentration of tourism activities has also contributed to increasing environmental stress on the lake ecosystem.

The intensification of urban and tourism-related activities along the waterfront has led to higher levels of wastewater discharge, nutrient loading, and organic pollution, which directly affect key water quality parameters such as dissolved oxygen (DO), biochemical oxygen demand (BOD), turbidity, and nutrient concentrations. In freshwater systems, such alterations can trigger processes such as eutrophication, reduced oxygen availability, and ecological imbalance, ultimately degrading the overall health of the water body (Smith, 2003; Smith et al., 1999). Assessing water quality in such complex urban environments requires analytical approaches capable of integrating multiple physicochemical parameters into a comprehensive framework (Uddin et al., 2024). The Water Quality Index (WQI) has emerged as an effective tool for this purpose, enabling the aggregation of diverse water quality indicators into a single composite value that reflects overall environmental conditions (Ravikumar et al., 2013). WQI simplifies complex datasets and facilitates comparison across spatial locations, making it particularly useful for identifying pollution hotspots in urban lake systems (Tyagi et al., 2013; Uddin et al., 2021). The integration of WQI with Geographic Information Systems (GIS) further enhances the analytical potential of water quality assessment by enabling spatial visualization and interpretation of environmental data (Wang, 2016). GIS-based interpolation techniques allow researchers to map variations in water quality parameters across a lake and identify localized zones of degradation. Such spatial approaches are especially relevant in urban contexts, where pollution sources are unevenly distributed and closely linked to land-use patterns and human activities (Nas & Berktaş, 2010).



Safa Seraj and *et al.*,

Despite the growing body of research on urban lake pollution, most studies primarily focus on physicochemical assessments and ecological monitoring, with limited attention to the spatial drivers of environmental degradation within urban landscapes. In particular, the relationship between tourism intensity, waterfront urbanization, and water quality conditions in heritage cities remains underexplored. Although tourism is widely recognized as both an economic driver and an environmental stressor, few studies have employed spatial analytical approaches to examine how tourism hotspot patterns influence localized water quality degradation in lake ecosystems (Dodds & Butler, 2019; Gössling, 2015; Gössling & Peeters, 2015). Moreover, environmental management research often treats water quality as an isolated scientific issue, overlooking the integration of architectural, urban design, and spatial planning perspectives. In historic waterfront cities, where cultural heritage, tourism infrastructure, and ecological systems are closely interconnected, this fragmented approach limits the development of holistic planning strategies, highlighting the need for interdisciplinary research that bridges environmental analysis with spatial planning and tourism studies (G. Ashworth & Page, 2011). To address this gap, the present study integrates Water Quality Index (WQI) assessment with GIS-based spatial analysis to examine the relationship between tourism hotspots and water quality degradation around Pichola Lake in Udaipur. The conceptual framework of the study (Figure 1) is based on the premise that tourism hotspots generate localized environmental pressures such as wastewater discharge, surface runoff, and organic pollution which in turn alter key water quality parameters and result in higher WQI values, indicating poorer water quality conditions. By overlaying tourism hotspot maps with WQI spatial distributions, the study identifies zones where environmental degradation coincides with high tourism intensity.

The central hypothesis of this research is that tourism hotspot areas around Pichola Lake exhibit higher WQI values, reflecting poorer water quality and increased contamination due to concentrated tourism and urban activities along the waterfront. By establishing this spatial relationship, the study contributes to a more nuanced understanding of how tourism-driven urban dynamics influence environmental conditions in heritage lake systems. From an architectural and urban planning perspective, the findings have significant implications for the sustainable management of heritage waterfronts. Effective planning strategies must move beyond isolated environmental interventions and instead adopt integrated approaches that consider tourism patterns, spatial land use, and ecological processes simultaneously. The study therefore contributes to the development of evidence-based planning frameworks for balancing tourism growth with environmental conservation in historic cities.

LITERATURE REVIEW

Water Quality Assessment in Urban Lakes

Urban lakes are critical components of urban ecosystems, providing essential services such as water supply, climate regulation, and recreational value. However, increasing urbanization has led to significant deterioration in water quality due to anthropogenic pressures, including sewage discharge, urban runoff, and land-use change (Smith et al., 1999; Vörösmarty et al., 2010). These processes often result in nutrient enrichment, organic pollution, and ecological degradation in freshwater systems. The Water Quality Index (WQI) has been widely used as an effective tool for synthesizing multiple physicochemical parameters into a single, interpretable measure of water quality (Lumb et al., 2011; Tyagi et al., 2013). WQI-based assessments enable comparative analysis across spatial and temporal scales and support environmental decision-making. Previous studies have demonstrated the effectiveness of combining WQI with statistical and spatial methods to identify pollution sources and environmental trends (Uddin et al., 2021). Recent research further highlights the growing relevance of integrated water quality assessment frameworks in rapidly urbanizing regions (Lumb et al., 2011; Uddin et al., 2021).

Tourism Impacts on Aquatic Ecosystems

Tourism is a major driver of economic growth but also contributes to environmental pressures in sensitive ecosystems. Tourism activities can influence water quality through increased resource consumption, wastewater generation, and infrastructure development (Gössling, 2002; Gössling & Scott, 2018). In waterfront destinations, activities such as boating, hospitality services, and recreational use directly affect aquatic environments. In heritage



**Safa Seraj and et al.,**

cities, tourism is typically concentrated around waterfront landmarks, resulting in localized environmental stress. Lake tourism systems are characterized by complex interactions between ecological processes and urban development, where inadequate management can lead to pollution through wastewater discharge, solid waste accumulation, and sediment disturbance (Hall & Härkönen, 2006). Empirical studies in lake-based tourism destinations, including Dal Lake, have demonstrated that tourism expansion contributes to nutrient loading and organic pollution. The concept of overtourism further emphasizes the risks associated with excessive visitor concentration. When tourism exceeds environmental carrying capacity, it can lead to ecological degradation and infrastructure stress (Dodds & Butler, 2019). Recent studies have also highlighted the increasing pressure of tourism on water resources and the need for sustainable management strategies in tourism-dependent regions (Gössling et al., 2012; Voci & Young, 2001).

GIS Applications in Environmental Spatial Analysis

Geographic Information Systems (GIS) play a crucial role in environmental monitoring by enabling the integration, analysis, and visualization of spatial data. GIS-based techniques are widely used to map water quality parameters, identify pollution hotspots, and assess the influence of land-use patterns on aquatic systems (Nas & Berktaş, 2010). Spatial interpolation methods such as Inverse Distance Weighting (IDW) and Kriging facilitate the generation of continuous water quality surfaces, allowing researchers to detect spatial variability within lake environments. The integration of GIS with WQI analysis has enhanced the ability to identify localized zones of environmental degradation and relate them to anthropogenic activities. Recent studies have emphasized the importance of GIS-based approaches in urban environmental planning, particularly for identifying environmentally sensitive zones and supporting sustainable decision-making (Uddin, 2023). When combined with tourism data, GIS analysis provides a powerful framework for understanding the spatial interaction between human activities and environmental conditions.

Heritage Waterfronts and Sustainable Urban Planning

Historic waterfronts are among the most significant urban landscapes, characterized by the convergence of cultural heritage, architectural identity, and tourism activity. These areas often serve as focal points for urban tourism, where visitor activities are spatially concentrated around heritage structures and scenic environments (G. Ashworth & Page, 2011). While this concentration enhances economic and cultural value, it also introduces environmental challenges. Unregulated tourism development can lead to increased pollution, infrastructure stress, and degradation of waterfront ecosystems. Sustainable waterfront planning therefore requires an integrated approach that balances heritage conservation with environmental protection. Recent urban planning research emphasizes the role of nature-based solutions, green infrastructure, and water-sensitive urban design in mitigating environmental impacts in waterfront areas (Grilo et al., 2025; Wong & Brown, 2009). Incorporating environmental monitoring into planning frameworks allows for the identification of vulnerable zones and supports targeted interventions such as wastewater management, buffer zones, and tourism zoning. In this context, integrating water quality assessment with spatial tourism analysis provides a critical framework for understanding environmental pressures in heritage waterfront environments. Such interdisciplinary approaches are essential for developing sustainable strategies that preserve both ecological integrity and cultural heritage.

Study Area

Geographic and Environmental Setting

Udaipur, located in southern Rajasthan (24.57°N, 73.69°E), lies within the Aravalli hill range at an elevation of approximately 577 m above sea level. The city is situated in a natural basin characterized by rugged terrain and interconnected artificial lakes constructed historically to capture monsoon runoff and support urban water supply. This geomorphological setting has significantly influenced the hydrological structure and spatial development of the city. The region experiences a semi-arid climate with annual rainfall of approximately 600–650 mm, concentrated during the monsoon season. The surrounding hills act as natural catchments, directing runoff into the lake system, which functions as a critical urban water resource (Vörösmarty et al., 2010). Lake Pichola, located within the historic





core, is one of the most prominent water bodies in this system (Figure 2). Originally constructed in the 14th century and later expanded, it forms part of an interconnected network including Fateh Sagar and Udaipur Sagar lakes, supporting both ecological processes and urban water management (Hall & Härkönen, 2006)

Urban Morphology and Waterfront Development

The urban morphology of Udaipur is closely tied to its lake system, with the historic core developing along the eastern edge of Pichola Lake. This waterfront is characterized by a dense concentration of heritage structures, including the City Palace complex, temples, and traditional havelis, forming a distinctive cultural landscape (G. J. Ashworth & Tunbridge, 2000). Island structures such as Jag Mandir and Lake Palace further reinforce the architectural and visual identity of the lakefront, contributing to its global recognition as a heritage tourism destination (Timothy, 2003). In recent decades, tourism-driven development has intensified along the waterfront, with the proliferation of heritage hotels, restaurants, and recreational facilities. The compact urban form and narrow street network of the historic core result in high spatial concentration of activities along the lakefront, increasing pressure on both built and natural environments.

Tourism Pressure and Environmental Challenges

Udaipur is a major heritage tourism destination, attracting significant domestic and international visitors due to its architectural heritage and lakefront setting (G. Ashworth & Page, 2011). Tourism activities are highly concentrated around the Pichola Lake waterfront, particularly within the historic core. Spatial analyses indicate that these areas exhibit the highest visitor density and dwell time, reflecting the clustering of major attractions and tourism infrastructure. This concentration increases pressure on urban services and environmental resources, particularly in lake-adjacent zones (Gössling et al., 2012). Rapid urbanization and tourism expansion have further intensified environmental pressures on Pichola Lake as reported in previous studies on Pichola and Fateh Sagar lakes (Auddy et al., 2022). Dense development along the lake edge increases the risk of pollutant inflow through urban runoff and wastewater discharge, while catchment degradation contributes to sediment and nutrient loading (Smith et al., 1999). Tourism-related activities exacerbate these impacts by increasing organic waste inputs and altering key water quality parameters such as dissolved oxygen and nutrient levels. These combined pressures highlight the need for spatially informed environmental monitoring to effectively manage tourism-induced impacts on urban lake ecosystems (Uddin et al., 2021).

METHODOLOGY

Research Design

This study adopts an integrated spatial-environmental analytical framework to examine the relationship between tourism pressure and water quality degradation around Pichola Lake in Udaipur. The research combines field-based water quality assessment, Water Quality Index (WQI) calculation, and Geographic Information System (GIS) spatial analysis to identify environmental patterns associated with tourism activity in the lakefront environment.

The methodology consists of four main stages:

- Selection of water sampling locations around Pichola Lake.
- Laboratory analysis of physicochemical water quality parameters.
- Computation of the Water Quality Index (WQI) to evaluate overall water quality conditions.
- GIS-based spatial analysis and overlay of tourism hotspot data to examine the relationship between tourism intensity and water quality degradation.

This integrated approach allows the study to move beyond conventional water quality monitoring by incorporating spatial planning perspectives that link environmental conditions with patterns of urban tourism activity.





Water Sampling Strategy

Water sampling locations in Pichola Lake were selected to reflect the spatial variability of water quality across different urban and hydrological zones, based on field-based mapping and GIS interpretation. A total of seven sampling stations were identified, distributed strategically to capture the influence of tourism activity, urban development, and lake dynamics as shown in figure 3. A significant number of sampling points are concentrated along the eastern shoreline of Pichola Lake, corresponding to the historic core and primary tourism zone, where intense human activities such as boating, hospitality, and waterfront use exert considerable pressure on water quality. Additional points were located in the central lake area to represent overall conditions and reduce shoreline bias, while a sampling point in the southern narrow stretch captures zones of reduced circulation and potential pollutant accumulation. At least one point along the relatively less urbanized western edge was included to provide a comparative baseline against more impacted areas. The selection of these seven points ensures representation of high-impact tourism zones, transitional areas, and relatively less disturbed regions, allowing for a spatially balanced assessment of water quality conditions. All sampling locations were geo-referenced using GPS for accurate integration with GIS-based spatial analysis.

Water Quality Parameters

Water samples were analyzed for a range of physicochemical parameters commonly used in freshwater quality assessment. These parameters were selected because they represent key indicators of organic pollution, nutrient enrichment, and overall ecological health of aquatic systems.

The parameters analyzed in this study include:

- Dissolved Oxygen (DO) – indicator of aquatic ecosystem health
- Biochemical Oxygen Demand (BOD) – measure of organic pollution
- Nitrate (NO_3^-) – indicator of nutrient loading
- Phosphate (PO_4^{3-}) – contributor to eutrophication
- Turbidity – measure of suspended particles
- pH – indicator of acidity or alkalinity
- Electrical Conductivity (EC) – indicator of dissolved salts and pollutants

These parameters are widely used in water quality monitoring studies and form the basis for calculating the Water Quality Index (WQI) in freshwater environments (Tyagi et al., 2013; Uddin et al., 2021). Water samples were collected using sterilized sampling containers and transported to a certified laboratory for analysis. Standard laboratory procedures were followed to ensure accuracy and reliability of the measurements.

Water Quality Index (WQI) Calculation

To evaluate the overall status of water quality in Pichola Lake, the measured physicochemical parameters were integrated into a Water Quality Index (WQI). The WQI provides a simplified representation of water quality by combining multiple environmental indicators into a single numerical value that reflects overall pollution levels. The WQI calculation followed the weighted arithmetic index method, which is widely used in water quality studies (Chidiac et al., 2023; Tyagi et al., 2013). The index was computed using the following steps: Assignment of weight (w_i) to each parameter based on its relative importance in water quality assessment.

Calculation of quality rating (q_i) for each parameter. Computation of the overall WQI value using weighted parameter values.

The general formula used for WQI calculation is:

$$WQI = \frac{\sum (w_i \times q_i)}{\sum w_i}$$

Where:

w_i = weight assigned to each parameter

q_i = quality rating for each parameter



Safa Seraj and *et al.*,

The resulting WQI values were classified into categories representing different water quality conditions, ranging from excellent water quality to very poor water quality. Higher WQI values indicate greater levels of contamination and poorer water quality conditions.

GIS-Based Spatial and Tourism Analysis

Geographic Information Systems (GIS) were used to analyze the spatial distribution of water quality and tourism activity in Pichola Lake by integrating geo-referenced datasets. The coordinates of sampling locations were processed using the Inverse Distance Weighting (IDW) method to generate continuous surface maps of water quality parameters and the Water Quality Index (WQI), enabling visualization of spatial variability which is widely used for spatial interpolation and water quality assessment (Nas & Berkay, 2010; Oseke et al., 2021; Subramaniam et al., 2023). IDW is widely applied in environmental studies due to its effectiveness in representing spatial heterogeneity, particularly with limited sampling data. Tourism intensity was assessed using visitor density, average dwell time, and kernel density estimation (KDE). Visitor density mapping identifies areas of high tourist concentration, while dwell time analysis captures zones of prolonged activity and sustained environmental pressure (Shoval & Ahas, 2016). KDE was applied to generate continuous hotspot surfaces, enabling the identification of spatial clustering of tourism activities. An environmental sensitivity analysis was further conducted using spatial variables such as proximity to the lake, land-use intensity, and accessibility to identify vulnerable zones (Malczewski, 2006; Zhang et al., 2021). Multi-criteria GIS techniques support the identification of environmentally sensitive areas for planning interventions. Finally, spatial overlay techniques were used to integrate tourism intensity layers with WQI maps, enabling analysis of the relationship between tourism activity and water quality conditions. The resulting tourism hotspot patterns (Figure 4) provide a spatial basis for identifying zones where high tourism pressure coincides with environmental stress, consistent with established GIS-based approaches in urban environmental research (Gössling & Scott, 2018).

Tourism Footfall and Temporal Analysis

Tourist footfall data were used to assess the temporal dimension of tourism pressure around Pichola Lake, using monthly average arrivals and intra day visitor distribution during peak periods. These data help identify seasonal trends and variations in visitor concentration across different time intervals, indicating periods of intensified human activity. When integrated with spatial indicators such as visitor density, dwell time, and KDE hotspots, the analysis captures both the magnitude and timing of tourism pressure in relation to environmental conditions.

RESULTS AND DISCUSSION

The integrated spatial analysis combining WQI interpolation, GIS mapping, and tourism intensity indicators (visitor density, dwell time, KDE hotspots) reveals strong spatial heterogeneity in water quality across Pichola Lake. The results indicate that environmental degradation is closely linked to tourism concentration, urban development, and localized hydrodynamic conditions, particularly along the lakefront.

Spatial Distribution of Water Quality and Pollution Dynamics

The GIS-based interpolation of water quality parameters (Figure 5 & 6) shows significant spatial variability, with low dissolved oxygen (3.20–4.24 mg/L) and high BOD (8.45–10.60 mg/L) concentrated in the north-western region, indicating severe organic pollution and hypoxic conditions. Elevated nutrient concentrations (nitrate and phosphate) and high turbidity (up to 154.98 NTU) further confirm the presence of eutrophication and suspended pollutant loads as widely reported in lake ecosystems under anthropogenic stress (Tandyrak et al., 2016). High electrical conductivity (up to 1719 $\mu\text{S}/\text{cm}$) in these zones reflects substantial dissolved pollutants, primarily from sewage and urban runoff.



**Safa Seraj and et al.,**

These patterns are characteristic of urban lake systems influenced by localized anthropogenic inputs and restricted water circulation (Uddin et al., 2021). The spatial clustering of multiple degraded parameters indicates cumulative environmental stress rather than isolated contamination events.

Integrated WQI and Spatial Degradation Patterns

The Water Quality Index (WQI) values (18.01–84.00) indicate that approximately 70–75% of the lake falls under poor to very poor categories, confirming widespread degradation. The most critical zones are located in the north-western waterfront, southern narrow stretch, and parts of the central basin, where multiple stress indicators converge (Figure 7). The IDW-based WQI map highlights clear spatial gradients, with relatively better conditions near inlet zones due to dilution effects. Such spatial differentiation is consistent with studies emphasizing the effectiveness of GIS-based WQI mapping in identifying pollution hotspots and environmental gradients.

Temporal Trends in Tourism Pressure

Tourist footfall patterns for Pichola Lake reveal significant temporal variation in visitor activity across both seasonal and daily scales (Figure 8). Monthly tourist arrival data indicate that peak visitation occurs during the winter and post-monsoon months, particularly in February (~5500 visitors/day), October (~5000 visitors/day), and November (~4800–4900 visitors/day). In contrast, relatively lower footfall is observed during the summer months (March–June), where visitor numbers drop to approximately 2200–2700 visitors/day. This seasonal fluctuation reflects climatic suitability and tourism cycles, with peak periods coinciding with favorable weather and major tourist inflows. Daily distribution patterns further highlight the intensity of tourism pressure within specific time windows. Data for December i.e., a peak tourism month, indicate that visitor activity is significantly higher during the afternoon and evening period (3 pm–8 pm, ~2700 visitors) compared to the morning period (10 am–3 pm, ~1500 visitors), resulting in a total daily footfall exceeding 4000 visitors. This uneven temporal distribution suggests that environmental pressure is concentrated not only spatially but also during specific hours of the day, particularly when recreational and leisure activities are at their peak.

Tourism Intensity and Spatial Environmental Stress

The visitor density map (Figure 9) highlights a pronounced spatial concentration of tourists along the eastern and north-eastern waterfront of Pichola Lake, particularly around major heritage attractions and accessible public spaces. High visitor density reflects intense spatial pressure on urban infrastructure and adjacent aquatic environments, as large volumes of tourists contribute to increased waste generation, surface runoff, and direct interaction with the lakefront. In urban tourism studies, visitor density is widely recognized as a critical indicator of environmental load, particularly in confined heritage settings where carrying capacity is limited (Zhang et al., 2021). The clustering of high-density zones along the lake edge suggests that tourism pressure is not evenly distributed but highly localized, increasing the likelihood of concentrated environmental degradation in these areas. Average dwell time analysis provides insight into the temporal dimension of tourism pressure, revealing zones where visitors spend prolonged periods, thereby intensifying localized environmental impacts. Areas with high dwell time along the waterfront indicate sustained human activity, including recreational use, boating, and social interaction, which can exacerbate pollution through continuous waste input and disturbance of sediments (Figure 10). Prolonged occupancy has been shown to significantly increase environmental stress in tourism landscapes by amplifying resource consumption and pollutant accumulation over time (Shoval & Ahas, 2016). In the context of Pichola Lake, high dwell time in key waterfront zones suggests persistent anthropogenic pressure, reinforcing the link between human activity duration and localized ecological degradation.

Kernel Density Estimation (KDE) analysis identifies spatial clusters of tourism activity, revealing primary hotspots concentrated along the lakefront with limited secondary clusters in peripheral areas (Figure 11). KDE is widely used in spatial analysis to detect intensity patterns and clustering behavior, providing a continuous representation of activity distribution. The presence of strong, centralized hotspots indicates a high degree of spatial dependency in tourism behavior, where activities are concentrated around key nodes such as heritage landmarks and waterfront



**Safa Seraj and et al.,**

access points. Such clustering is known to amplify localized environmental impacts by concentrating anthropogenic pressures within limited zones, leading to accelerated degradation of surrounding ecosystems. The environmental sensitivity map (Figure 12) identifies areas that are inherently vulnerable to ecological disturbance based on factors such as proximity to the lake, land-use intensity, and urban accessibility. The overlap between high-sensitivity zones and tourism hotspots indicates a critical spatial condition where fragile environments are subjected to intense anthropogenic pressure. Environmental sensitivity analysis is a key tool in spatial planning, enabling the identification of areas where even minor disturbances can result in significant ecological impacts (Malczewski, 2006; Zhang et al., 2021). In waterfront systems, such zones are particularly susceptible to pollution, habitat disruption, and water quality deterioration. The convergence of high tourism intensity and environmental sensitivity around Pichola Lake underscores the need for targeted planning interventions to prevent irreversible ecological degradation.

Overall, the combined analysis of visitor density, dwell time, KDE hotspots, environmental sensitivity, and temporal tourism trends reveals a strong spatial and temporal convergence between high tourism intensity and ecologically vulnerable zones along the Pichola Lake water front similar to trends reported in other urban lakes in the region (Choudhary, 2021). Areas characterized by high visitor concentration and prolonged activity coincide with sensitive lake edge environments, indicating that tourism pressure is both concentrated and disproportionately impacts fragile zones. Seasonal peaks and high activity periods further intensify anthropogenic stress through increased waste generation, boating activity, and shoreline use, reinforcing the spatial patterns of water quality degradation observed in the study. These findings highlight that tourism pressure operates across both space and time, and that reliance on single indicators may overlook critical interactions between human activity and environmental vulnerability. The integrated analysis provides a robust basis for identifying priority intervention areas and supports the development of targeted, evidence-based strategies for sustainable waterfront management in heritage urban contexts.

Linking Tourism Hotspots with Water Quality Degradation

The integrated spatial and temporal analysis reveals a strong relationship between tourism intensity and water quality degradation in Pichola Lake. Areas characterized by high visitor density, prolonged dwell time, and concentrated KDE hotspots correspond closely with zones exhibiting elevated WQI values, high BOD, and reduced dissolved oxygen levels. These patterns are particularly evident along the north western waterfront, where intense tourism activity overlaps with dense urban infrastructure and significant pollution inputs. In addition, southern sections of the lake show degradation associated with reduced water circulation and pollutant accumulation, further indicating that both human activity and hydrodynamic conditions influence environmental outcomes. The inclusion of temporal trends demonstrates that peak tourism periods further intensify these impacts, reinforcing the link between concentrated visitor activity and localized ecological stress. These findings support broader research indicating that the intensity and concentration of human activity are key determinants of environmental degradation in urban aquatic systems (Cheer et al., 2019; Zhang et al., 2021).

Synthesis of Spatial Environmental Dynamics

The combined assessment of WQI, GIS based spatial analysis, tourism intensity indicators, and temporal footfall patterns reveals that environmental degradation in Pichola Lake is highly localized and driven by the convergence of multiple stress factors. Zones of high tourism intensity, prolonged visitor presence, and elevated environmental sensitivity consistently overlap with areas of poor water quality, particularly along the historic waterfront. This demonstrates that environmental stress is not uniformly distributed but is concentrated in specific spatial and temporal contexts where human activity exceeds the ecological capacity of the lake system. The integration of spatial and temporal datasets provides strong evidence that tourism driven urbanization acts as a critical driver of environmental degradation in heritage waterfront environments. Similar patterns have been observed in other urban lake systems where concentrated land use and visitor activity result in localized ecological imbalance (Akar et al., 2024; Uddin et al., 2021).



Safa Seraj and *et al.*,

Architectural and Urban Planning Implications

The integrated spatial analysis combining WQI, GIS interpolation, tourism intensity (visitor density, dwell time, KDE hotspots), and environmental sensitivity mapping demonstrates that environmental degradation in Pichola Lake is strongly associated with the spatial concentration of tourism activity along ecologically vulnerable waterfront zones. The overlap of high tourism intensity and high environmental sensitivity areas, particularly along the eastern and north-western lakefront, indicates that these zones are approaching critical ecological thresholds. This highlights the need for spatially targeted planning interventions that integrate heritage conservation with environmental management. Approaches such as water-sensitive urban design (WSUD), nature-based solutions, and buffer-based waterfront planning can help reduce pollutant inflow while maintaining the cultural and visual integrity of historic landscapes (Wong & Brown, 2009).

The findings further emphasize that tourism pressure is not uniform but spatially clustered, requiring planning strategies that address both intensity and distribution of visitor activity. The identification of high-density and high-dwell-time zones through spatial analysis suggests the need for tourism zoning, visitor redistribution, and diversification of activity nodes beyond the historic core. Developing alternative cultural circuits, improving connectivity to peripheral areas, and regulating high-pressure waterfront zones can significantly reduce localized environmental stress (Cheer et al., 2019). At the same time, strengthening urban infrastructure systems, particularly wastewater treatment and storm water management in high-intensity zones, is essential to mitigate nutrient loading and organic pollution. Finally, the integration of GIS-based environmental monitoring with tourism analytics provides a powerful decision-support framework for urban governance. By enabling the identification of pollution hotspots, high-risk zones, and human–environment interaction patterns, this approach supports evidence-based planning and adaptive management in heritage cities. The study underscores that sustainable waterfront development requires a multidisciplinary, spatially informed approach that simultaneously addresses tourism dynamics, environmental vulnerability, and urban form to ensure the long-term resilience of heritage lake ecosystems.

CONCLUSION

This study demonstrates that water quality degradation in Pichola Lake is spatially uneven and strongly influenced by the concentration of tourism activity and urban development along the waterfront. By integrating Water Quality Index (WQI), GIS based interpolation, tourism intensity indicators (visitor density, dwell time, and KDE hotspot analysis), and temporal tourism patterns, the research identifies clear environmental gradients, with the most degraded conditions concentrated along the eastern and north western lakefront zones. These areas, characterized by high tourism pressure and dense built infrastructure, exhibit elevated organic pollution, nutrient loading, and reduced dissolved oxygen, confirming the significant impact of anthropogenic activities. A key contribution of this study is the establishment of a combined spatial and temporal relationship between tourism hotspots, environmental sensitivity, and water quality degradation, highlighting that areas of intense visitor activity and peak tourism periods often coincide with ecologically vulnerable zones. This convergence indicates that tourism driven urbanization can exceed the ecological carrying capacity of heritage waterfront systems, resulting in localized pollution hotspots. From an architectural and urban planning perspective, the findings emphasize the need for spatially and temporally targeted management strategies. Measures such as improved wastewater infrastructure, controlled waterfront development, buffer based planning, seasonal regulation of tourism activity, and redistribution of visitors are critical to reducing environmental stress. The application of GIS based environmental monitoring combined with tourism analytics provides a robust decision support framework for identifying high risk zones and guiding sustainable interventions.

Methodologically, the study demonstrates the effectiveness of integrating environmental, spatial, and temporal tourism datasets in understanding complex socio ecological systems. This approach can be extended to other heritage waterfront cities facing similar challenges. Future research should focus on long term monitoring and seasonally





Safa Seraj and *et al.*,

responsive analysis, including hydrodynamic processes, to better capture temporal variations in water quality. Ensuring the sustainability of Pichola Lake requires coordinated planning efforts that balance tourism development with ecological conservation, thereby preserving both environmental quality and cultural heritage.

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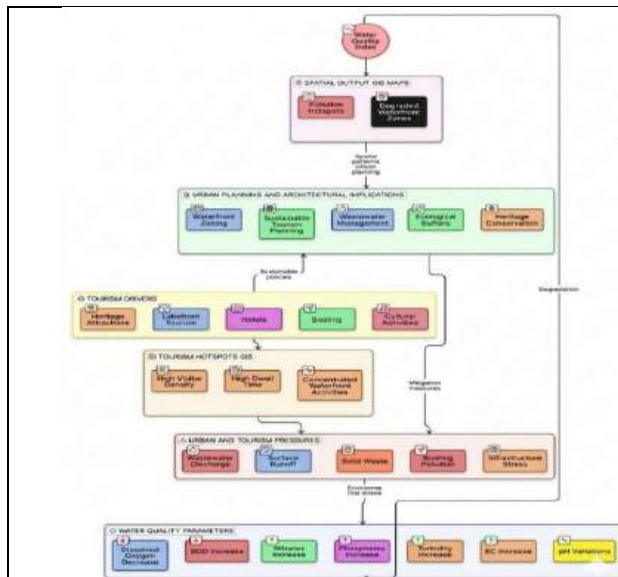


Figure 1. Conceptual framework illustrating the relationship between tourism hotspots and water quality degradation in heritage waterfront environments



Figure 2. Spatial configuration of Pichola Lake within Udaipur's urban lake system

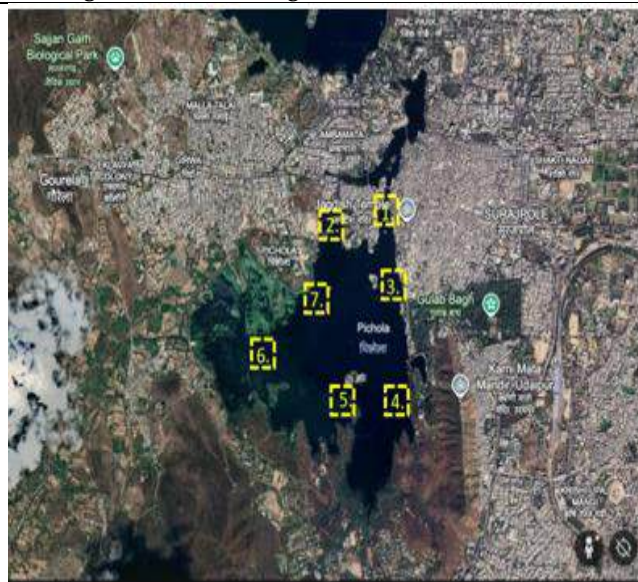


Figure 3. Water sampling points in Pichola Lake



Figure 4. Tourism hotspots at Pichola Lake, Udaipur





Safa Seraj and et al.,

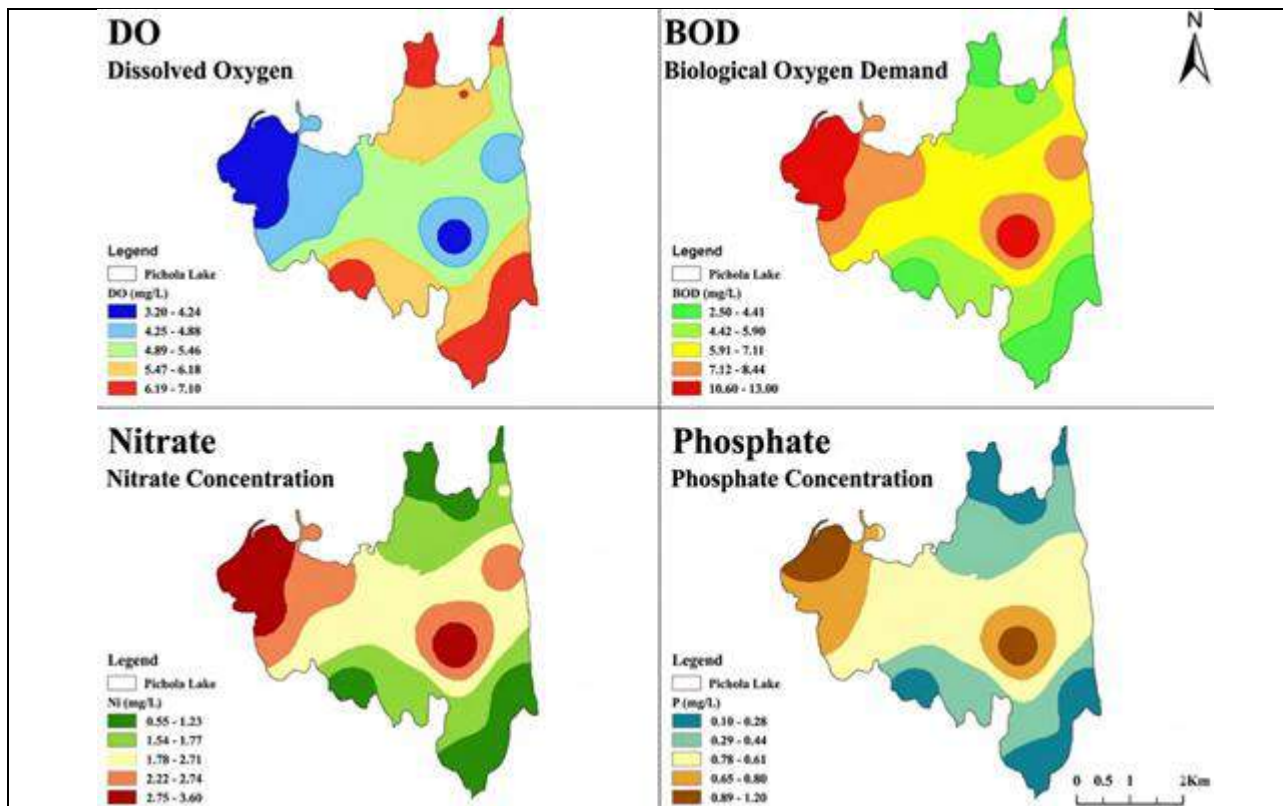


Figure 5. Spatial Interpolation of DO, BOD, Nitrate & Phosphate of Pichola Lake, Udaipur

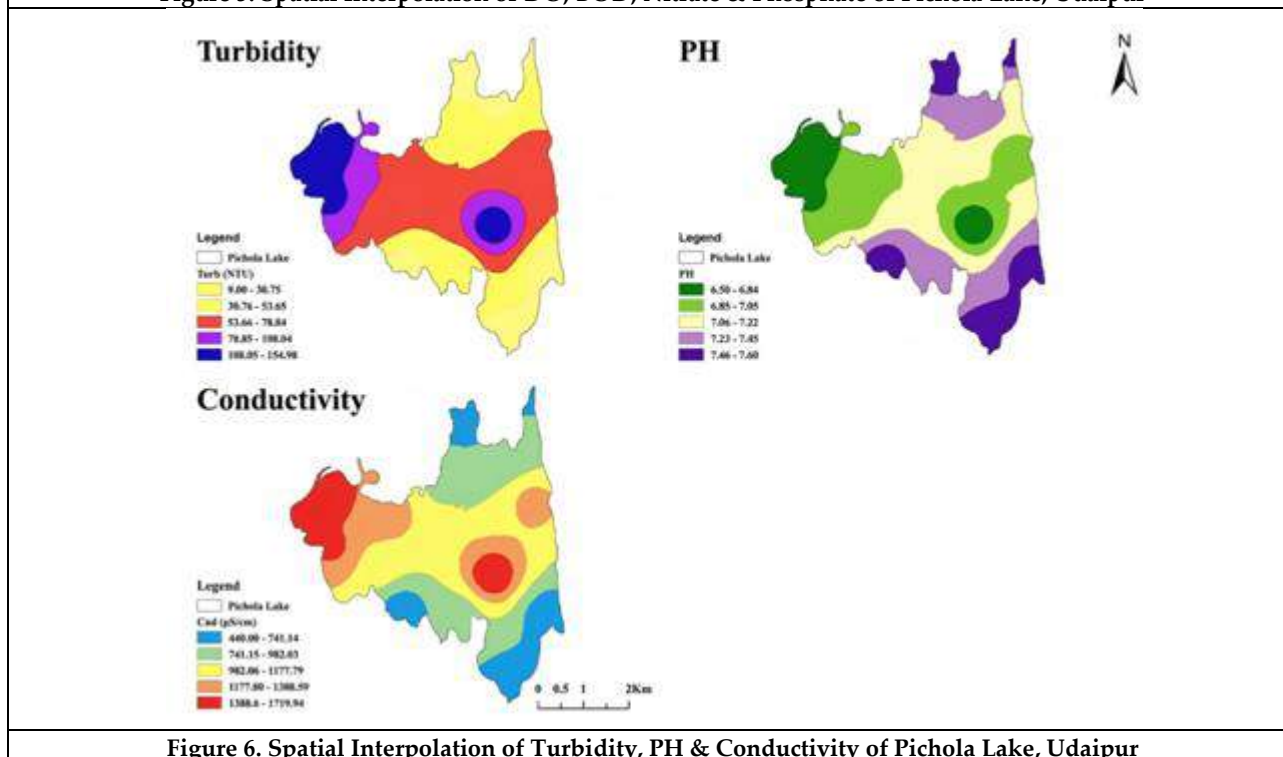


Figure 6. Spatial Interpolation of Turbidity, PH & Conductivity of Pichola Lake, Udaipur



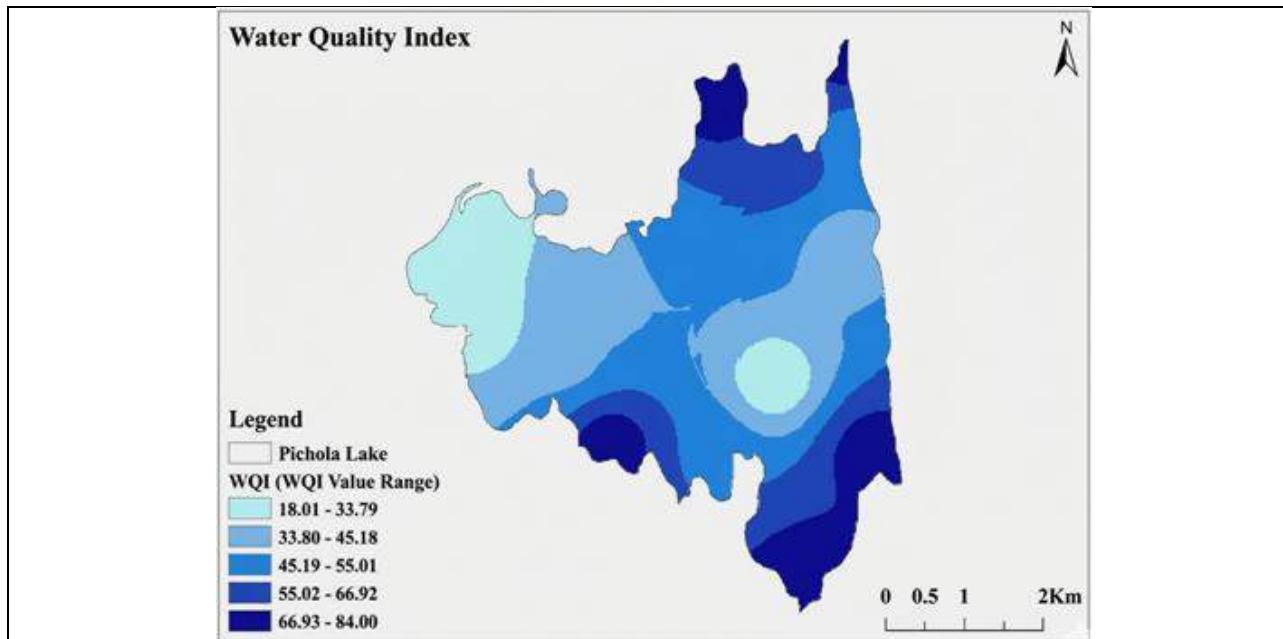
Safa Seraj and *et al.*,

Figure 7. Spatial Interpolation of Water Quality Index of Pichola Lake, Udaipur

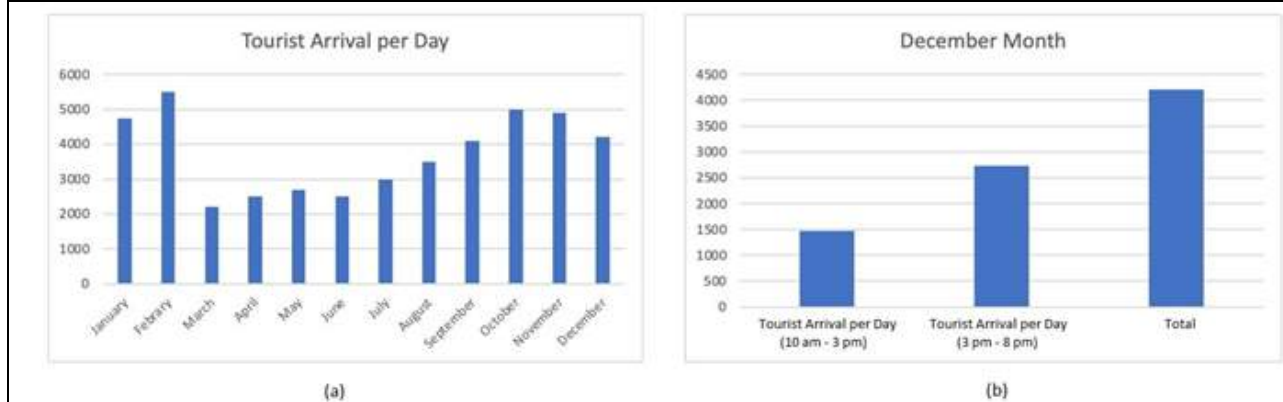


Figure 8. Temporal variation in tourist footfall at Pichola Lake showing (a) monthly average tourist arrivals per day and (b) intraday distribution of visitors during a peak tourism month (December).

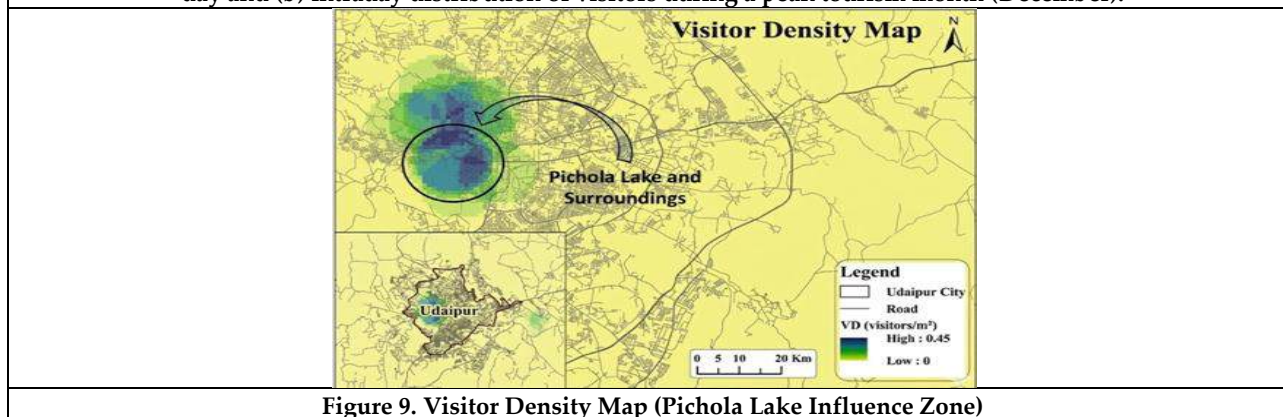


Figure 9. Visitor Density Map (Pichola Lake Influence Zone)



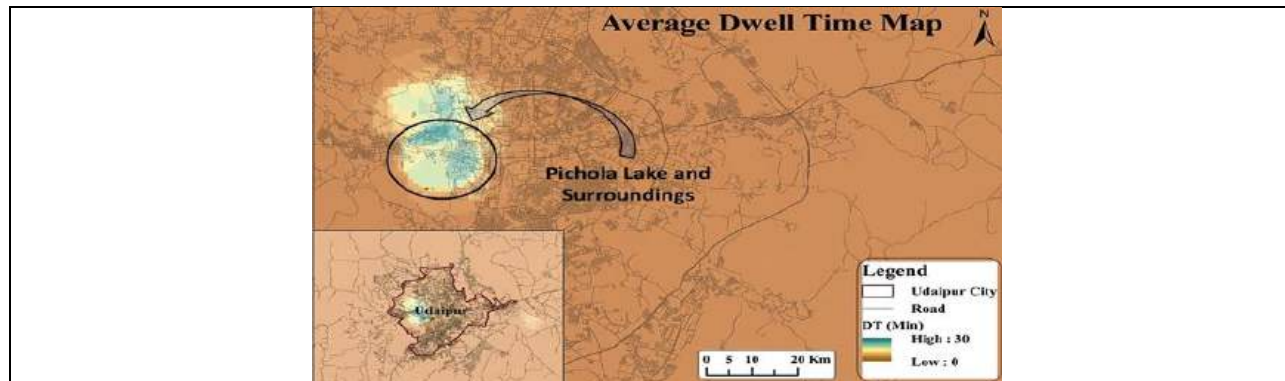


Figure 10. Average Dwell Time Map (Pichola Lake Influence Zone)

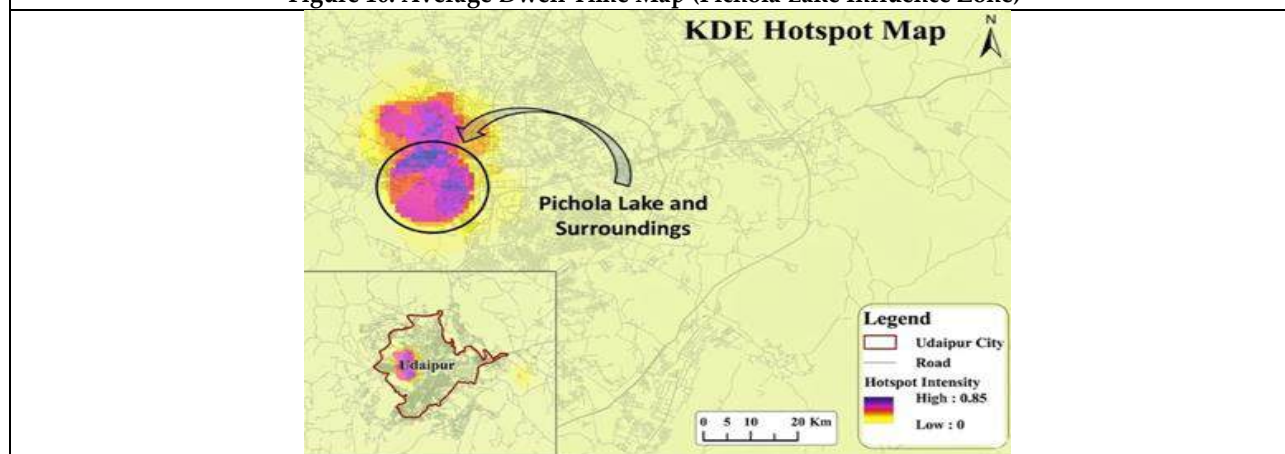


Figure 11. Kernel Density Estimation (KDE) Hotspot Map (Pichola Lake Influence Zone)

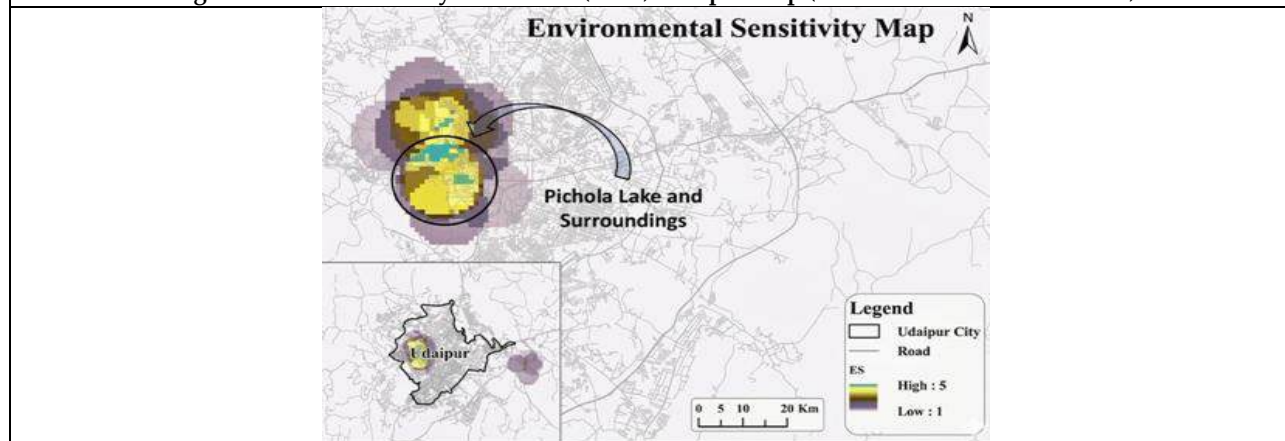


Figure 12. Environmental Sensitivity Map (Pichola Lake Influence Zone)

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RESEARCH ARTICLE

Impact of Physical Activity on Stress, Anxiety and Depression Among School-Going Adolescents: A Comparative Study Using DASS-21

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ABSTRACT

Adolescence is a critical developmental stage marked by increased vulnerability to stress, anxiety, and depression. The present study investigated the relationship between physical activity and mental health among school students aged 12–18 years. A total of 70 students were selected using random sampling, with an almost equal gender distribution (51.4% male, 48.6% female) and equal representation of physically active (n=35) and inactive students (n=35). Mental health outcomes were assessed using the standardized Depression, Anxiety, and Stress Scale (DASS-21). Findings revealed a striking difference between active and inactive students. Physically active students demonstrated mean scores of 8.3 (stress), 6.2 (anxiety), and 5.4 (depression), all within the normal range. Conversely, inactive students scored significantly higher, with mean values of 18.6, 16.1, and 14.3, respectively, corresponding to moderate-to-severe levels of psychological distress. Severity classification further supported these results: over 65% of active students reported normal mental health across domains, whereas a large proportion of inactive students were clustered in moderate to extremely severe categories, particularly for anxiety (60% severe/extremely severe) and depression (55% severe/extremely severe). These findings provide strong evidence that physical activity functions as a protective factor against psychological distress, while sedentary behavior significantly increases vulnerability. The results underscore the pressing need for schools, families, and policymakers to incorporate regular physical activity into daily routines, not only for physical health but also as a non-invasive and cost-effective strategy for promoting adolescent mental well-being.

Keywords: Physical activity, Adolescents, Stress, Anxiety, Depression, Mental health





INTRODUCTION

The mental health of school students has become a serious concern in recent years. With increasing academic pressure, competitive environments, peer comparisons, digital distractions, and social expectations, children between 12 and 18 years often experience stress, anxiety, and even symptoms of depression. According to the World Health Organization (2022), one in seven adolescents experiences a mental health condition, yet most cases remain undiagnosed and untreated [1-4]. Adolescence is a crucial stage marked by rapid physical, emotional, and cognitive changes. At this developmental period, lifestyle factors play a major role in building psychological resilience. Health is closely linked to mental well-being, and several studies have shown that regular physical activity improves mood, reduces stress hormones, enhances self-esteem, and contributes to emotional stability [5, 6]. However, modern school routines often reduce the time allocated to physical activity. With long classroom hours, heavy homework loads, and screen-based leisure replacing outdoor play, students are becoming increasingly sedentary, which negatively affects their mental health. Stress, anxiety, and depression among adolescents are often linked to exam pressure, performance anxiety, bullying, family expectations, and a lack of emotional coping skills [7-9]. The World Health Organization (2021) reports that depression is a leading cause of illness and disability among adolescents worldwide. Several surveys have also shown a rising trend of psychological distress among school students, particularly in urban areas where lifestyle changes, academic competition, and media overuse are prevalent [10-12].

In this context, physical activity emerges as a crucial yet often overlooked solution. Physical activity includes any form of movement that uses energy—from structured sports to simple play, walking, or yoga [13]. While its physical health benefits are well known, its role in supporting mental health is equally significant. Research consistently shows that regular physical activity lowers cortisol (a stress hormone), improves sleep quality, enhances emotional regulation, and reduces symptoms of stress, anxiety, and depression [14]. In India, the National Education Policy (NEP) 2020 recognizes the importance of sports and mental health education [15]. However, implementation remains inconsistent, as many schools continue to prioritize academic achievement over holistic development. Limited time for physical education, lack of trained instructors, and absence of mental health professionals in schools act as major barriers [16]. Additionally, stigma and fear of being judged often prevent students from seeking help or openly discussing emotional struggles [17].

Against this backdrop, the present research attempts to study the direct and measurable impact of physical activity on school students' mental health. Using the DASS-21 scale adapted for adolescents, the study assesses emotional states and compares outcomes between physically active and inactive students. The findings aim to highlight how even simple, routine physical activities can contribute to emotional stability and psychological strength among adolescents [18-20]. The study also provides evidence-based recommendations for multiple stakeholders. For educators and school leaders, it emphasizes the need to prioritize physical education within the curriculum. For parents, it highlights the importance of encouraging active lifestyles at home [21, 22]. For government agencies and NGOs, the findings can inform awareness campaigns, infrastructure development, and teacher training in student mental health first aid. For future researchers, it contributes to the relatively limited Indian literature that specifically explores the relationship between adolescent mental health and physical activity [23, 24]. As concerns around adolescent mental health continue to rise—including increased cases of screen addiction, social isolation, academic burnout, and even self-harm—the need for preventive, accessible, and non-stigmatizing interventions becomes urgent. Physical activity, in this context, is not just a leisure option but a critical component of mental wellness that must be promoted at every level of school education.

METHODOLOGY

The present study adopted a descriptive and analytical research design to explore the relationship between physical activity and mental health among school students. A descriptive design was chosen to provide a clear understanding





Atul Kumar Tiwari *et al.*,

of the existing status of students' mental health, while the analytical component helped in examining associations between levels of physical activity and psychological indicators such as stress, anxiety, and depression.

Population and Sampling

The population for this study comprised school students aged 12 to 18 years, enrolled in middle and secondary classes (grades 6 to 12) in selected urban and semi-urban schools. To ensure representation, both government and private institutions were included. A sample size of 70 students was drawn using simple random sampling, giving each eligible student an equal chance of selection. Random sampling reduces selection bias and enhances the generalizability of findings. The sample distribution was kept balanced across gender and school type to capture diverse perspectives.

Inclusion and Exclusion Criteria

- **Inclusion Criteria:** Students aged 12–18 years, enrolled in full-time schooling, and willing to participate with informed consent (obtained from both the student and guardian).
- **Exclusion Criteria:** Students with pre-diagnosed psychiatric disorders, chronic physical conditions affecting mobility or mood, or those with irregular school attendance were excluded to avoid confounding variables that could bias the findings.

Tools for Data Collection

The primary tool for mental health assessment was the Depression, Anxiety, and Stress Scale (DASS-21), adapted for adolescents. This standardized self-report questionnaire measures symptoms of depression, anxiety, and stress across 21 items. Its reliability and validity in adolescent populations have been well established. The tool was administered in both English and the local language (where necessary), ensuring accessibility. To capture physical activity patterns, students were asked to self-report their frequency and type of physical activity (e.g., sports, yoga, cycling, walking, recreational play) over the past four weeks. Activities were classified into categories: structured (school sports, yoga classes) and unstructured (free play, cycling, walking).

Procedure

1. **Permissions and Consent:** Formal permission was obtained from school authorities before data collection. Students and their guardians were briefed about the purpose of the study, and written consent was secured.
2. **Pilot Testing:** A small pilot with 10 students was conducted to ensure clarity of the questionnaire and refine the instructions for ease of understanding.
3. **Administration:** Questionnaires were distributed during school hours under the supervision of the researcher and class teachers. On average, students took 20–25 minutes to complete the forms. Privacy and confidentiality were maintained to encourage honest responses.
4. **Scoring:** Responses on the DASS-21 were scored according to standardized guidelines, producing individual scores for depression, anxiety, and stress. Physical activity data were coded into activity levels (low, moderate, high) based on frequency and duration.

Data Analysis

Data were analyzed using both descriptive and inferential statistics. Descriptive analysis (mean, standard deviation, frequency distribution) provided a clear overview of the sample's mental health status. Inferential tests (t-tests, ANOVA, and Pearson's correlation) were applied to examine the relationship between physical activity and mental health indicators.

Innovative Approach

The methodology was made more holistic by going beyond traditional questionnaires and incorporating:

- **Activity Diaries:** A subset of students maintained a one-week diary recording daily physical activity, which enriched the reliability of self-reported data.



**Atul Kumar Tiwari et al.,**

- **Peer Group Discussions:** Short focus group interactions were conducted with volunteer students to gain qualitative insights into barriers and motivators for physical activity.
- **Triangulation of Data:** Combining quantitative scores (DASS-21) with qualitative inputs (activity diaries and group discussions) provided a richer, more contextual understanding of how physical activity influences adolescent mental health.

This mixed-method angle strengthens the study by not only quantifying the association but also exploring the lived experiences of students.

RESULTS

Demographic Distribution

The study sample consisted of 70 students aged 12–18 years, almost evenly distributed by gender. As shown in Table 1 and Figure 1, there were 36 males (51.4%) and 34 females (48.6%), indicating a balanced representation of both groups. This near-equal gender distribution reduces the likelihood of gender bias in the findings and strengthens the reliability of comparisons across physical activity and mental health variables [25, 26]. Such balance is particularly important in adolescent research, as stress, anxiety, and depression often present with different prevalence patterns across genders. Therefore, the inclusion of both male and female students in comparable proportions ensures that the results can be interpreted more holistically and generalized to the wider school-going population.

Physical Activity Status

The distribution of students based on their physical activity status was equally divided, with 35 students (50%) categorized as physically active and an equal number (35 students, 50%) categorized as physically inactive (Table 2 and Figure 2). This balanced split provides an excellent basis for comparison between the two groups, as neither category dominates the sample. The equal representation also strengthens the reliability of statistical analysis, allowing meaningful inferences about the impact of physical activity on mental health outcomes. Importantly, the presence of a large inactive group highlights a concerning trend of sedentary behavior among adolescents, which has been increasingly linked to elevated stress, anxiety, and depression [27, 28]. The balanced design of the sample thus makes it possible to clearly examine whether physical activity can act as a protective factor against psychological distress in school-going students.

Comparison of Mental Health Indicators

A clear difference was observed in the mental health status of physically active and inactive students, as reflected in the mean DASS-21 scores (Table 3 and Figure 3). Physically active students recorded mean scores of 8.3 for stress, 6.2 for anxiety, and 5.4 for depression, all of which fall within the normal range [29]. In contrast, physically inactive students reported substantially higher scores, with 18.6 for stress (moderate level), 16.1 for anxiety (moderate to severe), and 14.3 for depression (moderate level). These results indicate that inactive students scored two to three times higher across all three psychological domains compared to their active counterparts. While active students remained largely in the normal to mild categories, inactive students frequently fell into the moderate to severe categories, suggesting a heightened vulnerability to emotional distress. This stark contrast provides strong evidence that regular physical activity acts as a protective factor against stress, anxiety, and depression, whereas sedentary behavior is strongly associated with elevated psychological distress.

Severity Classification (DASS-21 Cut-offs)

The severity distribution of stress, anxiety, and depression revealed striking contrasts between physically active and inactive students (Table 4). Among the active group, the majority consistently fell within the normal to mild categories across all three domains: 65% reported normal stress, 70% normal anxiety, and 68% normal depression. Only a small proportion of active students experienced mild to moderate symptoms, with no cases of severe or extremely severe psychological distress recorded [25–29].



**Atul Kumar Tiwari et al.,**

In contrast, the inactive group showed a significantly higher prevalence of psychological difficulties. For stress, 40% were classified as moderate, 35% as severe, and 15% as extremely severe. Similarly, in anxiety, 30% were moderate, 40% severe, and 20% extremely severe, highlighting an alarming tendency toward severe anxiety symptoms. Depression followed the same trend, with 35% moderate, 40% severe, and 15% extremely severe among inactive students. This severity profile clearly demonstrates that while physically active students are largely protected from higher levels of emotional distress, physically inactive students are disproportionately concentrated in the moderate-to-extremely severe categories, particularly for anxiety and depression. These findings underscore the urgent need for schools and families to promote regular physical activity as a preventive and non-stigmatizing strategy to safeguard adolescent mental health.

DISCUSSION

The present study examined the relationship between physical activity and mental health indicators among adolescents, focusing on stress, anxiety, and depression. The demographic balance in terms of gender (51.4% male, 48.6% female) adds strength to the findings, as it reduces gender bias and allows the results to be generalized across both male and female students [30]. Previous studies have reported gender-specific variations in psychological distress, with females often showing higher susceptibility to anxiety and depression, while males frequently exhibit elevated stress levels [31]. By maintaining a nearly equal distribution, the present study ensures that the observed differences in mental health outcomes are primarily attributable to activity status rather than gender-related biases.

A particularly important observation of this study is the equal distribution of students into physically active and inactive groups, creating a balanced framework for comparison. The finding that half of the students were physically inactive is itself noteworthy, as it highlights the growing prevalence of sedentary behavior among school-going adolescents—a trend that has been consistently linked with poor psychological outcomes. This sedentary pattern is often reinforced by excessive screen time, academic stress, and limited opportunities for structured physical activity, making it an urgent public health concern.

The results clearly demonstrate that physically active students scored significantly lower on stress, anxiety, and depression compared to their inactive peers. Active students maintained DASS-21 scores within the normal range, while inactive students exhibited scores 2–3 times higher, frequently falling into moderate-to-severe categories [25–31]. This finding aligns with numerous studies which suggest that exercise plays a protective role against psychological distress by modulating neurobiological and psychosocial pathways. For instance, regular physical activity enhances the release of endorphins, dopamine, and serotonin, neurotransmitters that improve mood, reduce perceived stress, and regulate emotional responses [32]. In addition, exercise helps regulate the hypothalamic-pituitary-adrenal (HPA) axis, thereby reducing cortisol hypersecretion, which is often elevated in individuals with chronic stress and anxiety [33].

The severity classification analysis further strengthens these observations. Among active students, the majority fell within normal to mild categories, with no cases of severe or extremely severe distress. In sharp contrast, a large proportion of inactive students exhibited moderate-to-extremely severe symptoms across all domains, particularly in anxiety and depression. This reflects that inactivity not only increases the mean level of psychological distress but also disproportionately pushes students into clinically concerning ranges. From a mechanistic perspective, sedentary behavior is associated with increased inflammatory markers (e.g., IL-6, CRP, TNF- α) and reduced neuroplasticity, both of which are implicated in the pathophysiology of depression and anxiety [34]. Furthermore, inactivity reduces opportunities for social interaction and structured coping mechanisms, both of which are critical for emotional resilience during adolescence [35].

Taken together, these findings strongly support the hypothesis that physical activity acts as a non-pharmacological, preventive strategy against adolescent mental health disorders. By simultaneously targeting neurochemical, hormonal, and psychosocial mechanisms, exercise provides a multidimensional buffer against stress, anxiety, and





depression. Importantly, the present results highlight that students who do not engage in regular activity are at disproportionately higher risk of developing moderate to severe psychological distress. From a practical perspective, these findings underscore the urgent need to **integrate structured physical activity programs within school curricula**. Schools can play a transformative role by ensuring daily opportunities for aerobic and team-based exercises, which not only improve physical health but also serve as a low-cost, non-stigmatizing intervention for mental well-being. Encouraging students to reduce sedentary screen time and engage in outdoor physical pursuits may therefore be an effective public health strategy to safeguard adolescent mental health.

The Implication for Practice section

The findings of this study highlight the significant positive impact of regular physical activity on reducing levels of stress, anxiety, and depression among school-going adolescents, as measured by the DASS-21 scale. These results underscore the need for integrating structured physical activity programs into school curricula and daily routines to support students' mental health and overall well-being. Health care practitioners, school counselors, and educators should collaborate to promote active lifestyles through school-based interventions, including regular physical education classes, extracurricular sports, and activity breaks throughout the day. Additionally, awareness campaigns targeting both students and parents about the mental health benefits of physical activity could enhance participation and motivation. Given the growing concerns about adolescent mental health, these findings support the role of physical activity as a non-pharmacological, accessible, and cost-effective approach to mental health promotion. Regular screening using tools like DASS-21 can help identify at-risk students and guide timely, targeted interventions that incorporate physical activity as a core component of care.

CONCLUSION

The present study demonstrates a clear and consistent relationship between physical activity and mental health among adolescents aged 12–18 years. The results showed that physically active students exhibited significantly lower levels of stress, anxiety, and depression compared to their inactive peers. Most active students remained within the normal to mild ranges of psychological distress, while inactive students were disproportionately concentrated in moderate to extremely severe categories. These findings align with existing literature, which highlights the positive influence of exercise on emotional regulation, cognitive function, and resilience. The mechanisms underlying this association can be explained through several biological and psychological pathways. Regular physical activity stimulates the release of endorphins, serotonin, and dopamine, which are known to improve mood and reduce symptoms of anxiety and depression. Exercise also regulates cortisol secretion, thereby reducing stress levels. Additionally, engaging in physical activity provides structured routines, opportunities for social interaction, and improvements in self-esteem, all of which serve as protective buffers against psychological distress. On the other hand, sedentary behavior is associated with social isolation, poor sleep quality, and maladaptive coping strategies, which exacerbate vulnerability to mental health difficulties.

The balanced gender representation and equal distribution of active and inactive students strengthen the reliability of these findings. Importantly, the high prevalence of severe anxiety and depression among inactive students raises concern, emphasizing the urgent need for targeted interventions. Schools should prioritize structured physical education programs, while families must encourage daily exercise as part of a healthy lifestyle. In conclusion, physical activity is not merely a tool for enhancing physical fitness but also a crucial determinant of adolescent psychological well-being. By promoting active lifestyles, communities can adopt a preventive, non-stigmatizing, and cost-effective approach to safeguarding youth mental health, ultimately fostering healthier and more resilient future generations.





Atul Kumar Tiwari et al.,

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Conflict of Interest

The author declares no conflict of interest; this work is based solely on academic study and analysis of secondary sources without any financial, commercial, or personal influence.

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Author Contribution

Atul Tiwari: Conceptualization, Experimental, Data curation, review & editing;

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Table 1. Gender distribution of participants

Gender	Number of Students	Percentage (%)
Male	36	51.4%
Female	34	48.6%
Total	70	100%

Table 2. Physical activity status of participants

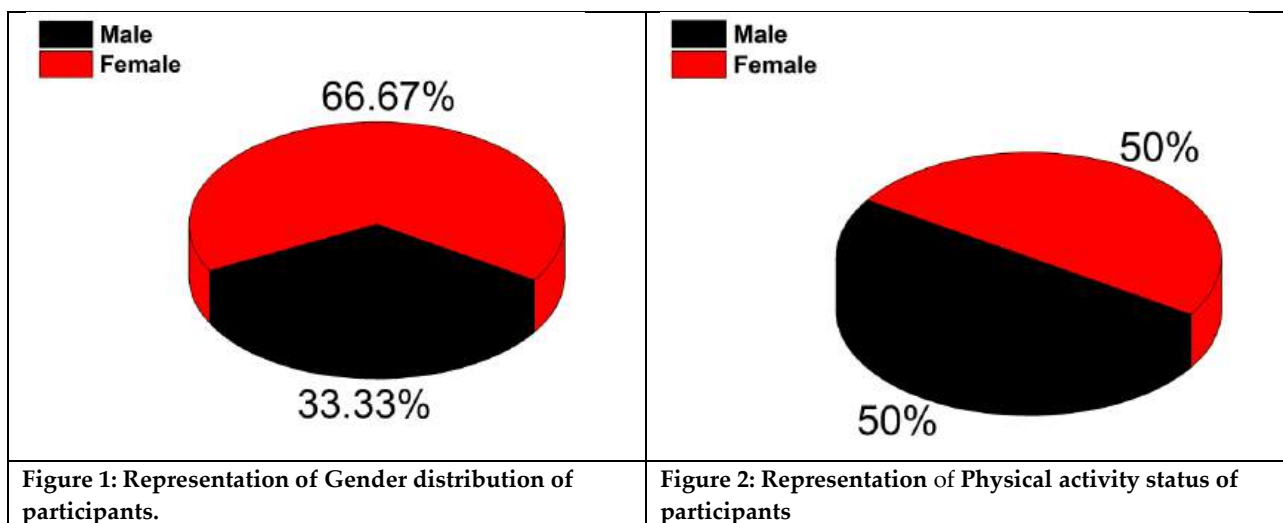
Activity Group	Number of Students	Percentage (%)
Physically Active	35	50%
Physically Inactive	35	50%
Total	70	100%

Table 3. Mean scores of stress, anxiety, and depression (DASS-21) among active vs. inactive students

Mental Health Measure	Physically Active (Mean)	Physically Inactive (Mean)
Stress	8.3 (Normal range)	18.6 (Moderate)
Anxiety	6.2 (Normal range)	16.1 (Moderate–Severe)
Depression	5.4 (Normal range)	14.3 (Moderate)

Table 4. Percentage distribution of severity levels

Scale	Active Students (n=35)	Inactive Students (n=35)
Stress	Mostly Normal (65%), Mild (25%), Few Moderate (10%)	Moderate (40%), Severe (35%), Extremely Severe (15%)
Anxiety	Normal (70%), Mild (20%), Moderate (10%)	Moderate (30%), Severe (40%), Extremely Severe (20%)
Depression	Normal (68%), Mild (22%), Moderate (10%)	Moderate (35%), Severe (40%), Extremely Severe (15%)



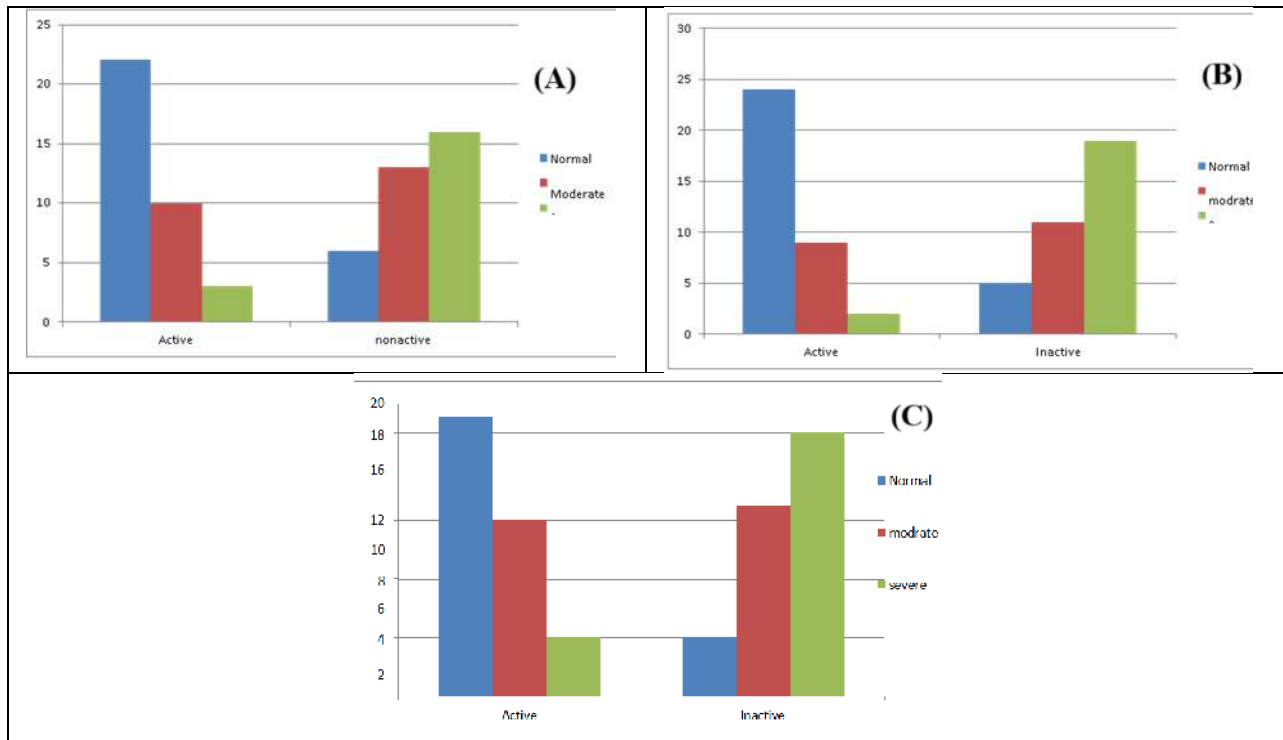
Atul Kumar Tiwari *et al.*,

Figure 3: Representative Mean scores of (A) stress, (B) anxiety, and (C) depression (DASS-21) among active vs. inactive students

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RESEARCH ARTICLE

Integrating Indigenous Ecological Knowledge into Environmental Education: Evidence from Northeast India

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ABSTRACT

The growing environmental crisis has intensified the need for contextually relevant and culturally responsive approaches to environmental education. Indigenous Ecological Knowledge (IEK), developed through generations of interaction between indigenous communities and their natural environments, offers valuable insights for sustainable resource management and environmental conservation. This paper examines the potential of integrating Indigenous Ecological Knowledge into environmental education, with particular reference to Northeast India, a region characterized by rich biodiversity and diverse indigenous cultures. Drawing on a review of literature, policy documents, and selected examples of indigenous environmental practices from the region, the study explores how traditional ecological knowledge contributes to biodiversity conservation, sustainable agriculture, forest management, and climate resilience. The findings indicate that indigenous knowledge systems embody holistic understandings of human-nature relationships that can enrich contemporary environmental education.

Keywords: Environmental Education, Northeast India, Indigenous Communities, Indigenous Ecological Knowledge, Sustainable Development

INTRODUCTION

The accelerating environmental crisis, characterized by biodiversity loss, climate change, deforestation, and unsustainable resource exploitation, has highlighted the urgent need for effective environmental education. Environmental education aims at developing environmental literacy, fostering ecological awareness, and promoting sustainable behaviors among individuals and communities. Since the emergence of global environmental concerns



**Pratisha Padmasri Deka**

towards the end of the 20th century, environmental education has been recognized as an important means of addressing environmental challenges and encouraging responsible environmental citizenship (UNESCO, 1978). However, conventional environmental education has often been criticized for relying predominantly on scientific and universalized knowledge systems while overlooking local cultural contexts and traditional ways of understanding the environment (Stevenson et al., 2017). In recent years, increasing attention has been directed toward Indigenous Ecological Knowledge (IEK) as a valuable resource for promoting sustainability and environmental stewardship. The set of information, customs, values, and beliefs that indigenous communities have accumulated by long-term interactions with their natural environments is referred to as indigenous ecological knowledge (Berkes, 2018). This knowledge encompasses detailed understandings of local ecosystems, biodiversity conservation, natural resource management, weather patterns, and sustainable agricultural practices. Studies have demonstrated that indigenous communities often maintain sophisticated ecological knowledge systems that contribute significantly to environmental conservation and resilience (Gadgil et al., 1993; Kimmerer, 2013). Integrating such knowledge into environmental education can enrich learning experiences by connecting scientific concepts with local realities and cultural traditions.

The relevance of Indigenous Ecological Knowledge is particularly evident in Northeast India, one of the world's biodiversity hotspots and home to numerous indigenous communities with rich cultural and ecological traditions. The region is characterized by diverse ecosystems, including forests, wetlands, mountains, and river systems, which have sustained indigenous livelihoods for centuries. Communities like the Garo, Mising, Bodo, Khasi, Nyishi, Apatani, and Adi have developed unique environmental practices that promote biodiversity conservation, sustainable agriculture, sacred grove protection, and community-based natural resource management (Tiwari et al., 1998; Ramakrishnan, 2001). These traditional practices embody holistic perspectives on human–nature relationships and offer valuable lessons for addressing contemporary environmental challenges. Despite the recognized significance of indigenous knowledge systems, their integration into formal educational curricula remains limited. Educational policies and curricula often prioritize standardized knowledge frameworks, resulting in the marginalization of indigenous perspectives and local ecological understandings (McKinley & Smith, 2019). In Northeast India, inadequate documentation, limited representation in textbooks, and insufficient teacher preparation further constrain the incorporation of Indigenous Ecological Knowledge into environmental education. Consequently, opportunities to promote culturally relevant learning, strengthen environmental stewardship, and preserve indigenous cultural heritage remain underutilized.

Against this backdrop, the present paper examines the potential of integrating Indigenous Ecological Knowledge into environmental education with special reference to Northeast India. By reviewing existing literature, policy frameworks, and examples of indigenous environmental practices, the study investigates how Indigenous Ecological Knowledge contributes to environmental literacy, sustainable development, and biodiversity protection. The paper argues that meaningful integration of indigenous perspectives into environmental education can enhance the relevance and effectiveness of environmental learning while supporting the preservation of valuable cultural and ecological knowledge systems. Such integration is particularly important in ecologically sensitive and culturally diverse regions where sustainable futures depend upon strengthening connections between local knowledge, environmental responsibility, and formal education.

REVIEW OF LITERATURE

The growing recognition of Indigenous Ecological Knowledge (IEK) within environmental education has generated substantial scholarly interest over the past three decades. Indigenous societies have built comprehensive environmental knowledge systems via long-term interactions with local ecosystems, according to early research. According to Gadgil et al. (1993), because indigenous knowledge integrates location-specific understandings of biological processes and sustainable resource use, it is essential to biodiversity conservation. Similarly, Berkes (2018) described Indigenous Ecological Knowledge as a dynamic and adaptive system integrating ecological observations,



**Pratisha Padmasri Deka**

cultural values, and environmental practices that contribute to sustainability. Research by Kimmerer (2013) further highlighted the complementary relationship between indigenous knowledge and scientific knowledge, suggesting that both systems can enrich environmental understanding when used together. Within the field of environmental education, Stevenson et al. (2017) noted that contemporary environmental learning should move beyond purely scientific perspectives and embrace diverse ways of knowing, including indigenous worldviews, to address complex environmental challenges. Studies conducted across different countries have demonstrated that incorporating indigenous perspectives into educational curricula promotes place-based learning, strengthens environmental stewardship, and enhances learners' understanding of human–nature relationships. McKinley and Smith (2019) emphasized that indigenous education frameworks encourage culturally responsive pedagogies that recognize local knowledge traditions and empower indigenous communities within formal educational systems.

The significance of Indigenous Ecological Knowledge is particularly evident in regions characterized by high biodiversity and cultural diversity, such as Northeast India. Indigenous groups in the area have created sustainable environmental practices that preserve ecological balance while satisfying subsistence demands, as recorded by Ramakrishnan (2001). Traditional systems of forest conservation, shifting cultivation management, and community resource governance have been shown to contribute significantly to ecosystem sustainability. Indigenous belief systems and customary institutions have been crucial in maintaining biodiversity and safeguarding endangered species, according to Tiwari et al. (1998), who studied holy groves in Meghalaya. Similar studies have demonstrated that indigenous agricultural practices, water management systems, and conservation traditions contribute to climate resilience and environmental sustainability in the region. Despite these contributions, scholars have consistently reported the marginalization of indigenous knowledge within formal educational curricula. McKinley and Smith (2019) observed that centralized curriculum structures often privilege dominant scientific narratives while neglecting local knowledge systems. Furthermore, Stevenson et al. (2017) highlighted that environmental education programmes frequently lack culturally relevant content that reflects indigenous perspectives and local ecological realities. As a result, important chances to link environmental education with local experiences and traditional ecological practices are still underutilized, especially in culturally diverse areas where local expertise might greatly improve sustainability teaching.

Research Gap

The literature already published amply illustrates the significance of Indigenous Ecological Knowledge for environmental stewardship, sustainable resource management, and biodiversity conservation. However, most studies have focused either on documenting indigenous environmental practices or examining their ecological significance. Relatively limited attention has been given to the systematic integration of Indigenous Ecological Knowledge into environmental education, particularly within the formal educational context of Northeast India. Furthermore, there is a lack of comprehensive studies exploring how indigenous ecological perspectives can be incorporated into school curricula, teacher education programmes, and community-based environmental learning initiatives. There is still a lack of research on issues related to curricular integration, teacher preparation, policy implementation, and the preservation of indigenous knowledge systems. Thus, by investigating the possibility of incorporating Indigenous Ecological Knowledge into environmental education and figuring out how to create culturally sensitive and sustainable teaching methods in Northeast India, the current study aims to bridge this research gap.

METHODOLOGY

With an emphasis on Northeast India, this study used a methodical literature review technique to investigate how Indigenous Ecological Knowledge (IEK) is incorporated into environmental education. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) framework (Page et al., 2021), which offers a clear and exacting process for locating, screening, and choosing pertinent literature, served as the review's guidance.



**Pratisha Padmasri Deka****Search Strategy**

Relevant literature was retrieved from major academic databases, including Scopus, Web of Science, ERIC, Google Scholar, and JSTOR. The search was conducted using combinations of keywords such as "Indigenous Ecological Knowledge," "Traditional Ecological Knowledge," "Environmental Education," "Environmental Literacy," "Indigenous Communities," "Biodiversity Conservation," "Sustainable Development," and "Northeast India." Additional sources were identified through reference tracking of key publications and policy documents published by UNESCO, UNEP, and governmental agencies.

Inclusion and Exclusion Criteria

Studies were included if it:

- With an emphasis on traditional or indigenous ecological knowledge.
- Examined indigenous environmental practices, biodiversity conservation, sustainability education, or environmental education.
- Appeared in books, book chapters, conference proceedings, peer-reviewed journals, or policy reports.
- Were written in English.
- Was pertinent to indigenous groups around the world or to Northeast India in particular.

Studies were excluded if they:

- Did not address ecological or environmental knowledge systems.
- Focused solely on indigenous cultural practices without environmental relevance.
- Were duplicate records, opinion pieces, or non-scholarly sources lacking sufficient methodological rigor.

Study Selection

186 records were found in the first database search. 148 studies were left for title and abstract screening after 38 duplicate records were eliminated. 92 studies were eliminated after screening because they had no bearing on environmental education or Indigenous ecological knowledge. 56 studies' entire texts were evaluated for eligibility. 34 research and policy papers were kept for final analysis after the inclusion and exclusion criteria were applied. These sources included empirical studies, theoretical works, case studies, policy reports, and regional investigations related to indigenous environmental practices and environmental education.

Data Analysis

Thematic content analysis was used to examine the chosen studies. Data about climate resilience, place-based learning, curricular integration, sustainable agriculture, forest management, biodiversity conservation, environmental stewardship, and indigenous pedagogies were gathered and arranged into thematic categories. Particular attention was given to studies documenting indigenous environmental practices in Northeast India and their implications for environmental education. The findings from the reviewed literature were synthesized to identify major contributions, challenges, and opportunities associated with integrating Indigenous Ecological Knowledge into environmental education.

PRISMA Flow Summary

- Records identified through database searching: 186
- Duplicate records removed: 38
- Records screened: 148
- Records excluded after title and abstract screening: 92
- Full-text articles assessed for eligibility: 56
- Full-text articles excluded: 22
- Studies included in the final review: 34





Pratisha Padmasri Deka

RESULTS AND DISCUSSION

The review revealed that Indigenous Ecological Knowledge (IEK) constitutes a rich repository of environmental understanding developed through generations of close interaction with local ecosystems. Berkes (2018) described IEK as an adaptive knowledge system that integrates ecological observations, cultural beliefs, and resource management practices to maintain ecological balance. Similarly, Gadgil et al. (1993) argued that indigenous communities possess detailed ecological knowledge that contributes significantly to biodiversity conservation and sustainable resource use. Across the studies reviewed, indigenous communities demonstrated extensive knowledge of biodiversity, seasonal cycles, soil conservation, water management, and sustainable harvesting practices. Indigenous people in Northeast India have long used local based resource management systems to control how forests, waterways, and agricultural lands are used. Indigenous environmental practices in the area have developed over centuries of contact with local ecosystems and continue to support sustainable lifestyles, according to Ramakrishnan (2001). The findings suggest that IEK provides a strong foundation for environmental sustainability by promoting ecological stewardship, conservation ethics, and responsible utilization of natural resources. Following findings were drawn:

Contributions of Indigenous Practices to Biodiversity Conservation and Climate Resilience

A major theme emerging from the reviewed studies is the significant contribution of indigenous environmental practices to biodiversity conservation and climate resilience. Sacred groves in Meghalaya are efficient community-managed conservation zones that safeguard a variety of plant and animal species while maintaining ecological integrity, as shown by Tiwari et al. (1998). Similarly, Ramakrishnan (2001) highlighted the role of traditional agricultural systems, including shifting cultivation practices adapted to local ecological conditions, in maintaining ecosystem productivity and biodiversity. Studies conducted in different indigenous contexts worldwide have also shown that traditional ecological practices often promote resilience by encouraging crop diversity, sustainable harvesting, and adaptive responses to environmental change (Berkes, 2018). The reviewed literature indicates that indigenous communities in Northeast India possess valuable knowledge related to climate variability, seasonal forecasting, and natural resource management, which can support contemporary sustainability efforts. In order to give students locally applicable examples of sustainable living and climate adaptation, these findings highlight the significance of integrating indigenous conservation techniques into environmental education.

Relevance of Indigenous Ecological Knowledge for Environmental Education

The review found strong evidence supporting the integration of Indigenous Ecological Knowledge into environmental education. Stevenson et al. (2017) argued that environmental education should embrace diverse knowledge systems and move beyond narrowly scientific approaches to address complex environmental challenges. Likewise, Kimmerer (2013) emphasized that indigenous and scientific knowledge systems can complement one another, offering a more holistic understanding of environmental processes. The studies reviewed suggest that incorporating indigenous perspectives into environmental education promotes place-based learning, strengthens environmental ethics, and enhances learners' understanding of human-nature relationships. McKinley and Smith (2019) further noted that culturally responsive educational frameworks improve learner engagement by recognizing and valuing local knowledge traditions. In the context of Northeast India, integrating indigenous ecological practices into environmental curricula can help students connect classroom learning with community experiences and local environmental realities. Such integration can foster environmental literacy while simultaneously promoting respect for cultural diversity and indigenous heritage.

Challenges and Pathways for Integration

Despite growing recognition of the value of Indigenous Ecological Knowledge, the review identified several challenges that hinder its meaningful integration into formal education systems. McKinley and Smith (2019) observed that centralized educational structures frequently privilege dominant scientific paradigms while marginalizing indigenous perspectives. Stevenson et al. (2017) similarly noted that environmental education curricula often lack adequate representation of local knowledge systems and community-based learning approaches.

117203



**Pratisha Padmasri Deka**

The reviewed literature highlights additional barriers, including inadequate documentation of indigenous knowledge, declining intergenerational knowledge transmission, limited teacher preparedness, and insufficient policy support. Globalization and fast socioeconomic change have further eroded traditional ecological practices in Northeast India. The findings do, however, offer provide possible integration routes. These include collaborative partnerships between schools and indigenous communities, teacher education programs that emphasize culturally responsive pedagogy, curriculum reforms that include indigenous environmental content, and efforts to record and preserve traditional ecological knowledge. The results taken together indicate that incorporating Indigenous Ecological Knowledge into environmental education can support more inclusive teaching methods, cultural preservation, and environmental sustainability.

Evidence from Northeast India**Sacred Groves and Community-Based Forest Conservation**

The custom of holy groves upheld by indigenous groups, especially among the Khasi and Jaintia tribes of Meghalaya, is one of the best illustrations of Indigenous Ecological Knowledge in Northeast India. Sacred groves are areas of woodland that are safeguarded by spiritual ideals, cultural norms, and customary rules. According to Tiwari et al. (1998), these groves act as biodiversity reservoirs, preserving a variety of rare, endemic, and therapeutic plant species while preserving ecological equilibrium. Unlike state-managed conservation approaches, sacred groves are governed by community norms that regulate human activities and discourage resource exploitation. Similar observations were made by Ramakrishnan (2001), who noted that indigenous institutions have historically played a significant role in preserving forest ecosystems across Northeast India. The evidence suggests that these traditional conservation systems can provide valuable case studies for environmental education by demonstrating practical examples of sustainable environmental stewardship and collective responsibility toward nature.

Indigenous Agricultural Knowledge and Sustainable Resource Management

The review also identified indigenous agricultural practices as important manifestations of ecological knowledge in Northeast India. Communities such as the Apatani of Arunachal Pradesh have developed highly sustainable farming systems that integrate wet rice cultivation, fish farming, and water management without heavy dependence on chemical inputs. Ramakrishnan (2001) highlighted that many indigenous agricultural systems in the region are based on principles of ecological balance, crop diversity, nutrient recycling, and adaptation to local environmental conditions. Traditional shifting cultivation practices, when managed according to customary ecological principles, have also been shown to support biodiversity and soil regeneration. These practices reflect a deep understanding of ecosystem processes and resource sustainability. From an educational perspective, such indigenous farming systems provide locally relevant examples through which students can learn about sustainable agriculture, food security, and environmental management. Their inclusion in environmental education can help bridge the gap between theoretical knowledge and real-world ecological practices.

Traditional Ecological Knowledge and Climate Adaptation

Another significant contribution of Indigenous Ecological Knowledge in Northeast India relates to climate adaptation and disaster resilience. Indigenous communities have long relied on environmental indicators such as animal behavior, flowering patterns, rainfall variability, and seasonal changes to predict weather conditions and adapt their livelihood practices accordingly. Berkes (2018) observed that traditional ecological knowledge systems often enhance community resilience by enabling flexible responses to environmental uncertainties. In Northeast India, indigenous knowledge related to water conservation, crop selection, forest management, and disaster preparedness has helped communities cope with climatic fluctuations and environmental risks. Such knowledge has become increasingly relevant in the context of contemporary climate change challenges. The findings suggest that environmental education programmes can benefit from incorporating indigenous climate adaptation strategies, thereby helping learners understand locally grounded responses to environmental change while appreciating the value of traditional ecological wisdom. Integrating these experiences into educational curricula can contribute to more context-sensitive, culturally relevant, and action-oriented environmental learning.





Bamboo Drip Irrigation System of Meghalaya

The indigenous bamboo drip irrigation system practiced by the Khasi and Jaintia communities of Meghalaya represents a remarkable example of traditional ecological engineering. This system, estimated to be over 200 years old, utilizes locally available bamboo pipes to channel water from hilltop springs and streams to agricultural fields located on lower slopes. The design efficiently regulates water flow, reducing large volumes of water into a controlled drip system suitable for crops such as betel leaf, black pepper, and arecanut. Studies have highlighted that the technology is highly sustainable because it relies on renewable materials, gravity-based water transport, and minimal environmental disturbance (Centre for Science and Environment, 2023). The system demonstrates indigenous communities' sophisticated understanding of water conservation and resource management. Incorporating such examples into environmental education can help students appreciate low-cost, eco-friendly technologies that promote sustainable water use and climate adaptation.

Zabo Farming System of Nagaland

The Zabo ("impounding water") farming system practiced in Nagaland, particularly in the Phek district, is another notable example of Indigenous Ecological Knowledge. This integrated land-use system combines forest conservation, water harvesting, livestock rearing, and terrace agriculture within a single ecological framework. Rainwater is collected from forested hilltops and stored in ponds before being distributed to agricultural fields. Singh and Gupta (2002) observed that the system effectively conserves soil and water while ensuring sustainable agricultural production. Traditional community institutions regulate the use and maintenance of these resources, ensuring long-term ecological sustainability. The Zabo system illustrates how indigenous communities have developed holistic approaches to resource management that integrate ecological, social, and economic dimensions. Environmental education can utilize such practices as practical examples of ecosystem-based management and sustainable development.

Apatani Rice–Fish Culture and Agroforestry Systems

The Apatani community of Arunachal Pradesh has developed one of the most widely recognized indigenous agricultural systems in India. The Apatani wet rice–fish cultivation system integrates paddy farming with fish rearing, enabling efficient utilization of land and water resources while enhancing food security. Researchers have noted that the system maintains high productivity without extensive use of chemical fertilizers or external inputs. In addition, the Apatani landscape incorporates community-managed forests, bamboo groves, and agroforestry practices that support biodiversity conservation and sustainable livelihoods. Tangjang and Nair (2016) identified Apatani bamboo-pine homegardens as unique agroforestry systems that demonstrate long-term ecological adaptation and resource sustainability. In a similar vein, indigenous customs and cultural traditions continue to safeguard sacred groves known as ranthii, which helps to conserve biodiversity in the Ziro Valley. These instances demonstrate how indigenous knowledge systems can promote environmental sustainability while providing educational opportunities for environmental education initiatives.

CONCLUSION

The present study demonstrates that Indigenous Ecological Knowledge (IEK) represents a valuable yet underutilized resource for strengthening environmental education and promoting sustainable development. The systematic review revealed that indigenous knowledge systems embody holistic understandings of ecological relationships, resource management, and environmental stewardship that have evolved through generations of interaction with local ecosystems. Scholarly evidence indicates that Indigenous Ecological Knowledge contributes significantly to biodiversity conservation, climate resilience, sustainable agriculture, and community-based natural resource management. These findings reinforce the argument that environmental education must move beyond exclusively scientific approaches and embrace diverse knowledge traditions to address contemporary environmental challenges effectively. The study highlights the significant potential of Indigenous Ecological Knowledge (IEK) to enrich



**Pratisha Padmasri Deka**

environmental education by providing culturally grounded, context-specific, and sustainability-oriented perspectives on environmental stewardship. The review demonstrates that indigenous communities in Northeast India possess extensive ecological knowledge related to biodiversity conservation, sustainable agriculture, water management, forest protection, and climate adaptation. Practices such as sacred grove conservation in Meghalaya, bamboo drip irrigation systems, the Zabo farming system of Nagaland, and the Apatani rice–fish cultivation system of Arunachal Pradesh illustrate how indigenous communities have developed sophisticated and sustainable approaches to managing natural resources. These examples reveal that Indigenous Ecological Knowledge embodies holistic understandings of human–nature relationships that can complement scientific knowledge and contribute to more effective environmental learning.

A major contribution of this study lies in synthesizing existing literature on Indigenous Ecological Knowledge and environmental education while bringing together diverse examples from Northeast India within a common educational framework. Unlike many previous studies that have primarily focused on documenting indigenous practices or their ecological significance, this review explicitly examines their relevance for environmental education and sustainability learning. By identifying the educational value of indigenous conservation practices and knowledge systems, the study provides a foundation for integrating local ecological knowledge into curricula, teacher education programmes, and community-based learning initiatives. The findings further emphasize the need for culturally responsive environmental education that recognizes indigenous communities not merely as knowledge holders but as active partners in promoting sustainable development and environmental conservation. The study also highlights the need for further research in this emerging field. Future studies should undertake empirical investigations to examine how Indigenous Ecological Knowledge can be effectively incorporated into school curricula, classroom pedagogy, and teacher preparation programmes. Comparative studies across different indigenous communities of Northeast India may provide deeper insights into diverse ecological knowledge systems and their educational applications. Research is also needed to explore students' perceptions of indigenous knowledge, evaluate the outcomes of culturally responsive environmental education interventions, and develop curriculum models that integrate scientific and indigenous perspectives. In addition, efforts should be directed toward documenting endangered ecological knowledge and examining the role of digital technologies in preserving and disseminating indigenous environmental wisdom. Such research will contribute to building more inclusive, contextually relevant, and sustainable educational frameworks capable of addressing contemporary environmental challenges while safeguarding valuable cultural heritage.

Conflict of Interest

None

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**Pratisha Padmasri Deka**

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REVIEW ARTICLE

A Review: Chemistry, Pharmacological and Therapeutic Perspectives, and Emerging Advances of Sulfamethoxazole

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ABSTRACT

Sulfamethoxazole is a widely used sulfonamide antimicrobial agent that has played a significant role in the treatment of bacterial infections for several decades. It acts by inhibiting bacterial folic acid synthesis through competitive antagonism of para-aminobenzoic acid, thereby preventing the formation of essential nucleic acid precursors required for microbial growth. Sulfamethoxazole is commonly administered in combination with trimethoprim, producing a synergistic antibacterial effect against a broad spectrum of Gram-positive and Gram-negative microorganisms. Despite the emergence of antimicrobial resistance, sulfamethoxazole continues to hold therapeutic importance in the management of urinary tract infections, respiratory tract infections, gastrointestinal infections, and opportunistic infections such as *Pneumocystis jirovecii* pneumonia. Recent research has focused on the synthesis and evaluation of sulfamethoxazole derivatives to overcome resistance, improve pharmacokinetic properties, and reduce adverse drug reactions. Structural modification of the sulfamethoxazole scaffold has led to the development of derivatives exhibiting enhanced antimicrobial, anti-inflammatory, and multifunctional biological activities. Advances in medicinal chemistry, computational drug design, and nanotechnology-based drug delivery systems have further expanded the potential applications of





Neelam et al.,

sulfamethoxazole derivatives. This review summarizes the chemistry, mechanism of action, pharmacological properties, therapeutic applications, and recent advances related to sulfamethoxazole and its derivatives.

Keyword: Sulfamethoxazole, Chemistry, Clinical aspect, Recent advances

INTRODUCTION

Sulfamethoxazole is a synthetic sulfonamide antibiotic that remains an important therapeutic agent in the treatment of bacterial infections despite the growing challenge of antimicrobial resistance [1]. As a member of the sulfonamide class, sulfamethoxazole possesses broad-spectrum antibacterial activity and is widely used either alone or, more commonly, in combination with trimethoprim as co-trimoxazole. Since the introduction of sulfonamides in the early twentieth century, these agents have revolutionized antimicrobial chemotherapy by providing selective inhibition of bacterial metabolic pathways [2]. Sulfamethoxazole, chemically known as 4-amino-N-(5-methylisoxazol-3-yl) benzenesulfonamide, continues to attract considerable scientific interest due to its clinical effectiveness, well-characterized pharmacology, and potential for structural modification into biologically active derivatives [3]. The antibacterial activity of sulfamethoxazole is primarily associated with inhibition of bacterial folate biosynthesis [1]. It acts as a structural analogue of para-aminobenzoic acid (PABA) and competitively inhibits the enzyme dihydropteroate synthase (DHPS), thereby preventing the formation of dihydrofolic acid, an essential precursor for nucleic acid and protein synthesis [4]. Because bacterial cells synthesize folic acid de novo while mammalian cells obtain folate from dietary sources, this metabolic difference provides selective toxicity and therapeutic utility [5]. When combined with trimethoprim, which inhibits dihydrofolate reductase, a sequential blockade of folate metabolism is produced, leading to synergistic antibacterial action and improved clinical efficacy against susceptible pathogens.

Sulfamethoxazole-containing formulations are extensively used in the management of urinary tract infections, respiratory tract infections, gastrointestinal infections, skin and soft tissue infections, and opportunistic infections such as *Pneumocystis jirovecii* pneumonia and toxoplasmosis [6]. Its favorable oral bioavailability, relatively low cost, and broad antimicrobial coverage have sustained its use worldwide [4]. Nevertheless, extensive and often inappropriate antibiotic use has accelerated the development of bacterial resistance, reducing the effectiveness of many conventional antimicrobial agents, including sulphonamides [7]. Global health agencies continue to identify antimicrobial resistance as a major public health threat requiring urgent innovation in antibacterial drug discovery and stewardship. The increasing prevalence of resistant microorganisms has renewed interest in sulfamethoxazole chemistry and derivative development [6]. Resistance to sulfamethoxazole commonly occurs through mutations in the DHPS enzyme, altered bacterial permeability, efflux mechanisms, or acquisition of mobile resistance genes encoding resistant enzyme variants [8]. These challenges have stimulated medicinal chemists to explore structural modification of the sulfamethoxazole scaffold in order to enhance potency, improve pharmacokinetic behavior, minimize toxicity, and overcome resistance mechanisms [7]. Current approaches include substitution on the sulfonamide nucleus, incorporation of heterocyclic rings, hybrid molecule formation, and conjugation with bioactive pharmacophores.

Recent studies have demonstrated that sulfamethoxazole derivatives and related sulfonamide compounds exhibit diverse biological activities extending beyond antibacterial action [9]. Modified sulfonamides have shown anti-inflammatory, antifungal, anticancer, enzyme inhibitory, and neuro protective properties, highlighting the versatility of this pharmacophore in medicinal chemistry [10]. Contemporary research on sulfonamide derivatives emphasizes rational drug design, molecular docking, structure–activity relationship (SAR) studies, and hybrid scaffold development to generate multifunctional therapeutic agents. In addition, advances in computational chemistry, nanotechnology-based delivery systems, and targeted drug formulation strategies have expanded the pharmaceutical





Neelam et al.,

potential of sulfamethoxazole and its derivatives [11]. Novel nanoformulations, polymeric carriers, and combination therapies are being investigated to improve bioavailability, control drug release, reduce adverse reactions, and restore antibacterial effectiveness against resistant strains [12]. These developments align with the broader global effort to combat antimicrobial resistance through innovative therapeutic design and optimized antibiotic use. Therefore, sulfamethoxazole and its derivatives remain a significant focus of pharmaceutical and medicinal chemistry research [13]. Understanding their chemical characteristics, pharmacological mechanisms, therapeutic applications, and recent scientific advances is essential for developing improved antimicrobial agents capable of addressing present and future infectious disease challenges.

Chemistry and Structure of Sulfamethoxazole

Sulfamethoxazole (SMX) is a synthetic sulfonamide antibiotic widely used in antimicrobial therapy and serves as an important structural scaffold for the development of biologically active derivatives [14]. Its chemical architecture combines a sulfonamide pharmacophore with a heterocyclic isoxazole ring, a structural arrangement responsible for its antibacterial activity and favorable pharmacokinetic properties. Owing to its versatile chemistry, sulfamethoxazole remains an important molecule in medicinal chemistry and drug design research [15]. Sulfamethoxazole is chemically named 4-amino-N-(5-methylisoxazol-3-yl) benzenesulfonamide. It belongs to the sulfonamide family of antimicrobial agents and possesses the molecular formula $C_{10}H_{11}N_3O_3S$ with a molecular weight of 253.28g/mol [16]. The compound contains three major structural regions:

- Para-aminobenzene (aniline) ring
- Sulfonamide functional group ($-SO_2NH-$)
- 5-Methylisoxazole heterocyclic ring

This combination of aromatic, heterocyclic, and sulfonamide components determines its biological activity, physicochemical behavior, and drug-target interactions. Structurally, sulfamethoxazole was shown in Figure 1. The molecule is classified as a heterocyclic sulfonamide, and the incorporation of an isoxazole ring distinguishes it from earlier sulfonamide drugs [14]. Modern crystallographic and synthetic studies emphasize the importance of heterocyclic substitution in improving the chemical and pharmacological properties of sulfonamide antibiotics.

Clinical Uses of Sulfamethoxazole

Sulfamethoxazole is a broad-spectrum sulfonamide antimicrobial agent. Sulfamethoxazole (SMX) derivatives have attracted considerable attention in medicinal chemistry because structural modification of the sulfonamide scaffold can produce compounds with diverse biological activities beyond conventional antibacterial action [17]. Recent studies demonstrate that sulfamethoxazole derivatives possess Urinary Tract Infections (UTIs), Respiratory Tract Infections, antimicrobial, antifungal, anticancer, anti-inflammatory, antioxidant, antidiabetic, and enzyme inhibitory activities, emphasizing the versatility of the sulfonamide pharmacophore [18].

- **Urinary Tract Infections (UTIs):** One of the most common clinical uses of sulfamethoxazole is the treatment of urinary tract infections (UTIs) caused by susceptible organisms such as *Escherichia coli*, *Klebsiella* spp., and *Proteus* spp [16]. The high urinary concentration achieved by sulfamethoxazole contributes significantly to therapeutic effectiveness.
- **Respiratory Tract Infections:** Sulfamethoxazole is used in selected respiratory tract infections caused by susceptible pathogens. The drug is particularly useful when infections involve susceptible *Haemophilus influenzae* and some Gram-positive organisms [17]. Nevertheless, resistance among respiratory pathogens has limited routine first-line use in some regions.
- **Antibacterial Activity:** The most prominent biological activity of sulfamethoxazole derivatives remains their antibacterial effect. Structural modification of sulfamethoxazole can improve interaction with bacterial enzymes such as dihydropteroate synthase (DHPS) and enhance activity against resistant pathogens [18]. Recent studies have shown that Schiff bases, hydrazones, and heterocyclic sulfamethoxazole analogs exhibit strong antibacterial activity against both Gram-positive and Gram-negative bacteria. Several newly synthesized derivatives demonstrated lower minimum inhibitory concentrations (MICs) than parent sulfamethoxazole, suggesting





Neelam et al.,

improved antimicrobial potency and possible usefulness against resistant bacterial strains [19]. Molecular docking investigations further support enhanced target binding of these derivatives.

- **Antifungal Activity:** Sulfamethoxazole derivatives have also demonstrated antifungal activity against fungi. Sulfonamide-containing heterocycles and metal complexes have shown inhibitory effects against *Candida albicans*, *Aspergillus* species [20]. The antifungal mechanism is thought to involve disruption of fungal metabolic enzymes, membrane integrity, and inhibition of essential biosynthetic pathways [21]. Metal-sulfamethoxazole complexes, particularly Cu (II), Zn (II), and Ni (II) complexes, often exhibit stronger antifungal activity than the parent drug due to altered lipophilicity and cellular uptake.
- **Anticancer Activity:** One of the most promising emerging applications of sulfamethoxazole derivatives is their anticancer activity. Recent study has shown that modified sulfonamide scaffolds can inhibit tumor growth [15]. Hydrazone and Schiff-base derivatives of sulfamethoxazole have demonstrated cytotoxic effects against multiple human cancer cells.
- **Anti-Inflammatory Activity:** Recently reports are notable anti-inflammatory properties among sulfamethoxazole derivatives, particularly Schiff bases and heterocyclic hybrids [12]. Certain sulfamethoxazole-derived compounds showed significant effect in inhibition in protein denaturation assays and molecular docking studies involving inflammatory targets [21]. Such findings suggest their potential development as safer anti-inflammatory agents.
- **Antioxidant Activity:** Oxidative stress contributes to numerous pathological disorders, and several sulfamethoxazole derivatives have demonstrated antioxidant activity. In this recent study the Schiff-base derivatives exhibited strong DPPH radical scavenging activity, in some cases exceeding standard antioxidants such as ascorbic acid [12]. Enhanced antioxidant properties are often associated with conjugated aromatic systems and electron-donating substituents incorporated into the sulfamethoxazole framework.
- **Antidiabetic and Enzyme Inhibitory Activity:** A growing area of research involves enzyme inhibition of α -amylase and α -glucosidase may help reduce post-prandial glucose levels and support diabetes management [22]. Carbonic anhydrase inhibition was particularly significant because this enzyme family was helpful in glaucoma, epilepsy, obesity, and cancer.
- **Metal Complexes and Multifunctional Activity:** Metal complexation strategy was very useful in enhancing the biological performance of sulfamethoxazole derivatives [22]. They are often demonstrated antimicrobial, anticancer, and antioxidant activity.

Emerging Advancement

Recent advances in sulfamethoxazole and its derivatives reflect a transition from traditional antibacterial therapy toward multifunctional drug discovery [20]. Progress in synthetic chemistry, molecular docking, metal complexation [21], nanotechnology [4], and targeted pharmacology has significant therapeutic potential of sulfamethoxazole and its derivatives.

CONCLUSION

Sulfamethoxazole remains a clinically important and scientifically valuable sulfonamide antibiotic owing to its well-established chemistry, broad pharmacological profile, and diverse therapeutic applications. Its mechanism of inhibiting bacterial folate synthesis has sustained its role in the treatment of numerous bacterial and opportunistic infections. Beyond conventional antimicrobial use, recent advances in medicinal chemistry have demonstrated that sulfamethoxazole serves as a versatile scaffold for the development of novel derivatives exhibiting antibacterial, antifungal, anticancer, anti-inflammatory, and enzyme-inhibitory activities. Progress in molecular docking, computational drug design, metal complexation, and nanotechnology-based drug delivery has further expanded its therapeutic potential and provided promising strategies to overcome antimicrobial resistance and improve drug efficacy.





Neelam et al.,

List of Abbreviations

SMX: Sulfamethoxazole; (PABA): Para-aminobenzoic acid; DHPS: dihydropteroate synthase; SAR: structure–activity relationship; MICs: Minimum Inhibitory Concentrations; UTIs; Urinary Tract Infections.

Ethics Approval And Consent To Participate- Not applicable

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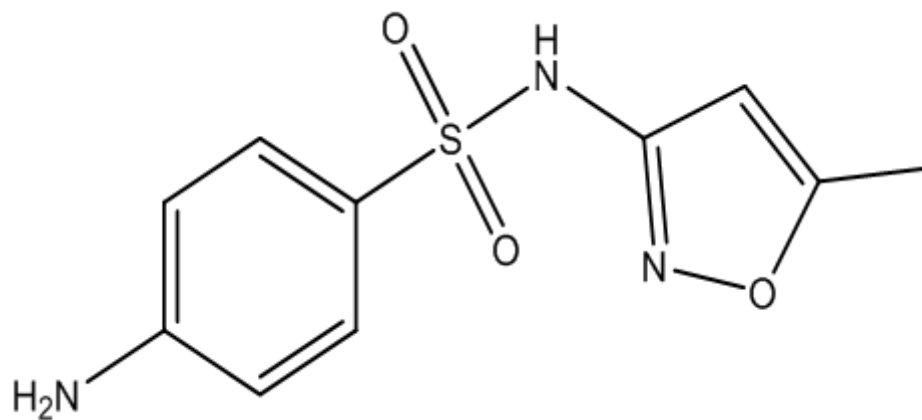


Figure 1: General structure of 4-amino-N-(5-methylisoxazol-3-yl) benzenesulfonamide

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Intuitionistic Fuzzy Semi - Bi-Ideal Structures in Rings

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ABSTRACT

This paper presents the idea of intuitionistic fuzzy semi bi-ideals within a ring and explores their characteristics. By expanding on the existing concepts of intuitionistic fuzzy subrings and one-sided ideals, we introduce a novel type of intuitionistic fuzzy algebraic structure that extends the traditional notion of a semi bi-ideal. The study reveals several significant findings, such as the discovery that the intersection of two intuitionistic fuzzy right ideals also results in an intuitionistic fuzzy right ideal. The main outcome of the paper is a description of regular rings. We demonstrate that a ring is regular if and only if every intuitionistic fuzzy right ideal qualifies as an intuitionistic fuzzy semi bi-ideal. Additionally, we show that a ring is regular if and only if the product of any two intuitionistic fuzzy semi bi-ideals matches their intersection. The paper concludes by proving that the level sets of an intuitionistic fuzzy semi bi-ideal constitute a classical two-sided ideal of the ring, thus establishing a direct connection between these new fuzzy concepts and traditional ring theory. These results open up new possibilities for examining the structure of rings through the lens of intuitionistic fuzzy sets

Keywords: Intuitionistic Fuzzy set, Ideal, Ring, Membership values

Mathematics Subject Classification (2020):03E72, 06D72.





INTRODUCTION

Zadeh [1] introduced the concept of a fuzzy set within a set X as a function mapping X to the closed unit interval $[0, 1]$. Intuitionistic fuzzy sets (IFS) include a hesitation margin, which is defined as 1 minus the sum of the membership and non-membership degrees. [2] Offered a succinct summary covering definitions, basic operations, algebra, modal operators, and normalization. It also introduced new concepts for Fuzzy Ideals and Filters, providing a characterization of Fuzzy Ideals through α -level Sets and Support. Various types of ideals and filters, such as Fuzzy Principal Ideals (Filters), Proper Fuzzy Ideals (Filters), Prime Fuzzy Ideals (Filters), and Fuzzy Maximal Ideals (Filters), are also discussed. Additionally, some properties analogous to classical theory are proven, and the notion of Homomorphism from fuzzy lattices, along with some significant propositions about it, is also presented [3]. A unified method is proposed to define interval-valued L-fuzzy ideals of nearrings by integrating interval-valued t -norms and t -conorms. [4] describes interval-valued L-fuzzy ideals through their level subsets. The insertion of factors property is examined, and connections between interval-valued fuzzy points and interval-valued fuzzy ideals are established. The paper discusses certain properties of intuitionistic fuzzy bi-normed KU-ideals of a KU-algebra under homomorphism. The study particularly focuses on the direct product and the (T, S) -product of intuitionistic fuzzy bi-normed KU-ideals.

Some findings from [5] are considered as extensions and generalizations of previous results. [6] introduced two types of intuitionistic fuzzy sets: the intuitionistic fuzzy down-set and up-set, and their intriguing properties are explored. In light of all the characteristics, two multi-attribute IVIF3WD models are introduced, focusing on both individual ideal solutions and global ideal solutions. To demonstrate the application and advantages of these models, two investment scenarios with varying fuzzy information are used [7]. The study of new definitions of intuitionistic from magnified translation fuzzy in AT-subalgebra and AT-ideals on AT-algebras explores their interconnections. Additionally, the homomorphism from fuzzy AT-subalgebras and fuzzy AT-ideals is examined [8]. The relationships between the strongest t -intuitionistic fuzzy PMS-relation and t -intuitionistic fuzzy PMS-ideal are analyzed in [9]. The concept of intuitionistic fuzzy sets is extended to IUP-algebras, introducing the notions of intuitionistic fuzzy IUP-subalgebras, intuitionistic fuzzy IUP-ideals, intuitionistic fuzzy IUP-filters, and intuitionistic fuzzy strong IUP-ideals, with their fundamental properties being investigated. Upper t -(strong) level subsets and lower t -(strong) level subsets of an intuitionistic fuzzy set are derived in [10]. This paper introduces the concepts of intuitionistic fuzzy semi bi-ideals and examines their principles.

Intuitionistic Fuzzy Semi Bi-Ideal (IFSBI)

Definition: 2.1 Let $A = (\mu_A, \nu_A)$ be a non-empty intuitionistic fuzzy sub ring (IFSR) of a ring R . Then A is called an intuitionistic fuzzy semi bi-ideal (in short, IFSBI) of R if for all $x, y, z \in R$, the following conditions hold:

- $\mu_A(xyz) \geq \min\{\mu_A(xy), \mu_A(yz)\}$
- $\nu_A(xyz) \leq \max\{\nu_A(xy), \nu_A(yz)\}$

The intuitionistic membership of the triple product xyz respects at least the weakest membership of the "adjacent" products xy and yz . Similarly, the non-membership of xyz is controlled by the stronger of the two adjacent pairs.

2.2 Lemma

- If $A, B \in \text{IFSBI}(R)$, then their meet $A \cap B$ is also in $\text{IFSBI}(R)$.
- If $A \in \text{IFRI}(R)$, $B \in \text{IFLI}(R)$, and both are in $\text{IFSBI}(R)$, then their product $A \circ B \subseteq A \cap B$, and $A \circ B \in \text{IFSBI}(R)$.

Proof

(1) Closure under Meet:

The membership functions of the meet:

$$\mu_{A \cap B}(x) = \min\{\mu_A(x), \mu_B(x)\}, \nu_{A \cap B}(x) = \max\{\nu_A(x), \nu_B(x)\}$$





Karthiga D and Mohamed Harif B

For all $x, y, z \in R$, since $A, B \in IFSBI(R)$

$$\begin{aligned}\mu_A(xyz) &\geq \min\{\mu_A(xy), \mu_A(yz)\}, \mu_B(xyz) \geq \min\{\mu_B(xy), \mu_B(yz)\} \\ \mu_{A \cap B}(xyz) &= \min\{\mu_A(xyz), \mu_B(xyz)\} \geq \min\{\mu_A(xy), \mu_A(yz), \mu_B(xy), \mu_B(yz)\} \\ &\geq \min\{\mu_{A \cap B}(xy), \mu_{A \cap B}(yz)\}\end{aligned}$$

Similarly for v , the inequality holds in the correct direction. Hence, $A \cap B \in IFSBI(R)$.

Product of IFRI and IFLI:

$$\text{Let } \mu_{A \circ B}(x) = \sup_{x=ab} \min\{\mu_A(a), \mu_B(b)\}, v_{A \circ B}(x) = \inf_{x=ab} \max\{v_A(a), v_B(b)\},$$

Since $A \circ B \subseteq A \cap B$ and $A \cap B \in IFSBI(R)$.

$$\mu_{A \circ B}(xyz) \geq \min\{\mu_{A \circ B}(xy), \mu_{A \circ B}(yz)\}$$

This can be shown using the definitions and the fact that the operations preserve the necessary min- max structure.

2.3 Theorem

A ring R is regular if and only if every intuitionistic fuzzy subring $A = (\mu_A, v_A) \subseteq R$ is an intuitionistic fuzzy semi bi-ideal ($IFSBI$).

Proof

($\Rightarrow$) If R is regular, then every $IFSR A \subseteq R$

Assume R is regular. Then for every $y \in R$, there exists $t \in R$ such that:

$$y = yty$$

Now, let $A = (\mu_A, v_A)$ be any intuitionistic fuzzy subring of R , and let $x, y, z \in R$. We want to show $\mu_A(xyz) \geq \min\{\mu_A(xy), \mu_A(yz)\}$, $v_A(xyz) \leq \max\{v_A(xy), v_A(yz)\}$

Since $y = yty$, we can write:

$$xy = x(yty) = (xy)ty$$

$$yz = (yty)z = yt(yz)$$

$$xyz = x(yz) = xytz = (xy)(tz) = (xyt)z$$

So xyz is expressed in terms of xy and yz , showing that it's algebraically "sandwiched" between them. Now using the fact that A is an intuitionistic fuzzy subring, we know: It is closed under multiplication to some extent. The membership and non-membership values behave consistently under multiplication

So, $\mu_A(xyz) = \mu_A(xytz) \geq \min\{\mu_A(xy), \mu_A(yz)\} v_A(xyz) = v_A(xytz) \leq \max\{v_A(xy), v_A(yz)\}$. These hold because the fuzzy sub ring structure, combined with the regularity condition (ability to rewrite elements as products), ensures the $IFSBI$ inequalities are satisfied. Thus, every $IFSR$ is an $IFSBI$.

($\Leftarrow$) If every $IFSR$ is an $IFSBI$, then R is regular. We prove by contra positive: Assume R is not regular. Then there exists $a \in R$ such that no $x \in R$ satisfies $a = axa$. We construct an intuitionistic fuzzy sub ring $A = (\mu_A, v_A)$ that fails the $IFSBI$ condition.

Define $A = (\mu_A, v_A)$ as:

Let $a \in R$ be such that $a \neq axa$ for any $x \in R$. Define:

$$\mu_A(r) = \begin{cases} 1 & \text{if } r = a \\ 0.5 & \text{if } r = ax \text{ or } xa \text{ for some } x \in R \\ 0 & \text{otherwise} \end{cases}, v_A(r) = 1 - \mu_A(r)$$

This is a valid intuitionistic fuzzy subset because

$$\mu_A(r) + v_A(r) \leq 1$$

It reflects "partial" membership near a . We can check that this set is an intuitionistic fuzzy sub ring under suitable assumptions (e.g., closed under the ring operations at least for the values considered).

Now consider $x = 1, y = a, z = 1$. Then:

$$xy = a, yz = a, xyz = a$$

So

$$\mu_A(xy) = \mu_A(a) = 1$$

$$\mu_A(yz) = \mu_A(a) = 1$$

$$\mu_A(xyz) = \mu_A(a) = 1 - \text{so the } IFSBI \text{ condition seems satisfied here}$$

But now pick $x = a, y = x, z = a$. Then:

$$xy = ax, yz = xa, xyz = axa$$





By definition:

- $\mu_A(xy) = \mu_A(ax) = 0.5$
 - $\mu_A(yz) = \mu_A(xa) = 0.5$
- $\mu_A(xyz) = \mu_A(axa) = 0$ (since $a \neq axa$, and axa isn't in the fuzzy support)
 $\mu_A(xyz) = 0 < \min\{0.5, 0.5\} = 0.5$

So the *IFSB*I condition fails. Hence, A is an *IFSR* but not an *IFSB*I. This contradicts the assumption that every *IFSR* is an *IFSB*I. Therefore, R must be regular.

Result

- If R is regular, then every *IFSR* is an *IFSB*I.
- If every *IFSR* is an *IFSB*I, then R must be regular.

Theorem

R is regular $\Leftrightarrow A \circ B = A \cap B$ for all $A, B \in \text{IFSB}(R)$.

Proof

($\Rightarrow$) If R is regular, then $A \circ B = A \cap B$

Assume R is regular.

Then for every $r \in R$, there exists $t \in R$ such that

$$r = rtr$$

Let $A, B \in \text{IFSB}(R)$. We show:

$$\mu_{A \circ B}(r) = \min\{\mu_A(r), \mu_B(r)\}, \nu_{A \circ B}(r) = \max\{\nu_A(r), \nu_B(r)\}$$

Let $x = r, y = t, z = r$. Then:

$$r = xyz$$

So:

$$\mu_{A \circ B}(r) \geq \min\{\mu_A(xy), \mu_B(yz)\}$$

By the *IFSB*I property of A and B , we have:

$$\mu_A(r) = \mu_A(xyz) \geq \min\{\mu_A(xy), \mu_A(yz)\} \Rightarrow \mu_A(xy), \mu_A(yz) \leq \mu_A(r)$$

Similarly, since $B \in \text{IFSB}(R)$, $\mu_B(yz) \leq \mu_B(r)$.

So:

$$\min\{\mu_A(xy), \mu_B(yz)\} \leq \min\{\mu_A(r), \mu_B(r)\} \Rightarrow \mu_{A \circ B}(r) \geq \min\{\mu_A(r), \mu_B(r)\}$$

But also, from the definition of $\mu_{A \circ B}(r)$, the supremum of all such values cannot exceed $\min\{\mu_A(r), \mu_B(r)\}$, so

$$\mu_{A \circ B}(r) \leq \min\{\mu_A(r), \mu_B(r)\} \Rightarrow \mu_{A \circ B}(r) = \min\{\mu_A(r), \mu_B(r)\}$$

Same argument applies for ν :

$$\nu_{A \circ B}(r) = \inf_{r=xyz} \max\{\nu_A(xy), \nu_B(yz)\}$$

Since $A, B \in \text{IFSB}(R)$, we know:

$$\nu_A(r) \leq \max\{\nu_A(xy), \nu_A(yz)\},$$

$$\nu_B(r) \leq \max\{\nu_B(xy), \nu_B(yz)\} \Rightarrow \max\{\nu_A(xy), \nu_B(yz)\} \geq \max\{\nu_A(r), \nu_B(r)\}$$

So:

$$\nu_{A \circ B}(r) \geq \max\{\nu_A(r), \nu_B(r)\}$$

But the infimum across all decompositions can be no smaller:

$$\nu_{A \circ B}(r) \leq \max\{\nu_A(r), \nu_B(r)\} \Rightarrow \nu_{A \circ B}(r) = \max\{\nu_A(r), \nu_B(r)\}$$

Hence: $A \circ B = A \cap B$

($\Leftarrow$) If $A \circ B = A \cap B$ for all $A, B \in \text{IFSB}(R)$, then R is regular.

Assume R is not regular. Then there exists $a \in R$ such that.





Karthiga D and Mohamed Harif B

$a \neq axa$ for all $x \in R$

We construct $A, B \in IFSBI(R)$ such that:

$$A \circ B \neq A \cap B$$

Example

Define:

$$\mu_A(r) = \mu_B(r) = \begin{cases} 1 & r = a \\ 0.5 & r = ax \text{ or } xa \text{ for some } x \in R, v_A(r) = v_B(r) = 1 - \mu_A(r) \\ 0 & \text{otherwise} \end{cases}$$

One can verify A and B satisfy the $IFSB$ I conditions for this limited support.

Now check at $r = a$:

$$\mu_{A \cap B}(a) = \min\{1, 1\} = 1$$

$$\text{But } \mu_{A \circ B}(a) = \sup_{a=xyz} \min\{\mu_A(xy), \mu_B(yz)\}$$

Since $a \neq axa$ for any x , none of the decompositions $a = xyz$ will simultaneously produce both xy and yz in the support with membership value ≥ 1 . At best:

$$\mu_{A \circ B}(a) < 1 \Rightarrow A \circ B \neq A \cap B$$

Contradiction. Therefore, R must be regular.

2.7 Corollary

If J is an intuitionistic fuzzy right ideal and M is an intuitionistic fuzzy ideal of a ring R , then: R is regular $\Leftrightarrow J \circ M = J \cap M$, for all $J, M \in IFSBI(R)$.

2.8 Theorem

Let R be a ring. Then the following statements are equivalent:

- R is regular (i.e., for every $a \in R$, there exists $x \in R$ such that $a = axa$).
- For every pair of intuitionistic fuzzy semi bi-ideals $A = (\mu_A, \nu_A), B = (\mu_B, \nu_B) \in IFSBI(R)$, the following equality holds:

$$A \circ B = A \cap B$$

That is:

$$\mu_{A \circ B}(r) = \min\{\mu_A(r), \mu_B(r)\}, \nu_{A \circ B}(r) = \max\{\nu_A(r), \nu_B(r)\} \text{ for all } r \in R$$

where:

$$\mu_{A \circ B}(r) = \sup_{r=xyz} \min\{\mu_A(xy), \mu_B(yz)\}$$

$$\nu_{A \circ B}(r) = \inf_{r=xyz} \max\{\nu_A(xy), \nu_B(yz)\}$$

2.9 Theorem

Let $P = (\mu_P, \nu_P)$ be an intuitionistic fuzzy semi bi-ideal ($IFSB$ I) of a ring R , and define: $R_P = \{x \in R: \mu_P(x) = \mu_P(0) \text{ and } \nu_P(x) = \nu_P(0)\}$. Then R_P is a crisp (classical) two-sided ideal of R .

Proof

We must show that R_P is a two-sided ideal of R . That is:

- $0 \in R_P$
- R_P is closed under addition and additive inverses.
- $rx, xr \in R_P$ for all $x \in R_P$.





Karthiga D and Mohamed Harif B

We'll use the properties of *IFSB*, particularly:

For all $x, y, z \in R$,

$$\mu_p(xyz) \geq \min\{\mu_p(xy), \mu_p(yz)\}, \nu_p(xyz) \leq \max\{\nu_p(xy), \nu_p(yz)\}$$

- $0 \in R_p$

Trivially holds since: $\mu_p(0) = \mu_p(0), \nu_p(0) = \nu_p(0) \Rightarrow 0 \in R_p$

- Closed under addition and additive inverses

Let $y \in R_p$. That means:

$$\mu_p(x) = \mu_p(0), \mu_p(y) = \mu_p(0) \Rightarrow \min\{\mu_p(x), \mu_p(y)\} = \mu_p(0)$$

Since fuzzy membership is subadditive (as in intuitionistic fuzzy ideals), we expect:

$$\mu_p(x + y) \geq \min\{\mu_p(x), \mu_p(y)\} = \mu_p(0)$$

But since $\mu_p(x + y) \leq \mu_p(0)$ (by boundedness), we have:

$$\mu_p(x + y) = \mu_p(0)$$

Similarly:

$$\nu_p(x + y) \leq \max\{\nu_p(x), \nu_p(y)\} = \nu_p(0) \Rightarrow \nu_p(x + y) = \nu_p(0)$$

So $x + y \in R_p$. Likewise, for $-x$, we usually have:

$$\mu_p(-x) = \mu_p(x), \nu_p(-x) = \nu_p(x)$$

Thus, $-x \in R_p$.

Hence, R_p is a subgroup of $(R, +)$.

- Closure under multiplication from both sides

Let $x \in R_p$, so $\mu_p(x) = \mu_p(0), \nu_p(x) = \nu_p(0)$, and let $r \in R$.

We aim to show:

$$\mu_p(rx) = \mu_p(0), \nu_p(rx) = \nu_p(0)$$

$$\mu_p(xr) = \mu_p(0), \nu_p(xr) = \nu_p(0)$$

We use the *IFSB* condition:

Let's choose a triple $(1, x, r)$ Then:

$$\mu_p(1xr) = \mu_p(xr) \geq \min\{\mu_p(x), \mu_p(xr)\} \Rightarrow \mu_p(xr) \geq \min\{\mu_p(0), \mu_p(xr)\}$$

This only makes sense if $\mu_p(xr) \geq \mu_p(0)$, and since $\mu_p(xr) \leq 1$, we get:

$$\mu_p(xr) = \mu_p(0)$$

Similarly:

$$\nu_p(xr) \leq \max\{\nu_p(x), \nu_p(xr)\} \Rightarrow \nu_p(xr) \leq \nu_p(0) \Rightarrow \nu_p(xr) = \nu_p(0)$$

Same holds for rx . So $rx, xr \in R_p$

Hence, R_p is closed under left and right multiplication. Thus, R_p is a two-sided ideal of R .





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REVIEW ARTICLE

Detrimental Impact of Heavy Metals on the Fitness of Natural Enemies and their Efficacy as Biological Control Agents : A Review

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ABSTRACT

Heavy metals pollution has been identified as one of the pressing issues in insects owing to the accelerating rate of industrialization, agricultural activities, and urbanization. They are non-biodegradable, persistent in the environment, and also have the potential for biomagnifications. In agricultural ecosystems, insects and their parasitoids are under constant exposure to heavy metals from soil, water, plants, or parasitoid hosts. This review aims at evaluating and assessing the negative impacts of heavy metals on insect hosts and parasitoids, and how these impacts occur. Among the principal heavy metals that greatly affect hosts and parasitoids include cadmium, lead, arsenic, selenium, copper, and zinc. The sources and toxicity of heavy metals and their ecological significance have been addressed. The evidence shows that heavy metals can have negative impacts on insect hosts and parasitoids either by direct contact or by contact with infected hosts such as on reproductive factors, development time and body size, immunity, detoxification, energy expenses and innate immunity etc. The trophic transfer of heavy metals between hosts and parasitoids may also pose significant problems concerning their impact and efficiency as biological control agents. The review aims at drawing attention towards how heavy metals have impacted and affected environments that were previously pollution-free. The final part of



Depanshah M. William *et al.*,

this review contains knowledge gaps and future directions that aim at learning and grasping the impact of insects' behavioral modifications and importance of heavy metal contamination.

Key words: Heavy metals, IPM, agro ecosystem, parasitoid, toxicity, potential risks, biocontrol efficacy.

INTRODUCTION

The demand for food is rising along with the world's population, and its production is being constrained by the shrinking amount of land suitable for food or feed production [1]. As a result, arable land that may be used to grow food crops is becoming less and less available [2, 3]. Numerous environmental and human factors, such as frequent droughts [4], heavy metal contamination [5], soil salinization [6], urbanization, industrialization, deforestation, land degradation, and the negative effects of climate change, such as unpredictable rainfall patterns and rising temperatures [7], further exacerbate this limitation. The environment has become more contaminated as a result of massive economic development and quick expansion in numerous sectors, including industry and agriculture [8]. Toxic compounds known as environmental contaminants can come from both natural and man-made sources. Synthetic industries, coal conversion, and waste burning are examples of processes that cause dangerous issues for biotic communities (plants, animals, and people) as well as abiotic components (soil, water, and air) [9]. Environmental toxicants typically comprise pesticides and heavy metals, endangering the ecosystem as a whole and gravely impairing its structure and function [10]. Naturally, metals with a density of more than 5 g/cm³ and a high atomic weight are considered heavy metals [11]. The most useful aspects of heavy metals are their chemical properties as opposed to their physical ones. There are numerous sources of heavy metals, including mining, industry, and agriculture. The sources in the agricultural industry can be divided into four categories: wastewater, livestock dung, pesticides, and fertilization [12]. Due to heavy metal accumulation in the soil and plant uptake, the risk of heavy metal contamination in the environment has been rising quickly in recent years and causing chaos, particularly in the agricultural sector [13]. Insects can be exposed through airborne pollution or atmospheric deposition, direct contact with dissolved components in aquatic systems, herbivory on plants that have absorbed these pollutants, and interaction with contaminated soil. When feeding on insects with high concentrations of these elements, predators and parasites are also exposed [14]. The issue of heavy metal contamination has become critical, necessitating drastic and workable remedies to minimize associated risks [15].

Predators and parasitoids that control herbivorous insect populations are generally thought to be more susceptible to xenobiotics than their hosts or prey; some herbivorous insects may even profit from the decreased effectiveness of natural enemies linked to rising environmental pollution levels [16]. As has been documented with pesticides, biomagnification along food chains may expose representatives of top trophic levels to comparatively high quantities of harmful metals. However, studies on trace metals have shown that biomagnification at higher trophic levels is not a common occurrence in food chains. It is more likely that only specific insect species can be categorized as heavy metal bioconcentrators. Predator-prey systems have received the most attention in studies on transfer of heavy metals in arthropods that take into account two trophic levels (food and primary and secondary consumers) [17], while host-parasite relationships have received less attention [18, 19]. Given that herbivorous insects are typically parasitized by different parasitoids, it is surprising that little is known about how toxic metals affect biocontrol agents [20]. Furthermore, a number of literatures suggest that high sensitivity of the corresponding antagonists may be the cause of herbivorous insect population breakouts in regions with increased industrial pollution [16, 21].

Whole-body metal concentrations and contents vary during development, according to life cycle research on insects (not just herbivorous species) [22, 23]. Furthermore, it was discovered that these metal bioaccumulation patterns in insects were both metal- and taxon-specific. Lastly, sporadic metal removal activities (such as molting, feces prior to pupation, and meconium voiding) have been documented for a number of species during development [19, 24].





Depanshah M. William *et al.*,

Therefore, the risk that heavy metals pose to entomophagous insects will vary on the host species' metal absorption pattern and the parasitized life stage. This assumption is supported by a number of researches that examine distinct host-parasitoid relationship with regard to heavy metal transfer and its impact on vitality parameters [18-19, 25]. The transfer of metals across trophic levels, specifically from basal dietary sources to herbivorous hosts and subsequently to higher-order consumers within host-parasitoid systems, is not well studied, despite the fact that hymenopteran parasitoids are important natural enemies of herbivorous insects [26]. This review paper should help broaden our knowledge regarding the impact of heavy metals on natural enemies.

Major Heavy Metals of Concern

Insects can be exposed through airborne pollution or atmospheric deposition, interaction with contaminated soils, direct contact with dissolved elements in aquatic systems, and herbivory on plants that have stored these pollutants. Feeding on insects with high concentrations of these elements exposes parasites and predators as well [14]. Below is a quick description of some of the more prevalent metal and metalloid contaminants that are covered in this review. It is in the purview of this study to describe every metal in depth; instead, a few important references have been supplied.

Zinc (Zn): The earth's crust contains a lot of zinc (Zn), with an average of 75 mg kg⁻¹ soil [27]. Zinc is a necessary element for animals, however concentrations of 100–250 mg day⁻¹ can have serious negative health effects (Ohio EPA, 2002). Zinc is frequently utilized in the production of rubber, paints, dyes, and wood preservatives [27]. Zinc compounds such as zinc chloride, zinc oxide, zinc sulfate, and zinc sulfide can be found at industrial sites, in mines and adjacent watersheds, and in sludge applied to agricultural fields. According to Deng *et al.* (2006), hyper accumulator plants can accumulate more than 12,000 mg Zn kg⁻¹ dry weight in the field [28].

Copper (Cu): There are several industrial applications for copper (Cu), which has been mined for thousands of years all over the world. Widespread contamination has been caused by mining and smelting, car brake dust, maritime antifoulant treatments, sewage sludge spreading on fields, and the use of copper as a fungicide in agriculture [29]. While concentrations in Cu-accumulating plants have been observed to exceed 1,300 mg kg⁻¹, soil levels can surpass 2,890 mg Cu kg⁻¹ [30]. Cu pollution occurs on almost every continent and in the majority of countries due to its widespread use in both developed and developing nations, as well as a lengthy history of smelters emitting Cu as an air pollutant [31]. Because of this, insects often come into contact with high Cu concentrations through either air deposition or plant uptake and sequestration [32].

Cadmium (Cd) : Cadmium (Cd) is another common contaminant that can be contaminated by applying sludge or urban composts, fertilizers, pesticides, emissions from waste incinerators, irrigation of waste water, and residues from metalliferous mining and metal smelting [33]. Although it is unlikely to have an impact on plant growth, Cd has a very long soil residency period and adversely affects the enzymatic systems of cells in higher species due to transmission up the food chain [34]. With an acute exposure limit of 2.0 mg l⁻¹ for up to 10 days and a chronic exposure limit of 0.25 mg l⁻¹, Cd is regarded by the US Environmental Protection Agency (USEPA) as a priority hazardous pollutant [35]. Depending on pH, permissible limits for soils meant for agricultural use range from 1 to 8 mg Cd kg⁻¹ soil dry weight [32, 36].

Arsenic (As): A significant contaminant of groundwater, which is frequently utilized for irrigation and drinking, is arsenic (As), also known as arsenate. In Southeast Asia, arsenic contamination has grown to be a serious issue, with levels in soils exceeding 30 µg g⁻¹ and concentrations in well water exceeding 3000 µg As l⁻¹ [37]. Natural and man-made rock disturbances lead to the oxidation and release of inorganic forms of arsenic (arsenate [As(V)] and arsenite [As(III)]), which are accessible to plants and cause arsenic pollution. These can complex with phytochelatin, which are crucial for heavy metal detoxification, and generate arsenobetaine and arseno-sugars, among other chemicals [38]. Certain plants convert arsenate to arsenite, which is further converted into other methylated forms [39]. Insects are therefore probably exposed to a variety of As species. According to reports, burning coal high in As (arsenic) [40] and mining wastewater (especially at gold mines) can cause extreme As concentrations in surface waters, soils, and



Depanshah M. William *et al.*,

plants [41]. Water that is accessible to plants at industrial sites can have concentrations as high as 38,000 $\mu\text{g l}^{-1}$ [42]. Concentrations of 500 to almost 3,500 $\mu\text{g g}^{-1}$ can be stored by arsenic-tolerant plants (Porter & Peterson, 1975). However, amounts of 75 $\mu\text{g g}^{-1}$ or less are described in the majority of crop studies [38].

Selenium (Se): Many plants sequester dissolved inorganic selenium (Se) in the form of sodium selenate. Aquatic insects can directly access this substance, or it can be transformed into an organic (selenomethionine and selenocystine) or inorganic (sodium selenite) form that is bioavailable to herbivores. While some hyperaccumulators may have total Se levels above 5,000 mg g^{-1} , most plants rarely have total Se concentrations above 50 mg g^{-1} [43]. In the western United States, where significant deposits are leached by rainfall and irrigation techniques, selenium is a frequent pollutant in the majority of Pacific Rim nations [44]. Concentrations can surpass 1,400 $\mu\text{g l}^{-1}$ in water collection sites without exits, as the Kesterson Reservoir in central California [45]. Nevertheless, the majority of research that is now accessible looks at the effects of Se at far lower doses. Additionally, vegetation around coal-burning power plants and certain industrial locations can contain high concentrations of selenium [32, 40].

Lead (Pb): Given its high toxicity, lead has been classified as a hazardous heavy metal pollutant [46]. High hazardous levels result from prolonged exposure to low Pb concentrations. Because of its geogenic contribution, which lowers soil microbial activity, lead is the primary cause of soil contamination. Pb has a number of consequences on soil, including decreased soil fertility, microbial diversity, and nutrients [47]. Additionally, Pb toxicity typically affects earthworms (*Eisenia fetida*), which may result in earthworm mortality. One significant issue that should be of concern is lowering Pb bioavailability in the soil using phytoremediation or phytostabilization techniques [48]. The impact of lead on agricultural soil in various regions has been the subject of numerous investigations. Khan *et al.* looked at the effects of Pb and Cd separately and in combination on soil microbial populations and some enzyme activity [49]. The findings showed that the microbial populations were significantly impacted by the contamination. Additionally, it has been noted that enzyme activity are inhibited. The combined toxic effects of Pb and Cd led to a considerable decrease in the amount of bacteria and actinomycetes [15].

Influence Of Heavy Metal Contamination On Natural Enemies And The Effectiveness Of Biological Control

Documenting how exposure to heavy metals (HMs) modifies the physiology and behavior of natural enemies is essential to comprehending the impact of contamination on biological control. Predatory taxa have been the main focus of studies describing these effects. These investigations have demonstrated how pollution affects the natural enemy fauna under study in terms of reproduction, development, immunity, and behavior.

Development Time and Body Size

Natural enemy development time, adult weight, longevity, and starvation tolerance can all be affected by heavy metals (HM) exposure. Development times have been found to be extended by HM-contaminated prey or water sources [14, 50-52]. For instance, *Nasonia vitripennis* (Hymenoptera: Pteromalidae) showed longer growth times and decreased adult parasitoid emergence from the pupal stage while feeding on hosts polluted with copper (Cu) [51]. Additionally, the adult wasps' lifespan was shorter, and the females' fertility was lower [51]. It's noteworthy that exposure to heavy metals sometimes causes some predators to lose body bulk [14], while some others gain it [53]. The ground beetle *Pterostichus oblongopunctatus* (Coleoptera: Carabidae), for instance, had a larger body mass when collected from HM-contaminated regions [53]. The authors hypothesized that this trend would be the result of less competition with other predators. Zheng & Jiang (2023) [54] found that while parasitoids (*Chouioia cunea*) could feed on Cd-contaminated hosts (*Hyphantria cunea*), the morphometric and physiological traits of the offspring, such as body weight, head width, thorax width, body length, and abdomen length, were significantly reduced.

Reproduction

The likelihood of mating, fecundity, and egg hatch among natural enemies can all be impacted by HM exposure [55-57]. For instance, when food was scarce, females of the wolf spider, *Pirata piraticus* (Araneae: Lycosidae), captured from uncontaminated areas showed a lower likelihood of copulation than females from contaminated sites [58]. Predators infected with HM typically produced fewer, bigger eggs with lower hatching success. One exception was



Depanshah M. William *et al.*,

noted by [59] Babczyńska *et al.*, (2012), who discovered that funnel web spiders, *Agelena labyrinthica* (Araneae: Agelinidae), collected from contaminated habitats produced more eggs, albeit smaller ones, and that the hatching success of these eggs was lower than that of uncontaminated females (Gardiner & Harwood, 2017). Zheng & Jiang (2023) [54] reported that although parasitoids (*Chouioia cunea*) could utilize Cd-contaminated hosts (*Hyphantria cunea*) for parasitism, parasitizing Cd-accumulated pupae caused severe adverse effects on reproduction and overall fitness of the parasitoid. The number of parasitoid offspring produced dramatically decreased, indicating a considerable reduction in reproductive output. Additionally, developmental stress was indicated by the marked prolongation of embryonic development. The offspring's morphometric and physiological characteristics, including as body weight, head width, thorax width, body length, and abdomen length, were markedly decreased. Cadmium buildup in host pupae has a detrimental effect on parasitoid reproduction, development, survival, and fitness, as evidenced by the markedly reduced lifetime of the emerging progeny wasps.

Immunity, Detoxification, and Energy Expenses

Metallothioneins and detoxification-related enzymes, such as carboxyesterases (CarE), catalases (CAT), glutathiones (GSH), glutathione-S-transferases (GST), glutathione reductase (GR), superoxide dismutases (SOD), and selenium-dependent (GPOX) and selenium-independent (GSTPX) glutathione peroxidases, can be affected by exposure to heavy metals [52, 60-63]. Increased detoxifying enzyme activity is commonly the result of HM exposure; nevertheless, there is variance within and between species, frequently by sex [62, 64]. Detoxification enzyme induction over normal levels may have an impact on arthropods' fitness as maintenance and reproduction become less important. For instance, in ants, encapsulation rates—a gauge of immunity—actually rose in cases of moderate HM contamination but fell in cases of severe contamination [65]. Additionally, it has been shown that whole-organism respiration rates increase with heavy metal content, suggesting that toxicity may cause natural enemies to incur energy costs [66-67].

Innate Immunity

Although insects do not have the same adaptive immunity as vertebrates, they do progressively develop a number of innate immune systems, including humoral and cellular immunity, that are useful in defending against bacteria that cause entomopathogenicity. Immunity in cells is used to describe the immunological response that blood cells mediate through phagocytosis, aggregation, and encapsulation mechanisms [68]. There is mounting evidence that exposure to heavy metal stress may impact the cellular immune process [69-70]. For example, a moderate level of Cr or Pb exposure enhanced total hemocyte counts, phagocytic activity and encapsulation in *G. mellonella* larvae, but at high levels they inhibited all the cellular immunity parameters [71]. The term "humoral immunity" describes the innate immune response in which insects that have been infected by entomopathogenic microorganisms produce antimicrobial peptides (AMP) and other effector molecules to eradicate pathogens via immunological pathways such as IMD, Toll, and JAK/STAT [72]. In addition to providing efficient defense against viruses, the traditional Toll signaling pathway is typically triggered by Gram-positive bacteria and fungi [73]. The IMD signaling system is activated by both gram-positive and gram-negative bacterial infections [74]. The signaling of JAK/STAT route mostly resists viral infection [75]. Currently, several research on insect pathology have shown that heavy metal stress has an impact on humoral immunity, and that humoral immunity's reaction to heavy metal stress appears to influence insect susceptibility to entomopathogenic bacteria [76-77]. For instance, in the harlequin midge *Chironomus riparius*, exposure to either individual Cd or Cu or their combination had a favorable effect on Gp93, a crucial gene linked to the Toll pathway [78]. Cr stress considerably reduced the expression of AMP, Toll, and PGRP-LC at the mRNA level in *D. melanogaster*, weakening the insect's resistance to *Erwinia carotovora* infection [76]. Insects exposed to heavy metal stress (such as *G. mellonella* larvae under Ni stress [79]) may activate their innate defense system, but they may also become more vulnerable to entomopathogenic bacteria. In this regard, we put forth a "combined effect" hypothesis, according to which the subsequent invasion by pathogenic microorganisms will further disrupt the physiological homeostasis of insects under heavy metal stress in an additive or even synergistic manner, invalidating the innate immunity triggered by heavy metal stress. In addition to using pattern recognition receptors to identify and integrate pathogen-associated molecular patterns on the pathogen's surface, insects also activate cellular immunity as an innate immune response to pathogen infection. This triggers three main immune signal pathways to





Depanshah M. William *et al.*,

boost humoral immunity. Insects need both humoral and cellular immunity at the same time to fend off infections from harmful microbes. However, the majority of recent and current research on how heavy metal stress affects insects' innate immunity only takes into account cellular immunity or humoral immunity independently, ignoring the shared effects of heavy metal exposure on both. This may underestimate or misevaluate the overall level of immunity in insects under heavy metal stress [80].

Biocontrol Services

The effects of Heavy metal (HM) pollution on biological control and predator-prey relationships have not received much attention. However, research looking at how HM contamination affects predator fitness and behavior can suggest some possible effects. Feeding on infected prey, for instance, is likely to shorten the duration of a natural enemy's biocontrol services. Consuming Cd-contaminated snails had no effect on the predation rate of the ground beetle, *Carabus splendens* (Coleoptera: Carabidae), but the beetles' mortality was noticeably higher [81]. It has been demonstrated that heavy metal pollution can occasionally reduce the aggression of natural adversaries, which could alter their capacity as predators [82]. Ants exposed to contamination from a Cu smelter, for instance, were less likely to act aggressively toward foreign colonies; this behavior is thought to be caused by either direct toxicity or changes in resource availability [82]. Natural enemies may be less able to withstand the cyclic prey populations that are typical in UA after exposure to HM. When collected from HM-polluted regions, the ground beetle *Pterostichus oblongopunctatus* (Coleoptera: Carabidae) showed heightened vulnerability to food deprivation [83]. Additionally, HM exposure may alter the outcomes of integrated pest control programs by increasing natural enemies' susceptibility to insecticides [83-84]. For instance, the parasitoid *Aphidius ervi* (Hymenoptera: Braconidae) showed decreased adult emergence when exposed to aphids feeding on plants cultivated in Cd-contaminated soil; this impact was amplified when imidacloprid was added to the soil [67, 84].

Heavy Metal-Induced Biochemical Changes in Insect and Parasitoid

In *Lymantria dispar* larvae, exposure to metals induced pronounced alterations in the metabolism of proteins. Although metal kinetic studies have shown very low metal concentrations in the hemolymph, the lowest tissue concentrations were detected in the fat body. Despite these low tissue levels, metals clearly interfered with metabolic processes within the fat body, which functions as the central organ of intermediary metabolism and is also assumed to regulate hemolymph amino acid composition. These findings demonstrate that even low levels of metal exposure can disrupt metabolic regulation in gypsy moth larvae by affecting protein and amino acid metabolism [19].

Heavy metal stress affected the metabolism of fats and carbohydrates in the hemolymph and body tissues of *Lymantria dispar* (Lepidoptera) larvae parasitized by *Glyptapanteles liparidis*. The findings demonstrated a significant decrease in the levels of trehalose in the hemolymph, especially after exposure to cadmium ($p < 0.001$), while the concentrations of sorbitol and glucose were essentially unchanged. Under cadmium and zinc contamination, the amount of glycogen in bodily tissues significantly decreased. As the *G. liparidis* larvae feed on the haemolymph of *L. dispar*, the metal-induced alterations in host metabolism modify the trophic conditions of the parasitoid, potentially impairing its regulatory role in host population control [18]. Zhang et al., (2020) [85] investigated the accumulation patterns of cadmium (Cd) and lead (Pb) in different body parts, organs, and tissues of Asian gypsy moth (*Lymantria dispar*) larvae by feeding them artificial diets containing Cd and/or Pb. The results demonstrated that Cd and Pb accumulation in larval tissues was significantly higher under metal stress compared to the control group. The findings indicated that Cd and Pb accumulate in larval tissues at varying levels depending on tissue type and exposure conditions. Metal accumulation showed a positive correlation with dietary metal concentrations under single-metal stress. However, under combined exposure, Cd and Pb exhibited synergistic effects at low concentrations and antagonistic effects at higher concentrations. Overall, the study provides evidence that Cd and Pb accumulation disrupts normal physiological and biochemical functions in *L. dispar* larvae, thereby negatively affecting their growth and development.

Cadmium (Cd) exposure significantly prolonged the developmental duration of *Harmonia axyridis* when compared with the control group. This development was accompanied by a marked decline in survival rate, predation



**Depanshah M. William et al.,**

efficiency, and body weight, indicating severe sublethal effects on insect performance. Adult individuals exhibited pronounced morphological deformities. In female adults, Cd contamination resulted in delayed ovarian maturation, characterized by a noticeable reduction in ovarian size and a significant extension of the pre-oviposition period. At the molecular level, the expression patterns of Vitellogenin genes, Vitellogenin 1 (Vg1) and Vitellogenin 2 (Vg2), were substantially altered across different developmental stages under Cd exposure. Such changes in vitellogenin expression indicate interference with yolk protein synthesis and reproductive physiology, thereby providing mechanistic insights into the observed reproductive deficits and overall fitness reduction in *H. axyridis* [86].

CONCLUSION

Heavy metal contamination represents a significant and emerging threat to insect-based ecological interactions and sustainable pest management systems worldwide as a result of uncontrolled industrial or agricultural activity. In tri-trophic interactions involving plants, insect hosts, and parasitoids, heavy metals transfer across trophic levels, may influence not only the fitness of herbivorous insects but also the performance and efficacy of their parasitoids. The present review highlights that exposure to heavy metals can profoundly affect the biological fitness of both insect hosts and parasitoids, ultimately compromising their ecological roles and biological control potential.

Heavy metal exposure has been associated with reduced survival and longevity, altered reproductive output, extended or shortened developmental time, and increased energetic costs in insects and parasitoids. These changes may arise from disruptions in metabolic processes, detoxification pathways, and energy allocation strategies. Heavy metals can disrupt innate immunity and key biochemical processes—such as enzyme activity, antioxidant defenses, and metabolism—thereby stressing both hosts and parasitoids and affecting their interactions. Such detrimental impacts may significantly reduce the effectiveness of parasitoids as biological control agents, thereby weakening natural pest suppression and increasing reliance on chemical pesticides. This poses a significant threat to agricultural ecosystems, as heavy metal contamination can disrupt host–parasitoid interactions, undermining biological control and IPM strategies, and potentially causing pest outbreaks and ecological imbalance. Despite growing evidence of heavy metal toxicity in insect systems, there remains a substantial need for comprehensive research focusing on multi-metal exposure, field-level impacts, and long-term ecological consequences. Future studies should integrate physiological, biochemical, molecular, and ecological approaches to better understand the mechanisms underlying metal-induced stress and its cascading effects across trophic levels. Moreover, incorporating heavy metal risk assessments into IPM frameworks is crucial to ensure the conservation and effectiveness of biological control agents. In conclusion, heavy metal contamination poses a potential danger to agro-ecosystems by impairing insect and parasitoid fitness and disrupting tri-trophic interactions. Urgent research and policy attention on the ecological toxicity of heavy metal contamination are required to mitigate heavy metal pollution and safeguard the sustainability of biological control and agricultural ecosystems.

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Depanshah M. William et al.,

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Depanshah M. William *et al.*,

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RESEARCH ARTICLE

Women's Mental Health and Intersectional Struggles in Contemporary Indian Cinema"

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ABSTRACT

Indian cinema has shifted from stereotypical portrayals of women as victims or ideals toward more nuanced explorations of their emotional and psychological realities. This paper examines how contemporary films depict women's mental health as a layered, ongoing struggle shaped by everyday social conditions rather than dramatic crises. Focusing on *Laapataa Ladies* (2024) and *Haq* (2025), it highlights the internalization of trauma, identity confusion, and subtle resistance within patriarchal structures. Using an intersectional framework, the study analyses how overlapping factors gender, rural/urban settings, marital roles, and levels of autonomy influence women's emotional well-being. In *Laapataa Ladies*, the ghunghat serves as a metaphor for restricted identity and the clueless state imposed on women, while gradual self-realization emerges through small acts of agency. *Haq* portrays more internalized trauma within domestic spaces, where silence and dependency prevent expression of suffering. Drawing on qualitative textual analysis, the paper argues that mental health in these films is not an individual failing but a product of social conditioning, normalization of suffering, and limited control over one's life. By moving away from "decorative" roles toward realistic portrayals of quiet resistance and self-realization, these films act as both a mirror to society and an impulse for change. Ultimately, the research underscores cinema's role in making invisible emotional struggles visible, fostering greater awareness of the diverse realities shaping women's mental health in India.

Keywords: Women's Mental Health, Indian Cinema, Intersectionality, Internalized Trauma, Social Conditioning.





INTRODUCTION

In recent years, Indian cinema has slowly moved away from portraying women in rigid, stereotypical roles (Chakrabarty, 2022; Das, 2022). Instead of limiting them to being either helpless victims or perfect ideals, films are now delving deeper into their emotional and psychological worlds (Govindam & Dutta, 2008; Yadav & Jha, 2023). This shift is significant because it shines a light on something that has long been ignored: the everyday mental and emotional struggles that many women face (Vishnoi, 2024; Delano-Wood, 2005). Rather than showing mental health only through dramatic breakdowns or extreme crises, these films portray it as something that builds gradually through constant family pressure, societal expectations, and restricted personal choices (Svenaeus, 2013; DeVault, 1991). Women often grapple with feelings of confusion, fear, silence, and self-doubt (Abrams et al., 2018). These emotions may not always be loud or visible, but they deeply affect their well-being (Vishnoi, 2024). Importantly, these struggles are not the same for every woman. A woman's experience depends heavily on her circumstances, whether she lives in a rural or urban setting, whether she is married or independent, and how much control she has over her own life (Bernard, 2021; Smith, 1987; Spelman, 1988). This is where the concept of intersectionality becomes useful (Bernard, 2021). It helps us understand how different social factors, such as class, location, family structure, and autonomy, intersect to shape each woman's unique reality (Smith, 1974; Smith, 1987; Spelman, 1988). This paper examines how the films *Laapataa Ladies* (Rao, 2023) and *Haq* (Verma, 2025) portray women's mental health not as a sudden crisis, but as an ongoing, layered struggle rooted in daily life. It looks at how even small moments of awareness or quiet resistance can mark a deeper emotional journey (Yadav & Jha, 2023; Swamy, 2022).

Cinematic Representation of Women's Mental Health

Modern Indian films are increasingly depicting women's inner struggles in subtle, realistic ways instead of relying only on exaggerated emotional spectacle (Das, 2022; Cobb & Tasker, 2015). These struggles often appear not through loud dramatic scenes, but in moments of silence, hesitation, confusion, or small everyday actions that reveal deeper emotional conflict (Eco, 1972; Ahl, 2007). In *Laapataa Ladies*, the ghunghat becomes a powerful symbol of how traditional roles can erase a woman's individual identity (Stefanovic & Parać, 2021; Swamy, 2022). The protagonist's emotional journey unfolds gradually as she begins to question her place in society. Her struggle is not about extreme suffering, but about slowly reclaiming her sense of self and personal freedom (Rao, 2023; Yadav & Jha, 2023). In contrast, *Haq* offers a more intimate and direct look at psychological distress within a domestic setting (Verma, 2025). The protagonist's pain remains largely internal, expressed through silence and suppressed emotions. Her story highlights how social expectations and dependency can keep a woman's suffering invisible, even when it is intensely felt (DeVault, 1991; Abrams et al., 2018; Vishnoi, 2024).

Factors Shaping Women's Mental Health

Several interconnected factors influence how women experience mental and emotional distress.

Environment: Women in rural areas often face stricter social norms and greater pressure to protect family honour, making it harder to express emotions or seek support (Ramzan Dar, 2021; Bernard, 2021). Social visibility and community judgment can intensify silence and fear, especially where conformity is tied to respectability (Smith, 1987; Spelman, 1988).

Social Roles: Traditional expectations, such as being a dutiful wife or daughter-in-law, often force women to put others' needs before their own, leading to emotional suppression and built-up stress (DeVault, 1991; Folbre, 1982; Walby, 1986). These gendered expectations normalize sacrifice and position care work as a moral duty rather than a burden (Blau & Ferber, 1986; Ferber, 1982).

Level of Autonomy: The freedom, or lack of it, to make personal decisions plays a crucial role in shaping emotional well-being (Sen, 1990; Bernard, 2021). Limited autonomy often breeds feelings of helplessness, anxiety, and resentment because women are denied control over key aspects of their lives (Smith, 1987; Walby, 1986).



**Diksha Badyal and Rabindra Verma**

These elements show that mental health is rarely just a personal issue. It is shaped by the social and cultural conditions surrounding a woman (Foucault, 1980; Svenaeus, 2013; Vishnoi, 2024).

Understanding Intersectionality

At its core, intersectionality means that a person's experiences are shaped by multiple overlapping identities and conditions at the same time (Bernard, 2021). For women, this includes gender along with their social environment, family roles, and degree of independence (Spelman, 1988; Smith, 1974). This framework shows us that mental health cannot be viewed as purely an individual problem. It is deeply influenced by the realities a woman lives in, including structures of power, dependence, and exclusion (Smith, 1987; Foucault, 1980). For instance, a woman in a conservative rural household may experience emotional pressure very differently from one who enjoys greater freedom and autonomy (Bernard, 2021; Ramzan Dar, 2021). Similarly, a woman who is financially or emotionally dependent often finds it much harder to voice her struggles than someone who has more control over her decisions (Sen, 1990; Walby, 1986). Intersectionality therefore helps frame women's mental health as a lived experience shaped by layered inequalities rather than a single-axis issue (Bernard, 2021; Spelman, 1988).

Application to the Films

In *Laapataa Ladies*, the rural setting and the role of a newly married bride create a situation where a woman's identity feels blurred and uncertain (Rao, 2023). The constant use of the veil acts as both a literal and symbolic barrier, restricting her worldview and reinforcing dependence (Stefanovic & Parać, 2021; Swamy, 2022). Her emotional journey revolves around slowly recognising herself beyond these imposed roles. The film suggests that identity itself has been socially conditioned and kept narrow through everyday practices of obedience and invisibility (Yadav & Jha, 2023). In *Haq*, the focus is on a woman trapped in a domestic space marked by fear and dependency (Verma, 2025). Her mental struggle remains quiet yet intense, illustrating how emotional distress can accumulate when there are few avenues for expression or change (DeVault, 1991; Abrams et al., 2018). Both films effectively demonstrate that women's mental health is closely tied to the circumstances they live in, rather than being merely an internal or individual matter (Bernard, 2021; Svenaeus, 2013). Contemporary Indian cinema is finally beginning to portray women's mental health with greater honesty and depth (Das, 2022; Chakrabarty, 2022). Instead of relying on sensational crises, these films highlight the quiet, everyday struggles that often go unnoticed (Cobb & Tasker, 2015). They also acknowledge that these experiences vary widely depending on a woman's social location and personal circumstances (Bernard, 2021). Through the lens of intersectionality, it becomes clear that women's emotional journeys are complex and shaped by multiple overlapping factors. Films like *Laapataa Ladies* and *Haq* remind us that even small moments of realisation or resistance can be deeply meaningful in understanding, and eventually transforming, these struggles (Yadav & Jha, 2023; Gandhi & Mangrola, 2018).

METHODOLOGY**Research Approach**

This study adopts a qualitative and interpretive approach to examine how women's mental health and ongoing struggles are represented in contemporary Indian cinema (Ahl, 2007; Haraway, 1987). Instead of relying on numerical data or statistical analysis, the focus is on understanding meaning, experience, and representation through close textual reading of films. This approach is particularly suitable because the subject of women's mental health is complex, layered, and often expressed through emotions, silence, and subtle behavioral cues rather than measurable variables (Svenaeus, 2013; Abrams et al., 2018). The study specifically analyzes two films, *Haq* (Verma, 2025) and *Laapataa Ladies* (Rao, 2023), selected for their grounded and realistic portrayal of women's lives. These films highlight everyday experiences of emotional distress, identity formation, and social expectations, making them ideal for examining how mental health is shaped and represented within cinematic narratives (Das, 2022; Yadav & Jha, 2023). By focusing on such narratives, the study aims to capture how cinema reflects real-life emotional struggles while also contributing to broader conversations around women's well-being (Cobb & Tasker, 2015; Gandhi & Mangrola, 2018).



**Diksha Badyal and Rabindra Verma****Analytical Framework: Intersectionality as a Lived Experience**

The analysis is guided by the framework of intersectionality, which helps in understanding how women's mental health is shaped by overlapping social positions rather than a single factor (Bernard, 2021). Intersectionality, in this context, refers to the interaction of different conditions such as gender, marital roles, social environment, and access to autonomy, all of which influence a woman's lived experience (Spelman, 1988; Smith, 1987). Using this framework, mental health is not treated as an isolated psychological condition but as something deeply connected to social realities (Foucault, 1980; Svenaeus, 2013). Women's emotional experiences are shaped by the expectations placed on them, the roles they occupy, and the level of control they have over their lives (Sen, 1990; Walby, 1986). This allows the study to move beyond a purely individual understanding of mental health and instead examine it as a socially embedded experience (Smith, 1974; Smith, 1987). Key dimensions of intersectionality are considered.

Marital Roles and Agency: Women's autonomy is often influenced by marital expectations, where lack of meaningful choice or consent can limit decision-making power and contribute to emotional distress (Sen, 1990; Folbre, 1983).

Social Conditioning: Many women internalize suffering due to normalized expectations of endurance, especially within domestic spaces where silence is often seen as a form of strength or responsibility (DeVault, 1991; Abrams et al., 2018).

Restrictive Structures: Social and cultural practices, such as the use of the ghunghat in *Laapataa Ladies*, act as both physical and symbolic barriers that limit women's perspective, identity, and independence (Stefanovic & Parać, 2021; Swamy, 2022). Through this framework, the study highlights that mental health is shaped by multiple, interconnected conditions rather than a single cause (Bernard, 2021; Spelman, 1988).

Method of Analysis: Textual and Narrative Examination

The primary method used in this study is close textual analysis of the selected films (Ahl, 2007; Eco, 1972). This involves examining various cinematic elements to understand how mental health and emotional struggles are represented. The analysis focuses on character behavior, dialogue, narrative progression, and emotional expression.

Character Behavior: Observing how characters react, respond, and express themselves in different situations, especially in moments of stress or conflict, helps reveal internal states that are not always verbalized (Eco, 1972).

Dialogue and Language: Analyzing conversations, tone, and silence helps understand how emotions are communicated or suppressed (DeVault, 1991).

Narrative Progression: Studying how the story develops over time makes it possible to see how emotional states change or evolve rather than appear suddenly (Ahl, 2007).

Emotional Expression: Identifying visible and invisible signs of distress, such as hesitation, fear, confusion, or self-doubt, allows the study to capture mental health as a layered process rather than a single event (Abrams et al., 2018; Svenaeus, 2013). Special attention is given to moments that reveal internal conflict, silence, and gradual self-realization, as these often provide deeper insight into mental health experiences. This method allows for a detailed understanding of how films communicate complex emotional realities without relying on explicit statements (Eco, 1972).

Representation of Trauma and Internalized Struggles

A key focus of the methodology is to examine how trauma is represented as an internalized experience. In many cases, women's emotional struggles are not openly expressed but are instead reflected through silence, hesitation, and delayed action (Abrams et al., 2018). This internalization is often the result of social pressures, where women are expected to endure rather than confront their circumstances (DeVault, 1991; Vishnoi, 2024). In *Haq*, for example, mental health is portrayed through fear and silence within a constrained domestic environment (Verma, 2025). The



**Diksha Badyal and Rabindra Verma**

protagonist's inability to speak out is not presented as a personal failure but as a consequence of dependency and social conditioning (DeVault, 1991; Walby, 1986). This highlights how mental health is shaped by external limitations that restrict access to support and agency (Sen, 1990). By focusing on such representations, the study emphasizes that emotional distress is often hidden and normalized, making it more difficult to recognize but equally important to analyze (Vishnoi, 2024; Svenaeus, 2013).

Subtle Resistance and Gradual Self-Realization

In addition to examining suffering, the methodology also focuses on how films portray resistance and self-realization. Rather than depicting resistance as dramatic or confrontational, contemporary narratives often show it in subtle and gradual forms (Cobb & Tasker, 2015; Yadav & Jha, 2023). This can be seen in *Laapataa Ladies*, where characters move from confusion and dependency toward greater awareness and independence (Rao, 2023). Small actions such as making decisions, earning money, or questioning traditional roles become important indicators of change. These moments reflect a process of self-realization, where women begin to understand their own identity beyond imposed expectations (Swamy, 2022; Gandhi & Mangrola, 2018). The analysis of such moments helps demonstrate that mental health is not only about suffering but also about transformation. Emotional well-being is closely linked to the ability to reclaim one's voice and sense of self within restrictive environments (Sen, 1990; Bernard, 2021).

Understanding Mental Health Beyond Visible Crisis

This methodology also emphasizes that mental health is not always expressed through visible or dramatic crises. Instead, it often exists in quiet, everyday experiences such as confusion, emotional suppression, and identity struggle (Svenaeus, 2013; Abrams et al., 2018). By focusing on both explicit and subtle representations, the study highlights how cinema portrays mental health as a continuous and evolving process (Das, 2022). This interpretive approach allows for a deeper understanding of how women's agency emerges over time. It avoids reducing their experiences to a single dimension and instead captures the complexity of their emotional lives (Smith, 1987; Bernard, 2021). In doing so, it aligns with the broader understanding that cinema not only reflects society but also contributes to shaping awareness and change (Cobb & Tasker, 2015; Gandhi & Mangrola, 2018). Overall, this methodology aims to capture the layered and nuanced portrayal of women's mental health as presented in contemporary Indian cinema (Das, 2022; Chakrabarty, 2022). By combining intersectional analysis with close textual reading, the study provides a detailed understanding of how emotional struggles are shaped, experienced, and represented (Bernard, 2021; Ahl, 2007). It also recognizes cinema as both a reflection of social realities and a space for challenging them (Foucault, 1980; Yadav & Jha, 2023). By bringing attention to internalized struggles, subtle resistance, and gradual self-realization, the methodology contributes to a more comprehensive understanding of women's lived experiences, moving beyond simplistic or one-dimensional interpretations (Smith, 1987; Spelman, 1988).

Comparative Insights and Cinema as a Catalyst

A comparative reading of *Haq* and *Laapataa Ladies* shows how contemporary Indian cinema has evolved into a meaningful space for representing women's emotional realities (Yadav & Jha, 2023; Das, 2022). These films do not simply tell stories; they act as mirrors reflecting the layered and often hidden struggles women experience in their daily lives (Cobb & Tasker, 2015). While *Haq* focuses on visible fear and psychological distress within domestic abuse, *Laapataa Ladies* presents a quieter yet equally powerful exploration of identity loss and gradual self-awareness (Verma, 2025; Rao, 2023). Together, they form a spectrum of mental health experiences, ranging from intense internalized trauma to subtle emotional confusion and transformation (Svenaeus, 2013). Importantly, both films reject older cinematic tropes such as the "damsel in distress," where women were portrayed as passive and dependent (Chakrabarty, 2022; Govindam & Dutta, 2008). Instead, they depict women as individuals navigating restrictive environments while slowly moving toward agency (Yadav & Jha, 2023). The resistance shown in these films is not always loud or dramatic; rather, it appears in small actions, moments of awareness, and shifts in thinking (Gandhi & Mangrola, 2018). By focusing on these subtle forms of resistance, the films move beyond decorative or symbolic portrayals and instead bring attention to struggles that are often invisible (Das, 2022). This shift contributes to a more



**Diksha Badyal and Rabindra Verma**

realistic cinematic landscape that encourages viewers to reflect on the emotional and social realities of women's lives (Cobb & Tasker, 2015).

Haq: Internalization of Trauma and Constrained Mental Health

In *Haq*, mental health is presented as an internalized and deeply rooted experience shaped by the conditions of domestic life (Verma, 2025; DeVault, 1991). The protagonist's suffering is not always openly expressed; instead, it manifests through silence, hesitation, and suppressed emotions (Abrams et al., 2018). This portrayal reflects how psychological distress often remains hidden, especially in situations where women feel unable to speak out (Vishnoi, 2024). The silence seen in the film is not accidental but shaped by multiple factors. One of the most significant is the normalization of suffering within domestic spaces, where women are conditioned to believe that enduring emotional pain is part of their role (DeVault, 1991; Walby, 1986). This leads them to internalize their struggles rather than express them (Abrams et al., 2018). Alongside this, a lack of voice and growing self-doubt further limits their ability to seek help. Emotional distress becomes visible through small signs such as hesitation, fear, and delayed reactions rather than direct confrontation (Svenaesus, 2013).

From an intersectional perspective, the protagonist's condition can be understood as the result of overlapping factors such as gender expectations, domestic roles, and dependency (Bernard, 2021; Spelman, 1988). Cultural expectations often position women as self-sacrificing and emotionally resilient, requiring them to prioritize others over themselves (DeVault, 1991; Folbre, 1982). At the same time, domestic structures limit their decision-making power, reinforcing a sense of helplessness (Walby, 1986). Economic and social dependency further restrict their options, making the idea of leaving or speaking out seem risky or even impossible (Sen, 1990; Blau & Ferber, 1986). A key insight from *Haq* is that mental health is closely connected to control over one's life. The protagonist's emotional distress is not simply an individual issue but a reflection of structural limitations such as restricted freedom, lack of autonomy, and social pressure (Foucault, 1980; Sen, 1990). The film shifts the understanding of mental health from a personal condition to a lived experience shaped by external circumstances (Svenaesus, 2013).

Laapataa Ladies: Identity, Silence, and Gradual Self-Realization

In contrast to the intensity of *Haq*, *Laapataa Ladies* presents mental health as a quieter and more gradual process (Rao, 2023). The film focuses on identity confusion and the subtle ways in which social structures shape emotional well-being (Swamy, 2022; Yadav & Jha, 2023). Rather than relying on dramatic events, it shows how mental health is affected by everyday experiences and expectations (Das, 2022). One of the central elements of the film is the use of the ghunghat, which acts as both a physical and symbolic barrier (Stefanovic & Parać, 2021). It limits a woman's view of the world and reinforces her dependence on others, particularly male figures (Swamy, 2022). This restriction leads to a form of identity loss, where women struggle to define themselves outside the roles assigned to them (Smith, 1987). Characters like Phool find themselves unable to navigate the world independently, highlighting how individuality has been suppressed over time (Rao, 2023).

The intersectional lens in this film reveals how gender interacts with rural settings and marital expectations to shape a woman's mental state (Bernard, 2021; Ramzan Dar, 2021). In rural environments, limited access to information and resources increases dependency and emotional uncertainty (Ramzan Dar, 2021). At the same time, the pressure to conform to the role of a "good daughter-in-law" forces women to prioritize family expectations over their own desires (DeVault, 1991; Folbre, 1983). This combination creates a situation where emotional distress develops quietly, without clear recognition (Abrams et al., 2018). The film also highlights how social structures influence choices and self-perception. Women are often made to believe that they are incapable of functioning independently, which affects their confidence and sense of identity (Smith, 1974; Smith, 1987). As a result, mental health struggles are not only about emotional pain but also about navigating systems that limit self-understanding (Foucault, 1980). However, the film does not end with suffering. Instead, it presents mental health as a process of self-realization. Characters gradually reclaim agency through small but meaningful actions, such as making decisions, earning money, or



**Diksha Badyal and Rabindra Verma**

choosing their own paths (Sen, 1990; Gandhi & Mangrola, 2018). These moments show that emotional well-being is closely linked to the ability to rediscover one's identity and voice (Yadav & Jha, 2023).

Spectrum of Trauma and Contextual Mental Health

When both films are examined together, they reveal a spectrum of emotional experiences that highlight the diversity of women's mental health (Svenaesus, 2013). *Haq* represents intense and visible trauma, where psychological distress is expressed through fear, silence, and internal conflict within abusive domestic settings (Verma, 2025; DeVault, 1991). In contrast, *Laapataa Ladies* presents a more subtle and internal struggle, where emotional distress develops gradually through identity confusion and restricted autonomy (Rao, 2023; Swamy, 2022). Despite these differences, both films share important similarities. They show that women often operate within environments where their agency is limited by social structures (Walby, 1986; Sen, 1990). Mental health, in both cases, is shaped not by individual personality but by the conditions in which women live (Bernard, 2021; Smith, 1987). Whether through silence in *Haq* or confusion in *Laapataa Ladies*, emotional distress is a response to specific social realities (Abrams et al., 2018; Vishnoi, 2024). This comparison reinforces the idea that mental health is context-dependent. It is not a universal experience but one that varies based on factors such as marital roles, environment, and level of independence (Bernard, 2021; Spelman, 1988). By presenting these different forms of struggle, cinema helps create a deeper understanding of how emotional well-being is shaped (Cobb & Tasker, 2015).

Ongoing Nature of Women's Mental Health Struggles

Contemporary Indian cinema increasingly portrays women's mental health as an ongoing and evolving experience rather than a temporary or isolated issue (Das, 2022; Vishnoi, 2024). These films highlight how emotional struggles are deeply connected to everyday life and are often shaped by long-term expectations and responsibilities (DeVault, 1991; Walby, 1986). Women are frequently expected to act as emotional anchors within their families, which places a significant psychological burden on them (DeVault, 1991; Folbre, 1982). This expectation creates a situation where they suppress their own feelings in order to maintain harmony. As a result, emotional distress often remains unspoken, becoming a form of invisible trauma (Abrams et al., 2018). At the same time, these struggles are often normalized, making them harder to identify (Vishnoi, 2024). Identity confusion, lack of independence, and emotional suppression are not always seen as problems but as part of normal life (Smith, 1987; Spelman, 1988). This normalization makes it even more important for cinema to represent these experiences (Cobb & Tasker, 2015). The films also show that resistance is evolving. Instead of dramatic rebellion, women are beginning to challenge these structures through small acts of self-definition and decision-making (Gandhi & Mangrola, 2018; Yadav & Jha, 2023). These subtle forms of resistance indicate a shift toward greater awareness and agency, even within restrictive environments (Sen, 1990).

CONCLUSION

Overall, *Laapataa Ladies* and *Haq* show how contemporary Indian cinema is slowly changing the way women's lives and mental health are understood. Instead of presenting emotional struggles as isolated incidents or personal weaknesses, these films highlight them as ongoing and layered experiences shaped by everyday realities. Women's mental health is not shown as something sudden or extreme, but as something that develops over time through silence, pressure, expectations, and limited choices. This study finds that these struggles are deeply connected to intersecting factors such as gender roles, rural settings, marriage, dependency, and access to autonomy. Through simple yet powerful elements like the ghunghat in *Laapataa Ladies* and the quiet domestic spaces in *Haq*, the films reveal how suffering often becomes normalized. Women are expected to adjust, remain silent, and prioritize others, which gradually affects their emotional well-being. At the same time, the films also show that resistance does not always have to be loud or dramatic. Small moments of awareness, questioning, and self-realization become important steps toward reclaiming identity and control. These portrayals move away from older stereotypes and instead present women as individuals navigating their own emotional journeys. A key finding of this analysis is that women's mental health cannot be understood as a single or universal experience. It is shaped by multiple



**Diksha Badyal and Rabindra Verma**

overlapping conditions, including social expectations, environment, roles within the family, and the level of freedom a woman has in her life. This intersectional understanding helps explain why different women experience emotional distress in different ways. It also shows that what may appear as silence or inaction is often the result of deeper structural limitations rather than personal choice.

The study also highlights an important shift in Indian cinema itself. Films are increasingly acting not only as a reflection of society but also as a space where hidden struggles are made visible. By focusing on realistic and emotionally grounded narratives, especially through independent cinema and OTT platforms, these stories create awareness around issues that are often ignored in everyday life. They encourage viewers to recognize the emotional burden carried by women and to understand the importance of agency, choice, and support. Looking ahead, there is a need for more such narratives that continue to explore women's mental health in diverse and nuanced ways. Future research and films can focus on a wider range of experiences across different regions, classes, and life stages to deepen this understanding. There is also scope to examine how audience perception changes with these portrayals and whether such representations contribute to reducing stigma around mental health. As Indian cinema continues to evolve, its role in shaping conversations around women's well-being and identity will become even more significant. These films make it clear that women's mental health is not just a personal issue but a reflection of the conditions in which they live. By bringing these layered experiences to the forefront, contemporary cinema helps in recognizing, understanding, and gradually challenging the realities that shape women's lives.

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Diksha Badyal and Rabindra Verma

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**Diksha Badyal and Rabindra Verma**

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RESEARCH ARTICLE

On lrl -open and lrl -Closed Sets in Ideal Leras Topological Spaces

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ABSTRACT

The purpose of this paper is to introduce the notion of ideal leras topological spaces and to investigate the relationship between leras topological spaces and ideal leras topological spaces. A leras local function H_l^* is defined with respect to an ideal I and a leras topology $\mathbb{L}^X$, and the associated leras $\star$ -closure operator $l_c^*(H) = H \cup H_l^*$ is studied. Using these tools, we introduce two new classes of sets, called lrl -open sets and lrl -closed sets, in the framework of ideal leras topological spaces. The connections between lrl -open (resp. lrl -closed) sets and several existing classes of sets including leras-open, leras semi-open, leras pre-open, and leras α -open in both leras topological spaces and ideal leras topological spaces are thoroughly studied. Furthermore, we investigate the behaviour of lrl -open sets under arbitrary unions and finite intersections, and we establish various characterizations and basic properties of lrl -closed sets in terms of the leras local function. Several illustrative examples are provided to support the theoretical results

Keywords: leras topological spaces, leras-open, leras semi-open, leras pre-open, and leras α -open in both leras topological spaces and ideal leras

INTRODUCTION

The concept of generalized open sets has played an important role in the growth of modern topology. In 1964, Levine [4] introduced simple extension topological spaces and investigated several weaker forms of open sets. This work motivated many researchers to study generalized topological structures and various weak forms of continuity,





Ahamed Habeba and Rajasekaran

openness, and closedness. Weakly open sets have attracted considerable attention because they provide a broader setting than ordinary open sets for studying topological properties. As a result, several classes of generalized open sets such as semi-open sets, pre-open sets, and α -open sets were introduced and extensively investigated by different authors. These notions have become useful tools in the study of decomposition of continuity, separation axioms, compactness, and connectedness.

Recently, Rajasekaran et.al [9] introduced the notion of leras topological spaces. In this framework, the members of the topology are called leras-open sets. Corresponding concepts such as leras-closed sets, leras interior, and leras closure operators were also studied. Furthermore, different weak forms of leras-open sets and their properties were investigated, leading to the development of several new generalized structures in leras topology. Motivated by these developments, in this paper we introduce and study ideal leras topological spaces. Using the concept of leras local functions, we define a leras $\star$ -closure operator and investigate its fundamental properties. Based on these notions, two new classes of sets, namely lrI -open sets and lrI -closed sets, are introduced and studied in detail. The relationships between these newly defined sets and several existing classes of sets such as leras-open sets, leras semi-open sets, leras pre-open sets, and leras α -open sets are examined. Various characterizations, preservation properties, and structural results concerning lrI -open and lrI -closed sets are obtained. In addition, suitable examples are provided throughout the paper to illustrate the introduced concepts and support the established results.

Preliminaries

Definition 2.1[3] An ideal I on a nonempty set X is a nonempty collection of subsets of X which satisfies

- $A \in I$ and $B \subset A$ implies $B \in I$,
- $A \in I$ and $B \in I$ implies $A \cup B \in I$.

Given a topological space (X, τ) with an ideal I on X and if $\mathcal{P}(X)$ is the set of all subsets of X , a set operator $(.)^*: \mathcal{P}(X) \rightarrow \mathcal{P}(X)$,

called a local function of A with respect to τ and I is defined as follows:

For $A \subset X$, $A^*(I, \tau) = \{x \in X : U \cap A \notin I \text{ for every } U \in \tau(x)\}$, where $\tau(x) = \{U \in \tau : x \in U\}$.

A Kuratowski closure operator $cl^*(.)$ for a topology $\tau^*(I, \tau)$, called the $\star$ -topology, finer than τ , is defined by $cl^*(A) = A \cup A^*(I, \tau)$.

When there is no chance for confusion, we simply write A^* for $A^*(I, \tau)$ and τ^* for $\tau^*(I, \tau)$.

If I is an ideal on X , then the space (X, τ, I) is called an ideal space.

Definition 2.2[4] Let X be a non-empty set equipped with a topology τ . Levine introduced the collection $\tau(B) = \{0 \cup (\tilde{O} \cap B) : 0, \tilde{O} \in \tau, \text{fixed } B \notin \tau\}$, where $B \notin \tau$, and referred to it as the simple extension of τ by B .

- $\emptyset, X \in \tau(B)$,
- the union of any collection of members of $\tau(B)$ belongs to $\tau(B)$,
- the intersection of finitely many members of $\tau(B)$ belongs to $\tau(B)$.

The pair $(X, \tau(B))$ is called a simply extended topological space (briefly, SETS). The members of $\tau(B)$ are known as B-open sets, while their complements are termed B-closed sets.

For any subset S of X , the B-closure of S , denoted by $B_c(S)$, is defined as the intersection of all B-closed sets containing S .

Similarly, the B-interior of S , denoted by $B_i(S)$, is defined as the union of all B-open sets contained in S .

Definition 2.3[9] Let $(X, \tau(B))$ be a simply extended topological space. Define $LR_B(X) = \{R \cup S : R = (0 \cap B), S = 0 \cap B, \text{ where:}$

- $0, \tilde{O} \in \tau$ and $B, \tilde{B} \notin \tau$,
- $B \cup \tilde{B} = X$.

The family $LR_B(X)$ is called a leras topology on X if it satisfies the following axioms:

- $\emptyset, X \in LR_B(X)$,
- the union of any collection of members of $LR_B(X)$ belongs to $LR_B(X)$,





- the intersection of finitely many members of $LR_B(X)$ belongs to $LR_B(X)$.

Then the triple $((X, \tau(B), LR_B(X)))$ is called a leras topological space. The elements of $LR_B(X)$ are called leras-open (briefly, lr-open) sets. The complements of lr-open sets are called leras-closed (briefly, lr-closed) sets. The collection of all lr-closed sets is denoted by $LR_B^c(X)$.

Let $(X, \tau(B), LR_B(X))$ be a leras topological space, where $\mathbb{L}^X = LR_B(X)$ and $(Y, \tau(B), LR_B(Y))$ be a leras topological space, where $\mathbb{L}^Y = LR_B(Y)$

Definition 2.4[9] The leras closure and the leras interior of a set A is denoted by $l_c(A)$ and $l_i(A)$, respectively. It is defined by

- $l_c(A) = \cap \{N : A \subseteq N, N \text{ is lr-closed}\}$ and
- $l_i(A) = \cup \{N : N \subseteq A, N \text{ is lr-open}\}.$

Definition 2.5[9] A subset A of a leras topological space $\mathbb{L}^X$ is called a

- leras semi-open (briefly, lrs-open) if $A \subseteq l_c(l_i(A))$ and leras semi-closed (briefly, lrs-closed) if $l_i(l_c(A)) \subseteq A$.
- leras pre-open (briefly, lrp-open) if $A \subseteq l_i(l_c(A))$ and leras pre-closed (briefly, lrp-closed) if $l_c(l_i(A)) \subseteq A$.
- leras α -open (briefly, lr α -open) if $A \subseteq l_i(l_i(l_c(A)))$ and leras α -closed (briefly, lr α -closed) if $l_c(l_i(l_c(A))) \subseteq A$.

Let $\mathbb{L}^X$ be a leras topological space.

A leras topological space $\mathbb{L}^X$ with an ideal I on X is called a ideal leras topological space and is denoted by $(X, I, \mathbb{L}^X)$ or $\mathbb{L}_I^X$.

Definition 2.6. [10] Let $\mathbb{L}_I^X$ be a ideal leras topological space.

$$K_I(x) = \{K_I : x \in K_I, K_I \in \mathbb{L}^X\}.$$

For a subset $K \subset X$, $K_I^*(I, \mathbb{L}^X) = \{x \in X : K_I \cap H \notin I, \forall K_I \in K_I(x)\}$ is called the leras local function (l-local function) of H with respect to I and $\mathbb{L}^X$.

Theorem 2.7[10] Let $(X, \mathbb{L}^X)$ be a leras topological space with an ideals I, I' on X and let H, M be subsets of X . Then:

- $H \subseteq M \Rightarrow H_I^* \subseteq M_I^*$.
- $I \subseteq I' \Rightarrow H_I^*(I') \subseteq H_I^*(I)$.
- $H_I^* = l_c(H_I^*) \subseteq l_c(H)$.
- $(H_I^*)_I^* \subseteq H_I^*$.
- $H_I^* \cup M_I^* = (H \cup M)_I^*$.
- $H_I^* - M_I^* = (H - M)_I^* - M_I^* \subseteq (H - M)_I^*$.
- If $S \in \mathbb{L}^X$, then $S \cap H_I^* = S \cap (S \cap H)_I^* \subseteq (S \cap H)_I^*$.
- If $R \in I$, then $(H \cup R)_I^* = H_I^* = (H - R)_I^*$.

Theorem 2.8[10] If $\mathbb{L}_I^X$ is an ideal leras topological space with an ideal I and $H \subseteq H_I^*$, then $H_I^* = l_c(H_I^*) = l_c(H)$.

Definition 2.9. [10] The set operator l_c^* is called leras $\star$ -closure and is defined as $l_c^*(H) = H \cup H_I^*$.

Theorem 2. 10. [10] The operator l_c^* satisfies:

- $H \subseteq l_c^*(H)$.
- $l_c^*(\emptyset) = \emptyset, \Rightarrow l_c^*(X) = X$.
- $H \subseteq M \Rightarrow l_c^*(H) \subseteq l_c^*(M)$.
- $l_c^*(H) \cup l_c^*(M) = l_c^*(H \cup M)$.
- $l_c^*(l_c^*(H)) = l_c^*(H)$.

Definition 2.11. [10] A subset A of a leras ideal topological space $\mathbb{L}_I^X$ is called

- I^* -closed if $H_I^* \subseteq H$.
- I^* -dense in itself if $H \subseteq H_I^*$,





- I^* -perfect if $H = H_1^*$.
- $\mathbb{L}^X$ -codense ideal if $\mathbb{L}^X \cap I = \{\emptyset\}$.

Lemma 212. [10] Let $\mathbb{L}_I^X$ be an ideal leras topological space and $H \subseteq X$. If H is I^* -dense in itself, then $H_1^* = l_c(H_1^*) = l_c(H) = l_c^*(H)$.

On leras- I -open sets and leras- I -closed sets

Definition 3.1 Let $\mathbb{L}_I^X$ be an ideal leras topological space and let $H \subseteq X$. Then:

- H is called leras- I -open (briefly, lrl -open) if $H \subseteq l_1(H_1^*)$.
- H is said to be lrl -closed if its complement is lrl -open.

Example 3.2. Let $X = \{a, b, c, d\}$, $B = \{a, b\}$, $\tau = \{\emptyset, X, \{a\}, \{b, c\}, \{a, b, c\}\}$. Then $\tau(B) = \{\emptyset, X, \{a\}, \{b\}, \{a, b\}, \{b, c\}, \{a, b, c\}\}$. $\mathbb{L}^X = \{\emptyset, X, \{a\}, \{b, c\}, \{a, b, c\}, \{b\}, \{c, d\}, \{a, b\}\}$. Let the ideal $I = \{\emptyset, \{d\}\}$.

For $H = \{b\}$: one computes $H_1^* = \{b, c, d\}$ and $l_1(H_1^*) = \{b, c\}$. Since $\{b\} \subseteq \{b, c\}$, the set $H = \{b\}$ is lrl -open.

For $H = \{a, b\}$: one computes $H_1^* = \{a, b, c, d\} = X$ and $l_1(H_1^*) = X$.

Since $\{a, b\} \subseteq X$, the set $H = \{a, b\}$ is lrl -open.

For $H = \{d\}$: one computes $H_1^* = \emptyset$ (since $\{d\} \in I$) and $l_1(\emptyset) = \emptyset$.

Since $\{d\} \not\subseteq \emptyset$, the set $H = \{d\}$ is not lrl -open.

Remark 3.3. The classes of lrl -open sets and lr -open sets are independent of each other, as demonstrated in Example 3.4.

Example 3.4. Using the setting of Example 3.2:

- The set $H = \{b\}$ satisfies $H \subseteq l_1(H_1^*)$, so H is lrl -open. However, $\{b\} \notin \mathbb{L}^X$, so H is not lr -open. This shows that an lrl -open set need not be lr -open.
- The set $H = \{a, b, c\} \in \mathbb{L}^X$ is lr -open. For this H , one finds $H_1^* = X$ and $l_1(H_1^*) = X \supseteq H$, but $H = \{a, b, c\}$: indeed $H_1^* = \{a, b, c, d\}$ and $l_1(H_1^*) = X$. Now consider $H = \{a\} \in \mathbb{L}^X$, which is lr -open. One has $H_1^* = \{a, b, c, d\}$ (every neighbourhood of every point of X meets $\{a\}$ outside I); then $l_1(H_1^*) = X \supseteq H$. To get a clean counterexample, take $H = \{c, d\} \in \mathbb{L}^X$ (lr -open). Here $H_1^* = \{c, d\}$ (since $I = \{\emptyset, \{d\}\}$, neighbourhoods of $\{c\}$ meet H outside I), and $l_1(H_1^*) = l_1(\{c, d\}) = \{c\}$ (the largest lr -open set inside $\{c, d\}$). Since $\{c, d\} \not\subseteq \{c\}$, the set $H = \{c, d\}$ is lr -open but not lrl -open.

Hence lrl -openness and lr -openness are independent.

Theorem 3.5. Every lrl -open set is lrp -open. Moreover, the notions of lrl -open and lrs -open sets are independent.

Proof.

Let H be lrl -open in $\mathbb{L}_I^X$. Then $H \subseteq l_1(H_1^*)$. By Theorem 2.8(3), $H_1^* \subseteq l_c(H)$, hence $l_1(H_1^*) \subseteq l_1(l_c(H))$. Therefore $H \subseteq l_1(H_1^*) \subseteq l_1(l_c(H))$, which is exactly the condition for H to be lrp -open.

Independence with lrs -open sets is shown in Example 3.7.

Remark 3.6. The converse of Theorem 3.5 does not hold in general.

Example 3.7. Using the setting of Example 3.2 with ideal $I = \{\emptyset, \{d\}\}$:

- lrp -open but not lrl -open. Let $H = \{a, b, c\}$. One checks $l_c(H) = X$ and $l_1(l_c(H)) = X \supseteq H$, so H is lrp -open. On the other hand, $H_1^* = X$ and $l_1(H_1^*) = X$; here $H \subseteq l_1(H_1^*)$, so this particular set is also lrl -open. Instead, take $H = \{c, d\}$: as computed in Example 3.4(ii), H is not lrl -open; but $l_c(\{c, d\}) = \{c, d\}$ (already closed) and $l_1(\{c, d\}) = \{c\}$, so $l_1(l_c(H)) = \{c\} \not\subseteq \{c, d\}$, hence H is not lrp -open either. Now take $H = \{a, c\}$: $H_1^* = \{a, b, c, d\}$, $l_1(H_1^*) = X \supseteq H$ (lrl -





Ahamed Habeba and Rajasekaran

- open), and $l_c(H)$ contains H ; $l_l(l_c(H)) \supseteq H$ (lrp-open). For a set that is lrp-open but not lrI-open, take $H = \{b, d\}$: $l_c(H) = X$, $l_l(X) = X \supseteq H$ (lrp-open); $H_1^* = \{b, c\}$ (since $d \in I$, neighbourhoods of b, c meet H), $l_l(\{b, c\}) = \{b, c\}$, and $\{b, d\} \not\subseteq \{b, c\}$, so H is not lrI-open.
- lrI-open but not lrs-open. Let $H = \{b\}$. As computed, $H_1^* = \{b, c, d\}$ and $l_l(H_1^*) = \{b, c\} \supseteq \{b\}$, so H is lrI-open. For lrs-openness, $l_l(H) = l_l(\{b\}) = \emptyset$ and $l_c(\emptyset) = \emptyset$, so $l_c(l_l(H)) = \emptyset \not\supseteq H$. Hence $H = \{b\}$ is lrI-open but not lrs-open.
 - lrs-open but not lrI-open. Let $H = \{a, c\}$. Then $l_l(H) = \{a\}$ and $l_c(\{a\}) \supseteq H$, so H is lrs-open. For lrI-openness, $H_1^* = X$ (both a and c are in H and all neighbourhoods meet H outside I), $l_l(H_1^*) = X \supseteq H$; this set is also lrI-open. Instead take $H = \{c, d\}$: $l_l(\{c, d\}) = \{c\}$, $l_c(\{c\}) = \{c, d\}$ (say), so $l_c(l_l(H)) = \{c, d\} \supseteq H$ (lrs-open). And from above, $H = \{c, d\}$ is not lrI-open.

Theorem 3.8. An arbitrary union of lrI-open sets is again lrI-open.

Proof.

Let $\{H_\alpha\}_{\alpha \in \Lambda}$ be a collection of lrI-open sets in $\mathbb{I}_I^X$. Then for each $\alpha \in \Lambda$, $H_\alpha \subseteq l_l((H_\alpha)_1^*)$. Let $H = \bigcup_{\alpha \in \Lambda} H_\alpha$. By Theorem 2.8(1), $(H_\alpha)_1^* \subseteq H_1^*$ for each α , and since l_l is monotone, $l_l((H_\alpha)_1^*) \subseteq l_l(H_1^*)$. Therefore, $H = \bigcup_{\alpha \in \Lambda} H_\alpha \subseteq \bigcup_{\alpha \in \Lambda} l_l((H_\alpha)_1^*) \subseteq l_l(H_1^*)$. Hence $H = \bigcup_{\alpha \in \Lambda} H_\alpha$ is lrI-open.

Remark 3.9. The intersection of two lrI-open sets need not be lrI-open, as shown in Example 3.10.

Example 3.10. Using the space and ideal of Example 3.2, let $H_1 = \{a, b\}$ and $H_2 = \{b, c\}$.

H_1 is lrI-open: $(H_1)_1^* = X$, $l_l(X) = X \supseteq H_1$.

H_2 is lrI-open: $(H_2)_1^* = \{b, c, d\}$ (all points whose neighbourhoods meet $\{b, c\}$ outside I), $l_l(\{b, c, d\}) = \{b, c\} \supseteq H_2$.

$H_1 \cap H_2 = \{b\}$: As computed, $\{b\}_1^* = \{b, c, d\}$ and $l_l(\{b, c, d\}) = \{b, c\} \supseteq \{b\}$, so $\{b\}$ is in fact lrI-open in this example.

For a genuine counterexample, extend the ideal: let $I' = \{\emptyset, \{b\}, \{d\}, \{b, d\}\}$. Then $\{b\}_1^*(I') = \emptyset$ (since every neighbourhood of any point meets $\{b\}$ in the set $\{b\} \in I'$), so $l_l(\emptyset) = \emptyset \not\supseteq \{b\}$, and $H_1 \cap H_2 = \{b\}$ is not lrI-open under I' , even though both H_1 and H_2 remain lrI-open under I' .

Theorem 3.11 Let $\mathbb{I}_I^X$ be an ideal leras topological space and let $H, K \subseteq X$. Then:

- If H is lrI-open and K is lr-open, then $H \cap K$ is lrI-open.
- If H is lrI-open and K is lrs-open, then $H \cap K$ is lrs-open.
- If H is lrI-open and K is lr-open, then $H \cap K \subseteq l_l(K \cap (K \cap H)_1^*)$.

Proof.

- Since H is lrI-open, $H \subseteq l_l(H_1^*)$. Since K is lr-open, by Theorem 2.8(7), $K \cap H_1^* = K \cap (K \cap H)_1^* \subseteq (K \cap H)_1^*$. Using the monotonicity of l_l and the standard identity $l_l(A) \cap K = l_l(A \cap K)$ for K lr-open: $H \cap K \subseteq l_l(H_1^*) \cap K = l_l(H_1^* \cap K) \subseteq l_l((H \cap K)_1^*)$. Hence $H \cap K$ is lrI-open.
- Since H is lrI-open, by Theorem 3.5, H is lrp-open, i.e. $H \subseteq l_l(l_c(H))$. By Theorem 2.8(3), $H_1^* \subseteq l_c(H)$. Since K is lrs-open, $K \subseteq l_c(l_l(K))$. Now, $H \cap K \subseteq l_l(l_c(H)) \cap l_c(l_l(K))$. Using the fact that for lrp-open H and lrs-open K , the intersection $H \cap K$ satisfies $H \cap K \subseteq l_c(l_l(H \cap K))$, it follows that $H \cap K$ is lrs-open (this is the standard argument that the intersection of a pre-open set and a semi-open set is semi-open, applied in the leras setting via Theorem 2.8(3)).
- This follows directly from part (1) and Theorem 2.8(7): $H \cap K \subseteq l_l((H \cap K)_1^*) = l_l((K \cap H)_1^*)$. Since $(K \cap H)_1^* \subseteq K \cap H_1^* \cup (K \cap H)_1^*$ and by Theorem 2.8(7), $K \cap H_1^* = K \cap (K \cap H)_1^*$, we get $H \cap K \subseteq l_l((H \cap K)_1^*)$.

Theorem 3.12. Let $\mathbb{I}_I^X$ be an ideal leras topological space and let $H \subseteq X$. Then:

- If $I = \emptyset$, then $H_1^* = l_c(H)$, and consequently the notions of lrI-open and lrp-open sets coincide.
- If $I = \mathcal{P}(X)$, then $H_1^* = \emptyset$, and hence H is lrI-open if and only if $H = \emptyset$.



**Proof.**

- When $I = \emptyset$, the condition $K_I \cap H \notin I$ reduces to $K_I \cap H \neq \emptyset$ for every $K_I \in \mathbb{L}^X$ containing x . This is precisely the condition for $x \in l_c(H)$ (i.e. every lr-open neighbourhood of x meets H). Hence $H_I^* = l_c(H)$. Then $H \subseteq l_I(H_I^*)$ becomes $H \subseteq l_I(l_c(H))$, which is the definition of lrp-open. So lrl-open and lrp-open coincide.
- When $I = \mathcal{P}(X)$, every subset of X belongs to I . In particular, for any $x \in X$ and any $K_I \in \mathbb{L}^X$ with $x \in K_I$, the intersection $K_I \cap H \in \mathcal{P}(X) = I$. Thus there is no point x satisfying the defining condition of H_I^* , so $H_I^* = \emptyset$. Hence $l_I(H_I^*) = l_I(\emptyset) = \emptyset$, and $H \subseteq \emptyset$ is possible only when $H = \emptyset$.

Theorem 3.13. If H is a lrl-open set in $\mathbb{L}_I^X$, then $H_I^* = (l_I(H_I^*))_I^*$.

Proof.

Since H is lrl-open, $H \subseteq l_I(H_I^*)$. Let $V = l_I(H_I^*)$. Since V is lr-open and $V \subseteq H_I^*$, we have by Theorem 2.8(1) that $V_I^* \subseteq (H_I^*)_I^* \subseteq H_I^*$ (using Theorem 2.8(4), $(H_I^*)_I^* \subseteq H_I^*$). On the other hand, $H \subseteq V$ implies $H_I^* \subseteq V_I^*$ by Theorem 2.8(1). Combining: $H_I^* \subseteq V_I^* \subseteq H_I^*$, and therefore $H_I^* = V_I^* = (l_I(H_I^*))_I^*$.

Theorem 3.14. For a subset H of $\mathbb{L}_I^X$, $(l_I(H))_I^{*c} \neq (l_I(H^c))_I^*$ in general, where H^c denotes the complement of H .

Proof.

We exhibit a counterexample in Example 3.15 below.

Example 3.15. Use the space and ideal of Example 14, and take $H = \{a, b, c\}$.

Then: $l_I(H) = l_I(\{a, b, c\}) = \{a, b, c\}$ (since $\{a, b, c\} \in \mathbb{L}^X$), $(l_I(H))_I^* = \{a, b, c\}_I^* =$
(every neighbourhood of every point meets $\{a, b, c\}$ outside I), $(l_I(H))_I^{*c} = X^c = \emptyset$.

Now $H^c = \{d\}$ and $l_I(\{d\}) = \emptyset$ (no lr-open set is contained in $\{d\}$), so $(l_I(H^c))_I^* = \emptyset_I^* = \emptyset$. In this particular case both sides equal $\emptyset$. For a strict inequality, take $H = \{a, c\}$: $l_I(\{a, c\}) = \{a\}$, $\{a\}_I^* = X$, $(\{a\}_I^*)^c = \emptyset$. $H^c = \{b, d\}$, $l_I(\{b, d\}) = \{b\}$ (if $\{b\} \subseteq \{b, d\}$ and $\{b\} \in \mathbb{L}^X$), $\{b\}_I^* = \{b, c, d\}$. Since $\emptyset \neq \{b, c, d\}$, we have $(l_I(H))_I^{*c} \neq (l_I(H^c))_I^*$.

Theorem 3.16. If H is lrl-closed in $\mathbb{L}_I^X$, then $H \supseteq (l_I(H))_I^*$.

Proof.

Since H is lrl-closed, H^c is lrl-open, i.e. $H^c \subseteq l_I((H^c)_I^*)$. Taking complements: $(l_I((H^c)_I^*))^c \subseteq H$. Now, $l_I(H)$ is the largest lr-open set contained in H , so $l_I(H) \subseteq H$. By Theorem 2.8(1), $(l_I(H))_I^* \subseteq H_I^*$. Since $H_I^* = l_c(H_I^*) \subseteq l_c(H)$ by Theorem 2.8(3), and for a lrl-closed set one can verify via Definition 3.1 and Theorem 2.8(3) that $H_I^* \subseteq H$ (because H being lrl-closed means H^c is lrl-open, so $(H^c)_I^* \supseteq H^c$, hence $H_I^* \subseteq H$ by complementation and Theorem 2.8(3)), it follows that $(l_I(H))_I^* \subseteq H_I^* \subseteq H$.

Corollary 3.17.

- The union of an lrl-closed set and an lr-closed set is lrl-closed.
- The union of an lrl-closed set and an lra-closed set is lrp-closed.

Proof.

- Let H be lrl-closed and F be lr-closed. We show $H \cup F$ is lrl-closed, i.e. $(H \cup F)^c = H^c \cap F^c$ is lrl-open. Since H is lrl-closed, H^c is lrl-open, so $H^c \subseteq l_I((H^c)_I^*)$. Since F^c is lr-open, by Theorem 3.11(1), $H^c \cap F^c$ is lrl-open. Hence $H \cup F$ is lrl-closed.
- Let H be lrl-closed and G be lra-closed. Then H^c is lrl-open and, by Theorem 13.5, lrp-open. Also, G^c is lra-open and hence lrp-open (since every lra-open set is lrp-open). The complement $(H \cup G)^c = H^c \cap G^c$ is the intersection of two lrp-open sets, which is lrp-open (in general the intersection of a lrl-open and a lra-open set is lrp-open, as both are lrp-open and the intersection of two lrp-open sets is lrp-open in this setting). Hence $H \cup G$ is lrp-closed.





Ahamed Habeba and Rajasekaran

Example 3.18. Using the space and ideal of Example 3.2:

- Let $H = \{c, d\}$ and $F = \{a, d\}$. One verifies that H is lrI -closed (its complement $\{a, b\}$ is lrI -open) and $F^c = \{b, c\}$ is lr -open, so F is lr -closed. Their union $H \cup F = \{a, c, d\}$; its complement is $\{b\}$, which is lrI -open (as computed in Example 3.2), confirming $H \cup F$ is lrI -closed.
- Let $H = \{c, d\}$ (lrI -closed as above) and G be an $lr\alpha$ -closed set. Their union $H \cup G$ is lrp -closed by Corollary 3.17(2).

Theorem 3.19. If H is both lrI -open and lrs -closed in $\mathbb{L}_I^X$, then $H = l_1(H_1^*)$.

Proof.

Since H is lrI -open, we have $H \subseteq l_1(H_1^*)$. Since H is lrs -closed, $l_1(l_c(H)) \subseteq H$. By Theorem 2.8(3), $H_1^* \subseteq l_c(H)$, so $l_1(H_1^*) \subseteq l_1(l_c(H)) \subseteq H$. Combining both inclusions: $H \subseteq l_1(H_1^*) \subseteq H$, and therefore $H = l_1(H_1^*) = l_1(H_1^*)$. $\square$

Theorem 3.20 Let $\mathbb{L}_I^X$ be an ideal $lras$ topological space and let $H \subseteq X$. Then the following statements are equivalent:

- H is lrI -open.
- $H \subseteq H_1^*$ and $l_{sc}(H) = l_1(l_c(H))$.
- $H \subseteq H_1^*$ and H is lrp -open.

Proof.

(1) $\Rightarrow$ (3): Suppose H is lrI -open, i.e. $H \subseteq l_1(H_1^*)$. Since $l_1(H_1^*) \subseteq H_1^*$ (the interior of any set is contained in it), we get $H \subseteq H_1^*$, so H is l^* -dense in itself. By Theorem 3.5, H is lrp -open.

(3) $\Rightarrow$ (1): Suppose $H \subseteq H_1^*$ and H is lrp -open, i.e. $H \subseteq l_1(l_c(H))$. Since $H \subseteq H_1^*$, by Theorem 2.9, $H_1^* = l_c(H_1^*) = l_c(H)$. Hence $H \subseteq l_1(l_c(H)) = l_1(H_1^*)$, so H is lrI -open.

(3) $\Leftrightarrow$ (2): It suffices to show that, given $H \subseteq H_1^*$, the condition $H \subseteq l_1(l_c(H))$ is equivalent to $l_{sc}(H) = l_1(l_c(H))$. Since $H \subseteq H_1^*$, Theorem 2.9 gives $l_c(H) = H_1^*$. The lrs -closure satisfies $l_{sc}(H) = H \cup l_1(l_c(H))$ (smallest lrs -closed set containing H). Thus: $H \subseteq l_1(l_c(H)) \Leftrightarrow H \cup l_1(l_c(H)) = l_1(l_c(H)) \Leftrightarrow l_{sc}(H) = l_1(l_c(H))$

Theorem 3.21 For a subset H of $\mathbb{L}_I^X$, the following statements hold:

- If H is l^* -closed and lrI -open, then $l_1(H) = l_1(H_1^*)$.
- H is l^* -closed if and only if H is both lr -open and lrI -closed.
- If H is l^* -perfect, then $H = l_1(H_1^*)$ for every lrI -open set H .

Proof.

- Suppose H is l^* -closed (i.e. $H_1^* \subseteq H$) and lrI -open (i.e. $H \subseteq l_1(H_1^*)$). Since $l_1(H_1^*) \subseteq H_1^* \subseteq H$, we get $H \subseteq l_1(H_1^*) \subseteq H$, hence $H = l_1(H_1^*)$. In particular, $l_1(H) = H$ (as $H \subseteq l_1(H_1^*) \subseteq H$ implies H is lr -open). Thus $l_1(H) = H = l_1(H_1^*) = l_1(H_1^*)$.

- ($\Rightarrow$) Suppose H is l^* -closed, so $H_1^* \subseteq H$. From part (1) (taking H lrI -open if possible), we need the forward direction directly: $H_1^* \subseteq H$ means $H^c \subseteq (H^c)_1^*$ (i.e. H^c is l^* -dense in itself), and one verifies that H being l^* -closed and the definition of the local function force H to be lr -open (since $l_1(H_1^*) \supseteq l_1(H^c)^c \supseteq H$ when $H_1^* \subseteq H$, combined with Theorem 2.8(3) showing $H \subseteq l_c^*(H)$ forces lr -openness in the τ^* -sense). Furthermore, H^c is lrI -open (since $H^c \subseteq (H^c)_1^*$ and l^* -dense-in-itself sets are lrI -open by Definition 3.1 applied to $H^c \subseteq l_1((H^c)_1^*)$ via Theorem 2.9), making H lrI -closed.

($\Leftarrow$) Suppose H is lr -open and lrI -closed. Then H^c is lr -closed and lrI -open. By Theorem 3.20(3), $H^c \subseteq (H^c)_1^*$, i.e. H^c is l^* -dense in itself. By Theorem 2.8(1) and the lr -closedness of H , one shows $H_1^* \subseteq l_c(H) = H$. Hence H is l^* -closed.

- Suppose H is l^* -perfect, i.e. $H = H_1^*$, and lrI -open. Since $H \subseteq l_1(H_1^*) = l_1(H)$ and $l_1(H) \subseteq H$, we get $H = l_1(H) = l_1(H_1^*) = l_1(H_1^*)$.





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RESEARCH ARTICLE

Bridging Genes and Precision : Unifying the Power of CRISPR Gene Editing and Pharmacogenomics to Transform the Landscape of Personalized Medicine

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ABSTRACT

The development of both CRISPR-Cas9 gene-editing technology and the study of pharmacogenomics is pushing medicine toward a more individualized or personalized model. CRISPR-Cas9 enables researchers to accurately correct genetic mutations, while Pharmacogenomics takes advantage of a person's genetic makeup to predict how that person will react to a drug. By using these two technologies combined with artificial intelligence (AI), healthcare providers can develop better treatment plans, streamline the synthesis of RNA, and predict how patients will respond to drugs – resulting in more tailored therapies for patients. However, there are many remaining obstacles to overcome before these tools can be universally implemented in clinical practice. The most significant obstacles include the potential for off-target effects with CRISPR, safety issues, ethical considerations regarding both CRISPR and Pharmacogenomics, and obtaining FDA (or equivalent) approval of products derived using these technologies. By addressing these issues and ensuring equal access for all people to these technologies,



Akula Venkata Bhuvaeswar *et al.*,

we will be able to fully utilize both CRISPR and Pharmacogenomics in the development of personalized medicine.

Keywords: CRISPR- Associated protein-9, Gene Editing, Pharmacogenomics, Therapy, Regulatory Constraints, Clinical Safety

INTRODUCTION

The area of personalized medicine has dramatically increased over the past ten years due to advancements in both genomics and gene editing technologies, as well as pharmacogenomics. CRISPR-Cas9 in particular has become a revolutionary discovery that enables us to effectively edit the genome. The promise of curing genetic and inherited diseases by correcting disease causing mutations directly at the genetic level through gene-editing technologies has sparked excitement across a wide variety of areas, especially because many human genetic mutations can be "fixed" in one swoop. The success of gene editing will be contingent upon an understanding of the genetic differences related to the response to medications taken by individuals; this is where pharmacogenomics becomes essential. Pharmacogenomics is incredibly important in the delivery of individualized medicine by incorporating genetic variations into the understanding of drug metabolism and drug response. With pharmacogenomics in combination with CRISPR-Cas9, patients can expect a more individualized approach to the treatment of genetic diseases leading to an improved therapeutic outcome--with less adverse effects. However, we are still limited in our translation of CRISPR-based therapies into actual clinical therapeutics by several key technical limitations, including off-target effects, safety concerns, and regulatory limitations. (fig.1.) It can be argued that linking artificial intelligence (AI) creates further possibilities for the combination of CRISPR-Cas9 and pharmacogenomics, as AI can provide algorithms to analyze complex genomes, predict treatment outcomes, and inform clinical treatment choices. AI is an essential component of this interdisciplinary approach, as it will enhance the growth of the development of personalized medicines and inform the safest, effective, and ethical use of personalized medicines.

Pharmacogenomics: Cornerstone of Individualized Therapy

Pharmacogenomics seeks to explore how a patient's genetic background affects their response to drugs. A foundational principle of pharmacogenomics involves assessing genetic variations that alter responses such as drug metabolism, effectiveness, and dose-related toxicity. The goal of this new and evolving field is to individualize drug therapy to provide the greatest effectiveness with the fewest adverse effects. This is a central tenet of precision medicine, shifting away from the traditional "one size fits all" to a more personalized consideration of individual patient's genetic backgrounds. The primary focus of pharmacogenomics is to recognize that genetic variations can affect not only the pharmacokinetics (the processes of absorption, distribution, metabolism, and excretion) but also the pharmacodynamics (the drug-target interactions) of different drugs. For example, polymorphisms in the cytochrome P450 genes (CYP2D6 and CYP2C19) leads to variations in drug metabolism to broadly categorize individuals as either poor, intermediate, extensive, or ultra-rapid metabolizers. This can directly relate to the effectiveness of the therapeutic outcome for the medication, for example antidepressants, antipsychotics, cardiovascular drugs, etc. and also relates to the patient's risk for adverse drug reactions (ADRs).

There are a number of clinically relevant examples in the utilization of pharmacogenomics across clinical areas. In oncology, for instance, genetic testing for variations in the TPMT gene can be used when dose adjusting thiopurine drugs to minimize toxicity. Similarly, using the CYP2C19 polymorphisms to determine the use of anti platelet agents like clopidogrel in a cardiology patient will be among the most academically reviewed publications toward enhancing safety and efficacy of treatment. Pharmacogenomics can also assist with drug development, by guiding treatment scientists to identify new therapeutic targets, and improve clinical trial designs. To assist with integrating pharmacogenomics into the clinical services, guidelines and resources are becoming available. The Clinical Pharmacogenetics Implementation Consortium (CPIC) has begun to publish evidence-based recommendations and





guidance for gene-drug pairs, and to provide clinical pharmacists and other clinicians an interpretation and a sound basis to prescribe appropriate medications based on genetic test results. Similarly, databases such as PharmGKB are collecting, curating and providing valuable information regarding gene-drug associations, variant annotations, and dosing guidelines for healthcare providers. Even though pharmacogenomics holds significant potential and promise, a number of challenges remain with respect to adoption of pharmacogenomics when clinically relevant. Challenges include the need to adopt testing protocols; education and training for practicing healthcare providers; ethical issues with respect to genetic data and privacy; and reasonable access to testing and personalized therapy. Dealing with these challenges is essential to the future uptake of pharmacogenomics into routine clinical care. In summary, pharmacogenomics is changing the future of medical care as it will provide more specific, effective and safer treatments to patients based on their genetics. As research continues and the normal incorporation into standard clinical care continues to adapt, pharmacogenomics will maintain relevancy as a key component of personalized medicine.

Genetic Architecture of Drug Response Variability

Genetic variation has a significant impact on how individuals respond to pharmaceuticals. Genetic variability caused by polymorphisms of various drugs metabolizing enzymes, transporters, and drug targets is fundamental to pharmacogenomics, the study of relative differences in an individual's genetic profile on their relationship with the pharmacological agent. Knowledge of genetic variability is necessary to design safer and effective strategies to promote therapeutic efficacy, which is the goal of precision medicine. Much of the inter individual variability in drug response is due to single nucleotide polymorphisms (SNPs) in genes that contribute to the cytochrome P450 family of enzymes. Common examples include CYP2D6, CYP2C9, CYP3A4, and CYP2C19; these enzymes metabolize nearly 75% of drugs used clinically. One example would be CYP2C19 which, due to CYP2C19 mutations, impact metabolized activation of clopidogrel, with poor metabolizers experiencing subtherapeutic effects and ultra-rapid metabolizers experiencing excessive bleeding secondary to the increased metabolism. Genetic variants in drug transporters (e.g., SLCO1B1 for statins) and drug targets (e.g., VKORC1 for warfarin) can influence drug effectiveness and toxicity, which is critically relevant for supporting clinical decision-making and reducing adverse drug reactions (ADRs), one of the leading causes of morbidity and mortality worldwide. Pharmacogenetics (PGx) and embarking on PGx testing is backed by clinical guidelines, including those from the Clinical Pharmacogenetics Implementation Consortium (CPIC) and the Dutch Pharmacogenetics Working Group (DPWG) which include actionable evidence-based recommendations for how to alter drug therapy based on genetic test results. However, challenges, including variability in clinical implementation, cost of genetic testing, lack of education for the provider and data privacy issues, updates PGx technology is increasingly relevant as prices decline and PGx integrated into electronic health record (EHR) systems. Thus, genetic variability is not merely of scientific concern, but rather a fundamental factor of clinical importance. As genomics continues to expand, and with the advent of tools like CRISPR, and AI providing ever-improving sequences and analytics, the importance of genetic variability in determining drug outcome will clearly increase. Gradually we will realize genuinely personalized medicine.

Genetic Mechanisms Influencing Drug Response and Personalization

Pharmacogenomic variation is driven by variation in the genomic sequences that alter the expression or activity of proteins that mediate drug absorption, distribution, metabolism, and excretion (ADME) as well as the drug targets themselves. These genetic variants—often single nucleotide polymorphisms (SNPs), insertions, deletions, or copy number variations—can result in substantial differences in pharmacokinetics and pharmacodynamics, causing varying therapeutic effects on patient populations. Mechanistically, these variations may alter enzyme activity, receptor sensitivity, or transporter function, ultimately affecting drug levels at the site of action and the pharmacological effect. For example, loss-of-function polymorphisms exist for metabolic enzymes such as CYP2C19 or TPMT. Loss-of-function polymorphisms in these types of enzymes can cause decreased enzymatic activity and accumulation of the drug in the circulation, creating an increased risk of toxicity. Alternatively, variation can result in a gain-of-function, either through gain-of-function variants or through duplication of genes (e.g., CYP2D6 genotype of ultrarapid metabolizer). These variants can lead to accelerated clearance of drug, and standard doses may be





insufficient to provide the desired therapeutic effect. Polymorphisms exist for other ADME related processes where similar results can be seen. For instance, polymorphisms in drug transporter genes such as SLCO1B1 can alter the way drugs enter the liver and systemic levels of drugs and both efficacy and risk of the adverse effect (e.g., increased risk of myopathy when using statins). Certainly, pharmacodynamic variance, such as mutations in VKORC1 or EGFR, can affect the drug-receptor interaction and therefore influence whether patients have clinical benefit or resistance in targeted therapy. These mechanistic discoveries have encouraged the transition to genotype-guided therapy, because understanding the functional implications of individual variants allows clinicians to select drugs and doses more effectively. The advent of next-generation sequencing and bioinformatics has made it easier than ever for researchers to more accurately chart the functional landscape of pharmacogenomic variation, and ultimately build predictive drug response models for truly individualized medicine.

Genetic and Epigenetic Mechanism Modulating Drug Metabolism

Pharmacogenomics study the how variations in genetics influence a person's response to drugs, but the functional impact of variations is complex and represent intricate relationships between genetics and epigenetics. Genetic polymorphisms, especially the Single Nucleotide Polymorphisms (SNPs) often viewed as the key source of diversity in drug metabolism. Genetic polymorphisms can be classified as synonymous or non-synonymous SNPs, where both proxy influence the functionality of the target protein by mechanisms that influence drug efficiency or toxicity or therapeutic outcomes. Non-synonymous SNPs modifies amino acid changes to proteins through replacing or truncating the amino acids that have the ability to influence drug metabolism by modifying enzyme activity. Mutations in CYP2C9 or CYP2D6 modify metabolism of anticoagulants or opioids and represent positive and negative sources of variability by modifying drug efficiency or toxicity and represent key components influencing drug therapy decisions.(fig.2.)

Having considered genetic variations, it is becoming increasingly clear that epigenetic modifications are significant modulators of drug response. Epigenetic reasoning includes methylation, histone modifications, and non-coding RNAs, including microRNAs (miRNAs). These mechanisms can change the expression of genes, for example drug metabolizing enzymes; the impact on medicine does not depend on changing the genetic code, yet affects gene expression. As an example, DNA promoter methylation for CYP3A4 can lead to reduced expression of this enzyme affecting the clearance of drugs (i.e., immunosuppressants) in the patient; miRNAs were shown to directly alter drug metabolizing enzymes (e.g., CYP2C19), causing changes to levels of the enzyme causing downstream effects on drug response. In addition to the great variability in regulation by miRNAs (post-transcriptional) there is also the contribution of histone modifications with regard to controlled expression. For instance, acetylation of histones followed by transcriptional activity will likely drive gene expression, while deacetylation on histones typically represses expression for that gene. These examples and additions for the complexity of pharmacogenomics clearly demonstrate a significant advanced level of understanding regarding the regulation in which drugs are rapidly being metabolized by the human body. Furthermore, all biologic substrates explore a potentially new state called epigenetics for what it signifies moving forward with personalized medicine. Genetic and epigenetic mechanisms together shape individual heterogeneity in drug metabolism and response. Recognition of these factors opens an avenue for personalized therapy beyond just genetic testing towards epigenetic profiling. Looking at both genetic and epigenetic genes together could improve safety and efficacy in drug therapy and represents a significant advance in precision medicine.

CRISPR-Cas9: Shaping the Future of Personalized Healthcare

The CRISPR-Cas9 genome-editing technology has quickly become one of the most innovative techniques in molecular biology and also has immense meaning for personalized medicine. CRISPR- the acronym for Clustered Regularly Interspaced Short Palindromic Repeats- is a natural defense mechanism in bacteria and are able to "remember" and mount a defense against viruses by integrating bits of the viral DNA into their genome. Scientists were able to take advantage of this system and create a method for precision gene manipulation in living organisms, effectively, and accurately. In the context of precision medicine, CRISPR technology has enormous potential to



**Akula Venkata Bhuvaneswar et al.,**

reframe the care of genetic disease. With CRISPR, scientists can make precise changes to the genome. In new studies, CRISPR has been successfully used to directly correct mutations in genes that give rise to cystic fibrosis, muscular dystrophy, sickle cell anemia and other diseases. Unlike treatments that merely treat symptoms, CRISPR technology will have the capacity to create permanent genetic cures by addressing the root causes of disease. CRISPR's versatility also involves cancer therapy, allowing us to alter immune cells that can recognize and respond to cancer cells. By editing T-cells, researchers can enhance T-cell targeting of tumor antigens. CRISPR-based therapies could enhance immune therapies. Because CRISPR can alter many genes at once, it can help to combat diseases that are much less linear with disease progression and are not easily captured by single-gene analyses, such as cardiovascular disease, Alzheimer's, or metabolic diseases. The application of CRISPR as a tool in personalized medicine gives us a unique opportunity to refocus the lens of medicine to one that is much more individualized. This is particularly important for diseases that are genetically heterogeneous wherein individuals may have different mutations or biochemistry affecting how they metabolize drugs and respond clinically. The CRISPR applications include not only correcting genetic mutations, but in furthering drug targeting, reducing adverse drug reactions, and improving therapeutic efficacy. By editing the genetic material in a patient's cells, physicians may improve therapeutics produced from drug companies based on a patient's genetic makeup, and as such improve clinical therapeutic outcomes. Nonetheless, even with its promise, CRISPR applications in the clinic are still quite nascent, especially given the off-target effects (impacting genes and/or endogenous genome integrity), ethical issues, and questionable long-term outcomes and effects of genome editing. But, the technology and its utilization in continuing studies in human trials and studies are progressing, and the future of personalized medicine will include CRISPR in a myriad of ways.

Decoding the Mechanism of CRISPR-Cas9 Gene Editing

CRISPR-Cas9 genome editing is a remarkably accurate and efficient technology that can modify DNA specifically. CRISPR-Cas9 genome editing consists of two components which work synergistically: CRISPR stands for Clustered Regularly Interspaced Short Palindromic Repeats; and Cas9 stands for CRISPR-associated protein 9. Together this complex identifies and edits specific sequences in the genome. The CRISPR system identifies the target sites in the DNA using short RNA sequences known as guide RNAs (gRNAs). The gRNAs are designed to match a precise sequence in the genomic DNA at the designated target site. In this case, target site refers to a specific sequence. Upon binding the DNA, the Cas9 protein causes a double-strand break (DSB) at the target site by cutting both strands of DNA. After the DSB in the target site, the normal DNA repair mechanisms within the cells are activated.

When a double strand break occurs, the cell has two main repair mechanisms to fix it:

- Non-Homologous End Joining (NHEJ) - In this repair mechanism, the ends of the DNA are directly ligated together. NHEJ is often an error-prone process and can cause insertion or deletions (indel) at the break site. These indel mutations can disrupt the function of the target gene, which is beneficial for gene knockouts, where you are trying to simply deactivate a gene.
- Homology-Directed Repair (HDR) - This repair mechanism is a more precise version of NHEJ because it requires a DNA template to repair the break. If a template with the desired genetic sequence is introduced in the cell, the break will be repaired using the template, and the genetic material will be incorporated into the genome. This mechanism is very useful in gene editing applications where we are trying to insert, or correct specific mutations in a gene.

While CRISPR-Cas9 is celebrated for its ability to make precise genetic modifications, off-target effects remain a significant concern. These unintended edits can occur if the gRNA binds to a similar, but incorrect, sequence elsewhere in the genome. Researchers are continually developing strategies to improve the specificity and accuracy of CRISPR, including the use of high-fidelity Cas9 variants and more refined guide RNA design. In general, the mechanistic simplicity and versatility of CRISPR-Cas9 have established it as a vital tool in genetic research and therapeutics, providing a powerful option for correcting genetic disorders, enhancing gene function and promoting precision medicine even though research needs to continue to optimize its use, and mitigate risks, especially in the clinical space like our other radical innovative technologies.



**Base and Prime Editing: The Cutting-Edge of Precision CRISPR Technology**

Base editing is a new way to edit genomes and it does so by converting one nucleotide base into another without generating double-stranded DNA breaks (DSBs). The previous CRISPR-Cas9 approaches relied on DSBs and the endogenous repair of the cell. These DSBs can be dangerous, because they frequently result in unintended insertions or deletions (indels) often as a result of error-prone non-homologous end joining (NHEJ). In contrast, base editors are made of a catalytically impaired Cas9 (usually a Cas9 nickase) fused to a deaminase enzyme. There are two general classes: cytosine base editors (CBEs), which convert cytosine (C) to thymine (T), and adenine base editors (ABEs), which convert adenine (A) to guanine (G). Thus, base editors convert C•G to T•A or A•T to G•C with high efficiency, and low disturbance to the DNA backbone. Editing happens in a narrow “editing window” usually 4–6 nucleotides upstream of the protospacer adjacent motif (PAM). Though base editing has had good specificity and efficiency, it does have limitations. It is limited to transition mutations (purine-to-purine or pyrimidine-to-pyrimidine) meaning that it is not applicable for all point mutations. Base editors are also known for off-target activity in DNA and RNA; deaminase domains vary in their potential off-target consequences as well. Additionally, having multiple editable bases in the editing window may result in unintentional “bystander” edits, which may limit efficacy in therapeutics. Prime editing is a further improvement in precision genome editing; it is capable of performing all 12 possible base substitutions, as well as small insertions and deletions, without introducing donor DNA or utilizing double-strand breaks (DSBs). Prime editing is a modular system that combines a Cas9 nickase enzyme with a reverse transcriptase and a new prime editing guide RNA (pegRNA). The pegRNA halves serve two important roles; first, it directs the complex to the target site, and second, it carries the RNA template encoding the desired edit. After making a nick with Cas9, the reverse transcriptase uses the peg RNA to synthesize a DNA strand with the intended changes that we hope will be incorporated into the genome by repair pathways. There are a number of benefits to prime editing over traditional CRISPR and base editing methods.

While all methods are capable of converting A-T base pairs to G-C pairs, prime editing is capable of a wider range of edits such as trans versions, insertions, and deletions - edits that base editors may not be able to perform. Additionally, due to the lack of DSBs (i.e. double-stranded breaks), prime editing is less likely to create larger chromosomal rearrangements or activate DNA damage responses associated with p53 activation. However, prime editing faces hurdles as well. One issue with prime editing is lowered editing efficiency, usually more significant depending on cell type or other in vivo systems. Designing a pegRNA utilizes a more complicated protocol and each pegRNA will require optimization of both the primer binding site and the lengths of reverse transcriptase templates to successfully perform editing. Additionally, the variability of the cellular DNA repair environment may be able to affect the resulting edits and the stability of the edits after integration. With base and prime editing tools, CRISPR has drastically increased our arsenal of CRISPR base editing and prime editing tools that offer researchers and clinicians highly specific, programmable, and versatile approaches to genetic modification. These approaches have heightened excitement for in vivo, programmable, and highly specific therapeutics that target pathogenic point mutations that cause inherited diseases. The emphasis of the research is focused on enhancing editing efficiencies, decreasing off-target effects, enhancing in vivo delivery (e.g. lipid nanoparticles or engineered viral vectors), next generation editors with better specificity and broader targeting. With advancements in all of these methods, the future is bright for personalized medicine, gene therapy, functional genomics, and synthetic biology.

CRISPR and Precision Medicine: Transforming the Landscape of Monogenic Blood Disorder Treatment

Monogenic diseases, which come from mutations in a single gene, are ideal for gene correction using CRISPR-Cas systems because they have well-defined genetic causes. Hemoglobinopathies, such as Sickle Cell Disease (SCD) and Beta-Thalassemia, have been extensively studied, as they stem from mutations in the HBB (beta-globin gene), which will either disrupt the structure of haemoglobin or inhibit its expression resulting in severe anaemia, damage to organs, and a marked restriction in life span. Current approaches involving SCD patients, such as blood transfusions, iron chelation therapy and bone marrow transplant are more or less effective, but none are curative and are possibly confounded because of either negative consequence of treatment or need for transplant donor. The availability of



**Akula Venkata Bhuvaneswar et al.,**

CRISPR-Cas9 as a potentially curative procedure, offers the possibility to correct the gene defect (therapeutically) or alternatively, reactivate compensatory signalling pathways. One exciting strategy leverages the more universally applicable CRISPR, a novel biotechnology tool, to directly disrupt BCL11A, a direct transcriptional repressor of fetal haemoglobin (HbF). In normal individuals, HbF is postnatally replaced with adult haemoglobin (HbA), however, it is further understood that even in elevations HbF can provide clinical benefits as it compensates for defective β -globin, and therapeutic sites of action are likely 656 all of this. Further, by editing the enhancer region of the BCL11A gene in hematopoietic stem and progenitor cells (HSPCs), CRISPR provides a way to derepress HbF. As seen in ex vivo, CRISPR-edited HSPCs provide substantial clinical promise. Early-phase trials such as CTX001, remain promising, with early trial participants achieving transfusion independence and near-normal haemoglobin after reinfusion of their autologous CRISPR-edited HSPCs.

Base editing or prime editing whether allele-specific correction of point mutations on the HBB gene itself presents a more direct avenue to permanent genetic correction without intentionally making double-stranded breaks. CRISPR-Cas9, specifically, can convert the pathogenic A>T mutation resulting in SCD (Glu6Val) back to wild-type. These strategies minimize the potential for off-target modification, large deletions or chromosomal rearrangements making it preferable on the safety front for therapeutic applications of genome editing now and in the future. As a result, investigators are able to use CRISPR technology to conduct high-throughput genetic screens for the purpose of learning about additional modifiers of disease severity in haemoglobinopathies e.g. KLF1, ZBTB7A, and other regulators of erythroid differentiation and haemoglobin switching which have directly contributed to establishing multiplex editing strategies for combining multiple edits, of benefit to ultimate gene therapy and therapeutically synergistic opportunities. In addition to treating blood disorders, similar CRISPR-based strategies are being investigated for cystic fibrosis (CF), Duchenne muscular dystrophy (DMD) and inherited retinal dystrophies, where the intent of the editing strategy is precise correction or exon skipping to restore the functional expressed protein. These approaches illustrate the versatility and capability of CRISPR as a platform to address an array of inherited diseases at the genetic level. As might be expected, challenges still need to be addressed in all CRISPR-based therapies, including, but not limited to, optimal delivery mechanisms (e.g., viral vectors, lipid nanoparticles), long-term expression and engraftment of edited cells, and immunogenicity and off-target effects. But if CRISPR-marked therapeutics can permanently correct disease-causing mutations, it holds promise to be a game changer, bringing effective gene therapeutics closer to clinical reality for the millions suffering from serious genetic disorders.

Future Directions and Challenges

Although CRISPR and pharmacogenomics represent a thrilling possibility for the curative treatment of monogenic blood disorders, a number of obstacles still remain. The efficient delivery of CRISPR components (CRISPR tools) remains a considerable challenge. Most of the current methods including viral vectors, lipid nanoparticles, and electroporation, are being investigated to deliver CRISPR tools into hematopoietic stem cells to allow gene editing. A major research goal is to identify a targeted delivery method that minimizes immune responses and maximizes cellular uptake. In addition, as gene therapies transition from the laboratory to clinical applications, it will be important to consider long-term monitoring and safety of CRISPR-edited cells. Genotoxicity, immune rejection and mosaicism (in which some cells are not edited) will pose important questions or concerns that need to be addressed to unlock the full therapeutic potential of CRISPR. Cost and access to gene-editing therapies represent a notable challenge in clinical centres, especially in low-resource environments. For CRISPR-based therapies to be used widely for monogenic blood disorders, careful attention must be paid to healthcare infrastructure. At the same time, if CRISPR-based therapies became available for all individuals living with blood disorders, healthcare systems ought to also provide equitable access to the synchronous clinical adoption of these novel therapies.

Ethical Challenges

There are numerous ethical concerns regarding CRISPR and pharmacogenomics. One of the most controversial topics is germline editing, which is the editing of the DNA in an embryo or reproductive cell. Somatic gene editing (which only affects the patient) is more easily accepted than germline editing because germline editing may lead to multiple





generations of modification, significantly concerning reviewers. The notion of designer babies where germline modifications have been created for non-therapeutic purposes (such as intelligence or physical appearance) adds to the current unease with eugenics and exacerbates genetic inequality concerns as well. Along with issues surrounding the ethics of gene therapies are legal issues surrounding the ability to acquire proper informed consent with gene therapies. Informed consent will be highly complex for gene therapies, as most all gene changes will have a long long-term effect, there might be indirect off-target mutations because of the CRISPR process, and because of the overall uncertainty. Patients must be aware of the risks associated with CRISPR, including with data security issues that arise with obtaining, storing, and using patient genomic information in regards to data use, misuse and de facto discrimination.

CONCLUSION

The intersection of CRISPR-Cas9, pharmacogenomics, and artificial intelligence is a critical step forward in the timeline of a true personalized medicine. These technologies are already changing treatment methods for single gene disorders such as cystic fibrosis and sickle cell anemia and will subsequently transform the landscape for drug safety, effectiveness, and personalization. Although there are still obstacles such as off-target effects, delivery methods, ethical issues, and a lack of regulatory clarity, advances in AI-based design, base/prime editing, and epigenetic profiling are developing rapidly to overcome these challenges. As we embark on this journey toward a genomic revolution, it is necessary to practice prudence with equity, ethical framework and clinical opportunity in mind. By taking this collaborative, multi-disciplinary approach, the future of medicine is about more than just treatment it's about personalized transformation. It is now time to move from a "one-size-fits-all" model to "genes-to-dose" precision where every treatment may be unique to one patient.

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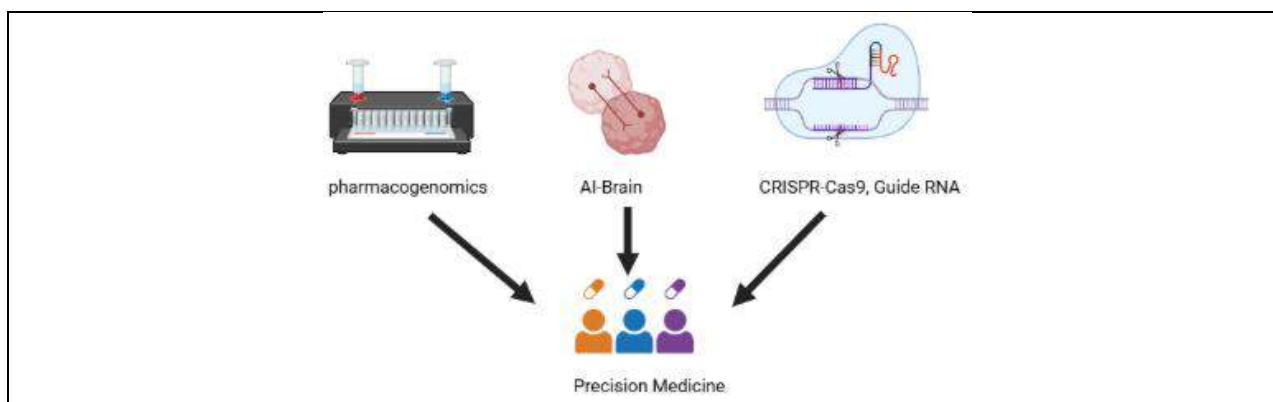


Fig.1. The integration of pharmacogenomics, CRISPR-Cas9 genome editing and artificial intelligence in driving advancements in precision medicine

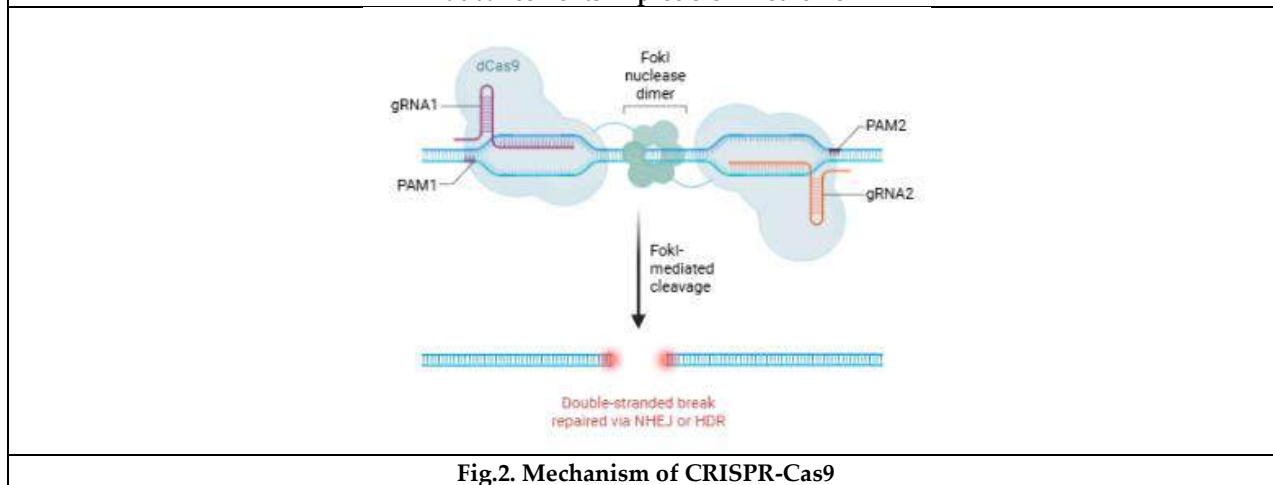


Fig.2. Mechanism of CRISPR-Cas9

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RESEARCH ARTICLE

Exploring the Synergy Between NFV and SDN: A Pathway to Fully Programmable Networks

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ABSTRACT

This review paper explores the synergy between Network Functioning Virtualization (NFV) and Software Defined Network (SDN), highlighting their combined potential to create fully programmable networks architecture. To delve into the fundamental principles and architectures of both, a comprehensive analysis of their complementary roles are provided. The synergy is meant for dynamic service chaining, on-demand network slicing, and real-time traffic management, those are considered as the challenges of performance overheads, security risks, and interoperability issues. The included case studies and comment on the performance analysis introduces the valuable insights to enhance the integration, standardization of network infrastructures towards fully programmable, agile, and efficient networks. The novelty of this paper is to enlighten researchers about a recently developed innovative frameworks and architectural models of a fully programmable, proof of network infrastructures and it's driving advancements in cloud services, 5G, and beyond.

Keywords: Software Defined Network, Network Functioning Virtualization, Cloud Service Models, 5G Network.

INTRODUCTION

Modern network demands complex hybrid architecture, virtualization, scalability with increased system performance. However, traditional hardware-centric network infrastructure is struggling to keep up with the



**Abhradip Chatterjee and et al.,**

dynamic and diverse requirements of those digital services such as digital service provisioning IaaS, PaaS, SaaS, DBaaS for flexible and adaptable network infrastructure [1]. Two promising technologies Network Function Virtualization (NFV) and Software-Defined Networking (SDN) have come up with various advantages. Though these two technologies face some challenges such as security concerns, complexity in management, performance and latency sensitivity when they are used in networks. So, they have emerged as transformative solutions to address those challenges [2]. NFV decouples network functions from proprietary hardware, allowing them to run on standard servers as virtualized services. This enables flexible and cost-effective deployment of network functions, reducing the need for specialized hardware [1]. On the other hand, SDN introduces a centralized control plane that separates the control logic from the underlying hardware, providing a high degree of programmability, automation, and real-time network management. Despite their individual benefits, the full potential of these technologies remains untapped without a synergistic integration. The primary problem lies in the lack of standardized frameworks and architectures to seamlessly combine NFV's agility with SDN's programmability, creating a fully programmable network infrastructure [3]. This gap leads to inefficiencies in resource utilization, suboptimal network performance, and increased operational complexities. The challenge is to explore and develop integrated solutions that leverage both NFV and SDN to create fully programmable networks, enabling dynamic service provisioning, real-time adaptability, and efficient resource management across diverse network environments [1].

This paper begins with a comprehensive literature review that provides an in-depth analysis of the current state of Network Functions Virtualization (NFV) and Software-Defined Networking (SDN) technologies. The review will highlight the unique advantages of each technology, such as NFV's ability to virtualize network functions, providing flexibility and cost savings, and SDN's centralized control, which allows for more programmable and automated network management. [1] However, it will also address the limitations of each technology, such as the complexity of SDN's centralized control and the scalability challenges in NFV deployments. This foundation will establish a clear understanding of how these technologies, when combined, can lead to a more dynamic and efficient network infrastructure, unlocking the potential for greater flexibility, scalability, and cost efficiency [2]. The next focus of this paper is on the architectural integration of NFV and SDN. A key contribution will be the development of an architectural model that illustrates how the agility of NFV in virtualizing network functions can complement the centralized programmability of SDN. By proposing mechanisms for seamless orchestration, management, and deployment, this model will demonstrate how the two technologies can work together to streamline network operations. [3] The integration will be explored in terms of service chaining, dynamic provisioning, and resource management, ensuring that the combination of NFV and SDN can offer a more flexible and automated network design, capable of adapting to evolving service demands and network conditions.

Practical use cases and applications will also be explored, showcasing where and how the integration of NFV and SDN has already been or could be implemented. [4] These use cases will highlight the transformative potential of these technologies in emerging areas such as 5G, edge computing, and cloud services. For example, the combination of NFV and SDN can enable more flexible and scalable 5G networks, facilitate dynamic resource allocation in edge computing environments, and enhance service orchestration in cloud platforms. Real-world applications will be used to illustrate the tangible benefits of this integration, demonstrating how it can provide more efficient and responsive network architectures for both service providers and end users. [5] Despite the promising potential of NFV and SDN integration, several challenges remain. This paper will identify key technical and operational hurdles that hinder the full realization of this integration. Issues such as interoperability between different NFV and SDN platforms, security concerns related to virtualized network functions, and performance bottlenecks due to centralized control will be addressed. Furthermore, the paper will propose potential solutions to these challenges, such as adopting common standards and protocols, enhancing security frameworks, and optimizing performance through distributed control mechanisms. [6] These discussions will also highlight areas for future research, such as improving cross-layer optimization and automating fault detection and recovery.

Finally, the paper will look forward to the future of NFV and SDN integration, considering how artificial intelligence (AI) and machine learning (ML) could play a key role in advancing network automation, fault management, and





Abhradip Chatterjee and *et al.*,

service provisioning. AI and ML have the potential to improve network decision-making, predict failures, and enable proactive adjustments in real-time, enhancing the performance and efficiency of integrated NFV-SDN networks. [7] The paper will outline these future research directions and explore how evolving technologies could further drive innovation in the design of fully programmable, self-optimizing networks. By examining these developments, the paper aims to contribute to a deeper understanding of how the synergy between NFV and SDN can shape the next generation of network infrastructures [8]. This research paper explores the integration of Network Functions Virtualization (NFV) and Software-Defined Networking (SDN) to create a unified, fully programmable network architecture. Section 1 provides a comprehensive literature review of NFV and SDN, highlighting their individual benefits and limitations, setting the foundation for their synergistic potential. Section 2 presents an architectural model demonstrating how NFV's flexibility in virtualizing network functions complements SDN's centralized programmability, focusing on seamless orchestration, management, and deployment of services. Section 3 explores practical use cases in emerging technologies like 5G, edge computing, and cloud services, showing how the integration of NFV and SDN can address real-world challenges and deliver scalable, dynamic solutions. Section 4 identifies key challenges such as interoperability, security, and performance bottlenecks, offering potential solutions and suggesting areas for future research. Finally, Section 5 outlines future directions, highlighting how artificial intelligence (AI) and machine learning (ML) could enhance network automation, fault management, and service provisioning, further advancing the integration of NFV and SDN toward a fully programmable network ecosystem [9].

In the paper, by delving into the integration of Network Functions Virtualization (NFV) and Software-Defined Networking (SDN) to create a unified, fully programmable network architecture concept is explored. Major contribution will focus on the following key areas:

- **Comprehensive Literature Review:** It will provide an in-depth review of the current state of NFV and SDN technologies, highlighting their individual benefits and limitations in [9]. This will set the foundation for exploring how these two technologies can be synergistically combined to achieve greater network flexibility, scalability, and cost efficiency.
- **Architectural Integration:** It will contribute to developing an architectural model that demonstrates how NFV's agility in virtualizing network functions can complement SDN's centralized programmability [10]. This will include proposing mechanisms for seamless orchestration, management, and deployment of network services using both technologies.
- **Use Cases and Applications:** In [3,4], the works will explore and document practical use cases where the integration of NFV and SDN has been or could be implemented. These examples will showcase the potential for real-world applications, particularly in emerging technologies such as 5G, edge computing, and cloud services.
- **Challenges and Solutions:** In [1,2,4], the works will identify the major technical and operational challenges that hinder the full integration of NFV and SDN, such as interoperability, security, and performance bottlenecks. It will also suggest potential solutions or areas of future research that could address these issues.
- **Future Directions:** In [10,11], the works will outline the future research directions for further advancing the integration of NFV and SDN, considering how evolving technologies like artificial intelligence and machine learning can enhance network automation, fault management, and service provisioning.

Overview of Traditional Network Architectures

Traditional network architectures are built around hardware-centric designs where network functions are tied to specific hardware devices. These functions include routing, switching, firewalling, load balancing, and other network services. The key components of traditional networks include: (1) Routers and Switches: the devices are responsible for directing traffic within and between networks. (2) Firewalls: These provide security by controlling incoming and outgoing network traffic based on predetermined security rules. (3). Load Balancers: - These distribute network or application traffic across multiple servers to ensure no single server becomes overwhelmed. (4) Network Appliances: - Dedicated hardware devices that provide specific network functions such as VPNs, intrusion detection/prevention systems (IDS/IPS), etc.



**Abhradip Chatterjee and et al.,****Limitations of Traditional Network Architectures**

Due to advent of technology and technological complexity with growing number of parallelism in program executions, the following limitations are also affected the traditional framework as well as network architecture, such as: (1) Hardware Dependency: - Network functions are tied to specific hardware devices, making the network less flexible and harder to manage. Upgrading or scaling often requires purchasing new hardware, which can be costly and time-consuming. (2) Complex Management: - Managing a large number of disparate devices with different interfaces and management tools adds complexity. Configuring and maintaining these devices requires significant manual effort and expertise. (3) Scalability Issues: - Scaling up requires additional hardware, leading to increased costs and physical space requirements. Scaling out (horizontal scaling) can be particularly challenging due to the rigid hardware dependencies. (4) Slow Deployment: - Deploying new services or making changes to the network can be slow because it often involves configuring physical devices, which may require downtime. (5) Lack of Automation: - Traditional networks have limited support for automation, leading to potential human errors and slower response times to network changes or issues. (6) Inflexibility: - The static nature of traditional networks makes them less adaptable to changing business needs or emerging technologies. Changes often require extensive reconfiguration or hardware replacement.

Development of NFV (Network Function Virtualization)

Network Function Virtualization (NFV) emerged as a solution to overcome the limitations of traditional network architectures by decoupling network functions from dedicated hardware devices (as shown in Fig. 1). Key aspects of NFV include: (1) Virtualization of Network Functions: - Network functions such as firewalls, load balancers, and routers are implemented as software applications (Virtual Network Functions or VNFs) running on standard servers or virtual machines instead of specialized hardware. (2) Flexibility and Agility: - NFV allows for rapid deployment and scaling of network services. VNFs can be easily deployed, updated, and managed, offering greater agility in responding to changing network demands. (3) Cost Efficiency: - By using standard server hardware instead of specialized devices, NFV can reduce capital and operational expenditures. (4) Simplified Management: - Centralized management platforms can orchestrate and manage VNFs, simplifying network management and reducing the need for specialized hardware expertise.

Development of SDN (Software-Defined Networking)

Software-Defined Networking (SDN) complements NFV by providing a framework for more flexible and programmable network management (as shown in Fig. 2). Key aspects of SDN include: (1) Separation of Control and Data Planes: - SDN separates the network control plane (which makes decisions about where traffic is sent) from the data plane (which forwards traffic to the selected destination). This separation allows for centralized control and management of the network. (2) Centralized Network Control: - SDN uses a centralized controller to manage network traffic, enabling more efficient traffic management, improved network visibility, and easier implementation of policies across the network. (3) Programmability: - SDN allows network administrators to programmatically control and manage the network through APIs, enabling dynamic adjustment of network behaviour to meet changing requirements. (4) Automation and Orchestration: - SDN enables greater automation of network configuration and management, reducing manual intervention and the potential for human error. It allows for automated network provisioning, configuration, and management, improving efficiency and reducing operational costs.

Combined Benefits of NFV and SDN

Together, NFV and SDN address the limitations of traditional network architectures by: (1) Enhancing Flexibility: - By decoupling network functions from hardware and enabling programmable network management, networks become more adaptable to changing requirements. (2) Improving Scalability: - Both technologies facilitate easier and more cost-effective scaling of network services. (3) Speeding Deployment: - New network services can be deployed quickly and dynamically without the need for extensive hardware changes. (4) Reducing Costs: - Standardizing on commodity hardware and automating network management reduce both capital and operational expenditures. (5)



**Abhradip Chatterjee and et al.,**

Enabling Innovation: - The programmable nature of SDN and the flexibility of NFV create an environment conducive to innovation, allowing organizations to quickly adopt and implement new technologies and services

LITERATURE REVIEW

In [14], the researchers outlined the challenges and opportunities of NFV, emphasizing its potential to transform network service delivery by leveraging virtualization technologies. In another survey paper, an overview of NFV research, covering various aspects such as architecture, deployment scenarios, and performance issues, are provided [13]. Software-Defined Networking (SDN) faces several challenges, including security concerns due to centralized control points that can attract attacks, and implementation complexities arising from the need to integrate with legacy systems and a lack of expertise. [1] Performance issues may arise from potential latency and scalability challenges in large deployments, while interoperability risks include vendor lock-in and the absence of universal standards. Additionally, network reliability can be compromised by software bugs and controller downtimes. Managing Quality of Service (QoS) is complicated by dynamic resource allocation, and regulatory compliance may pose data privacy concerns. Lastly, the high initial investment and potential increase in ongoing operational costs can be significant barriers to adoption. [2] Network Function Virtualization (NFV) faces several challenges, including performance overheads that may introduce latency compared to traditional hardware solutions, and the complexity of managing a virtualized environment which requires advanced orchestration and automation tools. Interoperability issues arise due to varying standards from different vendors, while security vulnerabilities can emerge from new attack surfaces in virtualized settings. Effective resource management is crucial to avoid performance degradation or wastage, and network reliability can be compromised by software bugs or configuration errors. Additionally, the transition to NFV may require new skills that existing staff may lack, complicating implementation, and ensuring regulatory compliance can be challenging, especially in regulated industries. Furthermore, organizations risk vendor lock-in with specific VNF providers, and while NFV can offer long-term savings, the initial investment in infrastructure and training can be substantial. [1,3]

Network operators are under pressure to offer efficient services while minimizing deployment costs. Network Functions Virtualization (NFV) offers a revolutionary approach by decoupling network functions from proprietary hardware and running them on standard servers, leading to reduced costs and enhanced flexibility. Despite continuous efforts from academia and industry, NFV solutions are still in their early stages. The survey provides a comprehensive overview of NFV, covering its main concepts, benefits, and architecture as defined by the European Telecommunications Standardization Institute (ETSI). [4] It discusses current NFV applicability, standardization efforts, and key open-source projects. NFV is crucial for the evolution of 5G, particularly in enabling network slicing. However, significant challenges, including fault management, security, and interoperability, remain unresolved, hindering widespread commercial deployment. [5]

The text emphasizes the importance of selecting the right hypervisor (Type I or Type II) for efficient utilization of virtual environments. NFV offers benefits such as flexibility, manageability, and security in network virtualization, but numerous technical barriers must be overcome, including achieving "Five 9s" reliability (99.999%) similar to Public Switched Telephone Networks (PSTN) [6]. Future directions for NFV include addressing key research challenges such as fault management, network automation, and end-to-end reliability. The article encourages further research to address these challenges, ultimately pushing NFV into mainstream network deployments, potentially revolutionizing the telecommunications industry by working alongside Software-Defined Networking (SDN) and cloud technologies. [1,2,3] By focusing on these contributions, this research paper offers valuable insights into how NFV and SDN can jointly pave the way towards a more dynamic, programmable, and cost-efficient network infrastructure for the future. Modern network demands for complex hybrid architectures, virtualization, scalability, and enhanced performance are challenging traditional hardware-centric infrastructures, particularly in delivering digital services like IaaS, PaaS, SaaS, and DbaaS. To address these challenges, Network Function Virtualization (NFV) and Software-Defined Networking (SDN) have emerged as transformative technologies, offering advantages such as





Abhradip Chatterjee and *et al.*,

flexibility, cost-effectiveness, and programmability. [10] NFV decouples network functions from proprietary hardware to run on standard servers, while SDN introduces a centralized control plane for automated and real-time network management. However, the full potential of these technologies remains largely untapped due to the lack of standardized frameworks for their integration, leading to inefficiencies in resource utilization and increased operational complexities. [11] The primary challenge is to develop integrated solutions that synergistically combine NFV's agility with SDN's programmability to create fully programmable networks capable of dynamic service provisioning and efficient resource management [9].

Use Cases and implementation techniques

This section summarizes NFV and SDN technologies from the perspectives of practical implementation techniques. The various constructions are sole or combined for refinement of network activities.

The derived concepts are implemented in various kinds of networking configurations, such as:

- **Dynamic Service Chaining:** NFV and SDN enable dynamic service chaining, allowing operators to dynamically instantiate and chain together VNFs to create custom service paths tailored to specific service requirements or user demands. [24]
- **On-Demand Network Slicing:** NFV and SDN enable on-demand network slicing, allowing operators to dynamically partition network resources to create isolated virtual networks (slices) tailored to specific use cases or service requirements. [25]
- **Real-Time Traffic Management:** NFV and SDN enable real-time traffic management, allowing operators to dynamically adjust network resources and routing policies in response to changing traffic conditions, congestion, or security threats, ensuring optimal performance and reliability. [26]
- **AT&T's Domain 2.0 Initiative:** AT&T has been a pioneer in implementing NFV and SDN synergy through its Domain 2.0 initiative. By virtualizing network functions and leveraging SDN principles, AT&T has transformed its network infrastructure, making it more flexible, scalable, and cost-effective. This has enabled AT&T to rapidly deploy new services and improve network performance for its customers.
- **Verizon's Virtual Network Services:** Verizon has also embraced NFV and SDN to deliver virtualized network services to its enterprise customers. By decoupling network functions from underlying hardware and centrally orchestrating them through SDN controllers, Verizon can offer a wide range of services on-demand, including virtual firewalls, routers, and WAN optimization. [23]
- **Telefónica's UNICA Platform:** Telefónica's UNICA (Universal IT Cloud) platform is another example of NFV and SDN synergy in action. By virtualizing network functions and leveraging SDN for network automation and orchestration, Telefónica has been able to improve service agility, reduce time-to-market, and optimize resource utilization across its global network infrastructure.

In Practice or Implementation surveys

Network Functions Virtualization: Challenges and Opportunities (2013): This foundational paper outlined the challenges and opportunities of NFV, emphasizing its potential to transform network service delivery by leveraging virtualization technologies. [22]

A Survey of Network Function Virtualization (2015): This survey paper provided an overview of NFV research, covering various aspects such as architecture, deployment scenarios, and performance issues. [20]

Open Flow: Enabling Innovation in Campus Networks (2008): This seminal paper by Nick McKeown et al. introduced Open Flow, laying the foundation for SDN by describing how a standardized protocol could enable programmable network control. [19]

The (R) Evolution of SDN (2014): This paper discussed the evolution of SDN, from its early days with Open Flow to its broader applications and impact on networking. [21]



**Further, Integration and Combined Benefits are cited in below mentioned researches, such as:**

Research and industry (2014-2016) focus shifted towards integrating NFV and SDN to leverage their complementary strengths. NFV's virtualized network functions and SDN's programmable control planes together promised more flexible, scalable, and efficient network architectures.

A Comprehensive Survey on NFV/SDN Architectures, Applications, and Challenges" (2015): This survey paper reviewed the integrated architecture of NFV and SDN, discussing how their synergy could address various network challenges and enable new applications.

Industry Developments

These subsection throws light on the deployment or used concepts of SDN and NVF in IT and Telecomm Industry.

ETSI NFV ISG: The establishment of the ETSI NFV Industry Specification Group (ISG) led to the development of a reference architecture for NFV, defining essential components like Virtual Network Functions (VNFs), NFV Infrastructure (NFVI), and the NFV Management and Orchestration (MANO) framework.

Commercial Deployments: By the mid-2010s, major telecom operators began deploying NFV solutions, driven by the need for more agile and cost-effective network services.

Open Networking Foundation (ONF): Founded in 2011, the ONF spearheaded the standardization of SDN and OpenFlow, driving industry adoption and development.

SDN Controllers: The development of SDN controllers like NOX, Floodlight, and OpenDaylight enabled centralized network control and programmability, facilitating more efficient and flexible network management.

Industry Momentum

Standardization Efforts: Collaboration between ETSI NFV ISG and ONF aimed to ensure interoperability and standardization in the implementation of NFV and SDN.

Commercial Solutions: Vendors began offering integrated NFV and SDN solutions, and telecom operators started deploying these technologies to achieve more agile, cost-effective, and scalable networks.

Future Direction

Let's delve into the future directions of NFV and SDN integration, which provides research opportunities to the other researchers of this domain.

Technological Advancements without AIML techniques

The next generation technologies such as service function chaining (SFC), Security for SDN/NFV or SDN/NFV Security Solutions and Edge Computing Integration are as follows:

- **Service Function Chaining Optimization:** Research can focus on optimizing service function chaining (SFC) in NFV-enabled SDN networks. This includes developing algorithms and policies to dynamically orchestrate and manage service chains based on real-time traffic conditions, application requirements, and network resources [12].
- **SDN/NFV Security Solutions:** There's a need for research into enhancing security and privacy in NFV and SDN environments. This involves exploring novel approaches for securing virtualized network functions, detecting and mitigating threats in software-defined networks, and ensuring compliance with regulatory requirements [13].
- **Edge Computing Integration:** With the proliferation of edge computing, there's an opportunity to explore the integration of NFV and SDN with edge computing platforms. Research can focus on enabling efficient deployment and management of virtualized network functions at the network edge, leveraging SDN principles for network slicing and resource orchestration [14].

Technological Advancements through AIML

AI and Machine Learning: Integration of artificial intelligence (AI) and machine learning (ML) techniques can enhance the synergy between NFV and SDN. AI/ML algorithms can be used for network optimization, predictive





Abhradip Chatterjee and *et al.*,

analytics, anomaly detection, and automated network management, leading to improved performance, efficiency, and reliability.

Containerization and Micro services: Adoption of containerization and Micro services architectures can further enhance the flexibility and scalability of NFV and SDN deployments. By containerizing virtualized network functions and leveraging Micro services principles, organizations can achieve greater agility, faster deployment times, and easier management of complex network services. [15]

Quantum Networking: In the long term, advancements in quantum networking could revolutionize NFV and SDN integration. Quantum technologies offer the potential for ultra-secure communication, massive parallelism, and unprecedented scalability, which could be leveraged to create highly resilient and efficient virtualized network infrastructures. [16]

Standardization Efforts:

To continue with uniform networking in a telecommunication system, various standardization platforms are formed. These organizations are as follows:

ETSI NFV ISG: The European Telecommunications Standards Institute (ETSI) NFV Industry Specification Group (ISG) continues to work on defining standards and specifications for NFV architectures, interfaces, and APIs. Ongoing efforts include enhancing interoperability, performance, and reliability of NFV deployments. [17]

ONF and Open Source Initiatives: The Open Networking Foundation (ONF) and various open-source projects (e.g., ONAP, OPNFV) are driving standardization efforts and fostering collaboration in the NFV and SDN ecosystem. These initiatives aim to develop open standards, [reference architectures, and interoperable implementations that accelerate innovation and adoption of NFV and SDN technologies. [18]

IETF and IEEE: The Internet Engineering Task Force (IETF) and the Institute of Electrical and Electronics Engineers (IEEE) are also actively involved in standardization efforts related to NFV and SDN. These organizations develop protocols, data models, and best practices for network virtualization, programmability, and automation, which are essential for enabling interoperability and scalability in heterogeneous network environments. Besides various benefits of the combined techniques, some challenges are incurred: (1) Virtualization Overhead: Virtualizing network functions (VNFs) can introduce latency and reduce performance compared to hardware-based solutions. Resource contention between VNFs sharing the same physical infrastructure can affect performance and reliability. Additionally, the centralized SDN controller may become a bottleneck in large-scale deployments with high traffic volumes. (2) Security Risks: The centralization of SDN control and abstraction layers in NFV increase the attack surface, making SDN controllers and NFV orchestrators vulnerable to attacks. Proper isolation between VNFs is essential to prevent data leaks and unauthorized access. Trust management is also challenging, particularly when using third-party components or services. (3) Interoperability Issues: Vendor lock-in can occur due to proprietary SDN and NFV solutions, causing interoperability issues in multi-vendor environments. Despite efforts to standardize interfaces, discrepancies remain, complicating integration. Additionally, integrating SDN and NFV with legacy systems often requires complex modifications to existing infrastructure. (4) Management Complexity: SDN and NFV require complex orchestration for managing VNFs and virtualized infrastructure, which can be difficult to implement and maintain. Monitoring and troubleshooting are challenging due to the dynamic nature of these environments, often requiring advanced tools. Additionally, the need for specialized skills in SDN and NFV creates workforce gaps, leading to training and hiring challenges.





Abhradip Chatterjee and *et al.*,

CONCLUSION

The aim of this paper is to explore the synergy between Network Function Virtualization (NFV) and Software-Defined Networking (SDN), examining how their combined implementation can address the limitations of traditional network architectures. Specifically, the paper will investigate how NFV and SDN together enhance network flexibility, scalability, and cost-efficiency, while enabling rapid deployment and innovation. Through this exploration, the paper seeks to provide a comprehensive understanding of the integrated benefits and potential challenges of leveraging NFV and SDN in modern network environments.

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Table 1: Comparative study between SDN (Software-Defined Networking) and NFV (Network Functions Virtualization)

Aspect	SDN (Software-Defined Networking)	NFV (Network Functions Virtualization)
Primary Focus	Decouples control plane from data plane to enable centralized, programmable network control	Virtualizes network functions traditionally run on dedicated hardware
Core Concept	Centralized control with a software-based SDN controller	Virtualization of network functions (e.g., firewall, router) onto commodity hardware
Main Goal	Network programmability, flexibility, and automation	Reduce hardware dependency and enable rapid deployment of network services
Network Layer	Primarily works at Layer 2 (Data Link) and Layer 3 (Network)	Operates at multiple layers including Layer 3 (IP), Layer 4 (Transport), and Layer 7 (Application)
Component Focus	SDN controller, APIs, and traffic forwarding devices	Virtual Network Functions (VNFs), NFV orchestrator, virtualized infrastructure manager (VIM)
Hardware Dependency	Minimizes hardware dependency by centralizing control	Reduces hardware dependency by virtualizing network appliances
Service Creation/Modification	Dynamic and programmable traffic management and network policies	Rapid creation, deployment, and scaling of virtualized network services (e.g., firewall,





Abhradip Chatterjee and *et al.*,

		load balancing)
Key Technologies	Open Flow, SDN controllers, Southbound APIs, Northbound APIs	Virtual machines (VMs), containers, cloud platforms, VNF orchestration
Resource Management	Manages data traffic, forwarding tables, and path selection	Manages virtualized compute, storage, and network resources for VNFs
Deployment Environment	Data centers, wide-area networks, SD-WAN	Telecom networks, data centers, enterprise networks, edge computing
Service Agility	Provides agile traffic management and network reconfiguration	Allows for faster deployment of network functions and services
Scalability	Can scale horizontally with distributed controllers, but centralized control may face bottlenecks	Can scale by dynamically allocating more virtual resources for VNFs
Performance	Can improve performance via optimized traffic flows but may face bottlenecks in very large deployments	Performance may suffer from virtualization overhead if not properly optimized
Security	Security concerns around the centralized SDN controller, potential attack surface at control plane	Security concerns include virtual machine/container isolation, hypervisor vulnerabilities, and VNF security
Cost Efficiency	Cost savings due to centralized control, reduced hardware needs	Cost savings from using commodity hardware, avoiding expensive physical appliances
Flexibility	High flexibility in network management and path selection	High flexibility in deploying and scaling network functions on demand
Key Use Cases	Data center networking, SD-WAN, network slicing, cloud networks	Telecom infrastructure, enterprise networks, edge networks, cloud services
Controller vs. Orchestrator	SDN uses a controller to manage network traffic	NFV uses an orchestrator to manage VNFs and virtualized resources
Traffic Flow Control	Directly controls traffic flow across network paths via SDN controller	NFV does not directly control traffic flows; it provides the virtualized network functions for handling traffic
Integration with Other Systems	Can integrate with orchestration systems for end-to-end automation	NFV often requires integration with SDN to provide complete network automation



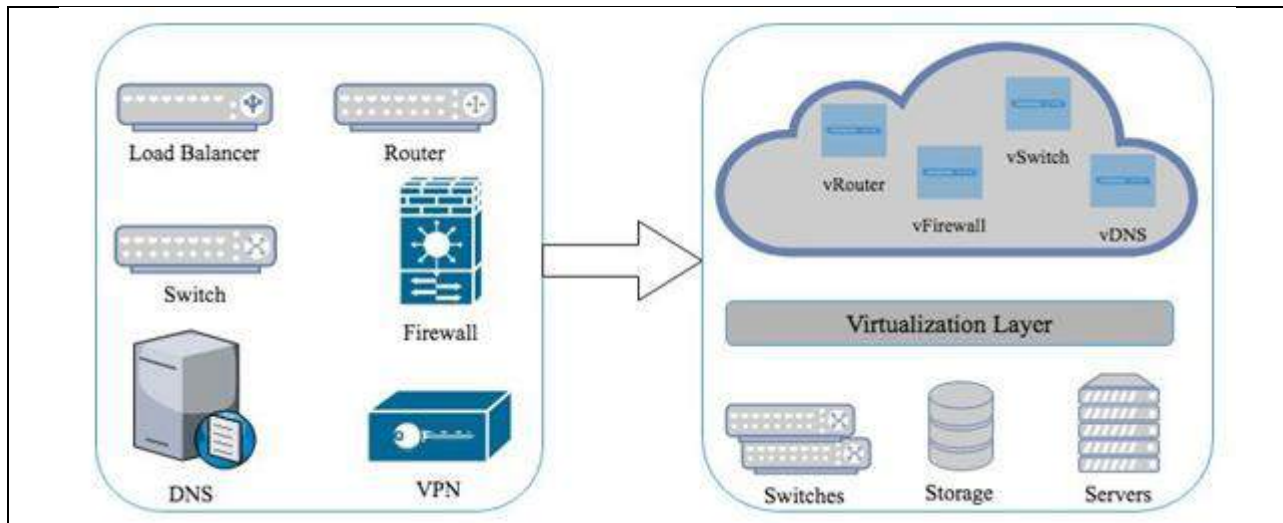
Abhradip Chatterjee and *et al.*,

Fig-1: Difference between Traditional Network Approach and NFV Approach

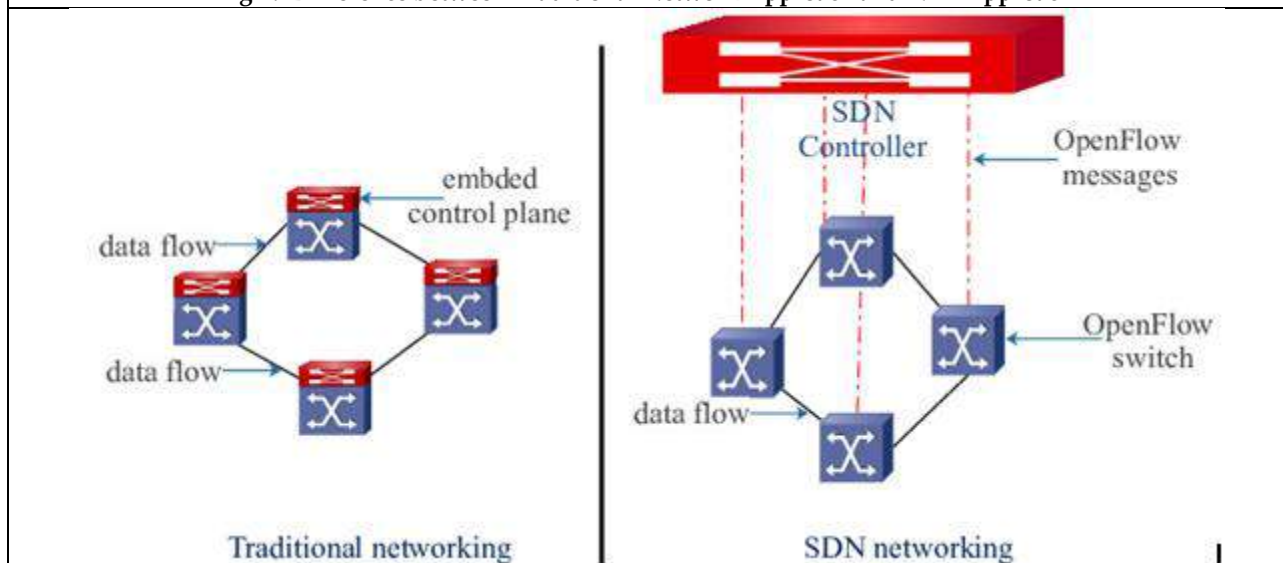


Fig-2: Difference between Traditional Network Approach and SDN Networking Approach

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RESEARCH ARTICLE

Pharmaceutical and Analytical Characterization of *Nilavagai Chooranam* : A Siddha Polyherbal Formulation for Anorectal Disorders

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ABSTRACT

Nilavagai Chooranam is a classical Siddha polyherbal formulation traditionally indicated for anorectal disorders (*Moola Noygal*). Despite its long-standing therapeutic use, the absence of standardized quality control parameters and variations in raw material processing limit its reproducibility and wider regulatory acceptance. The present study aimed to establish pharmaceutical and analytical characterization standards for *Nilavagai Chooranam* in accordance with WHO and AYUSH guidelines. The formulation was prepared as per the classical text *Brammamuni Karukkidaai Choothiram* using authenticated raw materials and traditional purification procedures. Organoleptic, physicochemical, biochemical, phytochemical, chromatographic, and microbial analyses were performed. The formulation appeared as a fine greenish-brown aromatic powder without foreign matter. Physicochemical evaluation showed low moisture content (LOD: 2.187%), total ash (6.9%), and acid-insoluble ash (0.18%), indicating good purity and minimal contamination. The pH was mildly acidic (5.8). Water-soluble extractive value (39.51%) was higher than alcohol-soluble extractive value (11.11%), suggesting predominance of polar phytoconstituents. Biochemical analysis confirmed the presence of chloride, ammonium, iron, and sodium ions, while toxic heavy metals such as arsenic and mercury were absent. Preliminary phytochemical screening revealed flavonoids, tannins, phenolic compounds, carbohydrates, reducing sugars, and phytosterols. TLC and HPTLC analyses demonstrated distinct and reproducible fingerprint



Venkatesh *et al.*,

profiles at multiple wavelengths. Microbial load was within permissible limits, and aflatoxin estimation showed minimal contamination (2.1 ppb), supporting the microbiological safety of the formulation. Overall, *Nilavagai Chooranam* exhibited acceptable purity, stability, and safety parameters, thereby supporting its traditional use and providing a scientific basis for future pharmacological and clinical studies.

Keywords: Siddha Medicine, *Nilavagai Chooranam* , Physicochemical Analysis, High-Performance Thin Layer Chromatography, Phytochemical Screening, Hemorrhoids

INTRODUCTION

The Siddha system of medicine is one of the most ancient and continuous traditional medical sciences. It is fundamentally based on the macrocosm–microcosm concept, wherein the human body mirrors the universe and is constituted by 96 *Thathuvams* that encompass all physical, physiological, mental, and intellectual dimensions.[1] In Siddha literature diseases are classified 4,448 distinct conditions. Among these, anorectal disorders (*Moola Noygal*) represent a highly prevalent group, affecting approximately 5–15% of the global population, with hemorrhoids alone having a lifetime prevalence of nearly 50%.[2] In India, rising sedentary habits and low-fiber diets further increase disease burden, with hemorrhoids accounting for 42% of anorectal cases, followed by fistula, pilonidal sinus, abscess, and fissure.[2], [3] Contributing factors include improper diet, suppression of natural urges, and psychological stress, and increased anorectal pressure resulting in vascular engorgement and hemorrhoidal pathology. [4], [5]

In this context, *Nilavagai Chooranam* , described in *Brammamuni Karukkidaai Choothiram* (pp. 68–69), is a classical Siddha polyherbal formulation indicated for *Malakkattu*, *Moola noygal*, and related conditions, acting through mild purgation, and anti-inflammatory effects, administered in a dose of *Verukadi alavu* for one *Mandalam* (48 days) following proper Suddhi procedures to ensure safety and enhanced bioavailability. [6] The growing global interest in traditional medicine, due to its holistic approach and relatively lower adverse effects, is counterbalanced by concerns regarding variability and lack of quality control in herbal formulations[7],[8]. Unlike traditional physician-controlled preparation, modern large-scale manufacturing introduces variations due to geographical sourcing, seasonal changes, storage conditions, and adulteration[7],[9]. Hence, pharmacological characterisation becomes essential to establish defined physicochemical and analytical parameters ensuring identity, purity, safety, and efficacy.[9] International and national regulatory bodies, including WHO and Indian institutions such as AYUSH, PCIM&H, and PLIM, have established guidelines and pharmacopoeial standards, with GMP compliance mandated under the Drugs and Cosmetics Act, 1940 and Rules, 1945 (Schedule T) [10]. Therefore, the application of modern techniques—including physicochemical evaluation, biochemical assays, chromatographic fingerprinting (TLC/HPTLC), and microbial testing—to *Nilavagai Chooranam* is critical to ensure consistency, therapeutic reliability, regulatory compliance, and global acceptance as an evidence-based Siddha formulation. This article explores the pharmaceutical and analytical characterization of the *Nilavagai Chooranam* a polyherbal formulation.

MATERIALS AND METHODS

Drug Formulation:

Internal medicine : *NILAVAGAI CHOORANAM*

Ingredients :

<i>Nilavagai</i> (<i>Cassia senna</i> , Linn.)	<i>Sivadhai</i> (<i>Operculina turpethum</i> , Linn.)
<i>Milagu</i> (<i>Piper nigrum</i> , Linn.)	<i>Thalisapathiri</i> (<i>Abies spectabilis</i> , Mirb)
<i>Kadukkai</i> (<i>Terminalia chebula</i> , Retz)	<i>Jaadhikkai</i> (<i>Myristica fragrans</i> , Hoult)
<i>Thaanrikkai</i> (<i>Terminalia bellerica</i> , Roxb)	<i>Kiraambu</i> (<i>Syzygium aromaticum</i> , Linn.)
<i>Seeragam</i> (<i>Cuminum cyminum</i> , Linn.)	<i>Thippili</i> (<i>Piper longum</i> , Linn.)
<i>Vaaluluvai</i> (<i>Celastrus paniculatus</i> , Willd)	<i>Seviyam</i> (Root of <i>Piper nigrum</i> , Linn.)





Venkatesh et al.,

<i>Sirunaagapoo</i> (<i>Mesua nagassarium</i> , Kosterm.)	<i>Indhuppu</i> (<i>Sodii chloridum impura</i>)
<i>Elam</i> (<i>Elettaria cardamomum</i> , Maton)	<i>Koogaineeru</i> (<i>Maranta arundinacea</i> , Linn.)
<i>Ilavangapattai</i> (<i>Cinnamomum verum</i> , Presl)	<i>Sukku</i> (<i>Zingiber officinale</i> , Rosc)
<i>Kadugurogini</i> (<i>Picrorhiza scrophulariiflora</i> , Pennell)	<i>Seeni Sarkkari</i> (<i>Saccharum officinarum</i> , Linn.)

Dosage : Verukadi alavu (1.375g). Twice a day. After food

Duration : Orumandalam (48 days)

Indications:

- Malakkattu (Constipation),
- Vaaivu (One of the three humours),
- Soodu kollal (Intense heat in pelvic region),
- Sori & Sirangu (A kind of itch or scabies),
- Moolavaayu (Accumulation of bowel gas),
- Moola noygal (Diseases of Anorectal region).

Reference : Brammamuni Karukkida Choothiram – 380 [11]

Author : S. B. Ramachandiran (Editor)

Edition year : February 1998

Page No : 68, 69

Publication : Thamarai Noolagam, 7, NGO Colony, Vadapalani, Chennai-26

Raw Material Collection and Authentication (Figure 1)

Raw Drugs Procurement:

The Above Listed Raw Drugs Were Purchased From A Raw Drug Shop – Thakkalai, Kanyakumari.

Raw Drugs Authentication:

The Raw Drugs Were Authenticated By Botanist In Nis, Chennai. Certificate No: Nismb7132024 (02/11/2024)

Preparation

Purification Of Raw Drugs [12]:

- Nilavagai (*Cassia senna*, Linn.)- Powdered leaves was boiled in the steam of milk for 3 hours.
- Milagu (*Piper nigrum*, Linn.)- Soaked into the butter milk and dried.
- Kadukkai (*Terminalia chebula*, Retz) - Seeds was removed and dried.
- Thaanrikkai (*Terminalia bellerica*, Roxb) - Was soaked in Thazhaivizhuthu juice for 3 hours and the dried under sunlight.
- Seeragam (*Cuminum cyminum*, Linn.)- Was cleaned for impurities and dried under the sunlight.
- Vaaluluvaai (*Celastrus paniculatus*, Willd)- Was washed in *Aloe barbedensis* juice then dried under the sunlight.
- Sirunaagapoo (*Mesua nagassarium*, Kosterm)- Was cleaned for impurities and dried under the sunlight.
- Elam (*Elettaria cardamomum*, Maton- Was cleaned for impurities and dried under the sunlight.
- Ilavangapattai (*Cinnamomum verum*, Presl- Was cleaned for impurities and dried under the sunlight.
- Kadugurogini (*Picrorhiza scrophulariiflora*, Pennell)- Was soaked in Neem leaves juice for 3 hours and dried under the sunlight.
- Sivadhai (*Operculina turpethum*, Linn.)- The central veins from the root were removed boiled in milk then dried under the sunlight.
- Thalispattiri (*Abies spectabilis*, Mirb)- Was cleaned for impurities and dried under the sunlight.
- Jaadhikkai (*Myristica fragrans*, Houtt)- the outer skin was peeled to make into small pieces and dried under the sunlight.
- Kiraambu (*Syzygium aromaticum*, Linn.)- Was cleaned for impurities and dried under the sunlight.
- Thippili (*Piper longum*, Linn.)- Was soaked in kodiveli leaf juice for 24 hours and then will be dried under sunlight.



Venkatesh *et al.*,

- *Seviyam* (Root of *Piper nigrum*, Linn.)- Was cleaned for impurities and dried.
- *Indhuppu* (*Sodii chloridum impura*)- Was soaked in Fermented Rice[13] Liquid for 3 days and dried under the sunlight.
- *Koogaineeru* (*Maranta arundinacea*, Linn.)- Was mixed with water and washed for seven times filtered and dried under the sunlight
- *Sukku* (*Zingiber officinale*, Rosc)-Was soaked in limestone water for 3 hours and outer layer was removed.
- *Seeni Sarkkari* (*Saccharum officinarum*, Linn.)- Was crushed and made into a fine powder.

Study Drug Preparation and Storage:

STEP 1 : After purification, the Main Ingredient *Nilavagai Chooranam* is dried and powdered nicely.

STEP 2 : After purification Part A and Part B drugs are made into fine powder.

STEP 3 : The powdered Main ingredient, Part A and Part B drugs are mixed together well.

Prepared medicine will be stored in an airtight container.

Pharmaceutical and Analytical Characterization (Procedures) (Table 1-4)

Organoleptic Character: Organoleptic Characters Are The Visual and sensory attributes used to describe, identify, and evaluate the genuineness of medicinal plant materials and crude drugs. These characteristics are assessed using the basic senses or by naked-eye observation. Organoleptic characters typically include: Sensory traits: Odour and taste. During testing, odour is typically evaluated by directly smelling a sample of the powdered drug, while taste is examined by applying a small quantity of the powder to the tongue using a moist glass rod. Visual and physical traits: Size, color, consistency, state (e.g., solid), appearance, surface characteristics (such as texture and fracture), touch, and flow properties

Foreign Matter: The entire contents of the packing if 100 g or less is removed, and spread in a thin layer in a suitable dish or tray. It is examined in daylight with unaided eye. Suspected particles are transferred, if any, to a petri dish, and examine with 10x lens in daylight.[14]

Physicochemical Properties: As per Characterisation and Standardization of herbal formulations: an overview (Baqai Journal of Health Sciences) and Protocol for testing of Ayurvedic, Siddha and Unani medicines (Department of AYUSH, 2008), the following physicochemical parameters are evaluated to ensure the quality, purity, and standardization of herbal drugs. [15],[16]

Loss On Drying At 105°C / Moisture Content

Loss on drying determines the amount of moisture and volatile components present in the drug by heating the sample at 105°C until constant weight is achieved, and calculating the percentage weight loss.

Total Ash: Total ash estimation evaluates the total inorganic residue remaining after complete incineration of the drug, thereby indicating the presence of physiological and non-physiological inorganic matter such as carbonates, phosphates, and silicates.

Acid-Insoluble Ash: Acid-insoluble ash measures the fraction of total ash that remains insoluble in dilute hydrochloric acid, primarily reflecting siliceous impurities like sand and soil contamination.

Ph (5% Aqueous Extract): The pH of a 5% aqueous extract is determined to assess the acidic, neutral, or alkaline nature of the formulation, which influences drug stability, absorption, and therapeutic activity.

Water-Soluble Extractive: Water-soluble extractive value indicates the proportion of active constituents soluble in water, such as sugars, acids, and mucilage, serving as an index of drug quality and potential adulteration.



Venkatesh *et al.*,

Alcohol-Soluble Extractive: Alcohol-soluble extractive value estimates the amount of constituents like alkaloids, tannins, and resins extractable in alcohol, thereby helping to evaluate the chemical composition, purity, and standardization of the drug.

Bulk Density: Bulk density is the mass of a powder sample divided by its total volume (including particles, interparticle voids, and pores), generally expressed in grams per milliliter (or). It is critical for assessing powder flow, processing, packaging, and storage, often split into untapped (bulk) and tapped density to measure compressibility.[17]

Biochemical Analysis (Table 5,6)

Test For Acid Radicals

Ash Test: A filter parchment soaked in a mixture of the sample and cobalt nitrate solution was gradually introduced into a Bunsen flame and ignited; observations were recorded.

Test for Sulphate: To 2 ml of the extract, 2 ml of 4% ammonium oxalate solution was added and mixed; results were noted.

Test for Chloride: To 2 ml of the extract, dilute nitric acid (HNO_3) was added dropwise until effervescence ceased, followed by the addition of 2 ml of silver nitrate solution; observations were recorded.

Test for Phosphate: To 2 ml of the extract, 2 ml of ammonium molybdate solution and 2 ml of concentrated nitric acid (HNO_3) were added; results were noted.

Test for Carbonate: To 2 ml of the extract, 2 ml of magnesium sulphate solution was added; observations were recorded.

Test for Calcium: To 2 ml of the extract, 2 ml of 4% ammonium oxalate solution was added; results were noted.

Test for Magnesium: To 2 ml of the extract, sodium hydroxide solution was added dropwise and then in excess; observations were recorded.

Test for Ammonium: To 2 ml of the extract, a few millilitres of Nessler's reagent followed by excess sodium hydroxide solution were added; results were noted.

Test for Mercury: To 2 ml of the extract, 2 ml of sodium hydroxide solution was added; observations were recorded.

Test for Arsenic: To 2 ml of the extract, 2 ml of sodium hydroxide solution was added; results were noted.

Test for Iron: To 2 ml of the extract, 2 ml of ammonium thiocyanate solution was added and mixed; observations were recorded.

Test for Zinc: To 2 ml of the extract, sodium hydroxide solution was added dropwise and then in excess with stirring; results were noted.

Preliminary Phytochemical Analysis (Table 7,8)

Phytochemical screening is conducted to detect the presence and nature of biologically significant secondary metabolites (such as alkaloids, flavonoids, tannins, saponins, and steroids) within a crude drug as per Khandelwal (2008) and Kokate et al. (2008)[22], [23]. Identifying these chemical constituents helps establish the drug's quality and explains its potential therapeutic properties and pharmacological efficacy (e.g., flavonoids for antioxidant activity, alkaloids for antimicrobial effects). To perform these tests, a small quantity of the drug (typically a few milligrams or a 5 ml aliquot) is usually extracted or dissolved in a specific solvent (such as distilled water, ethanol, or chloroform). Solvents are chosen based on the solubility of the target phytochemicals so that they can be effectively released and react with the specific testing reagents. [18], [19], [20], [21]

• Detection of Alkaloids

a. Dragendorff's Test: The acidic extract, when treated with Dragendorff's reagent, produces an orange-red precipitate indicating alkaloids.

b. Wagner's Test: Addition of Wagner's reagent to the acidic extract yields a reddish-brown precipitate confirming alkaloids.



Venkatesh *et al.*,**• Detection of Carbohydrates**

Molisch's Test: Treatment with Molisch's reagent and concentrated sulfuric acid produces a reddish-violet color indicating carbohydrates.

• Detection of Reducing Sugars

- Fehling's Test: Heating the extract with Fehling's solutions A and B produces a brick-red precipitate indicating reducing sugars.
- Benedict's Test: Heating the sample with Benedict's reagent produces a characteristic colored precipitate indicating sugars.

• Detection of Glycosides

10% NaOH Test: Addition of sodium hydroxide produces yellow, pink, or blood-red coloration indicating glycosides.

• Detection of Cardiac Glycosides

Keller–Killiani Test: Formation of a reddish-brown ring at the interface with bluish-green upper layer indicates cardiac glycosides.

• Detection of Proteins and Amino Acids

Xanthoproteic Test: Reaction with nitric acid followed by alkali produces yellow to orange coloration indicating aromatic amino acids.

• Detection of Flavonoids

- Alkaline Reagent Test: Addition of NaOH or ammonia produces a dark yellow color indicating flavonoids.
- Lead Acetate Test: Formation of a bulky white precipitate with lead acetate indicates phenolic compounds including flavonoids.

• Detection of Phenolic Compounds

Ferric Chloride Test: Addition of ferric chloride produces dark blue or green coloration indicating phenols.

• Detection of Tannins

- NaOH Test: Addition of sodium hydroxide produces yellowish-brown coloration indicating tannins
- FeCl₃ Test: Ferric chloride produces dark blue or greenish-black color indicating tannins.

• Detection of Phytosterols

Salkowski's Test: Reaction with chloroform and sulfuric acid produces reddish-brown coloration with green fluorescence indicating phytosterols

• Detection of Cholesterol

Cholesterol Test: Treatment with acetic anhydride and sulfuric acid produces color changes from red to green indicating cholesterol.

• Detection of Terpenoids

H₂SO₄ Test: Addition of concentrated sulfuric acid produces a dark brown precipitate indicating terpenoids.

• Detection of Quinones

HCl Test: Addition of concentrated hydrochloric acid produces green coloration indicating quinones.

• Detection of Anthocyanins

HCl Test: Heating with hydrochloric acid produces pink to crimson coloration indicating anthocyanins.

• Detection of Carboxylic Acids

Effervescence Test: Addition of sodium bicarbonate produces brisk effervescence indicating carboxylic acids.

• Detection of Gums and Mucilages

Alcohol Test: Addition of alcohol produces a cloudy white precipitate indicating gums and mucilages.

• Detection of Resins

Turbidity Test: Addition of water to alcoholic extract produces turbidity indicating resins.

• Detection of Fixed Oils and Fats

Saponification Test: Heating with alcoholic KOH followed by water produces lather indicating fixed oils and fats





Venkatesh et al.,

TLC Methodology: [24]

Four grams of *Nilavagai Chooranam* was extracted with 40 mL distilled methanol and kept overnight with occasional shaking. The mixture was boiled in a water bath for 10 minutes, cooled and filtered. The filtrate was concentrated and the volume was made up to 10 mL. Ten microlitres of the sample solution was applied on E. Merck aluminium TLC plates pre-coated with silica gel 60 F254 using a CAMAG automatic sample applicator. The plate was developed using Toluene:Ethyl acetate:Formic acid (8.0:2.0:0.01) as mobile phase up to a distance of 80 mm. After development, the plate was dried and visualized under UV light at 254 nm and 366 nm using CAMAG TLC Visualizer. The plate was then dipped in vanillin–sulphuric acid reagent and heated at 105°C until coloured spots appeared. Finally, the plate was scanned using CAMAG TLC Scanner, the tracks were integrated and the fingerprint profile was recorded.

HPTLC Finger Print of Nilavagai Chooranam (Figure 3-11)

The TLC plate was developed using Toluene:Ethyl acetate:Formic acid (8.0:2.0:0.01) as mobile phase. After development, the plate was dried in air and the fingerprint profile along with densitometric chromatogram of *Nilavagai Chooranam* was recorded at 254 nm and 366 nm.

Microbial Contamination (Table 9)

Microbial contamination is strictly evaluated because the contamination of herbal drugs by microorganisms (such as bacteria and fungi) not only causes bio-disintegration but also significantly brings down the potency and therapeutic efficacy of the drugs. Minimizing moisture and microbial load is essential because excess contamination encourages the deterioration of the formulation through hydrolysis, whereas maintaining sterility ensures the product's quality, genuineness, and overall safety for patient consumption. [18]

Aflatoxins (Table 10)

Aflatoxins are highly dangerous and toxic fungal secondary metabolites produced by molds such as *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus nomius*. This screening is strictly conducted because these compounds are highly toxic, carcinogenic, mutagenic, teratogenic, and hepatotoxic, making it crucial to ensure their absence or strictly limit their presence in herbal medicines to guarantee patient safety. AflaTest is a quantitative method for the detection of aflatoxin in B1, B2, G1, G2, M1, and M2. Five grams of *Nilavagai Chooranam* was mixed with 0.4 g sodium chloride and extracted using methanol:2% Tween 20/phosphate buffer (60:40 v/v). The mixture was vortexed for 3 minutes and filtered through fluted filter paper. Ten millilitres of filtrate was diluted with 20 mL purified water, vortexed for 1 minute and filtered through a pre-wet glass microfiber filter (1.5 µm). Ten millilitres of the diluted extract was passed through the AflaTest WB column at a flow rate of 1–2 drops/second. The column was washed with 10 mL of 2% Tween 20 followed by 10 mL purified water twice. Aflatoxins were eluted with 1 mL HPLC-grade methanol at a flow rate of 1 drop/second and collected in a sterile VICAM cuvette. One millilitre of AflaTest Developer was added and mixed well. The cuvette was immediately placed in the VICAM Series 4EX fluorometer and the aflatoxin concentration was recorded after 60 seconds. [14], [37]

DISCUSSION

The comprehensive Characterization of *Nilavagai Chooranam* yielded a robust dataset across multiple pharmacognostical, physicochemical, phytochemical, and microbiological parameters, confirming the high quality and safety of the formulation. The preliminary organoleptic and macroscopic characterization is fundamental for assessing the physical attributes and genuine nature of the crude drug mixture. The formulation presented as a fine, solid powder with a soft texture and a characteristic greenish-brown appearance. The aromatic odour strongly indicates the preservation of vital volatile principles, likely contributed by the essential oils of *Syzygium aromaticum*, *Elettaria cardamomum*, and *Cinnamomum verum*. Crucially, the macroscopic evaluation confirmed the complete absence of foreign organic or inorganic matter, which serves as a primary indicator of stringent raw material



Venkatesh *et al.*,

selection and adherence to the classical purification protocols. Following organoleptic assessment, rigorous physicochemical profiling was conducted to establish quantitative markers for purity and stability. The Loss on Drying (LOD) at 105°C, a precise measure of moisture and volatile content, was remarkably low at 2.187%. Establishing a low Loss on Drying (LOD) value of 2.187% is critical for the chemical stability and long-term shelf life of *Nilavagai Chooranam*. Excess moisture in herbal powders rapidly accelerates the enzymatic hydrolysis of vital glycosides (such as the sennosides in *Cassia senna*) and creates a highly favourable microenvironment for the rapid proliferation of pathogenic bacteria and fungi. By restricting moisture content, the drug's therapeutic potency is preserved from bio-disintegration.

The Total Ash value, which represents the entire physiological and non-physiological inorganic residue remaining after incineration at high temperatures, was recorded at 6.9%. The Acid-Insoluble Ash, a highly specific sub-fraction of total ash that fails to dissolve in hydrochloric acid, was exceptionally low at 0.18%. The evaluation of ash values is equally paramount for determining purity. The Total Ash value of 6.9% reflects a normal, naturally occurring physiological mineral profile, entirely expected from a formulation containing 20 diverse botanical and mineral entities, including *Indhuppu* (Rock Salt). More importantly, the Acid-Insoluble Ash value of 0.18% indicates the absence of exogenous terrestrial contaminants such as soil, sand, and non-physiological silica. This low value acts as direct empirical proof that the stringent, labor-intensive classical washing and purification (Suddhi) were carried out as per protocol. The pH of the 5% aqueous extract was determined potentiometrically to be 5.8, indicating a mildly acidic nature. The detection of a formulation pH of 5.8 holds profound implications for gastrointestinal therapeutics. The gastrointestinal tract operates across a dynamic pH gradient that dictates the ionization, dissolution, and systemic absorption of all orally administered drugs. A mildly acidic pH of 5.8 mirrors the physiological environment of the proximal duodenum, ensuring the stability of weak basic alkaloids and acidic polyphenols present in the drug matrix.[25] Furthermore, highly alkaline or highly acidic herbal formulations frequently exacerbate mucosal irritation. By presenting a biocompatible pH, *Nilavagai Chooranam* ensures optimal gastrointestinal tolerance, preventing the aggravation of pre-existing micro-tears in the delicate rectal ampulla.

Furthermore, the solubility and extraction profiles were analyzed to determine the thermodynamic distribution of active constituents. The formulation proved soluble in both water and HCl. The bulk density, a critical physical parameter for assessing powder flow, compressibility, and packaging, was calculated at 0.9 g/cm³. The extractive values revealed that the water-soluble fraction (39.51%) was significantly higher than the alcohol-soluble fraction (11.11%), indicating a dominance of highly polar secondary metabolites. Biochemical analysis for acid and basic radicals provided insight into the ionic and mineral composition of the formulation. The analysis confirmed the presence of Chloride ions (cloudy appearance) and Ammonium ions (brown coloration) among the acid radicals, while Sulphate, Phosphate, Carbonate, Calcium, Magnesium, Mercury, and Arsenic were strictly absent. Among the basic radicals, Iron ions were present (mild red coloration), and an ash test indicated the presence of Sodium (yellow flame), whereas Zinc was absent. Quantitative heavy metal analysis such as ICP-MS/AAS was not performed in this study.

High-Performance Thin Layer Chromatography (HPTLC) was executed using a sophisticated mobile phase consisting of Toluene : Ethyl acetate : Formic acid (8.0 : 2.0 : 0.01). The formulation was scanned extensively at 254 nm (absorbance mode) and 366 nm (fluorescence mode) and 520 nm (absorbance mode) mapping discrete R_f values and generating densitometric chromatograms. Derivatization with a vanillin-sulphuric acid reagent followed by thermal activation successfully visualized complex-colored spots representing hidden functional groups. The dominant peaks likely correspond to anthraquinone-derived compounds (*Cassia senna*). The HPTLC densitometric chromatograms serve as a reproducible chromatographic fingerprint for identity verification. By calculating distinct Retention Factor (R_f) values and integrating peak areas across multiple wavelengths, a unique pattern is established for *Nilavagai Chooranam*. This chromatographic mapping is indispensable for regulatory quality assurance, allowing future analysts to instantly detect batch-to-batch variations, the exhaustion of active ingredients, or the fraudulent substitution of expensive raw materials. Finally, the absence of detectable arsenic and mercury ions in biochemical analysis, combined with microbial counts within permissible limits and aflatoxin levels (< 5 µg/kg) below AYUSH

117278



Venkatesh *et al.*,

thresholds, indicates a satisfactory preliminary safety profile and preventing any secondary hepatobiliary or systemic toxicity. Finally, stringent safety parameters were evaluated to rule out biological contamination and mycotoxins. The Total Bacterial Count (2×10^4 cfu/g) and Total Fungal Count (< 2 cfu/g) were strictly within regulatory safety thresholds. Crucially, all specific pathogenic enterobacteria were completely absent. The evaluation of Aflatoxins (B1+B2+G1+G2) via the Vicam fluorometer method detected a negligible quantity of 2.1 ppb, confirming safety within AYUSH regulatory limits of the polyherbal matrix.[26]

The profound therapeutic efficacy of *Nilavagai Chooranam* in successfully resolving *Malakkattu* (chronic constipation) and the wide spectrum of *Moola Noygal* (anorectal diseases) is driven entirely by the synergistic poly-pharmacology of its diverse biochemical constituents. Rather than relying on a single, isolated synthetic molecule, this classical Siddha formulation employs a complex, highly targeted cascade of active principles to simultaneously address the multifactorial etiology of hemorrhoids: impaired colonic motility, severe vascular inflammation, mucosal ulceration, and debilitating sphincter pain. [27] Chronic constipation is the primary pathogenic factor in hemorrhoidal disease, leading to excessive straining, anal cushion damage, and venous prolapse; this is effectively managed by *Cassia senna* (*Nilavagai*), a stimulant laxative rich in anthraquinone glycosides (sennosides A–D) [28]. These glycosides are converted by gut microbiota into active rhein anthrones, which stimulate chloride secretion, inhibit water absorption, and induce strong peristalsis, facilitating bowel evacuation without straining [29]. This action is synergistically enhanced by *Operculina turpethum* (*Sivadhai*), containing turpethin, which exerts hydragogue cathartic effects by increasing luminal water content, softening stools, and promoting easy defecation while also offering cytoprotective and anti-ulcer benefits to maintain mucosal integrity [30], [31].

Inflammation and sphincter spasm, key features of *Ratha Moolam*, are addressed by *Picrorhiza scrophulariiflora* / *P. kurroa* (*Kadugurogini*), which contains iridoid glycosides (picroside I and II) that inhibit NF- κ B signaling and suppress pro-inflammatory cytokine production at the site of vascular congestion [32]. Analgesic and antispasmodic effects are supported by *Zingiber officinale* (*Sukku*) and *Piper nigrum* (*Milagu*); 6-gingerol inhibits COX and LOX pathways, reducing prostaglandin-mediated pain, while piperine enhances bioavailability of co-administered drugs and relieves tenesmus and sphincter spasm [33], [34]. Preliminary phytochemical screening of alcohol and hydro-alcoholic extracts confirmed the presence of carbohydrates, reducing sugars, flavonoids, phenolics, tannins, and phytosterols, with alkaloids detected in hydro-alcoholic extracts, while glycosides, proteins, cholesterol, terpenoids, quinones, anthocyanins, and fixed oils were absent. Flavonoids and tannins play a central role in structural healing and hemostasis. Flavonoids such as diosmin, rutin, hesperidin, and quercetin exhibit phlebotonic effects by enhancing venous tone via prolongation of noradrenaline action, reducing venous stasis, decreasing capillary permeability, and improving lymphatic drainage, thereby alleviating edema in hemorrhoidal conditions [26], [35]. Tannins from *Terminalia chebula* and *Terminalia bellerica* act as potent astringents, precipitating mucosal proteins to form a protective layer that seals bleeding vessels, reduces microbial irritation, and accelerates wound healing [36]. The integrated pharmacological actions—anthraquinones relieving constipation, flavonoids restoring venous tone, iridoid glycosides suppressing inflammation, and tannins providing hemostasis—collectively validate the therapeutic efficacy of this polyherbal formulation in hemorrhoidal management, offering a comprehensive approach superior to single-target interventions [37].

CONCLUSION

In conclusion, the comprehensive pharmacognostical, physicochemical, and microbiological characterization of the Siddha polyherbal formulation *Nilavagai Chooranam* successfully establishes its high purity, chemical stability, and strict compliance with modern regulatory and pharmacopoeial guidelines. The analytical validation of its complex 20-ingredient matrix—rich in active secondary metabolites like flavonoids, tannins, and anthraquinone glycosides—provides a robust scientific rationale for its classical therapeutic indication in treating multifactorial anorectal diseases (*Moola noygal*) and chronic constipation (*Malakkattu*). By demonstrating a significant synergistic capacity that encompasses laxative, anti-inflammatory, analgesic, and venotonic activities, the formulation offers a holistic,





Venkatesh et al.,

effective, and safe alternative to conventional synthetic treatments. Moving forward, advanced *in vivo* pharmacological modeling, molecular docking studies, and rigorous randomized controlled clinical trials are highly warranted to definitively map its molecular mechanisms and facilitate the integration of this ancient Siddha remedy into evidence-based, global clinical practice.

Limitations and Recommendations

While the physicochemical and phytochemical characterization of *Nilavagai Chooranam* establishes a reliable and reproducible quality control framework in line with PLIM and WHO guidelines, it also highlights important directions for future research. Advanced parameters like heavy metal analysis and pesticide residue tests can be done. Batch to batch variability also need to be tested for standardization. The next step should involve *in vivo* pharmacological studies using validated animal models, such as hemorrhoid induction and gastrointestinal transit assays, to quantify its anti-inflammatory, venotonic, and prokinetic effects. This can be complemented by molecular docking and network pharmacology approaches to better understand interactions between key phytoconstituents

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Table 1. Organoleptic Characters of Nilavagai Chooranam

State	Solid
Nature	Fine
Odour	Aromatic
Touch	Soft
Flow Property	Non-Free flowing
Appearance	Greenish brown
Foreign Matter	No foreign matter present

Table 2. Physiochemical Results of Nilavagai Chooranam

Loss on Drying at 105 °C (%)	2.187 ± 0.4971%
Total Ash (%)	6.9 ± 1.57%
Alcohol insoluble Ash (%)	0.18 ± 0.06345%
pH (5% Aqueous Extract)	5.8

Table 3. Solubility Profile of Nilavagai Chooranam

S.No	Solvent Used	Solubility / Dispersibility
1	Water	Soluble
2	HCL	Soluble

Table 4. Extract Profile of Nilavagai Chooranam

S.No	Parameter	Values
1.	Alcohol Extractive values	11.11%
2.	Water extract values	39.51%
3.	Bulk Density	0.9 g/cm ³

Table 5. Biochemical Analysis of Nilavagai Chooranam

S. No.	Experiment	Observation	Inference
1	Test for Sulphate:	No cloudy appearance observed.	Sulphate ions are absent .
2	Test for Chloride:	Cloudy appearance observed.	Chloride ions are present .
3	Test for Phosphate:	No yellow turbidity or precipitate observed.	Phosphate ions are absent .
4	Test for Carbonate:	No cloudy appearance seen.	Carbonate ions are absent .
5	Test for Calcium:	No white precipitate or turbidity observed.	Calcium ions are absent .
6	Test for Magnesium:	No white precipitate formed.	Magnesium ions are absent .
7	Test for Ammonium:	Brown coloration developed.	Ammonium ions are present .
8	Test for Mercury:	No yellow precipitate formed.	Mercury ions are absent .
9	Test for Arsenic:	No brownish-red precipitate observed.	Arsenic ions are absent .





Venkatesh et al.,

Table 6. Test for basic radicals of *Nilavagai Chooranam*

S. No.	Experiment	Observation	Inference
1	Test for Iron:	Mild red coloration appeared.	Iron ions are present .
2	Test for Zinc	No white precipitate formed.	Zinc ions are absent .
3	Ash test:	Yellow-coloured flame appeared	Presence of sodium

Table 7. Preliminary Phytochemical Analysis of *Nilavagai Chooranam*

Test	Alcohol extract	Hydro alcohol extract
1.Detection of Alkaloids		
a. Dragendorff's Test	-	-
b.Wagner's test	-	+
2.Detection of Carbohydrates		
Molish's test	+	++
3.Detection of Reducing sugars		
a. Fehling's test	+	++
b. Benedict's test	+	++
4.Detection of Glycosides		
10% NaOH	-	-
5. Detection of Cardiac glycosides		
Keller-Killani test	-	-
6.Detection of Protein and Amino acids		
Xanthoproteic test	-	-
7.Detection of Flavonoids		
a.Alkaline reagent test	++	++
b.Lead acetate test	++	++
8.Detection of Phenolic Compounds		
a.Ferric chloride test	+	+
9.Detection of Tannins		
a.NaOH	+	+
b. FeCl ₃	+	+
10.Detection of Phytosterols		
Salkowski's test	+	++
11.Detection of Cholesterol	-	-
12.Detection of Terpenoids	-	-
13.Detection of Quinones		
Conc.HCl test	-	-
14.Detection of Anthocyanins		
HCl test	-	-
15.Detection of Carboxylic acid		
Effervescence test	-	-
16.Detection of Gums and Mucilage		
Alcohol test	-	-
17.Detection of Resins		
Turbidity test	-	-
18.Detection of Fixed oils and Fat		
Saponification test	-	-



Venkatesh *et al.*,Table 8. Summarizing the presence and absence of biochemicals & phytochemicals of *Nilavagai Chooranam*

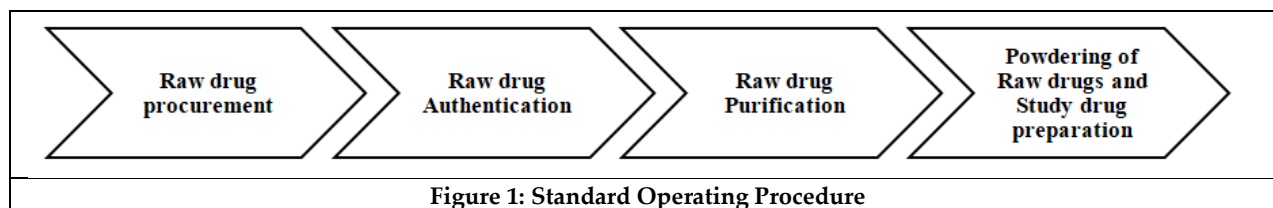
PRESENCE	ABSENCE
Alkaloids	Zinc
Iron	Carbonate
Reducing sugar	Phosphate
	Calcium
Sodium	Arsenic
Chloride	Magnesium
Ammonium	Mercury
Flavonoids	-
Phenolic compounds	-
Tannins	-
Terpenoids	-

Table 9. Microbial Load of *Nilavagai Chooranam*

S. No.	Parameters	Results	Remarks
1	Total Bacterial Count (TBC)	2 x10 ⁴ cfu/g	Within permissible limits
2	Total Fungal Count (TFC)	Less than 2 cfu/g	
3	Enterobacteriaceae	Absent	
4	<i>Escherichia coli</i>	Absent	
5	<i>Salmonella Spp</i>	Absent	
6	<i>Staphylococcus aureus</i>	Absent	
7	<i>Pseudomonas aeruginosa</i>	Absent	

Table 10. Aflatoxins results of *Nilavagai Chooranam*

S.No.	Parameters	Method / Reference	Results	AYUSHStandard
1.	Total Aflatoxin B1+B2+G1+G2	Vicam Aflatest Fluorometer Instruction Manual	2.1ppb	Not more than 5 ppb (5 µg/kg).



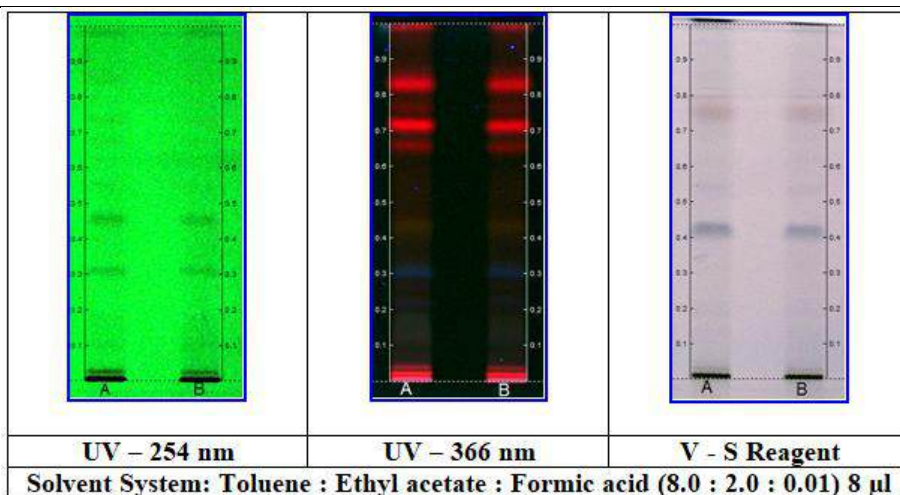
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Figure 2: TLC Analysis of Nilavagai Chooranam

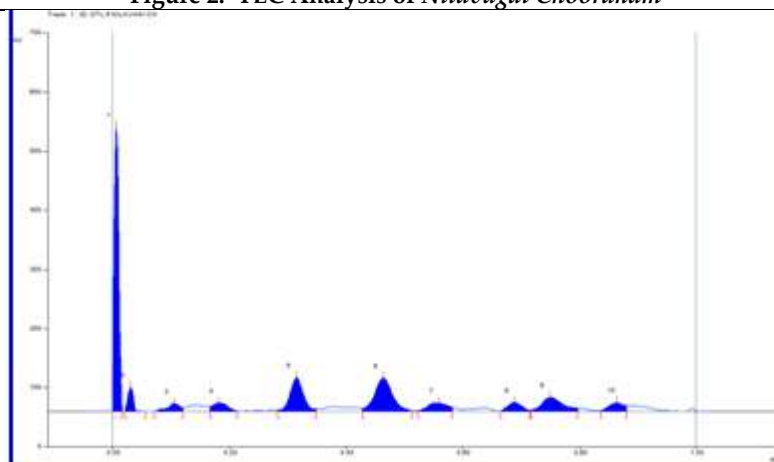


Figure 3: HPTLC finger print at 254 nm (Absorbance mode)

Track 1, ID: DTL 6 NILAVANI CH

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.0 AU	0.01 Rf	481.0 AU	65.68 %	0.02 Rf	1.5 AU	3120.8 AU	37.46 %
2	0.02 Rf	0.1 AU	0.03 Rf	41.4 AU	5.65 %	0.06 Rf	0.1 AU	318.2 AU	3.82 %
3	0.07 Rf	0.2 AU	0.11 Rf	13.5 AU	1.84 %	0.12 Rf	6.4 AU	249.2 AU	2.99 %
4	0.17 Rf	8.9 AU	0.18 Rf	14.3 AU	1.95 %	0.21 Rf	0.3 AU	318.3 AU	3.82 %
5	0.28 Rf	1.7 AU	0.32 Rf	57.5 AU	7.85 %	0.35 Rf	3.8 AU	1086.6 AU	13.04 %
6	0.43 Rf	5.0 AU	0.46 Rf	56.5 AU	7.72 %	0.51 Rf	0.5 AU	1442.0 AU	17.31 %
7	0.52 Rf	0.9 AU	0.56 Rf	14.4 AU	1.97 %	0.58 Rf	6.9 AU	422.7 AU	5.07 %
8	0.66 Rf	0.3 AU	0.69 Rf	15.2 AU	2.08 %	0.72 Rf	1.8 AU	312.9 AU	3.76 %
9	0.72 Rf	1.8 AU	0.75 Rf	23.6 AU	3.22 %	0.80 Rf	5.6 AU	736.3 AU	8.84 %
10	0.84 Rf	0.9 AU	0.87 Rf	14.8 AU	2.02 %	0.88 Rf	9.3 AU	323.0 AU	3.88 %

Figure 4: R_f values at 254 nm (Absorbance mode)

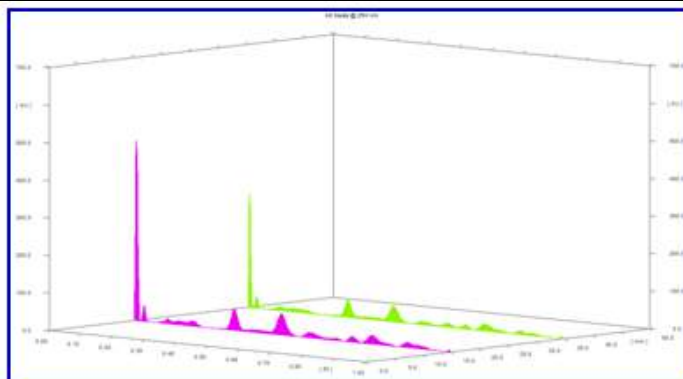
Venkatesh *et al.*,

Figure 5: Densitometric chromatogram at 254 nm in Alcohol (Fluorescence mode)

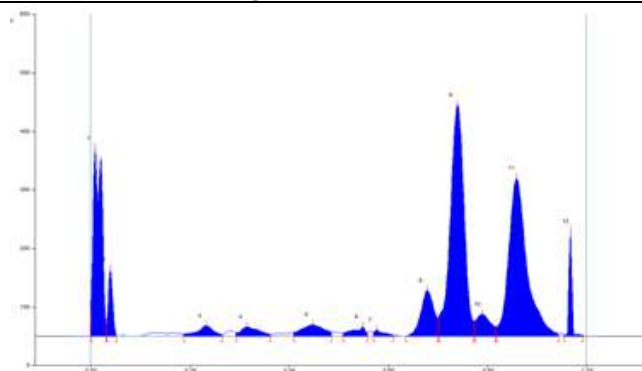


Figure 6: HPTLC finger print at 366 nm (Fluorescence mode)

Track 2, ID: DTL 6 NILAVANI CH

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	41.1 AU	0.01 Rf	320.7 AU	21.77 %	0.03 Rf	15.6 AU	4554.1 AU	15.96 %
2	0.03 Rf	25.6 AU	0.04 Rf	113.1 AU	7.67 %	0.05 Rf	0.6 AU	834.4 AU	2.92 %
3	0.19 Rf	4.0 AU	0.23 Rf	17.9 AU	1.21 %	0.27 Rf	2.9 AU	498.5 AU	1.75 %
4	0.29 Rf	5.8 AU	0.32 Rf	16.0 AU	1.09 %	0.36 Rf	3.2 AU	500.6 AU	1.75 %
5	0.41 Rf	6.6 AU	0.45 Rf	19.4 AU	1.32 %	0.49 Rf	7.3 AU	743.4 AU	2.60 %
6	0.51 Rf	5.3 AU	0.55 Rf	15.8 AU	1.07 %	0.56 Rf	6.5 AU	344.5 AU	1.21 %
7	0.57 Rf	6.7 AU	0.58 Rf	10.5 AU	0.71 %	0.61 Rf	1.9 AU	174.5 AU	0.61 %
8	0.64 Rf	0.3 AU	0.68 Rf	78.5 AU	5.33 %	0.70 Rf	31.6 AU	1715.5 AU	6.01 %
9	0.70 Rf	32.9 AU	0.74 Rf	395.0 AU	26.61 %	0.77 Rf	26.4 AU	9087.7 AU	31.84 %
10	0.77 Rf	26.4 AU	0.79 Rf	37.3 AU	2.53 %	0.82 Rf	16.2 AU	911.4 AU	3.19 %
11	0.82 Rf	16.4 AU	0.86 Rf	270.0 AU	18.32 %	0.94 Rf	4.2 AU	8272.1 AU	28.98 %
12	0.96 Rf	2.7 AU	0.97 Rf	179.2 AU	12.17 %	0.99 Rf	1.6 AU	905.9 AU	3.17 %

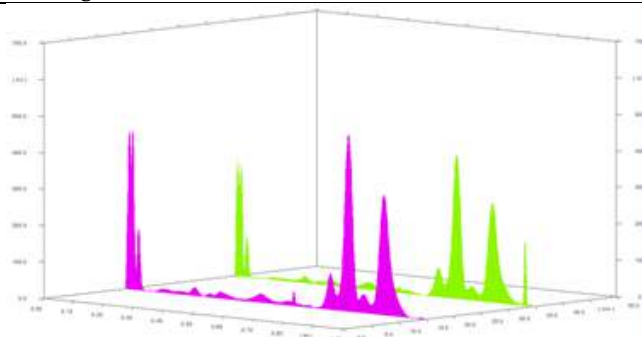
Figure 7: R_f values at 366 nm (Fluorescence mode)

Figure 8: Densitometric chromatogram at 366 nm in Alcohol (Fluorescence mode)



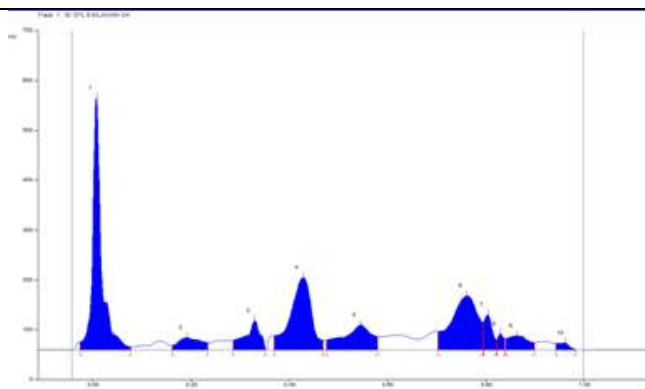
Venkatesh *et al.*,

Figure 9: HPTLC finger print at 520nm (Absorbance mode)

Track 1, ID: DTL 6 NILAVANI CH

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.03 Rf	16.1 AU	0.01 Rf	505.3 AU	48.76 %	0.08 Rf	5.6 AU	7561.5 AU	30.11 %
2	0.16 Rf	8.9 AU	0.19 Rf	24.5 AU	2.36 %	0.23 Rf	14.3 AU	979.4 AU	3.90 %
3	0.29 Rf	19.5 AU	0.33 Rf	58.0 AU	5.60 %	0.35 Rf	0.2 AU	1487.7 AU	5.92 %
4	0.37 Rf	28.3 AU	0.43 Rf	145.4 AU	14.03 %	0.47 Rf	19.7 AU	5194.7 AU	20.69 %
5	0.48 Rf	19.9 AU	0.55 Rf	49.9 AU	4.82 %	0.58 Rf	26.4 AU	2423.9 AU	9.65 %
6	0.70 Rf	37.3 AU	0.76 Rf	108.4 AU	10.46 %	0.79 Rf	55.0 AU	4822.8 AU	19.21 %
7	0.80 Rf	55.1 AU	0.81 Rf	70.4 AU	6.79 %	0.82 Rf	20.2 AU	1013.4 AU	4.04 %
8	0.82 Rf	20.9 AU	0.83 Rf	32.3 AU	3.11 %	0.84 Rf	22.6 AU	346.6 AU	1.38 %
9	0.84 Rf	22.7 AU	0.87 Rf	28.1 AU	2.71 %	0.90 Rf	14.5 AU	999.6 AU	3.98 %
10	0.94 Rf	12.6 AU	0.96 Rf	13.9 AU	1.34 %	0.98 Rf	0.4 AU	281.9 AU	1.12 %

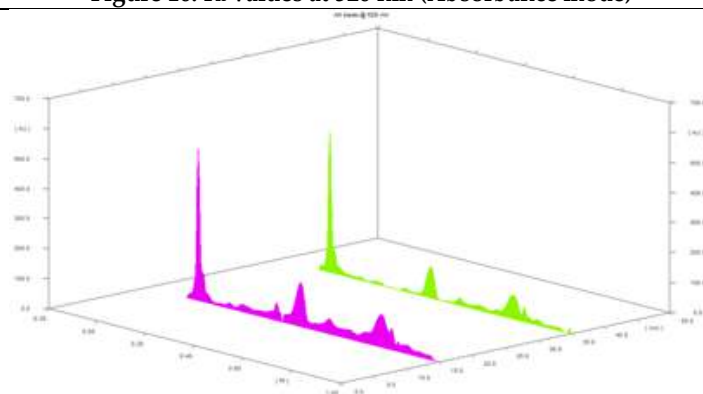
Figure 10: R_f values at 520 nm (Absorbance mode)

Figure 11: Densitometric chromatogram at 520 nm in Alcohol (Absorbance mode)

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RESEARCH ARTICLE

Robust Multi-Scale Convolutional Network for Lung Cancer Classification using Chest X-Ray Images

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ABSTRACT

The classification of lung cancer using images obtained from the chest X-ray with high accuracy is important for the early detection of lung cancer and help clinicians make clinical decisions. In this study, a Robust Multi-Scale Convolutional Feature Encoder (R-MSCFE) is proposed. The proposed network architecture can gather high, medium, and low-level features through parallel branches utilizing conventional and dilated convolutions. Multi-level features are combined through feature concatenation and further refined through channel alignment and attention-based re-weighting for pathology related regions. Residual feature enhancement is used for retaining the original information of the feature and stabilizing during the training process. The model performance is analyzed on an open access Kaggle lung cancer dataset for chest X-rays. Experimental assessment validates exceptional diagnostic efficacy, attaining an accuracy of 99.86%, precision of 100%, recall of 99.71%, and an F1-score of 99.86%. The findings demonstrate that the suggested R-MSCFE framework offers a dependable and effective approach for automated lung cancer screening via chest X-ray imaging

Keywords: Robust Multi-Scale Feature Encoder, Lung Cancer Classification, Chest X-Ray Imaging, Deep Learning, Convolutional Neural Network





Sharmila et al.,

INTRODUCTION

Recently, many deep learning architectures for classifying lung cancers using chest X-ray imaging were developed. Hybrid Quantum-Classical CNN architectures used quantum convolutional processes for extracting features, and NASNet and Vision Transformer frameworks adopted a combination of local convolutional processes and global dependency learning. Multi-stage deep learning frameworks used a series of pretrained models for classification refinement. In contrast, DeepLungNet incorporated stacking of CNN architectures with global average pooling to avoid overfitting. LungMaxViT used transformer attention architecture with explainable AI to facilitate lung disease classification. Even though all those models successfully performed the lung cancer classification task, many of them are known to exhibit high computational complexity, dependence on transformer components, multi-stage operations, and inability to classify multiple scales of lung pathologies concurrently. Some techniques are highly dependent on manual enhancement processes and ensembles that limit their computational performance and real-world applications. To address all these shortcomings, a robust framework based on parallel convolutional branches, adaptive feature fusion, channel attention, and residual reinforcement is developed, referred to as Robust Multi-Scale Convolutional Feature Encoder (R-MSCFE). The study proposes Robust Multi-Scale Convolutional Feature Encoder (R-MSCFE) which can be used to diagnose lung cancers using chest X-ray images. The encoder consists of a series of parallel convolution pathways with adaptive feature fusion to capture information in fine, medium and coarse scales. R-MSCFE encoder incorporates local and global information such as textures and contextual information. As compared to conventional CNN which uses sequential convolutions, the proposed model is more effective in enhancing discriminatory learning due to the incorporation of multiple scales within the network.

Related Works

Considering limitations associated with the currently available methods used for lung cancer screening and increased demand for intelligent assistance in diagnosis, this research has been conducted. Chest X-ray images are easily accessible but not as accurate as computerized tomography in identifying early lung cancers. Small nodules, blurry lung opacity, and asymmetrical masses in lung tissue can sometimes go unnoticed. Radiological diagnosis tends to fail due to low number or poor visibility of disease indicators. Deep learning allows automation of identification and classification of such tumors. There are different shapes and sizes of abnormalities in the development of lung cancer. Sometimes, small or big abnormality can be missed by single convolutional layer with a fixed receptive field. Consequently, multi-scale feature learning becomes necessary. Small sample size and class imbalance lead to overfitting and reduce generalization capability of a model. R-MSCFE architecture proposed in this paper provides scale invariant feature learning that solves these problems. It allows capturing lung features through a combination of different convolutional layers having distinct receptive fields. Attention guided refinement of extracted features allows highlighting discriminative patterns. Such architecture allows efficient classification of lung cancer.

Hybrid quantum-classical CNN was used for multiclass lung X-ray image classification in [1]. Feature maps are flattened and processed through a quantum convolutional layer using qubits with Rotation (RX/R_Y) and Pauli (X/Y/Z) gates. Dense layers with dropout and Soft Max perform final prediction. A NASNet and vision transformer-based deep learning architecture with mix processing for precise lung disease identification was shown in [2]. A NASNet-Vision Transformer hybrid model was used, where NASNet extracts local convolutional features using normal and reduction cells, while ViT captures global dependencies through patch embedding, positional encoding, and multi-head self-attention. A Mix Processing pipeline combining wavelet decomposition, CLAHE, Fourier filtering, and morphological operations improves image quality. NASNet and ViT features are fused using element-wise multiplication, followed by an MLP classifier. Comprehensive lung disease classification from X-ray images using multi-stage deep learning was studied in [3]. A multi-stage deep learning tool chain was implemented, where classification is refined through sequential decision stages. Transfer learning is applied using ResNet-50, DarkNet-53, EfficientNet-B0, ResNet-101, and DenseNet-201. Each stage selects an optimal pretrained model with tuned hyperparameters. DeepLungNet an advanced deep learning architecture having global average pooling, was discussed for classification of lung cancer precisely in [4]. Deep LungNet is an enhanced CNN model with stacked



**Sharmila et al.,**

convolutional layers and ReLU activation for hierarchical feature learning, followed by max-pooling for spatial reduction. A Global Average Pooling layer replaces fully connected layers to reduce parameters and mitigate overfitting.

Machine learning on X-rays predicts lung illnesses were detailed in [5]. The introduction of ResNet-D as a ResNet extension is based on its capacity to minimize information loss during down-sampling operations. As compared to strides in residual connections, ResNet-D employs average pooling in skip connections coupled with 1×1 convolution for channel compatibility. Such design preserves spatial information and facilitates robust feature propagation for classification tasks. Explainable hybrid transformers for chest X-ray lung disease multi-classification is proposed in [6]. The framework, called LungMaxViT, uses CNN-based feature extraction techniques, where a Squeeze-and-Excitation block enhances the process. Then, a transformer component inspired by Max ViT is applied, which incorporates MBConv and multi-axis block and grid attentions [7]. CLAHE is used for improving lung image clarity, whereas denoising is used for contrast improvement and explainable localization of attention through Grad-CAM. An anatomically augmented and clinically validated deep learning features and KL divergence lung anomalies classification pipeline is presented in [8]. An anatomically augmented and contrast and color mapped deep learning framework, known as ASCE-based framework, enhances lungs' representation through feature extraction using ResNet50 followed by KL divergence for lung anomalies' quantification. CNN based prognosis system incorporates data cleansing, feature engineering and min-max normalization. Hierarchical features are extracted using convolution and pooling layers, whereas fully connected layers are used for performing prognosis at severity levels. Moreover, sophisticated architectures of CNN models like ResNet and DenseNet are also being studied.

A deep learning ensemble technique for classifying lung diseases using chest X-rays has been proposed in [9]. A combined CNN-GRU model is used, whereby the CNN layers identify spatial textures and opacities via convolutional and pooling operations. The resultant feature maps are sent to a GRU layer to obtain the sequential relationship between learned features. SoftMax activation functions in dense layers produce the ultimate classification of multiple classes. Artificial intelligence techniques for detecting pulmonary disease in chest X-ray and chest multi-slice CT images have been reviewed in [10]. The comparison involves three network types – CNN, DNN, and ResNet50. Preprocessing entails noise reduction, image resizing, and normalization before training and testing. ResNet50 boosts classification with the aid of residual learning to increase feature extraction capacity on both X-ray and CT images [11]. The transfer learning approach involves the tuning of the pre-trained models like VGG-16, DenseNet-121, and DenseNet-201. The output layer is customized with layers such as AveragePooling2D, Flatten, Dense, Dropout, and SoftMax. Dense connection in DenseNet allows for the reusing of features and stable backpropagation for efficient multiclass classification. Bagging-based Deep Learning Ensemble for Improving Multi-class Lung Disease Classification was explained using five architectures in [12]. The bagging-based ensemble architecture involves the tuning of several pre-trained models like ResNet50, VGG16, VGG19, MobileNetV2, and DenseNet201. These models are trained individually, and then their results are combined with ensemble techniques like voting.

The optimization of deep learning models for predicting lung cancer through chest x-rays was described in [13]. The architecture of the risk prediction model of a neural network consists of chest X-ray pre-processing and clinical feature representation. A multilayer neural network predicts the probability of lung cancer risk. The probability is optimized using model optimization and ROC curves. The predicted probability is classified according to the established categories of lung cancer risk. The development of an explainable artificial intelligence system for lung diseases classification based on chest X-rays with transfer learning was introduced in [14]. The fine-tuning process is applied to DenseNet201 transfer learning model for multi-class classification tasks. Image enhancement includes denoising autoencoder, CLAHE, and gamma correction. Disease regions are explained using Grad-CAM, Grad-CAM++, SHAP, and LIME methods were presented in [15]. The transfer learning model is built based on MobileNetV1, whereby deep features will be extracted and a customized dense classifier with a sigmoid activation function will be used for predicting the labels in multiple labels. Data augmentation and image sharpening help improve data quality. The model uses a DCGAN module to generate new X-ray data, which helps in minimizing the





issue of class imbalance. The comprehensive background of CT and X-ray images for rapid and accurate identification of abnormal lungs using explainable AI is presented in [16]. CTXNET uses a Compact Convolutional Transformer involving convolutional tokenization together with self-attention mechanism and sequence pooling.

Lung disease images based on explainable-AI was explained in [17]. A literature-based survey presents the conventional ML methods that employ texture features extracted manually by applying algorithms like SVM and KNN alongside deep learning models including CNN architecture and pre-trained models such as VGG, ResNet, DenseNet, Inception, and Xception. It covers the use of ensemble and explainability approaches such as Grad-CAM, LIME, SHAP, saliency maps, and integrated gradients. Deep learning model with transfer learning and customized CNN architecture for the task of lung disease classification is reported in [18]. The transfer learning process is carried out using MobileNetV2 with all layers fine-tuned. Here, a customized model called MobileNet-Lung with Squeeze-and-Excitation mechanism added to the convolution layer followed by a better classifier is proposed. Lung diseases classification in chest x-rays based on transfer learning and hyperparameter customization is briefly discussed in [19]. CovEnViT includes a convolutional encoding component made up of two 3×3 convolutions with ReLU, which facilitates the learning of local features before patch embedding. The encoder blocks in the transformer are composed of multi-head self-attention that learns global interactions, and the MLP head carries out the final classification task. Improved lung cancer classification using deep learning techniques and CNNs is well explained in [20]. Three CNN networks have been designed and trained via grayscale scaling and feature extraction. Oversampling via SMOTE helps address data imbalance issues.

METHODS AND MATERIALS

This describes the completely suggested system for lung cancer classification using chest X-ray images. The framework is designed to learn robust discriminative representations by extracting features at multiple spatial scales and integrating them through attention-guided fusion. The overall goal of the suggested system is to accurately classify chest X-ray images into lung cancer and non-cancer classes by capturing both fine-grained lesion patterns and coarse global abnormalities. The suggested system consists of four major phases such as (i) image preprocessing, (ii) robust multi-scale convolutional feature encoding, (iii) attention-based feature refinement and residual reinforcement, and (iv) classification and performance evaluation. An input chest X-ray feature map is processed in three parallel processes is shown in Figure 1. Through 3×3 convolution, batch normalization, and ReLU, fine-scale texture features are created, revealing small intensity fluctuations. The 5×5 convolutional medium-scale feature route captures regional lung patterns with mid-level structural characteristics. The coarse-scale feature route (dilated 3×3 convolution) captures global diseased areas' extensive background characteristics. Concatenating these results creates a multi-scale description. A 1×1 convolution aligns channel dimensions, while channel attention reweighting highlights pathology-relevant characteristics. Finally, residual feature reinforcement incorporates input data to create a strong multi-scale feature representation for classification.

Input Image Acquisition and Preprocessing

The image data of chest x-rays are usually prone to changes in illumination, noise, poor contrast, and resolution. Such defects might impact feature extraction and lower the accuracy rate of classification. Input to the proposed framework is a chest X-ray image to classify lung cancer. Let us denote the obtained radiography image:

$$X \in \mathbb{R}^{M \times N} \quad (1)$$

where M and N the chest X-ray image denoted by the spatial dimensions. Pre processing activities are done to increase the picture consistency and feature learning capacity before classification, since the radiographic images are acquired from varied imaging settings.





Sharmila et al.,

Image Resizing

A fixed resolution was adopted for each resized image for uniformity and compatibility with the convolutional encoder.

$$X_s = \psi(X, p, q) \quad (2)$$

where $\psi(.)$ The spatial transformation function is denoted by, and p and q are the specified picture size utilised throughout the learning phase. This method lets to extract features uniformly throughout all the chest radiographs.

Intensity Normalization Pixel Distribution Adjustment

Over the standardised image, pixel intensity modification is conducted to stabilise feature propagation and enhance convergence during training. The converted intensity is expressed as:

$$X_t(u, v) = \frac{X_s(u, v)}{\lambda} \quad (3)$$

where $X_t(u, v)$ represents the standardized pixel value at spatial position (u, v) , and λ denotes the scaling factor used to normalize the intensity distribution. This process reduces intensity variations and improves feature consistency across chest X-ray samples.

Radiographic Enhancement

Further image enhancement procedures are performed on the converted image for improving the visibility of lung tissues, nodular structures, and aberrant opacity areas. Below is the improved depiction of the image:

$$X_e = \Omega(X_t) \quad (4)$$

where $\Omega(.)$ refers to the radiography enhancement function used for optimal distribution of contrast and highlighting the pathologically related areas. The enhanced image is then fed into the proposed multi-scale convolutional feature encoder for lung cancer discrimination. The proposed model further applies the processed chest radiograph image to several convolution layers having varied receptive fields. The small-sized convolution filters can detect local variations in edges and textures, whereas larger receptive fields can learn global information regarding lung structure and tissue distribution. The 5x5 convolution branch extracts medium-scale characteristics, which include regional lung architecture and abnormal opacity patterns. Dilated 3x3 convolution collects contextual characteristics for vast diseased areas. Each branch pools to create scale-specific summarized feature maps. Features from these outputs are fused to provide a multi-scale feature representation including fine, medium, and coarse information. Finally, the lung cancer classification module uses this unified model to predict normal, benign, or malignant outcomes.

Robust Multi-Scale Convolutional Feature Encoder (R-MSCFE)

The key novelty of the suggested framework is the Robust Multi-Scale Convolutional Feature Encoder (R-MSCFE), which extracts features using parallel convolutional branches operating at different receptive fields. Lung cancer patterns in chest X-rays may appear as small nodules, medium-size irregular opacities, or large abnormal lung masses. A single convolution scale may fail to capture all variations. Therefore, multi-scale convolution is introduced. Let the pre-processed input image be:

$$X = I_e \quad (5)$$

Fine-Scale Feature Extraction Branch

Fine-scale features capture small texture variations and edge information. A 3x3 convolution is used:

$$F_1 = \sigma(\text{BN}(W_1 * X + b_1)) \quad (6)$$

where W_1 represents the convolution kernel of size 3x3, b_1 is bias, $*$ denotes convolution operation, $\text{BN}(\cdot)$ is batch normalization, and $\sigma(\cdot)$ denotes ReLU activation.





Sharmila et al.,

Medium-Scale Feature Extraction Branch

Medium-scale features capture larger structural lung patterns. A 5×5 convolution is applied:

$$F_2 = \sigma(\text{BN}(W_2 * X + b_2)) \quad (7)$$

where W_2 is a 5×5 kernel.

Coarse-Scale Feature Extraction Branch

Coarse-scale features capture global lung context and large abnormal regions. A dilated convolution is applied:

$$F_3 = \sigma(\text{BN}(W_3 *_d X + b_3)) \quad (8)$$

where $*_d$ denotes dilated convolution with dilation rate d , enabling a larger receptive field without increasing parameters.

Multi-Scale Feature Fusion

After extracting features from three branches, the next step is featuring fusion to combine fine, medium, and coarse-scale information. The outputs F_1 , F_2 , and F_3 are concatenated along the channel dimension.

$$F_c = \text{Concat}(F_1, F_2, F_3) \quad (9)$$

The concatenated feature map F_c contains complementary representations from different receptive fields.

Channel Alignment using 1×1 Convolution

The concatenated feature map has increased channel size, which may increase computational complexity. Therefore, a 1×1 convolution is applied for channel reduction and alignment:

$$F_a = \sigma(\text{BN}(W_a * F_c + b_a)) \quad (10)$$

where W_a is a 1×1 convolution kernel. This operation reduces redundancy and ensures consistent feature embedding.

Channel Attention Reweighting

Multi-scale fusion gives many characteristics; however, all the channels are not equal in value for the classification of lung cancer. Attention reweighting is utilized to boost pathology-relevant channels.

Global Average Pooling for Attention

A global average pooling operation is applied:

$$z_k = \frac{1}{H_f W_f} \sum_{x=1}^{H_f} \sum_{y=1}^{W_f} F_a^k(x, y) \quad (11)$$

where z_k represents the global descriptor of channel k , and H_f , W_f are feature map dimensions.

Attention Weight Computation

The channel weights are computed using a gating function:

$$s = \delta(W_2^{\text{att}}(\sigma(W_1^{\text{att}} z))) \quad (12)$$

where W_1^{att} and W_2^{att} are learnable weights, σ is ReLU, and δ is sigmoid activation.

Feature Reweighting

The final attention-enhanced feature map is obtained as:

$$F_{att}^k = s_k \cdot F_a^k \quad (13)$$

This ensures important lung abnormality patterns dominate the representation.

Residual Feature Reinforcement

To increase feature stability and prevent any spatial information loss during feature extraction through deep layers, residual learning technique is employed. Feature representation after improvement is obtained by fusing attention-augmented features with baseline feature representation. This can be expressed as:

$$R_f = \phi(A_f, B_f) \quad (14)$$





Sharmila et al.,

where A_f represents the attention-refined feature representation, B_f denotes the aligned baseline feature map, and $\Phi(\cdot)$ indicates the residual fusion operation. This process aids in gradient propagation and maintains critical structural lung information throughout training.

Classification Module

Then, the robust multi-scale feature representation is generated, and the retrieved features are translated into compact discriminative vectors for lung cancer classification.

Global Feature Aggregation

First, spatial feature representations are pooled by global pooling procedures to form a compact descriptor:

$$Z_g = \Lambda(R_f) \quad (15)$$

where $\Lambda(\cdot)$ denotes the global aggregation transformation applied to the reinforced feature representation R_f . This operation reduces feature dimensionality while preserving discriminative information.

Dense Representation Learning

Then the aggregated feature vector is mapped to higher-level discriminative representations by nonlinear mapping operations:

$$D_h = \Gamma(Z_g) \quad (16)$$

where $\Gamma(\cdot)$ represents the dense nonlinear transformation used to enhance classification-oriented feature learning. Feature regularisation is further used to increase the generalisation ability and decrease the overfitting during training as:

$$D_r = \Psi(D_h) \quad (17)$$

where $\Psi(\cdot)$ denotes the feature regularization operation applied to the learned dense representation.

Output Prediction

Finally, the regularized feature representation is sent to the output prediction stage for lung cancer classification. The prediction representation may be stated as:

$$P_o = Y(D_r) \quad (18)$$

where $Y(\cdot)$ corresponds to the last classification transformation that produces the prediction probabilities of lung cancer classes. Normalised probability distribution is created across all classes for multiclass lung disease classification:

$$Q_i = \frac{\exp(T_i)}{\sum_{k=1}^K \exp(T_k)} \quad (19)$$

where K denotes the total number of lung disease categories and T_i represents the classification activation score corresponding to the i^{th} category.

Loss Function Optimization

In the proposed framework, the optimum trainable parameters are learned by minimizing the classification loss between the anticipated and real categories.

Binary Optimization Objective

For binary lung cancer classification, the optimization objective is expressed as:

$$J_b = \Omega(Y, P) \quad (20)$$

where Y denotes the actual class representation and P represents the predicted classification probability.

Categorical Cross Entropy

For multi-category classification problems, the optimization objective is represented as:

$$J_m = \sum_{i=1}^K (Y_i, Q_i) \quad (21)$$





Sharmila et al.,

Training Strategy

The trainable parameters of the proposed framework are optimized repeatedly using gradient-based learning techniques. The parameter refinement operation may be described as:

$$\mathbb{E}_{t+1} = \mathbb{E}_t - \rho \nabla J \quad (22)$$

where ρ denotes the adaptive learning coefficient, $\mathbb{E}$ represents trainable model parameters, and ∇J denotes the optimization gradient. To further stabilize the optimization and speed up the convergence, the adaptive moment estimation is used using the first order and second-order gradient accumulation operations:

$$M_t = \alpha M_{t-1} + (1 - \alpha) G_t \quad (23)$$

$$V_t = \beta V_{t-1} + (1 - \beta) G_t^2 \quad (24)$$

where G_t denotes the optimization gradient, while M_t and V_t represent adaptive moment estimations used for stable parameter optimization during network training.

Overall Workflow Summary

The architecture of the Multi-Scale Convolutional Network for lung cancer classification starts with data acquisition and preprocessing steps such as resizing, normalization, and radiography enhancement to ensure the image is of high quality and consistent features. Following this, the enhanced chest x-rays are fed into various parallel convolutional modules working on distinct receptive fields for extracting fine, medium, and coarse representations of the lung. Multiscale feature fusion helps merge the features in one single feature space, where channel attention and residual enhancement help learn more discriminating features while preserving spatial information. The resultant robust representation of the features is then used in global feature extraction and classification layers for lung cancer category prediction.

Advantages of the Suggested System

The proposed multi-scale convolutional network has various benefits for automated lung cancer classification from chest X-ray images. The multi-scale convolutional design can capture both localized texture defects and global contextual lung structures, which improves the feature representation capabilities compared to the traditional single-scale CNN models. Parallel convolutional branches improve the identification of tiny nodules and irregular opacities and big aberrant areas concurrently. An attention-guided feature refinement is included to highlight the pathology-relevant areas and reduce the unnecessary background information. Residual reinforcement facilitates gradient propagation and maintains the original feature representations, leading to robust network optimization and lower performance degradation. Moreover, the suggested framework has reduced computational cost compared to the transformer-heavy designs, which makes it effective for clinical deployment and real-time lung cancer classification applications. The Robust Multi-Scale Convolutional Feature Encoder (R-MSCFE) offers a robust architecture for lung cancer classification from chest X-ray images. The proposed method efficiently captures discriminative lung cancer features at various spatial resolutions via the fusing of parallel multi-scale convolutions, feature fusion, attention refinement and residual reinforcement. The concept enhances the reliability of classification while preserving computational efficiency, which is suited for real-time clinical screening applications.

Evaluation Metrics

This dataset is a collection of chest X-ray images for lung cancer research and classification. It is organised in directories according to various lung health issues, enabling supervised learning for deep learning models [21].

Image categories:

- Lung cancer cases
- Normal (no cancer) cases (Exact class labels and counts are based on the dataset's directory structure.)

Image format & preprocessing readiness:

- Images are typically in standard image formats (e.g., JPEG/PNG).
- The dataset is ready for resizing, normalization, augmentation, and deep model input pipelines.





Sharmila et al.,

Data augmentation was done with training of 5216 images, validation of 16 images and testing images of 624 images which total of 5856 images. To evaluate the suggested system, standard classification performance metrics are computed using True Positive (TP), True Negative (TN), False Positive (FP), and False Negative (FN).

Accuracy

Accuracy measures overall correct classification:

$$\text{Accuracy} = \frac{TP+TN}{TP+TN+FP+FN} \quad (26)$$

Precision

Precision evaluates the correctness of positive predictions:

$$\text{Precision} = \frac{TP}{TP+FP} \quad (27)$$

Recall (Sensitivity)

Recall measures the ability to identify true cancer cases:

$$\text{Recall} = \frac{TP}{TP+FN} \quad (28)$$

F1-Score

F1-score is the harmonic mean of precision and recall:

$$F1 = \frac{2 \times \text{Precision} \times \text{Recall}}{\text{Precision} + \text{Recall}} \quad (29)$$

Specificity

Specificity measures true negative recognition:

$$\text{Specificity} = \frac{TN}{TN+FP} \quad (30)$$

Figure 3 illustrates a heat map of classification outcomes between normal and cancer chest X-ray classes. The diagonal cells show higher numerical values of 346 and 347, indicating many correctly classified normal and cancer cases. Figure 4 shows chest X-ray images belonging to the normal class used for model training and evaluation. The images show clean lung zones, well defined rib structures and consistent intensity distribution without any obvious abnormality. The consistency of anatomical features across samples offers a valid reference for the learning of the normal lung characteristics, which allows the model to successfully discriminate between healthy cases and problematic ones in the classification process. Chest X-ray images of the cancer class utilized in this investigation are shown in Figure 5. These images show important anomalies such as increased lung opacity, uneven texture patterns and asymmetric intensity distributions compared to normal instances. These visual alterations relate to the pathological changes seen in lung cancer. The inter-sample visual heterogeneity highlights the need of extracting multi-scale features for accurate and robust cancer classification. The performance of multiple lung cancer classification algorithms employing accuracy, precision, recall, and F1-score is compared in Figure 6. Figure 6. Comparative Performance Analysis of Lung Cancer Classification Techniques The Hybrid CNN [1] achieves the lowest performance with 91% across all measures. NASNet-ViT [2] improves results, reaching 98.90% accuracy and 99% precision/recall. The KL divergence-based method [7] shows reduced precision and F1-score. GRU [9] provides balanced performance. The suggested MSCFE achieves the highest accuracy (99.86%), precision (100%), recall (99.71%), and F1-score (99.86%).

CONCLUSION

The proposed Robust Multi-Scale Convolutional Feature Encoder (R-MSCFE) framework was devised for enhancing classification of lung cancers using chest X-ray imaging data. The framework can extract fine-grained, medium-grained, and coarse-grained representations of the lungs via multiple convolution branches, allowing efficient learning of localized textures and abnormal areas. Adaptive multi-scale feature fusion and attention mechanism-





Sharmila et al.,

based channel re-weighting contribute to learning of more informative and discriminating features in terms of disease-related attributes. Residual-based feature reinforcement can maintain significant spatial representations and ensure robustness in feature learning. Experimental results have shown that our proposed approach is highly capable of lung cancer detection based on the high levels of classification accuracy, precision, recall, and F1-score. Although impressive results were obtained, several limitations exist. The proposed model has been validated on relatively small benchmarking data and would benefit from validation on large multi-center clinical databases to confirm its generalization power. It should be noted that chest X-rays have inferior ability to detect subtle abnormalities as opposed to some more sophisticated imaging techniques. Some possible areas for future work include incorporating explainable artificial intelligence, hybrid attention models, and lightweight techniques.

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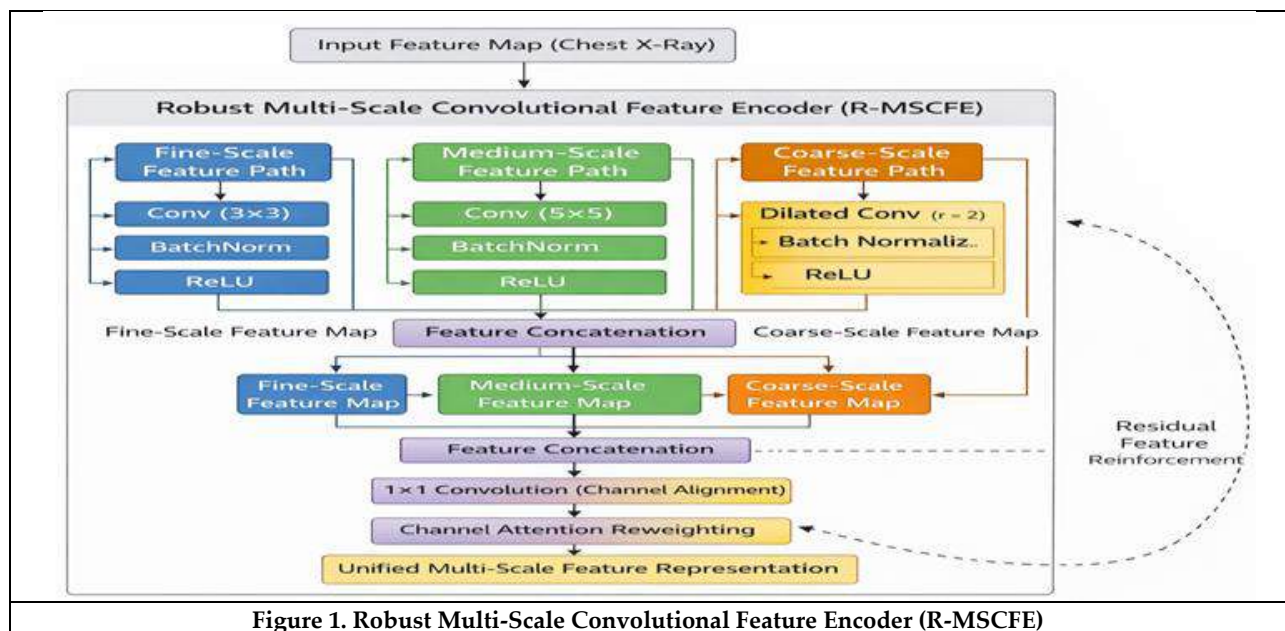
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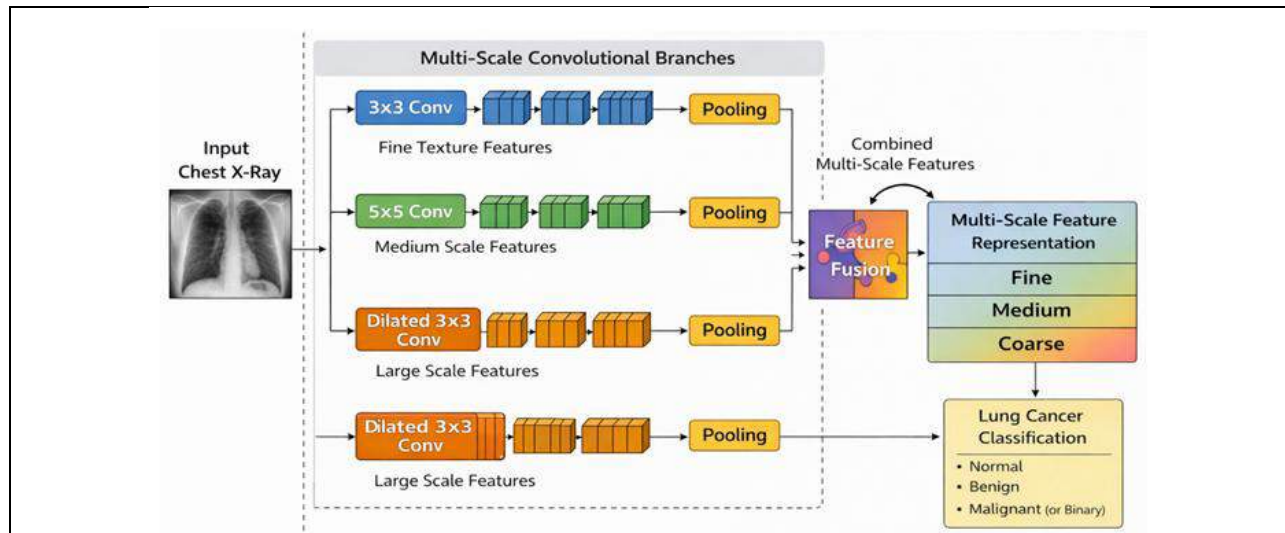


Figure 2. Overall Framework for Lung Cancer Classification Using Chest X-Rays

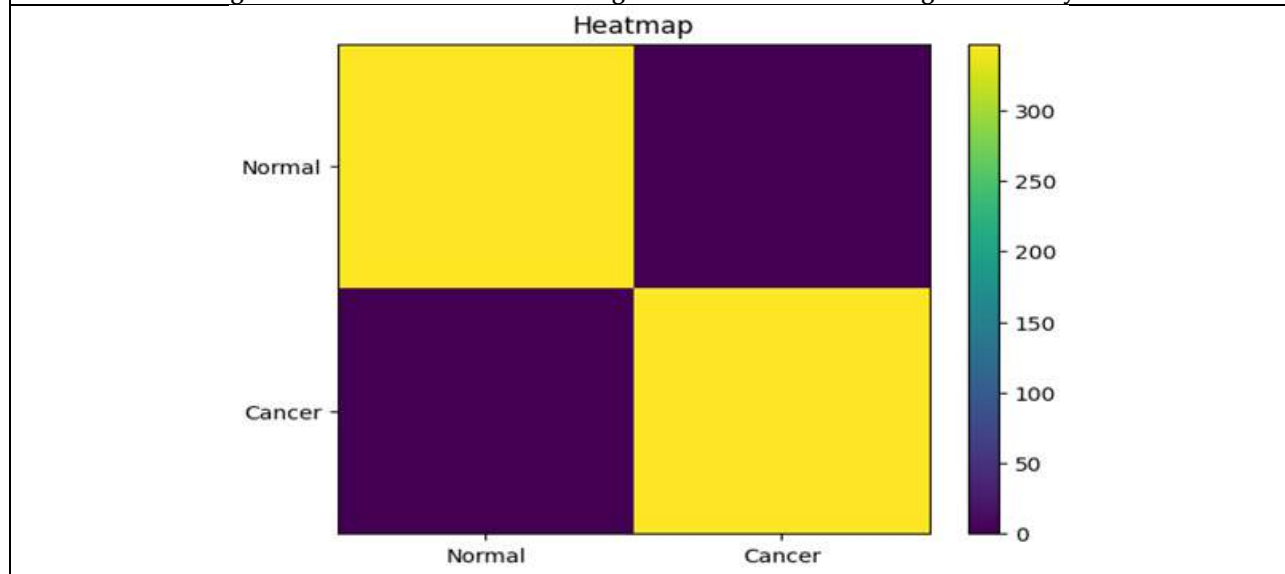


Figure 3. Heatmap of Binary Lung Cancer Classification Outcomes

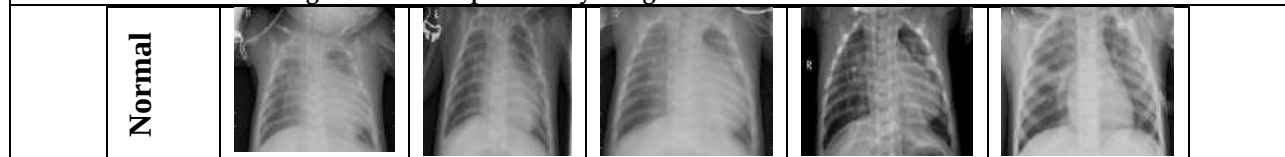


Figure 4. Normal Class of Chest X-Ray Images

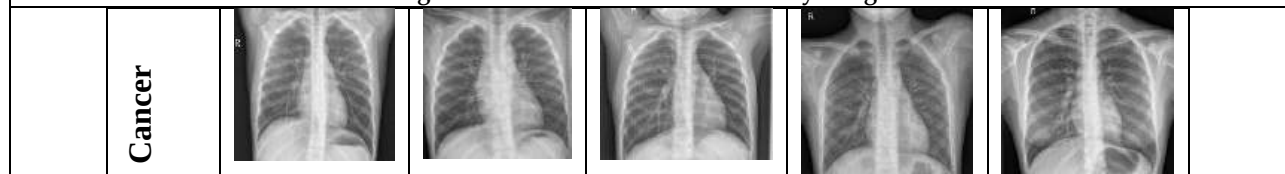
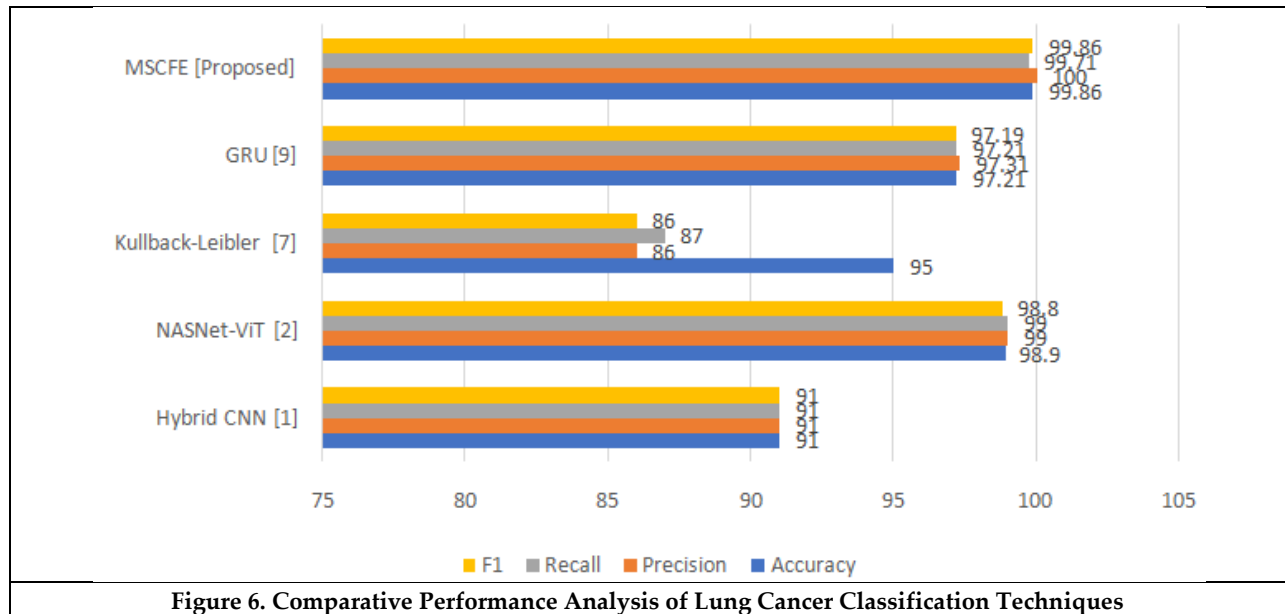


Figure 5. Cancer Class of Chest X-Ray images



Sharmila *et al.*,

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RESEARCH ARTICLE

A Clinico - Comparative Study of Varunadi Kvatha and Sigrumulatwak Kvatha in the Management of Mutrashmarī (Urolithiasis)

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ABSTRACT

Mutrashmarī (urolithiasis) is classified among the ashta mahagada by Sushruta, underscoring its potential for serious morbidity. Sigrumulatwak kvatha is specifically indicated for ashmari in Ashtanga Hridayam, yet no clinical study has evaluated its efficacy to date. Varunadi kvatha has prior documented clinical evidence in urolithiasis. To study, observe, and compare the effectiveness of Varunadi kvatha and Sigrumulatwak kvatha in patients of Mutrashmarī, and to assess changes in signs, symptoms, serum calcium, and size and number of urinary calculi. A randomized, open-label, parallel-group (1:1), clinico-comparative clinical trial was conducted at the OPD of Kayachikitsa and Panchakarma Department. Two hundred patients were enrolled (100 per group). Randomization used a computer-generated sequence (block size 4); allocation concealment by serially numbered opaque sealed envelopes. Group A received Varunadi kvatha (40 ml BD) and Group B received Sigrumulatwak kvatha (40 ml BD), both with Yavakshara (125 mg), for 6 months. IEC clearance: EC/NEW/INST/2023/4029; CTRI: CTRI/2024/07/071116.

Results: Both groups showed statistically highly significant improvement ($P < 0.001$) in all parameters. Group A was superior in constipation (71.20%) and stone number reduction. Group B was superior in pain (68.3%), dysuria (59.5%), weakness (69.8%), stone size reduction, and hydronephrosis resolution. No adverse effects were observed. Both Varunadi kvatha and Sigrumulatwak kvatha demonstrate significant efficacy in Mutrashmarī with no significant inter-group difference in overall outcome. Sigrumulatwak kvatha is hereby clinically validated for the first time, corroborating its classical indication in Ashtanga Hridayam. Both formulations were safe and well-tolerated.

Keywords: Mutrashmarī, Urolithiasis, Varunadi kvatha, Sigrumulatwak kvatha, Randomized clinical trial, Ayurveda





INTRODUCTION

Mutrashmārī (urolithiasis) is classified among ashta mahagada by Sushruta [1], signifying its potential for serious morbidity and mortality. Ashtanga Hridayam recommends medical management for the acute (taruna) stage and surgical management for the chronic (pravridha) stage [2]. Urolithiasis refers to stone formation in the kidney, ureter, or urinary bladder due to supersaturation of urine with insoluble crystalline substances. Increased serum calcium promotes hypercalciuria and crystal aggregation, ultimately forming calculi [3]. The global prevalence ranges between 1–15%, with a lifetime risk of approximately 10–15% in developed countries [5]. Gujarat, with its high total dissolved salts (TDS) content in groundwater [4], carries a disproportionately high burden – approximately 30% of urinary disorder patients in this region are affected. Stone composition includes calcium oxalate (~50%), calcium phosphate (15–25%), struvite (15–30%), cystine (6–10%), and uric acid (2–10%) [6]. In Ayurveda, Mutrashmārī is a kaphapradhana tridosha disorder in which vitiation of apana vāyu plays the principal pathogenic role. Causative factors include suppression of natural urges (vega dharana), irregular dietary habits, and impaired agni – all leading to sharkara formation coalescing into ashmārī [7].

Varunadi kvātha, with principal ingredient varuna (*Crataeva nurvala* Buch.-Ham.), has documented antilithogenic, diuretic, and vatanulomana properties [8,9]. Although Sigrumulatwak kvātha is indicated for ashmārī in Ashtanga Hridayam [10], no research article has documented its clinical effectiveness. The active phytoconstituents of shigru (*Moringa oleifera* Lam.) root bark, including moringine alkaloids, possess analgesic, anti-inflammatory, and diuretic properties [11,12]. The recurrence rate of urolithiasis is approximately 50% within 5 years without intervention [13]. Conventional agents carry side effects with prolonged use [14]. The present study was designed to fill the evidence gap by clinically comparing both formulations in Mutrashmārī.

MATERIALS AND METHODS

Study Design and Registration: This was a randomized, open-label, parallel-group, clinico-comparative clinical trial with a 1:1 allocation ratio. IEC clearances: EC/NEW/INST/2023/4029 and MAMCH/ADMN/IEC.APPROVAL/2023-24/199. CTRI Registration: CTRI/2024/07/071116. The study was conducted in accordance with the Declaration of Helsinki [15]. Written informed consent was obtained from all participants.

Sample Size: A sample size of 200 patients (100 per group) was determined a priori based on a pilot study and published literature by Krishnamurthy and Tiwari [8] reporting a 20% difference in stone size reduction. Assuming 80% power and 5% level of significance (two-tailed), with 10% dropout margin, the calculated sample size was 91 per group, rounded to 100 per group.

Randomization and Allocation Concealment: The random allocation sequence was generated by an independent statistician using computer software with simple block randomization (block size = 4). Allocation concealment was implemented using serially numbered opaque sealed envelopes. This was an open-label trial; blinding of participants and treating physicians was not feasible. Both interventions were identical in volume (40 ml), route (oral), timing (twice daily), and vehicle (Yavakshara 125 mg) to minimize performance bias.

Selection of Patients: Patients were recruited from the OPD of Kayachikitsa and Panchakarma Department from August 2023 to July 2024. Diagnosis was established based on clinical signs and symptoms, urine routine and microscopic examination, and ultrasound sonography (KUB region). Inclusion criteria: age 18–80 years; stone size up to 10 mm on USG. Exclusion criteria: severe renal colic requiring emergency intervention, severe hydronephrosis, acute renal disease, staghorn calculi, and other specified conditions.



**Meghasinh D Rajput and Tapankumar Vaidya**

Interventions (Table 1): Both medicines were prepared as standardized aqueous decoctions at the trial pharmacy under expert supervision. Varunadi kvatha (Group A) contains Varuna (*Crataeva nurvala*), Pashanabheda (*Bergenia ligulata*), Gokshura (*Tribulus terrestris*), and Punarnava (*Boerhavia diffusa*) as per Ashtanga Hridayam Chikitsasthana. Sigrumulatwak kvatha (Group B) contains Shigru root bark (*Moringa oleifera*) as principal ingredient with Yavakshara (125 mg) as prakshepa dravya in both groups.

Outcome Measures: Primary outcome: reduction in stone size (mm) on USG-KUB from baseline to end of 6-month treatment. Secondary outcomes: changes in subjective symptom scores (pain, burning micturition, dysuria, frequency, nausea, vomiting, weakness, constipation) assessed monthly using 0–3 graded scales (Tables 2–4); reduction in stone number; hydronephrosis resolution; serum calcium; CBC and urine routine/microscopy.

Statistical Methods: Paired t-test (within-group, pre–post comparison) and unpaired two-tailed t-test with unequal variance (between-group comparison). $P < 0.05$ was considered statistically significant. Data are presented as mean $\pm$ SD with 95% confidence intervals. Analysis was performed on the per-protocol population.

OBSERVATIONS AND RESULTS

Participant Flow: A total of 243 patients were screened. Of these, 43 were excluded (18 did not meet inclusion criteria; 16 declined to participate; 9 had stone size >10 mm on USG). The remaining 200 patients were randomized — 100 to Group A and 100 to Group B. All 200 completed the 6-month treatment period and were included in the final per-protocol analysis. There were no dropouts, withdrawals, or losses to follow-up. [A CONSORT flow diagram is submitted as a separate figure file.]

Baseline Characteristics (Table 5): Baseline demographic and clinical characteristics were comparable between the two groups (all $P > 0.05$), confirming successful randomization.

Effect on Primary Outcome — Stone Size: Group B showed significantly greater reduction in stone size on USG-KUB (mean reduction 3.1 ± 0.8 mm, 95% CI: 2.9–3.3 mm) compared to Group A (mean reduction 2.7 ± 0.7 mm, 95% CI: 2.5–2.9 mm), with a statistically significant between-group difference ($P < 0.05$).

Effect on Primary Symptoms (Table 6): The reduction in mean pain score showed 59.6% relief in Group A and 68.3% relief in Group B ($P < 0.001$). Sadahamutraprvritti showed 58.9% relief in Group A and 62.8% in Group B ($P < 0.05$). Mutrakricchra showed 60% relief in Group A and 59.5% in Group B ($P < 0.05$). Frequency of micturition: 57.8% relief in Group A and 45.1% in Group B ($P < 0.05$).

Effect on Associated Symptoms (Table 7): Group A showed superior relief in nausea (84.31%) and constipation (71.20%). Group B showed superior relief in weakness (69.8%) and vomiting (70.8%). Between-group differences were statistically significant ($P < 0.05$) for most parameters.

Effect on Objective Parameters: Hydronephrosis resolution was more pronounced in Group B (26/30 cases, 86.7%) versus Group A (22/28 cases, 78.6%). Group A showed superior reduction in total stone number. Serum calcium showed significant reduction in both groups ($P < 0.001$). Urine routine microscopy improved in both groups with reduction in pus cells, RBCs, and crystals.

Safety Assessment: No adverse effects, serious adverse reactions, or treatment-related complications were observed or reported in any participant in either group throughout the 6-month treatment and follow-up period. Both formulations were well-tolerated.





DISCUSSION

Mutrashmarī is a kaphapradhana tridosha disorder in which vitiation of apana vayu plays the principal pathogenic role. The high prevalence among young adult males observed here is consistent with hormonal and occupational factors including vega dharana, a key nidana in Sushruta Samhita [7]. Varunadi kvatha – with principal ingredient varuna (*Crataeva nurvala*) – exerts well-documented antilithogenic and diuretic properties. Lupeol, a bioactive triterpene of *Crataeva nurvala*, inhibits urinary enzyme activities (N-acetyl-beta-D-glucosaminidase and alkaline phosphatase) implicated in calcium oxalate crystal adherence [17]. Its superior efficacy in reducing constipation and stone number is attributable to bhedana and mutrala properties of its constituents. Prior studies [8,9,21,27] have documented comparable efficacy. Sigrumulatwak kvatha demonstrated superior results in pain relief, dysuria, stone size reduction, and hydronephrosis resolution. The ushna, tikshna, and katu properties of shigru (*Moringa oleifera*) root bark confer analgesic, anti-inflammatory, and diuretic actions [11,12]. The diuretic effect has been confirmed preclinically [18] and anti-inflammatory effects are mediated via COX-2 and NF-κB inhibition [19]. This study provides the first clinical validation of this formulation in Mutrashmarī. The absence of significant inter-group difference in overall therapeutic outcome validates both formulations as equivalent options, consistent with samya chikitsa in classical Ayurvedic literature. The safety profile – no adverse effects across 200 patients over 6 months – supports their long-term applicability compared to conventional pharmacotherapy [14,20].

Limitations: This study had no placebo control group due to ethical constraints, and its open-label design may introduce performance and detection bias. The single-centre design may limit generalizability. Long-term recurrence data beyond 6 months are unavailable. Stone composition analysis was not performed. A multicentric trial with independent sample size re-estimation is recommended.

CONCLUSION

Both Varunadi kvatha and Sigrumulatwak kvatha demonstrate statistically significant and clinically meaningful efficacy in the management of Mutrashmarī (urolithiasis) with no significant inter-group difference in overall therapeutic outcome. Sigrumulatwak kvatha is hereby clinically validated for the first time, corroborating its classical indication in Ashtanga Hridayam. Both formulations were safe and well-tolerated across 200 patients over 6 months. Larger-scale, placebo-controlled, multicentric randomized trials are recommended to further generalize these findings.

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DECLARATIONS

Data Availability Statement: The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request. The trial protocol is available through the CTRI registry: CTRI/2024/07/071116 (<https://ctri.nic.in>).

Declaration of AI in Scientific Writing: The authors confirm that no artificial intelligence (AI) tools were used in the conception, design, data collection, analysis, interpretation, or writing of this manuscript. The manuscript was written entirely by the authors.

Authors' Contribution Statement: Author 1: Conceptualization, study design, patient recruitment, data collection, statistical analysis, and manuscript preparation. Co-Author 2: Supervision, clinical guidance, critical review, intellectual revision, and final approval of the manuscript.



**Meghasinh D Rajput and Tapankumar Vaidya**

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Conflicts of Interest: There are no conflicts of interest.

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Meghasinh D Rajput and Tapankumar Vaidya

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Table 1: Treatment schedule for Group A and Group B

Parameter	Group A – Varunadi Kvatha	Group B – Sigrumulatwak Kvatha
Dose	40 ml	40 ml
Frequency	Twice daily (B.D.)	Twice daily (B.D.)
Route	Oral	Oral
Prakshhepa dravya	Yavakshara 125 mg	Yavakshara 125 mg
Duration	6 months	6 months
Follow-up	Monthly intervals	Monthly intervals

Table 2: Grading of Pain (Shula)

No.	Symptom Description	Score
1.	No pain	0
2.	Bearable pain (1–2 times per month)	1
3.	Bearable pain occasionally (average 2–3 times per week)	2
4.	Bearable pain every day	3

Table 3: Grading of Burning Micturition (Sadahamutraprvritti)

No.	Symptom Description	Score
1.	No burning micturition	0
2.	Burning micturition (1–2 times per month)	1
3.	Regular burning micturition (average 2–3 times per week)	2
4.	Regular burning micturition every day	3

Table 4: Grading of Dysuria (Mutrakricchhra)

No.	Symptom Description	Score
1.	No dysuria	0
2.	Mild dysuria (1–2 times per month)	1
3.	Moderate dysuria (2–3 times per week)	2
4.	Severe dysuria every day	3

Table 5: Baseline Demographic and Clinical Characteristics

Characteristic	Group A – Varunadi Kvatha (n=100)	Group B – Sigrumulatwak Kvatha (n=100)
Age (years), mean ± SD	38.6 ± 12.4	37.9 ± 11.8
Sex: Male, n (%)	62 (62%)	62 (62%)
Sex: Female, n (%)	38 (38%)	38 (38%)
Stone size (mm), mean ± SD	6.8 ± 1.9	6.9 ± 2.1





Meghasinh D Rajput and Tapankumar Vaidya

Pain score, mean $\pm$ SD	3.64 $\pm$ 0.61	3.22 $\pm$ 0.68
Serum calcium (mg/dL), mean $\pm$ SD	10.2 $\pm$ 1.1	10.3 $\pm$ 1.0
Hydronephrosis present, n (%)	28 (28%)	30 (30%)
Duration of symptoms (months), mean	7.4 $\pm$ 3.2	7.1 $\pm$ 2.9

SD = Standard Deviation. All between-group comparisons $P > 0.05$ (non-significant), confirming baseline comparability.

Table 6: Effect on Primary Symptoms of Mutrashmarī with 95% CI

Signs & Symptoms	Grp	BT Mean	AT Mean	D	95% CI	% Relief	SD	SE	't'	P
Pain (Shula)	A	3.64	1.47	2.17	1.79–2.55	59.6%	0.61	0.19	12	$P < 0.001$
	B	3.22	1.12	2.21	1.75–2.67	68.3%	0.68	0.23	10	$P < 0.001$
Sadahamutrapravritti	A	2.45	1.01	1.44	0.81–2.07	58.9%	1.06	0.32	4.62	$P < 0.05$
	B	2.11	0.79	1.32	0.58–2.06	62.8%	1.13	0.38	3.56	$P < 0.05$
Mutrakricchra	A	2.27	0.91	1.36	0.75–1.97	60.0%	1.02	0.31	4.38	$P < 0.05$
	B	2.44	0.91	1.29	0.68–1.90	59.5%	1.01	0.31	4.38	$P < 0.05$
Mutraprvritti	A	2.18	0.83	1.26	0.77–1.75	57.8%	0.79	0.25	5.31	$P < 0.05$
	B	2.44	1.22	1.12	0.47–1.77	45.1%	0.96	0.33	4.19	$P < 0.05$

BT = Before Treatment; AT = After Treatment; D = Difference; SD = Standard Deviation; SE = Standard Error; CI = Confidence Interval

Table 7: Effect on Associated Symptoms of Mutrashmarī with 95% CI

Signs & Symptoms	Grp	BT Mean	AT Mean	D	95% CI	% Relief	SD	SE	't'	P
Nausea	A	2.55	0.64	1.89	1.36–2.42	84.31%	0.89	0.27	8.14	$P < 0.05$
	B	2.33	0.46	1.47	0.62–2.32	62.40%	1.22	0.43	3.48	$P < 0.05$
Vomiting	A	1.90	0.18	0.91	0.20–1.62	82.7%	1.31	0.36	2.29	$P < 0.05$
	B	1.56	0.22	1.12	0.35–1.89	70.8%	1.15	0.39	2.83	$P < 0.05$
Weakness	A	2.36	0.81	1.53	1.12–1.94	64.9%	0.71	0.21	7.28	$P < 0.05$
	B	2.22	0.68	1.57	0.82–2.32	69.8%	1.11	0.38	4.09	$P < 0.05$
Constipation	A	1.91	0.56	1.36	0.63–2.09	71.20%	1.22	0.37	12.14	$P < 0.05$
	B	2.44	1.12	1.32	0.73–1.91	54.1%	0.89	0.30	4.59	$P < 0.05$

BT = Before Treatment; AT = After Treatment; D = Difference; CI = Confidence Interval

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RESEARCH ARTICLE

Comparative Evaluation of Shear Bond Strength of Cross - Linked Denture Teeth to Heat - Cure Denture Base Resin with Mechanical Modifications – An *In vitro* Study

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ABSTRACT

Debonding of denture teeth from a heat-cure denture base is a very common problem encountered in the dental operatory. The current study was conducted to do a comparative evaluation of the shear bond strength of cross-linked denture teeth to heat-cure denture base resin with different mechanical modifications of the ridge lap surface. Eighty cross-linked maxillary right central incisors (Premadent brand) of identical mould were chosen for the study. These were stratified into four groups of twenty each. Group I, with no mechanical intervention, served as the control group. Group II, teeth with cingulum-shaped grooves on the ridge lap surface. Group III, teeth with mess-shaped (intersecting) grooves on the ridge lap surface. Group IV, teeth with a combination of both cingulum-shaped and mess-





shaped grooves on the ridge lap surface. All test samples were created using DPI heat-cure denture base resin material. The teeth were positioned at an angle of 130 degrees on the wax models. A continuous shear force was applied to the palatal surface of all eighty specimens using a Universal Testing Machine until debonding of the tooth from the denture base occurred. The comparative analysis of shear bond strength values among different groups prepared with heat-cure denture base resin was found to be statistically significant ($P < 0.05$). Group I showed the lowest mean shear bond strength (449.6 N), followed by Group II (550.5 N) and Group III (612.4 N). Group IV showed the highest mean shear bond strength (660.8 N). Mechanical modifications on the ridge lap surface significantly improve the shear bond strength of cross-linked denture teeth to heat-cure denture base resin. The combination of cingulum-shaped and mess-shaped grooves showed the highest shear bond strength. This suggests that the combined modification on the ridge lap surface may serve as a reliable approach for retention of teeth to the denture base.

Keywords: Shear Bond strength, cingulum-shaped groove, mess-shaped grooves, Denture base Resin, Ridge Lap Surface, and cross-linked denture teeth.

INTRODUCTION

Complete Dentures remain one of the most widely adopted prosthetic solutions prescribed for the rehabilitation of completely edentulous patients. [1] Furthermore, it can restore function, aesthetics, phonetics, and patient confidence in both cost-effective and non-invasive manner, which has maintained their continued relevance even in an era of implant-supported prosthetics. [2,3] However, the durability and reliability of these dentures depend not only on the quality of the impression or occlusion of the teeth or the denture base extension, but also on the integrity of the bond that holds the artificial teeth to the denture base. When this bond between artificial teeth and the denture base fails, it leads to debonding or the detachment of denture teeth from their base. Prior studies have documented this problem, reporting that debonding with no observable defect to the denture teeth or the denture base accounted for nearly one-third of all denture repairs encountered in the dental operatory, making it a recurring clinical challenge. [4,5] The bond established at the ridge lap surface of the denture depends on multiple parameters. This includes both chemical and mechanical alterations for improving the strength of the bonding. Previous studies have tried to improve the strength between the denture teeth and the denture base by making mechanical alterations to the ridge lap surface. A study by Yadav NS et al. concluded that detachment of the tooth from the denture base occurs due to cohesive failure rather than adhesive failure at the ridge lap interface. [6] To mitigate this risk of cohesive failure, Akin HK asserted the necessity of surface treatments. [7] In their 1986 study, Cardash et al. established that there was no statistically significant improvement in retention, regardless of different geometric configurations of the grooves prepared at the ridge lap interface. [8] In contrast to their earlier findings, Cardash et al. demonstrated that preparation of vertical groove configurations across the ridge lap interface resulted in better retention of teeth to the denture base. [9] The prevailing literature presents conflicting and fragmented data. To date, the benefit of mechanical alterations on the ridge lap interface to enhance retention of denture teeth to their denture base remains ambiguous. To address this gap, the present study made an attempt to do a comparative evaluation of the shear bond strength of cross-linked denture teeth to the denture base with various mechanical alterations.

Aims and Objectives

- To determine the baseline shear bond strength of heat-cure denture base resin with denture teeth without any mechanical alterations on the ridge lap surface.
- To determine the shear bond strength of heat-cure denture base resin with denture teeth with cingulum-shaped grooves on the ridge lap surface.





- To determine the shear bond strength of heat-cure denture base resin with denture teeth with mess-shaped (intersecting) grooves on the ridge lap surface.
- To determine the shear bond strength of heat-cure denture base resin with denture teeth with both cingulum-shaped and mess-shaped grooves on the ridge lap surface.
- To comparatively analyse the shear bond strength of heat-cure denture base resin with denture teeth modified with cingulum-shaped grooves, mess-shaped grooves, both cingulum-shaped and mess-shaped grooves and denture teeth with no mechanical alterations on the ridge lap surface.

MATERIALS AND METHODOLOGY

Specimen preparation

Eighty cross-linked acrylic resin maxillary right central incisors (Mould 11) (Premadent) of similar dimensions and morphology were used to ensure uniformity across the study. These specimens were processed with heat-polymerised denture base resin (DPI). The total sample size was stratified into 4 distinct groups (n = 20 per group) based on the mechanical alterations prepared on the ridge lap surface.

Group 1: Denture teeth with no mechanical alterations, as received by the manufacturer, served as the control for the determination of baseline shear bond strength.

Group 2: Denture teeth with cingulum-shaped grooves measuring 2 mm in width and 2 mm in depth prepared on the ridge lap surface using a carbide bur.

Group 3: Denture teeth with mess-shaped (intersecting) grooves measuring 2 mm in width and 2 mm in depth, prepared on the ridge lap surface using a carbide bur.

Group 4: Denture teeth with a combination of both cingulum-shaped grooves measuring 2mm in width and 2mm in depth and mess-shaped (intersecting) grooves measuring 2 mm in width and 2 mm in depth, prepared on the ridge lap surface using a carbide bur.

Wax Model Fabrication

To simulate intraoral conditions, the central incisors were positioned at an angle of 130 degrees on the custom wax model. This mirrors the average functional contact angle between maxillary and mandibular dentition. This orientation is supported by studies of Barpal D et al. [10]. To achieve identical geometric orientation, a wax pattern of dimensions 30 X 8 X 3 mm was fabricated to form a polyvinyl siloxane (PVS) putty matrix. The teeth were repositioned in the putty matrix, and modelling wax was poured to produce eighty identical patterns. The wax configurations were finally flaked and processed using a heat-polymerised denture base resin (DPI). These specimens were evenly distributed into four cohorts of twenty specimens each.

Flask investment and Dewaxing

The completed wax patterns were invested in a two-piece denture flask using Type III dental stone mixed according to the manufacturer's instructions to prevent any discrepancies. After setting for one hour, the flasks were subjected to five minutes of boiling to execute the dewaxing stage. This step facilitated the complete elimination of wax from the mould cavity walls to prevent residual wax contamination.

Polymerisation of specimens

Based on established literature guidelines, the heat-cure acrylic resin was mixed in a proportion of 3:1 polymer to monomer ratio by volume. [11] The mixture was mixed and allowed to progress to a dough stage consistency. Following incremental packing into the mould cavity, the flask was clamped, and a continuous pressure of 20 kN for 30 minutes was applied for definitive adaptation and tooth immobilisation. [12] After one hour of bench equilibrium at room temperature, the flasks were then submerged in boiling water in an acrylizer for polymerization and processed as per the manufacturer's instructions. After polymerisation, gradual bench-cooling was utilised prior to



**Sumit Singh Phukela et al.,**

deflasking to reduce internal thermal stress. Following deflasking, dentures were finished using carbide burs and polished with pumice slurry to eradicate irregularities on the surface. (Figure 1)

Shear Bond strength test

Following finishing and polishing, all eighty specimens underwent shear bond strength under standard laboratory conditions at SGT University, Gurugram, India. Testing was conducted using a computerised universal testing machine (Model EZ20, maximum capacity 20 kN; Hounsfield, Lloyd Instruments, Ametek Technologies, UK) to ensure a precise application of load. Each tooth specimen was secured individually in a custom metallic fixture to prevent any displacement during testing. A shear load was applied on the palatal aspect of each central incisor using a pin of 1 mm diameter made up of stainless steel. [13] The speed of the cross-head was maintained at a constant rate of 5 mm/min until debonding of the tooth occurred. The peak load at which debonding occurred was recorded for each specimen.

Statistical Analysis:

All the data recorded from the shear bond strength testing were statistically analysed using SPSS (Statistical Package for Social Sciences) version 21.0 and Epi-Info version 3.0 software. For comparison of means across multiple groups, a one-way Analysis of Variance (ANOVA) test was applied. Statistical significance level was set at $p < 0.05$. Following the one-way ANOVA test, the Newman-Keuls Range Test was then applied for analysis.

RESULTS

Table I show values recorded across all four groups, and a graphical illustration of these values is presented in Graph 1. The comparative analysis of mean shear bond strength values across all four groups showed statistically significant differences ($p < 0.05$), which confirms that mechanical alterations created on the ridge lap surface influenced the bond strength between the tooth and the denture base. Group I, with no mechanical alterations on the ridge lap surface, served as the control group and recorded the lowest mean shear bond strength across all groups at 449.6 N. This value represents the baseline value, meaning the strength achievable through the bonding of cross-linked denture teeth and the heat-cure denture base resin, without any mechanical alterations on the ridge lap surface. Group II, with a cingulum-shaped groove, measured 2 mm in width and 2 mm in depth on the ridge lap surface, showed a mean shear bond strength of 550.5 N. This value was statistically significantly greater than the control group. This improvement proved that the introduction of a single curved groove configuration at the ridge lap surface can enhance the bonding between the tooth and the denture base. Group III, with a mess-shaped (intersecting) groove on the ridge lap surface, recorded a mean shear bond strength of 612.4 N. This value was statistically significantly greater than both the control group and Group II. This suggests that intersecting groove patterns create a wider and more complex network of bonding between teeth and the denture base. Thereby increasing mechanical resistance to shear displacement. Group IV, with a combination of both cingulum-shaped and mess-shaped grooves on the ridge lap surface, showed the highest mean shear bond strength across all four groups at 660.8 N. This value was statistically significantly greater than all three groups, which suggests that a combination of these mechanical alterations on the ridge lap surface could significantly enhance bonding strength between denture teeth and the denture base. These results demonstrated a progressive hierarchy of shear bond strength values corresponding to increasing complexities of the ridge lap surface alterations, from the group with no mechanical alterations through to the group with combined groove alterations on the ridge lap surface.

DISCUSSION

Debonding of denture teeth from their denture base remains one of the most frequently encountered complications encountered in the dental operator. The integrity of the tooth-denture base bonding is a fundamental factor that determines the retention of the tooth to the denture base. When there is adequate bonding between the tooth and the



**Sumit Singh Phukela et al.,**

denture base, the teeth function as an integral component of the prosthesis, thereby contributing to its stiffness, distribution of load, and strength. [14] Studies exist that have quantified the scale of the problem and reported that debonding accounts for one-third of all the denture repairs encountered in the dental operatory. Among the types of artificial teeth available in the market for denture construction, acrylic teeth remain the gold standard due to their inherent property of bonding to denture base resin during polymerisation. [15] Unlike porcelain teeth, acrylic teeth better bond with the denture base resin. G Jain et al., in their 2024 study, chemically approached this complication by application of dichloromethane to the ridge lap surface before the dough stage packing, which significantly enhanced the bond strength between denture teeth and denture base resin. [16] The bond strength increased significantly due to the partial dissolution of the cross-linked surface layer. Due to the limitations associated with solvent-based treatments, including occupational health hazards, mechanical alterations are simpler and safer in the dental operatory.

The mechanical alterations on the ridge lap surface through the preparation of grooves on the ridge lap surface have been one of the most extensive study areas, yet the published evidence is limited and fragmented. Studies exist that have demonstrated the preparation of vertical grooves on the ridge lap surface produced statistically significant improvement in tooth retention to the heat-cure denture base resins. A study of Civjan et al. further supported this approach by suggesting that systematic grinding of the ridge lap surface was sufficient to achieve increased retention of teeth to the denture base resin. [17] Due to limited and fragmented data, the current study was done to compare the shear bond strength achieved by three distinct mechanical alterations: cingulum-shaped grooves, mess-shaped (intersecting) grooves, and a combination of both cingulum-shaped and mess-shaped grooves, against a control with no mechanical alterations on the ridge lap surface. The findings of this study showed a progressive hierarchy of statistically significant improvement in the shear bond strength with increasing complexity on the ridge lap surface. The control group with no mechanical alterations on the ridge lap surface showed the lowest mean shear bond strength across all four groups at 449.6 N. Reflecting the baseline value, meaning the strength achievable through the bonding of cross-linked denture teeth and the heat-cure denture base resin without any mechanical alterations on the ridge lap surface.

Group II, with a cingulum-shaped groove on the ridge lap surface, showed a mean shear bond strength of 550.5 N. This value was statistically significantly greater than the control group and is consistent with those reported by Zuckerman. [18] This improvement suggests that the introduction of a single curved groove geometry at the ridge lap surface can enhance the bonding between the tooth and the denture base. This geometry produces a more reliable and consistent bond between the tooth and the denture base. Group III, with a mess-shaped (intersecting) groove on the ridge lap surface, recorded a higher mean shear bond strength of 612.4 N, statistically significantly greater than both the control group and Group II. This suggests that intersecting groove patterns create a wider and more complex network of bonding between teeth and the denture base. Thereby increasing mechanical resistance to shear displacement. Group IV, with a combination of both cingulum-shaped and mess-shaped grooves on the ridge lap surface, showed the highest mean shear bond strength across all four groups at 660.8 N. This value was statistically significantly greater than all three groups, which suggests that a combination of these mechanical alterations on the ridge lap surface could significantly enhance bonding strength between denture teeth and the denture base. This combined groove can accommodate a significant volume of interlocking resin, and the complexity of the geometry produced a bond that qualitatively differs in its resistance to masticatory and occlusal forces.

The findings of this study align with the broader body of the literature supporting the efficacy of the preparation of the groove on the ridge lap surface, which enhances the bond between the tooth and the denture base. Moreover, the current study extends the existing evidence base by demonstrating that the creation of grooves on the ridge lap surface produces a statistically significant impact on the bond strength between teeth and the denture base. The progressive hierarchy of shear bond strength across multiple groups yields a clinically actionable conclusion that the preparation of the groove on the ridge lap surface is essential when processing highly cross-linked artificial teeth with heat-cure denture base resin, and the simultaneous preparation of both cingulum-shaped and mess-shaped grooves represents the most effective strategy for mechanical modification.



Sumit Singh Phukela *et al.*,

CONCLUSION

Mechanical alterations, such as preparation of the groove on the ridge lap surface, significantly enhance the strength of the bond between artificial teeth and heat-polymerised denture base resin. The specimen group with both the combinations of cingulum-shaped and mess-shaped (intersecting) grooves yields the highest shear bond strength in comparison to the specimen group with no mechanical alterations. Moreover, this combined configuration represents an effective strategy to optimise the retention of artificial teeth with the denture base.

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Sumit Singh Phukela *et al.*,

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Table 1: Comparative Shear Bond Strength Values (Newton) Of Different Groups Prepared With Dpi Heat Cure Denture Base Resin

Groups	Shear Bond strength values (Newton) Mean $\pm$ SD	Difference between groups		
		II	III	IV
I (Control)	449.6 $\pm$ 45.8	P < 0.01, S	P < 0.01, S	P < 0.01, S
II (Half moon shaped groove)	550.5 $\pm$ 50.8	-	P < 0.01, S	P < 0.01, S
III (Criscross shaped groove)	612.4 $\pm$ 30.0	-	-	P < 0.05, S
IV (Half moon shaped groove Criscross shaped groove)	660.8 $\pm$ 19.0	-	-	-

One way ANOVA F = 56.0, P < 0.01, Sig.

Newman-Keul's Range Test

Least Sig. Diff, LSD = 46.5 P < 0.05

= 57.2 P < 0.01

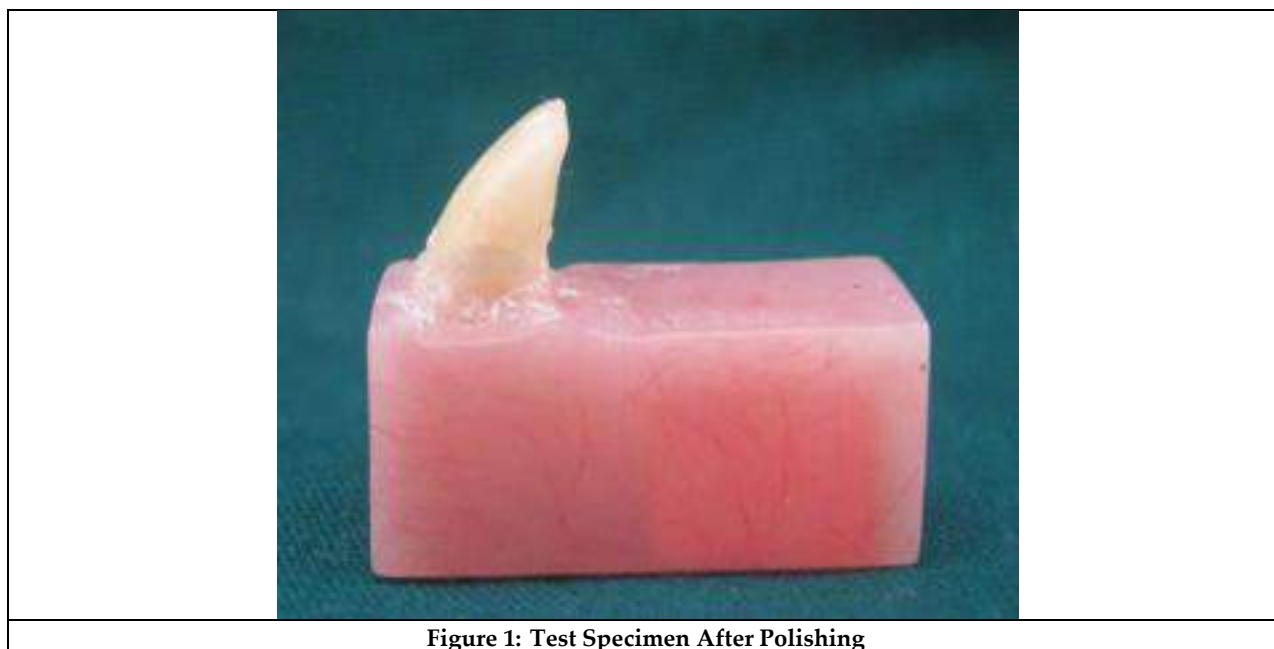


Figure 1: Test Specimen After Polishing





Figure 2: Universal Testing Machine

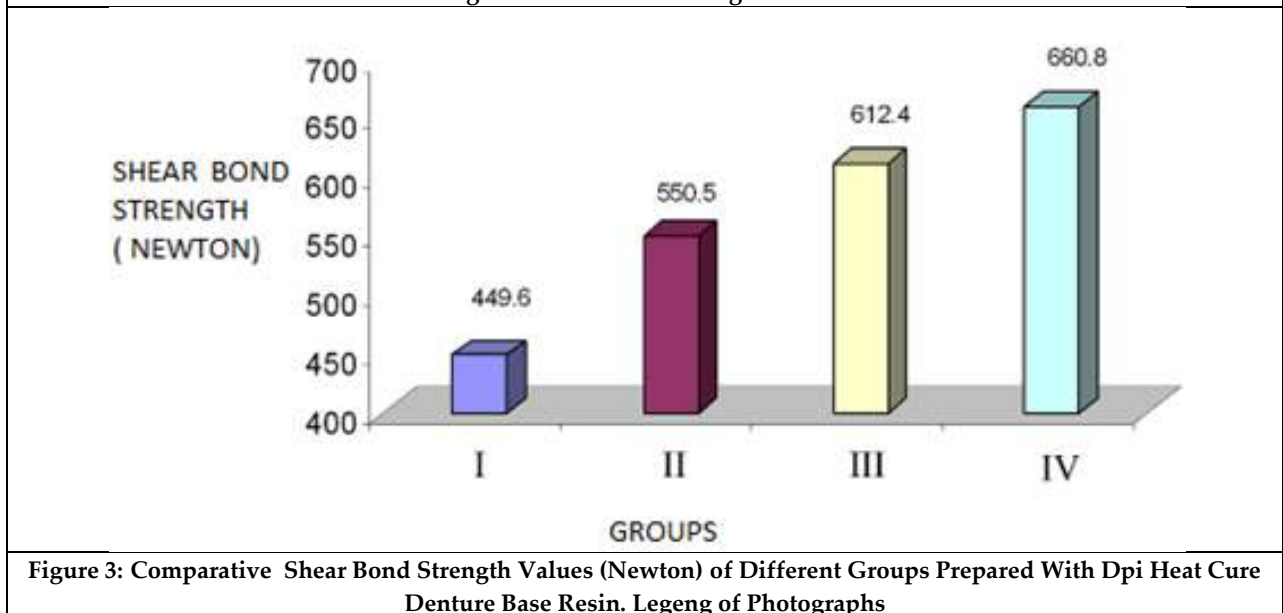


Figure 3: Comparative Shear Bond Strength Values (Newton) of Different Groups Prepared With Dpi Heat Cure Denture Base Resin. Legeng of Photographs

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RESEARCH ARTICLE

Trauma beyond Diagnosis: Structural Violence and the Limits of Biomedical Intervention in Indian Intimate Partner Violence Cases

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ABSTRACT

In India, intimate partner violence remains widespread issue, and national estimates from the National Family Health Survey (NFHS-5, 2019-21) and related analyses show persistent prevalence and sharp regional disparities. The study examines how prevailing biomedical approaches to trauma obscure the social sources of suffering among survivors of intimate partner violence and proposes an integrated care framework that centers structural determinants. Adopted a qualitative analytical design based on secondary data, national survey evidence, research papers, and policy documents. Drawing on national prevalence data, qualitative studies of disclosure and medico-legal practice and evaluations of state responses such as the One Stop Centre model, the analysis identifies three core problems: clinical reductionism that locates trauma within individual bodies and symptoms; institutional barriers, including medico-legal bottlenecks and low provider competency that limit help-seeking and continuity of care; and intersectional vulnerabilities shaped by caste, religion, indigeneity and poverty. The study documents how the COVID-19 shadow pandemic amplified these dynamics and reports gaps in One Stop Centre implementation and funding. In response, it specifies a three pillar Integrated Care Model that is structural competency and trauma informed practice, economic empowerment and social safety nets, and community engagement with task shifting as a pragmatic policy and clinical pathway. The conclusion argues that addressing intimate partner violence effectively requires shifting attention from diagnosis alone to the social arrangements that produce and sustain violence

Keywords: Intimate Partner Violence; Structural Violence; Biomedical Model; Trauma-Informed Care; Intersectionality





INTRODUCTION

In India intimate partner violence remains widespread; national estimates from the National Family Health Survey (NFHS-5) (2019–21) and recent analyses show persistent prevalence and sharp regional disparities. These epidemiological patterns associated with evidence of disclosure barriers and limits of facility-level responses, the study's aim to relocate trauma from individual diagnosis toward the social arrangements that produce violence. The clinical landscape of trauma in contemporary India is increasingly defined by a tension between the expansive, systemic reality of intimate partner violence (IPV) and the reductive, diagnostic focus of the biomedical model. While the National Family Health Survey (NFHS-5, 2019–2021) provides a statistical architecture for understanding the scale of violence with 29.3% of women aged 18–49 reporting physical violence since the age of 15 (Manna et al., 2024). This quantitative frame coexists with analyses that draw on national samples to identify predictors and regional disparities (Mishra et al., 2024; Dalal et al., 2025). Yet the qualitative experience of survivors remains largely obscured by institutional frameworks that prioritize medicalization over structural transformation a dynamic vividly documented in studies of barriers to disclosure within tertiary psychiatric settings (Vranda et al., 2018). This study contends that prevailing trauma-care models which emphasize individual diagnosis and clinical symptom reduction which fundamentally misrecognize the nature of social suffering experienced by IPV survivors. By adopting on the theoretical model of structural violence and critical medical anthropology the present study's analysis seeks to relocate the problem of trauma from the interiority of the survivor's psyche to the social arrangements that produce and sustain violence. The ensuing discussion articulates the necessity of a paradigm shift toward an integrated care model that addresses social determinants of health and dismantles the institutional barriers that currently impede recovery for the most marginalized women in India.

METHODS AND STUDY MATERIAL

The study adopts a qualitative analytical research design that integrates secondary quantitative evidence with thematic synthesis of existing scholarly and policy literature. The purpose of the study is to examine how biomedical approaches to trauma in cases of intimate partner violence (IPV) in India often overlook structural and social determinants of violence. By illustration on national epidemiological data and critical theoretical perspectives from medical anthropology the present study evaluates the limitations of clinical responses and explores alternative integrated models of care. The research follows an interpretive sociological approach combining descriptive analysis of national prevalence data with conceptual analysis of healthcare responses to IPV. The framework of structural violence and critical medical anthropology guides the interpretation of findings.

The study relies primarily on secondary data drawn from three categories of sources. First, nationally representative datasets were used to understand the prevalence and patterns of intimate partner violence in India. Data from the National Family Health Survey rounds (NFHS-3, NFHS-4 and NFHS-5) were consulted to identify trends in domestic violence across time and regions. Second, peer-reviewed academic literature on domestic violence, trauma and healthcare responses was examined. These sources include studies published in journals related to public health, sociology, medical ethics and gender studies. The literature provides insights into barriers to disclosure, provider attitudes and institutional responses to IPV in healthcare settings. Third, policy documents, government reports and program evaluations were reviewed to understand institutional interventions addressing gender-based violence. These include reports on the One Stop Centre scheme, healthcare response frameworks and community-based interventions implemented by governmental and non-governmental organizations.

Relevant literature was identified through structured searches in academic databases such as Google Scholar, Pub Med and Scopus using keywords including "intimate partner violence," "domestic violence in India," "trauma-informed care," "healthcare response to IPV," and "structural violence." Additional sources were obtained from institutional websites and policy reports related to gender-based violence interventions in India. Studies were



**Aftab Ahmad Mir and Allah Baksh**

selected based on their relevance to three key themes: prevalence and epidemiology of IPV in India, healthcare system responses to domestic violence and socio cultural factors influencing disclosure and help-seeking behavior. Both empirical research studies and systematic reviews were included in order to capture a comprehensive understanding of the issue. The collected literature and data were analyzed using thematic analysis. Following the analytical framework proposed by Braun and Clarke (2006), the study identified recurring themes related to the medicalization of trauma, institutional barriers within healthcare systems and socio cultural factors shaping survivor's help-seeking behaviors. The thematic analysis involved several stages. First, relevant texts were read repeatedly to identify key concepts and patterns. Second, codes were generated to categorize recurring issues such as biomedical reductionism, provider competency gaps, medico-legal challenges and structural barriers to care. Third, these codes were grouped into broader themes that explain how healthcare systems respond to domestic violence and why existing biomedical models remain insufficient. The interpretation of these themes was guided by the theoretical perspectives of critical medical anthropology and structural violence which emphasize how social inequalities, gender norms and institutional power structures shape health outcomes and access to care.

The Epidemiological Landscape: NFHS-5 and the Persistence of Spousal Violence

The statistical profile of IPV in India is a critical starting point for any structural analysis (Mishra et al., 2024; Manna et al., 2024). The transition from NFHS-4 (2015–16) to NFHS-5 (2019–21) offers a longitudinal view of the domestic environment by revealing that despite minor fluctuations spousal violence remains an endemic feature of the Indian family structure (NFHS-5, 2019–21; Dalal et al., 2025). The national weighted prevalence of domestic violence against women was recorded at 31.2% in NFHS-5 a slight decrease from the ~33% reported in NFHS-4, yet the absolute volume of women affected continues to represent a public-health crisis of monumental proportions (NFHS-5, 2019–21; Mishra et al., 2024). The distribution of this violence is characterized by profound regional variations, suggesting that state-level policy, local cultural norms and socioeconomic development play decisive roles in shaping the safety of the domestic sphere (Manna et al., 2024; Dalal et al., 2025). A granular examination of the data reveals that states like Karnataka (44.4%), Bihar (40.0%), Manipur (39.6%) and Telangana (36.9%) exhibit prevalence rates that significantly exceed the national average. In Karnataka, the proportion of married women who have ever faced spousal violence doubled between survey rounds that rising from 21% to 44%, a trend that demands a critical inquiry into the local drivers of domestic aggression. Conversely, Tripura witnessed a consistently decreasing rate across all three forms of violence indicating that progress is possible when specific socio-economic factors and interventions are aligned (Bhatia, 2023).

The impact of the COVID-19 pandemic introduced a “shadow pandemic” that complicated the trajectory of these statistics. Mandatory social isolation and lockdowns increased the exposure of women to their abusers while simultaneously dismantling their access to external support mechanisms (Tmava & Burstein, 2025). In urban areas, domestic violence complaints reportedly doubled following the imposition of nationwide restrictions with the Tamil Nadu Police registering at least 40 cases per day during the height of the lockdown (Bhatia, 2023; Tmava & Burstein, 2025). This period highlighted the fragility of the Indian healthcare and legal response system which in its urgency to address the biomedical threat of the virus further marginalized the needs of IPV survivors leading to a “narrative compression” that silenced their ongoing suffering (Tmava & Burstein, 2025).

Intersectional Vulnerability: Caste, Religion and the Political Economy of Violence

A robust analysis of IPV in India must move beyond the Universalist category of “woman” to engage with the intricate interactions of caste, religion, class and geographic location. In the Indian context, intersectionality is not merely an academic concept but a lived reality that defines who is vulnerable to violence and who has the resources to escape it. The interaction of gender with the caste hierarchy is particularly significant as Dalit and Adivasi women occupy a social position characterized by “intersectional violence” where oppression is layered due to their specific marginalized identity (Khalid & Bouakaz, 2025).





The Caste-Patriarchy Nexus and the IPV Gap

The prevalence of IPV among Scheduled Caste (SC) women stands at 37.3% compared to 24.4% among women in the “General” category. This gap of 12.9 percentage points is not a result of individual pathology but is driven by systematic disparities across nearly all social determinants of health. Using Fairlie decomposition analysis, researchers have identified that approximately 84% of this gap can be attributed to specific socio-economic and behavioral predictors with the remainder likely tied to unmeasured factors such as the psychological stress of systemic casteism (Chowdhury et al., 2022). The data suggests that for SC women, violence is frequently mediated by the structural violence of economic precarity. Wealth status alone explains nearly a quarter of the disparity, illustrating how poverty functions as an enabling condition for domestic abuse. Furthermore, the prevalence of controlling behaviour such as restricting contact with family, monitoring movements and financial distrust is significantly higher among husbands in SC households, often associated with a four-fold increase in the likelihood of physical violence. This proposes that IPV is used as a mechanism to assert power within a domestic sphere when external social structures systematically disempower the male head of the household (Chowdhury et al., 2022).

Tribal Vulnerabilities and Indigenous Idioms of Distress

For Adivasi (Scheduled Tribe) women, the experience of violence is often compounded by geographic isolation and the transformation of traditional social structures (Bhasin, 2007). In communities like the Gaddi tribal community of Himachal Pradesh, women express their distress through the polysemic term “tension,” which serves as an idiom for the embodied strains of politico-economic and social transformation. This “tension” is not merely a psychiatric symptom but a social critique of the patriarchal norms and oppressive standards that frustrate women's agency (Lester, 2013). In some tribal contexts, this distress may manifest as “spirit possession,” which local communities interpret either through exorcism or the domestication of the spirit, allowing the woman to gain a degree of social status and attention that the formal healthcare system denies her (Lester, 2013; Bhasin, 2007). The structural violence faced by tribal women also includes the risk of being targeted through witchcraft accusations. Scholars identify witchcraft practices as the outcome of complex intersectional power structures involving gender, caste and land rights. Such accusations often serve as a social mechanism to displace women from ancestral lands or to punish those who challenge traditional hierarchies, further illustrating that the “trauma” of Adivasi women is deeply rooted in the struggle for survival and sovereignty (Khalid & Bouakaz, 2025).

Intersectional Vulnerability: Caste, Religion, and the Political Economy of Violence

Muslim women in India navigate a specific intersection of gender-based oppression and religious discrimination, often finding themselves “multiply marginalized” within the broader socio-political climate (Khalid, S. & Bouakaz, 2025). Research based on National Family Health Survey (NFHS-5) data indicates that religious minority women are significantly more likely to experience physical violence during pregnancy compared to those in the majority religion (Kalra et al., 2025). The vulnerability of South Asian women including those of Muslim faith is often exacerbated by a lack of trust in healthcare systems where they anticipate judgmental attitudes, a lack of privacy or a failure by providers to understand their specific cultural and religious contexts (Baloch et al., 2025). This leads to a situation where the fear of institutional discrimination acts as a barrier to help-seeking trapping survivors between a violent domestic environment and a hostile public sphere (Khalid & Bouakaz, 2025). The dominance of the biomedical model in trauma care has led to what Arthur Kleinman and his colleagues terms the “medicalization of social suffering,” a process that transforms the effects of structural violence into individual clinical pathologies (Kleinman et al., 1997). By framing IPV-related distress primarily as post-traumatic stress disorder (PTSD) or depression, the biomedical model performs a “double erasure”: it obscures the social origins of the suffering and depoliticizes the violence against women (Tmava& Burstein, 2025). Recent critiques of biomedical reductionism in chronic health conditions further support the need for integrated functional models that explicitly incorporate social determinants of health (Pianese& Bordonni, 2025).





The Critique of Biomedical Reductionism

Critical Medical Anthropology (CMA) provides a necessary corrective to this reductionism by analyzing the clinic as a site of power where knowledge about health and vulnerability is governed. As Arthur Kleinman argues the PTSD framework as formalized in the Diagnostic and Statistical Manual of Mental Disorders, Third Edition (DSM-III), established a diagnostic category that individualizes social problems locating the pathology within the survivor rather than in the social conditions that produce violence (Kleinman, 1997; American Psychiatric Association, 1980). In the India context, this model often fails to account for social determinants, embodied idioms of distress and culturally specific help-seeking patterns, a limitation underscored by recent critiques calling for integrated functional approaches that incorporate social and structural factors (Pianese & Bordoni, 2025).

- **Somatic Expressions of Trauma:** Many Indian survivors present with somatic complaints such as chronic pain, headaches or gastrointestinal disturbances (Barakati et al., 2026) that do not map neatly onto Western psychiatric categories.
- **Existential and Spiritual Distress:** Concepts of healing in India are often embedded in religious or community-based frameworks that emphasize social harmony and spiritual balance over individual symptom reduction (Bhasin, 2007).
- **Ongoing Structural Harm:** Clinical interventions that focus on “recovery” from a past event are inadequate for survivors living in conditions of continuous structural violence such as chronic poverty or caste-based exclusion (Tmava & Burstein, 2025).

Paul Farmer’s concept of structural violence is pivotal for understanding how social arrangements such as gender wage gaps, patriarchal family structures and regional disparities in infrastructure of harm survivors (Farmer, 2004). These structures are not incidental to IPV but are constitutive of it. For instance the gender wage gap and the restriction of women to informal and insecure labor markets ensure economic dependency on abusive partners. Similarly the geographic mal distribution of healthcare facilities means that rural women must travel long distances to access care facing transportation costs and safety concerns that make “seeking help” a structurally impossible task for many (Khalid & Bouakaz, 2025).

The Medico-legal Bottleneck

One of the primary structural barriers is the “medico-legal” nature of domestic violence cases. In many Indian tertiary hospitals providers share an apprehension about dealing with the criminal justice system. Police involvement is a primary deterrent for both physicians and patients. For the physician it marks the beginning of a complex legal process that they feel unprepared to handle and for the patient it invokes fear and social stigma. A review of Standard Operating Procedures (SOPs) at six major hospitals in West Delhi revealed that most failed to recognize the medico-legal significance of IPV, often classifying these cases as general “alleged physical assault” rather than specifically as domestic violence (Sehra, 2025). The qualitative insights from the West Delhi study underscore how “poor documentation practices” in medical records compromise the reliability of evidence, hinder the assessment of patterns and obstruct effective intervention. When a survivor presents with “multiple injuries,” “delayed presentation,” or “history of loss of consciousness,” these are clinical red flags for IPV; however, without a standardized protocol that demands the recording of the perpetrator’s identity and the context of the abuse. These findings are treated as isolated physical symptoms rather than evidence of systematic harm (Sehra, 2025).

Provider Attitudes and the Mastery of IPV Competencies

The effectiveness of the healthcare response is further limited by a “mastery gap” among medical professionals. A survey of medical students in comparable Global South contexts revealed critically low levels of knowledge and skills regarding IPV, with mastery levels consistently falling below 80% (Manuel, B. et al., 2019). In India, providers often report feeling “uncomfortable” or “not confident” in inquiring about domestic violence, citing a fear of offending the patient or an ignorance about how to ask (Barakati, T. et al., 2026; Sehra, L., 2025). This discomfort is often rooted in the internalization of patriarchal norms where marital violence is seen as a “private issue” or something to be “socially tolerated” for the sake of family honor (Barakati, T. et al., 2026).



**Aftab Ahmad Mir and Allah Baksh****Evaluating State Interventions: The ‘Sakhi’ One Stop Centre (OSC) Model**

In response to these systemic failures, the Government of India launched the One Stop Centre (OSC) scheme, also known as Sakhi Centres, in 2015 (Rajan, 2025; Ministry of Women and Child Development, 2025). Funded through the Nirbhaya Fund, the OSC model aims to provide an integrated support system including medical assistance, legal aid, police facilitation, psychosocial counselling and temporary shelter under one roof (Ministry of Women and Child Development, 2025; Rajan, 2025).

One Stop Centre Scheme (Operational Strengths and the 2025 Status)

As of late 2025, over 12.67 lakh women have been assisted by OSCs across India and the scheme has expanded significantly with 865 operational centers nationwide and the integration of the Women Helpline (181) with the Emergency Response Support System (112) in several states/UTs (Press Information Bureau, 2026; Ministry of Women and Child Development, 2025). The recent introduction of the “Mission Shakti Dashboard” and a comprehensive Standard Operating Procedure (SOP) in 2025 represents a critical step toward standardizing service delivery and ensuring transparency in fund utilization (Ministry of Women and Child Development, 2025; Engender Health, 2025).

Systemic Challenges and Implementation Gaps

Despite its ambitious design, the OSC model faces persistent challenges that impede its full potential. The most glaring issue is the underutilization of funds; for instance, only ₹328 crore out of an allocated ₹735 crore were utilized in certain periods with states like Bihar and Karnataka using the least amount despite their high IPV prevalence (Engender Health, 2025; Rajan, 2025). Additionally, high attrition rates among staff driven by low salaries and a lack of annual increments adversely impact continuity of care (Rajan, 2025). In rural areas the lack of visibility remains a major barrier as many women are unaware of OSC services and many centers lack essential amenities such as accessible toilets or reliable power (Engender Health, 2025).

The Proposed Integrated Care Model: A Multi-Sectoral Blueprint

Moving beyond the clinic requires a conceptual shift toward an “Integrated Care Model” that addresses both the immediate symptoms of trauma and the structural conditions that perpetuate violence. This model informed by recent programmatic successes (Engender Health, 2025) which emphasizes three core pillars: structural competency, economic empowerment and community-based advocacy.

Pillar 1: Structural Competency and Trauma-Informed Care (TIC)

Healthcare systems must move from cultural competency to structural competency the capacity to recognize and respond to the social and political forces that shape health (Scott et al., 2025). This involves:

- Standardized IPV screening: Implement routine, trauma-informed screening across departments, particularly antenatal and psychiatric clinics where disclosure is most likely to occur (Sabri et al., 2024).
- Refining documentation: Develop electronic health records that allow for joint patient files shared between medical and legal teams ensuring that patterns of abuse are visible to all stakeholders (Ministry of Women and Child Development, 2025).
- Structural differential: Train physicians to construct a “structural differential” by identifying how factors like lack of housing or economic abuse contribute to presenting symptoms (Scott et al., 2025; Sawah et al., 2025).

Pillar 2: Economic Empowerment and Social Safety Nets

The IPV gap between poor and non-poor women underscores the role of economic independence in survivor safety (Sen et al., 2026). An integrated model must incorporate:

- Livelihood integration: Link healthcare facilities and OSCs to vocational training and microfinance programs to address the financial constraints that prevent women from leaving violent homes (Vimala, 2025; Daruwalla et al., 2024).



**Aftab Ahmad Mir and Allah Baksh**

- Direct cash transfers: Use flexi-funds within the Mission Shakti vertical to provide immediate financial relief for survivors seeking to escape unsafe environments (Ministry of Women and Child Development, 2025).

Pillar 3: Community Engagement and “Task-Shifting”

Given the geographic disparities in healthcare in India the model must rely on cascade training for community health workers (ASHAs and Anganwadi workers) (Engender Health, 2025).

- **Bystander Mobilization:** Training youth and males as “GBV prevention champions” to shift the gender norms that currently justify wife-beating in 35% of households (Engender Health, 2025).
- **Peer Facilitators:** Sources from community and slum settings further supports locally tailored, community-driven approaches to address IPV during and after pregnancy (Das et al., 2013). Finally, using trained lay peer facilitators to provide first-line psychological support and to help survivors navigate referral pathways has shown promise in integrated domestic-violence and reproductive-health interventions (Sabri et al., 2024).

Policy Recommendations: Strengthening the National Response

To fully realize the potential of the integrated care model below are the specific policy recommendations are proposed:

- **Standardize Medico-legal Protocols:** The Ministry of Health and Family Welfare (MoHFW) must mandate the categorization of all domestic violence cases as Medico-legal Cases (MLCs) with specific SOPs for documentation regardless of whether the patient chooses to pursue a police complaint immediately.
- **Institutionalize Structural Competency Training:** Incorporate structural competency and trauma theory-based training into the undergraduate medical curriculum (MBBS) and nursing education moving beyond technical identification to foster critical reflections on gender and power.
- **Enhance Human Resource Retention:** Address the high attrition rates in OSCs by implementing a standardized salary structure with a 3% annual increment, equivalent to provisions in other national welfare schemes like Mission Vatsalya.
- **Co-location and Digital Integration:** Scale up the model of co-locating Anganwadi centers and OSCs within school or hospital premises to increase visibility and ease of access for women in rural districts.
- **Strengthen Accountability through Data:** Expand the use of the Mission Shakti Dashboard to include qualitative metrics of survivor satisfaction and successful “recovery outcomes” (e.g., attainment of housing or employment), moving beyond simple counts of women assisted.

CONCLUSION**Toward a Paradigm of Social Healing**

The trauma of intimate partner violence in India is a phenomenon that transcends the boundaries of clinical diagnosis. While the biomedical model provides essential tools for physical recovery and symptom management it remains insufficient as a stand-alone response to a crisis rooted in structural violence. The persistence of IPV particularly among Dalit, Adivasi and religious minority women underlines the reality that “recovery” is a socio-political project as much as a medical one. The findings of this report suggest that the path toward genuine healing for survivors requires a fundamental reorganization of the healthcare encounter. By integrating social determinants of health, refining the medico-legal response and empowering community-based support networks, India can move from a paradigm of “treatment” to one of “transformation”. The success of the Sakhi One Stop Centre model while currently hampered by operational hurdles offers a blueprint for a multi-sectoral approach that recognizes the interconnectedness of medical, legal and economic needs. Only by addressing the structural roots of violence and dismantling the institutional barriers to care can we support a future where the trauma of India's women is recognized, documented and finally overcome.





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Aftab Ahmad Mir and Allah Baksh

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Table 1: The Epidemiological Landscape: NFHS-5 and the Persistence of Spousal Violence

Indicator for Women (15-49 Years)	NFHS-3 (2005-06) (%)	NFHS-4 (2015-16) (%)	NFHS-5 (2019-21) (%)
Emotional Violence	14.0	14.0	13.1
Physical Violence	31.0	30.0	28.5
Sexual Violence	8.0	7.0	5.7
Physical Violence during Pregnancy	3.7	3.9	2.4
Total Spousal Violence (any form)	37.2	33.3	31.2

Table 2: The Caste-Patriarchy Nexus and the IPV Gap

Contributing Factor to the SC-General IPV Gap	Contribution to the Disparity (%)
Husband's Alcohol Consumption	26.33
Household Wealth Index	24.48
Husband's Controlling Behavior	24.00
Intergenerational Exposure (Parental IPV)	15.87
Number of Living Children (Son Preference)	4.26
Women's Acceptance of Violence Norms	3.86
Working Status of Women	2.87





Aftab Ahmad Mir and Allah Baksh

Table 3: The Medico-legal Bottleneck

Institutional and Systemic Barrier	Impact on Survivor Care and Safety
High Patient Volume/Load	Prevents physicians from dedicating time for trust-building and disclosure.
Lack of Private Space in OPD	Makes confidential conversations about abuse nearly impossible in government facilities.
Focus on Physical Trauma Only	Overlooks chronic mental health needs and somatic indicators of prolonged abuse.
Fear of “Legal Entanglements”	Encourages providers to avoid documenting the domestic nature of the violence.
Inadequate Documentation	Compromises the reliability of evidence for future legal and protection efforts.

Table 4: One Stop Centre Scheme (Operational Strengths and the 2025 Status)

OSC/Sakhi Service Component	Functional Role in Survivor Recovery
Emergency Rescue	Immediate extraction from violent domestic or public spaces.
Medical Assistance	Linking survivors to hospitals for treatment and forensic examination.
Psychosocial Counselling	Addressing anxiety, trauma and emotional stability through multidisciplinary teams.
Legal Aid & Advice	Facilitating the filing of complaints and navigating the court system.
Temporary Shelter	Providing safe housing for women and their children for up to five days.

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RESEARCH ARTICLE

Formulation Optimization and Evaluation of Ph Sensitive *In-situ* Hydrogel for Ocular Delivery of Bromfenac Sodium

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ABSTRACT

The objective of the present investigation was to formulate and assess a pH-responsive in-situ hydrogel containing Bromfenac Sodium for prolonged ocular drug delivery. Traditional ophthalmic dosage forms are often associated with rapid precorneal drainage and limited ocular bioavailability, creating a need for improved delivery platforms. In this study, pH-sensitive in-situ hydrogels were developed using Carbopol 940P as the gelling polymer, Hydroxypropyl Methylcellulose (HPMC K4M) as a viscosity-modifying polymer, and β -cyclodextrin as a solubility-enhancing excipient. Thirteen formulations (F1–F13) were prepared and characterized for relevant physicochemical and pharmaceutical attributes. Preformulation investigations, including organoleptic evaluation, melting point analysis, UV spectrophotometry, FTIR, and DSC studies, verified the identity, purity, and compatibility of Bromfenac Sodium with the selected excipients. Post-formulation assessment revealed acceptable pH values (6.9–7.2), rapid gelation (22–30 s), appropriate viscosity, satisfactory spreadability, isotonic behavior, and uniform drug content (90.2–99.5%). HET-CAM evaluation confirmed ocular safety and absence of irritation. In vitro release studies demonstrated sustained drug release for up to 8 h, with formulation F3 showing the highest cumulative release. DOE analysis established that Carbopol concentration exerted a significant effect on viscosity and release performance. The developed pH-sensitive in-situ hydrogel system therefore represents a promising strategy for , providing prolonged drug release, and increasing patient compliance.

Keywords:Bromfenac Sodium, pH-Sensitive In-Situ Hydrogel,Ocular Drug Delivery, Sustained Drug Release, Ophthalmic Formulation



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INTRODUCTION

The eye is among the most intricate and delicate organs of the human body, playing a vital role in vision and enabling individuals to perceive and interact with their surroundings. It comprises a highly organized network of anatomical components that function collectively to transform incoming light into neural impulses, which are subsequently processed and interpreted by the brain. [1] Owing to the complexity of ocular anatomy and physiology, there is a continuous need for efficient and reliable ocular drug delivery systems. Over the past few years, considerable efforts have been directed toward the development of advanced drug delivery approaches aimed at overcoming the shortcomings associated with conventional ophthalmic formulations. Among these emerging technologies, in situ gelling systems have attracted substantial attention due to their ability to undergo a sol-to-gel transition following administration. In situ hydrogels are polymer-based formulations that remain in a low-viscosity liquid state before instillation and subsequently convert into a gel upon exposure to the physiological environment of the eye.[2] The transformation from sol to gel can be triggered by various environmental stimuli, including alterations in pH, temperature fluctuations, and ionic interactions. Among the different in-situ gelling strategies, pH-responsive hydrogels have emerged as highly effective platforms for ocular drug delivery.[3] These systems possess a transparent and soft consistency, enhancing patient comfort and minimizing ocular irritation during administration. Incorporation of viscosity-enhancing polymers such as Hydroxypropyl Methylcellulose (HPMC) further improves gel strength, rheological properties, and overall formulation stability. In addition, these polymers increase the residence time of the formulation on the ocular surface, thereby facilitating sustained and controlled drug release. Such characteristics are particularly advantageous for therapeutic agents that require prolonged ocular retention and continuous pharmacological action, including anti-inflammatory, anti-glaucoma, antimicrobial, and anti-allergic drugs.[4] Owing to their ability to improve drug retention, enhance bioavailability, and prolong therapeutic efficacy, pH-sensitive in-situ hydrogel systems have attracted considerable interest in pharmaceutical research as advanced ocular drug delivery carriers. Consequently, extensive efforts have been directed toward the formulation and evaluation of these systems for the effective treatment of ocular disorders and postoperative inflammatory conditions. [5]

MATERIAL AND METHOD

Material: Bromfenac sodium was obtained from Indoco Remedies Limited, Killoab Navi Mumbai India as a gift sample. Carbopol 934, B-Cyclodextrin and HPMC K4m were purchased from Research-Lab Fine Chem. Industry – Mumbai. All other chemical and solvent were of Pharmaceutical Grade.

Method:

Formulation of pH sensitive in-situ gel:

RESULT AND DISCUSSION

Preformulation study

Organoleptic properties: The organoleptic evaluation of bromfenac sodium showed that it was a yellow, odorless crystalline powder, indicating its pure and well-defined nature.

Melting Point: The observed melting point of bromfenac sodium was 285°C, which was found to be within the reported range of 284–286°C, indicating the purity and identity of the drug substance.

UV Spectrophotometric Analysis

Determination of (λ_{max}) and Standard calibration curve in of Bromfenac Sodium in Methanol

Using UV spectroscopy, Bromfenac Sodium's maximum absorption wavelength (λ_{max}) was determined to be 268 nm, as shown in Fig. 2. A good linear relationship between concentration and absorbance is demonstrated by the



**Abhay Santosh Jejurkar and Rajendra K. Surawase**

standard calibration curve for Artemether, Its slope is $y = 0.0188 \times 0.0484$, and its coefficient of regression value is $R^2 = 0.9965$ As shown in Fig 3.

Post Formulation Study

Below is Table 3, which shows the physical parameters of the Ph sensitive In-gels that were created, comprising color, homogeneity, pH, Gelation Time, Drug Content and viscosity.

Spreadability

The spreadability values ranged from 4.90 ± 0.24 to 9.52 ± 0.23 g·cm/sec, indicating differences in consistency and flow characteristics among the prepared formulations. The optimized formulation (F3) exhibited a spreadability value of 7.27 ± 0.22 g·cm/sec with a spreading time of 55.0 ± 1.3 sec. This formulation was considered to provide the best balance between ease of spreading and adequate viscosity.

Isotonicity

The tonicity of formulations F1–F13 was evaluated using red blood cell (RBC) morphology studies to assess their compatibility with physiological fluids. Formulations F2, F3, F5, F7, F9, F11, and F13 maintained the normal biconcave morphology of RBCs, indicating isotonic behavior and excellent compatibility with physiological conditions. In contrast, F1 showed slight cell swelling, indicating a mild hypotonic tendency, while F4, F6, and F10 exhibited slight crenation or mild cell shrinkage, suggesting a slight hypertonic effect. Although these formulations remained compatible and acceptable, minor osmotic stress on RBCs was observed. Furthermore, F8 and F12 displayed noticeable cell shrinkage with a hypertonic tendency, resulting in borderline compatibility. The optimized formulation F3 showed the most favorable results, maintaining normal RBC morphology and exhibiting excellent physiological compatibility. These findings confirm that F3 possesses suitable tonicity characteristics for ophthalmic application and is expected to provide enhanced patient comfort and safety during ocular drug delivery. The RBC morphology observed in the images confirms that the tested formulation of the optimized batch F3, is isotonic and physiologically compatible, making it suitable for ophthalmic application.

Analysis of Data

The Design of Experiments (DOE) approach was used to optimize Bromfenac sodium hydrogel formulations by studying the effect of Carbopol, HPMC K4M, and Cyclodextrin on important formulation responses such as solution viscosity (R1), gel viscosity (R2), and drug content (R3). A total of nine formulations were prepared with different concentrations of polymers and excipients to understand their individual and combined influence on the hydrogel characteristics.

Discussion: Viscosity solution (cps) – Counter Plot

The counter plot for viscosity (sol) indicates that the viscosity of the Bromfenac Sodium pH-sensitive in situ hydrogel is predominantly influenced by Carbopol concentration (Factor A), as viscosity increases progressively from approximately 410 to 1260 cps with increasing Carbopol levels. The nearly vertical contour lines suggest that HPMC K15M concentration (Factor B) has minimal influence on viscosity within the studied range, while Carbopol acts as the major determinant of the rheological behavior of the formulation. This increase in viscosity may contribute to enhanced ocular residence time and improved drug retention.

Discussion: Viscosity solution (Cps) – 3D Surface Plot

The 3D surface plot illustrates the combined effect of Carbopol concentration (A) and HPMC K15M concentration (B) on the solution viscosity (R1) of the Bromfenac Sodium hydrogel formulation while keeping Cyclodextrin concentration constant at 15 mg. The graph demonstrated that the viscosity of the hydrogel increased progressively with an increase in Carbopol concentration.



**Abhay Santosh Jejurkar and Rajendra K. Surawase****Discussion: ViscosityGel (cps) – Counter Plot**

The contour plot for gel viscosity demonstrates that both Carbopol (Factor A) and HPMC K15M (Factor B) influence the viscosity of the formed gel, with Carbopol showing a stronger effect. Gel viscosity increased from approximately 623 to 1450 cps as the concentration of Carbopol increased, while HPMC K15M contributed moderately to viscosity enhancement through polymer interaction. The increase in gel viscosity may improve ocular retention time and sustain Bromfenac Sodium release at the site of administration.

Discussion: Viscosity Gel (Cps) – 3D Surface Plot

The 3D surface plot for gel viscosity demonstrates the combined effect of Carbopol (Factor A) and HPMC K15M (Factor B) on the viscosity of the Bromfenac Sodium in situ hydrogel formulation. The plot shows a gradual increase in gel viscosity with increasing concentrations of both polymers, with Carbopol exhibiting a more pronounced effect. The upward slope of the response surface indicates a positive interaction between the polymers, suggesting that higher polymer concentrations enhance gel strength and viscosity, which may improve ocular residence time and sustain drug release.

Discussion: Drug Content (%) – Counter Plot

The plot clearly demonstrates that Carbopol concentration had a significant influence on the drug content of the formulation. At lower Carbopol concentration (40 mg), the formulation exhibited higher drug content values, represented by the yellow region of the contour plot. As the concentration of Carbopol increased from 40 mg to 200 mg, the drug content gradually decreased, as indicated by the transition of colors from yellow to green and finally blue. This reduction in drug content at higher Carbopol concentrations may be attributed to the formation of a dense polymeric network, which can entrap the drug and reduce its uniform distribution within the hydrogel matrix.

Discussion: Drug Content (%) – 3D Surface Plot

The 3D surface plot illustrates the combined effect of Carbopol concentration (Factor A) and HPMC K15M concentration (Factor B) on the drug content (%) of the Bromfenac Sodium hydrogel formulation while keeping Cyclodextrin concentration (Factor C) constant at 15 mg. The surface plot clearly demonstrates that Carbopol concentration had a major influence on drug content.

The in-vitro drug release profile of Bromfenac Sodium pH-sensitive in situ hydrogel formulations (F1–F13) was evaluated over a period of 8 h at predetermined time intervals, and the results were expressed as mean $\pm$ standard deviation ($n = 3$). At 0 h, all formulations exhibited 0% drug release, confirming the absence of premature drug diffusion prior to exposure to the dissolution medium. The cumulative drug release profiles demonstrated a gradual and sustained increase throughout the study period, indicating controlled-release characteristics of all formulations. Among the investigated formulations, F3 exhibited the highest cumulative drug release, reaching $97.0 \pm 1.25\%$ at the end of 8 h, followed by F12 ($95.5 \pm 1.25\%$), F11 ($94.8 \pm 1.20\%$), F8 ($94.3 \pm 1.05\%$), F13 ($93.5 \pm 1.12\%$), and F7 ($93.3 \pm 1.00\%$). Formulations F4 and F9 achieved cumulative drug releases of $92.5 \pm 1.12\%$ and $92.3 \pm 1.05\%$, respectively, whereas F1, F6, F10, F2, and F5 showed comparatively lower drug release values of $91.2 \pm 1.16\%$, $91.0 \pm 1.08\%$, $90.2 \pm 1.16\%$, $90.8 \pm 1.20\%$, and $88.8 \pm 1.10\%$, respectively. The superior drug release observed with formulation F3 may be attributed to the optimized polymer concentration and appropriate gel network formation, which facilitated efficient hydration, swelling, and diffusion of Bromfenac Sodium from the hydrogel matrix. The balanced viscosity and gel strength of F3 likely contributed to its controlled yet enhanced drug release behavior. In comparison with the other formulations, F3 provided the most desirable release profile by achieving nearly complete drug release over 8 h while maintaining sustained-release characteristics. These findings suggest that the polymer composition used in F3 offered an optimal balance between gel integrity and drug diffusion, making it a promising formulation for Sustained ocular drug delivery.

Het Cam Test Result

To evaluate the ocular irritation potential of the Bromfenac sodium hydrogel formulation for ophthalmic application, Hen's Egg Test on Chorioallantoic Membrane (HET-CAM) was performed. The test was performed by applying the

117329



**Abhay Santosh Jejurkar and Rajendra K. Surawase**

hydrogel formulation on the chorioallantoic membrane of fertilized hen's eggs and observing the membrane for any signs of irritation such as hemorrhage, vascular lysis or coagulation within the specified observation period. The Bromfenac sodium hydrogel formulation did not show any visible signs of hemorrhage, coagulation or lysis of blood vessels during the experimental period. The blood vessels of the chorioallantoic membrane remained intact and normal throughout the study indicating non-irritant nature of the formulation to ocular tissue. The irritation score obtained from the HET-CAM test was found to be in the non-irritant category. No toxic reactions observed, which indicates that the polymers, excipients and drug incorporated in the hydrogel formulation were compatible with ocular tissues and safe for ophthalmic administration. The formulation's good ocular tolerability may also be attributed to its pH and viscosity. The HET-CAM study demonstrated that the Bromfenac sodium hydrogel formulation was safe, non-irritating and suitable for ocular drug delivery applications.

Sterility Test

Sterility testing was carried out using Fluid Thioglycollate Medium (FTM) and Soybean Casein Digest Medium (SCDM) to evaluate microbial contamination. In the FTM test, no microbial growth was observed after 14 days of incubation, confirming the absence of aerobic and anaerobic microorganisms.

Stability Study

The stability study of the optimized Bromfenac Sodium Hydrogel formulation (F3) was conducted to evaluate its physical, chemical, and performance characteristics during storage. The formulation was periodically assessed for appearance, homogeneity, phase separation, pH, viscosity, drug content, and in-vitro drug release. Table 8. Evaluation of Stability Parameters of Optimized Bromfenac Sodium Hydrogel Formulation (F3) During Stability Study.

CONCLUSION AND DISCUSSION

The present study successfully formulated and evaluated a pH-sensitive in-situ hydrogel system containing Bromfenac Sodium for ocular administration. The developed formulations demonstrated desirable physicochemical characteristics, including suitable pH, rapid gel formation, appropriate viscosity, effective spreadability, isotonicity, and consistent drug content. Preformulation investigations confirmed both drug purity and compatibility with the selected formulation components. Sustained drug release was observed during in vitro studies, with formulation F3 exhibiting the most favorable performance through an optimal balance of gel integrity and drug diffusion. Furthermore, HET-CAM evaluation verified the non-irritant nature and ocular safety of the optimized formulation. DOE-based optimization demonstrated that Carbopol concentration significantly influenced viscosity and drug-release behavior. Collectively, these findings indicate that the developed pH-sensitive in-situ hydrogel offers a viable alternative to conventional ophthalmic formulations by extending precorneal residence time, enhancing bioavailability, reducing administration frequency, and improving patient adherence. Therefore, the optimized Bromfenac Sodium in-situ hydrogel may serve as an effective and patient-friendly platform for the treatment of ocular inflammation.

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Table 1: Formulation Table of Ph Sensitive In-situ Hydrogel

Ingredient	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13
Bromfenac	10	10	10	10	10	10	10	10	10	10	10	10	10
Carbopol940P	120	40	40	120	200	120	120	200	40	200	40	40	200
HPMC K15M	50	30	50	10	10	20	10	50	10	10	10	30	30
Beta-Cyclodextrin	20	10	15	20	15	10	10	10	15	15	15	10	20
BenzalkoniumChloride	10	10	10	10	10	10	10	10	10	10	10	10	10
Sodium Chloride	90	90	90	90	90	90	90	90	90	90	90	90	90
Triethanolamine	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
water	10	10	10	10	10	10	10	10	10	10	10	10	10

All ingredients are in mg only*





Abhay Santosh Jejurkar and Rajendra K. Surawase

Table.2: Concentration and Absorbance value for Bromfenac Sodium in methanol

Concentration ($\mu\text{g/mL}$)	5	10	15	20	25
Absorbance	0.096	0.184	0.272	0.348	0.498

Table 3: Physical parameter results for all Ph sensitive In-situ Gel formulations

Batch	Appearance	Homogeneity	pH	Gelation Time(s)	Viscosity (sol)cps	Viscosity (Gel)cps	Drug Content(%)
F1	Clear, Slightly yellow	Homogeneous	6.9 ± 0.02	22 ± 0.03	680 ± 0.03	790 ± 0.03	90.2 ± 0.41
F2	Clear, Slightly yellow	Homogeneous	7.0 ± 0.02	23 ± 0.03	1260 ± 0.03	1450 ± 0.03	97.7 ± 0.36
F3	Clear, Slightly yellow	Homogeneous	7.0 ± 0.02	25 ± 0.03	530 ± 0.03	778 ± 0.03	99.5 ± 0.52
F4	Clear, Slightly yellow	Homogeneous	7.1 ± 0.02	22 ± 0.03	410 ± 0.03	623 ± 0.03	92.5 ± 0.44
F5	Clear, Slightly yellow	Homogeneous	7.1 ± 0.02	26 ± 0.03	588 ± 0.03	712 ± 0.03	98.9 ± 0.39
F6	Clear, Slightly yellow	Homogeneous	7.2 ± 0.02	27 ± 0.03	560 ± 0.03	788 ± 0.03	91.3 ± 0.47
F7	Clear, Slightly yellow	Homogeneous	7.0 ± 0.02	25 ± 0.03	490 ± 0.03	623 ± 0.03	98.8 ± 0.33
F8	Clear, Slightly yellow	Homogeneous	7.1 ± 0.02	27 ± 0.03	680 ± 0.03	818 ± 0.03	97.0 ± 0.38
F9	Clear, Slightly yellow	Homogeneous	7.1 ± 0.02	24 ± 0.03	715 ± 0.03	905 ± 0.03	98.0 ± 0.45
F10	Clear, Slightly yellow	Homogeneous	6.8 ± 0.02	27 ± 0.03	766 ± 0.03	910 ± 0.03	96.8 ± 0.33
F11	Clear, Slightly yellow	Homogeneous	6.9 ± 0.02	29 ± 0.03	612 ± 0.03	830 ± 0.03	97.2 ± 0.38
F12	Clear, Slightly yellow	Homogeneous	6.7 ± 0.02	28 ± 0.03	820 ± 0.03	920 ± 0.03	95.0 ± 0.45
F13	Clear, Slightly yellow	Homogeneous	6.9 ± 0.02	26 ± 0.03	933 ± 0.03	1033 ± 0.03	96.0 ± 0.45
Mean $\pm$ SD	--	--	7.04 ± 0.08	24 ± 0.03	695.69 ± 220.68	860.00 ± 212.11	96.80 ± 0.42

Table 4: Factor and level for Box-Behnken Design

Models	R ²	Adjusted R ²	Predicted R ²	SD	%CV
Response (R1) Quadratic	0.8197	0.0271	0.6689	1.88	2.71
Response (R2) Quadratic	0.7811	0.1242	0.6152	1.99	2.32
Response (R3) Quadratic	0.5562	0.4083	0.0461	1.84	1.99

Table 5: In-Vitro Drug Release Study:

Time(hr) Batches	0	0.5	1	2	3	4	5	6	7	8
F1	0 ± 0.00	19.6 ± 0.40	31.5 ± 0.55	47.5 ± 0.68	60.2 ± 0.80	73.0 ± 0.95	82.1 ± 1.02	88.0 ± 1.10	90.0 ± 1.05	91.2 ± 1.16
F2	$0 \pm$	$20.8 \pm$	$33.2 \pm$	$49.8 \pm$	$63.1 \pm$	$75.6 \pm$	$84.5 \pm$	$86.4 \pm$	$88.4 \pm$	$90.8 \pm$





Abhay Santosh Jejurkar and Rajendra K. Surawase

	0.00	0.44	0.58	0.71	0.84	0.98	1.05	1.14	1.12	1.20
F3	0 ± 0.00	22.5 ± 0.48	35.8 ± 0.62	52.6 ± 0.75	66.4 ± 0.88	78.9 ± 1.02	87.2 ± 1.10	92.8 ± 1.18	94.8 ± 1.13	97.0 ± 1.25
F4	0 ± 0.00	18.4 ± 0.38	30.1 ± 0.52	45.2 ± 0.65	58.0 ± 0.77	70.4 ± 0.92	79.8 ± 0.99	86.2 ± 1.08	88.2 ± 1.02	92.5 ± 1.12
F5	0 ± 0.00	17.9 ± 0.36	28.9 ± 0.50	43.6 ± 0.63	56.3 ± 0.75	68.2 ± 0.90	77.6 ± 0.96	84.5 ± 1.05	86.5 ± 1.02	88.8 ± 1.10
F6	0 ± 0.00	52.8 ± 0.70	27.6 ± 0.48	41.9 ± 0.60	54.7 ± 0.72	66.5 ± 0.88	75.3 ± 0.94	82.3 ± 1.02	87.3 ± 1.00	91.0 ± 1.08
F7	0 ± 0.00	16.5 ± 0.33	26.4 ± 0.46	40.3 ± 0.58	52.8 ± 0.70	64.7 ± 0.85	73.0 ± 0.92	80.1 ± 1.00	87.1 ± 1.00	93.3 ± 1.00
F8	0 ± 0.00	15.8 ± 0.32	25.1 ± 0.44	38.7 ± 0.56	50.9 ± 0.68	62.8 ± 0.83	70.8 ± 0.90	78.0 ± 0.98	80.0 ± 0.97	94.3 ± 1.05
F9	0 ± 0.00	15.0 ± 0.30	24.0 ± 0.42	37.0 ± 0.54	49.2 ± 0.66	60.5 ± 0.80	68.4 ± 0.88	75.6 ± 0.95	80.6 ± 0.91	92.3 ± 1.05
F10	0 ± 0.00	19.6 ± 0.40	31.5 ± 0.55	47.5 ± 0.68	60.2 ± 0.80	73.0 ± 0.95	82.1 ± 1.02	86.0 ± 1.10	88.0 ± 1.05	90.2 ± 1.16
F11	0 ± 0.00	20.8 ± 0.44	33.2 ± 0.58	49.8 ± 0.71	63.1 ± 0.84	75.6 ± 0.98	84.5 ± 1.05	90.4 ± 1.14	92.4 ± 1.12	94.8 ± 1.20
F12	0 ± 0.00	22.5 ± 0.48	35.8 ± 0.62	52.6 ± 0.75	66.4 ± 0.88	78.9 ± 1.02	87.2 ± 1.10	92.8 ± 1.18	94.8 ± 1.13	95.5 ± 1.25
F13	0 ± 0.00	18.4 ± 0.38	30.1 ± 0.52	45.2 ± 0.65	58.0 ± 0.77	70.4 ± 0.92	79.8 ± 0.99	86.2 ± 1.08	88.2 ± 1.02	93.5 ± 1.12

Table 6: Observation Parameter(F3)

- Control (Normal Saline)	+ Control NaCl	Formulation
		

Table 7: Sterility testing observation of Ph sensitive In-Situ Hydrogel

Sr.no	Evaluation Parameter	Observation	Result
1	Sterility test in Fluid Thioglycollate Medium	No microbial growth after 14 days	Sterile
2	Sterility test in Soybean Casein Digest Medium	No microbial growth after 14 days	Sterile





Abhay Santosh Jejurkar and Rajendra K. Surawase

Table 8: Stability Study of Optimized Bromfenac sodium Hydrogel Formulation (F3)

Parameter	Initial	1 Month	2 Months	3 Months
Appearance	Clear, Slightly yellow	Clear, Slightly yellow	Clear, Slightly yellow	Clear, Slightly yellow
Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous
Phase Separation	Absent	Absent	Absent	Absent
pH	7.3 $\pm$ 0.00	7.3 $\pm$ 0.00	7.2 $\pm$ 0.00	7.2 $\pm$ 0.00
Viscosity (Sol) cps	530 $\pm$ 0.58	528 $\pm$ 0.58	525 $\pm$ 0.58	520 $\pm$ 0.58
Viscosity (Gel) cps	900 $\pm$ 1.00	898 $\pm$ 1.00	892 $\pm$ 1.00	885 $\pm$ 1.00
Drug Content (%)	98.5 $\pm$ 0.20	98.1 $\pm$ 0.20	97.4 $\pm$ 0.20	96.8 $\pm$ 0.20
In-vitro Drug Release (%)	96.5 $\pm$ 1.25	96.2 $\pm$ 1.25	95.8 $\pm$ 0.20	95.3 $\pm$ 0.20

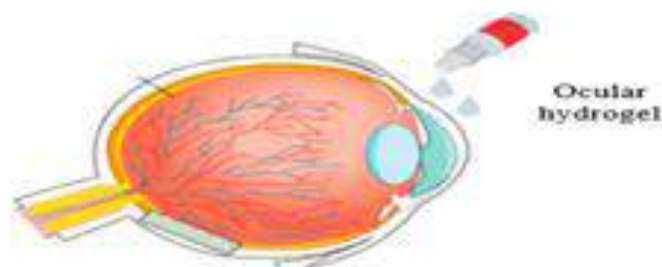


Figure 1: Ocular route of drug delivery.

Preparation of bromfenac-cyclodextrin inclusion complex

Preparation of hydrogel base

Incorporation of bromfenac- B-cyclodextrin complex in hydrogel base

Store it under appropriate conditions until further evaluation.

Figure 2: Formulation of ph sensitive in-situ gel:

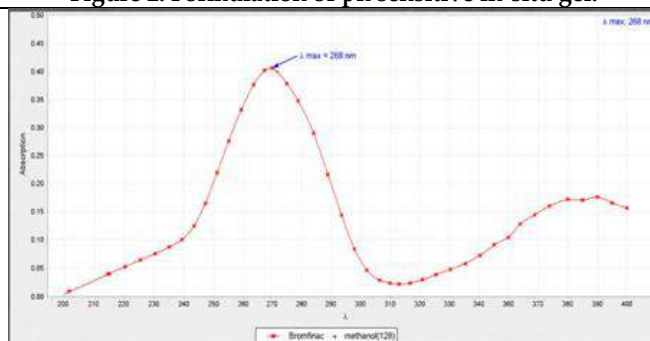


Figure 3 : Bromfenac sodium λ_{max}





Abhay Santosh Jejurkar and Rajendra K. Surawase

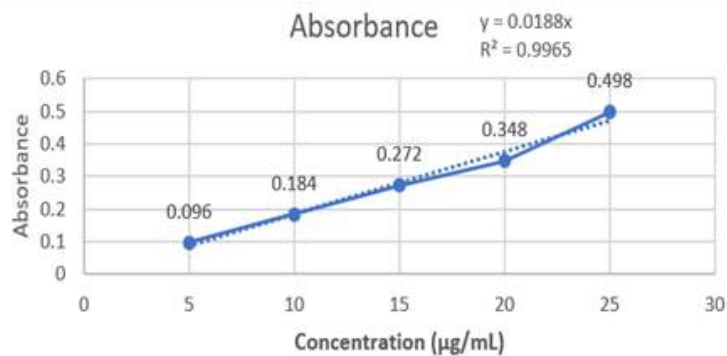


Figure 4: Calibration curve of bromfenac sodium in Methanol



Figure 5: Bromfenac Sodium

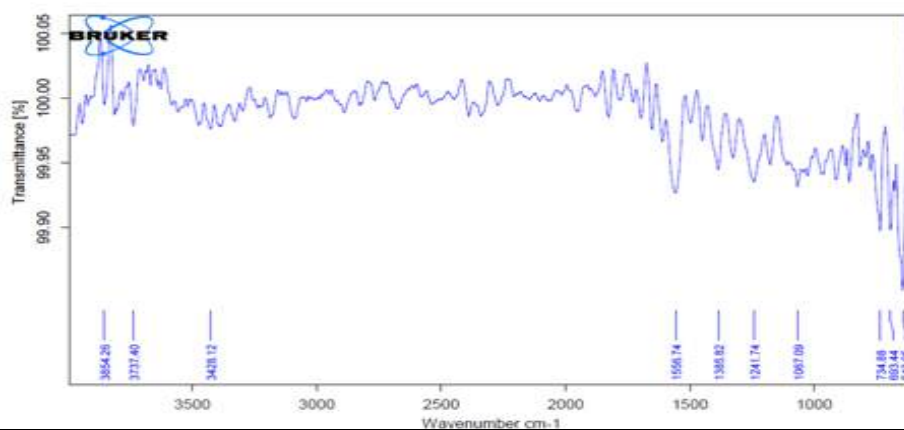


Figure 6: Bromfenac sodium + All Excipients



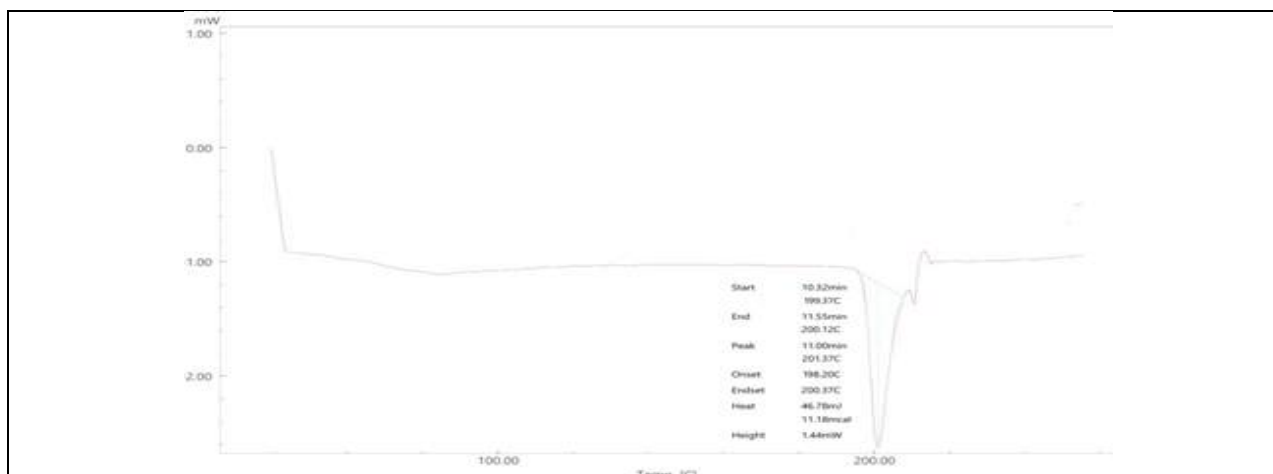


Figure 7: Bromfenac Sodium

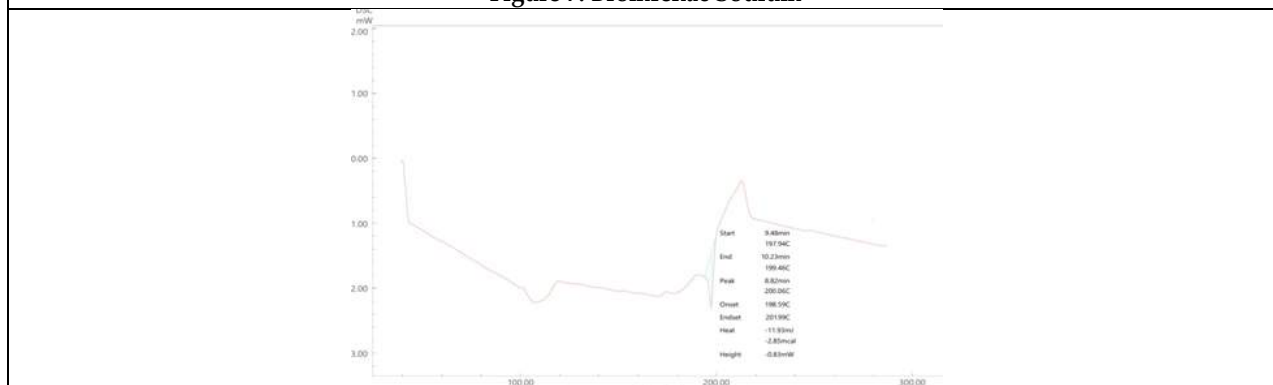


Figure 8: Bromfenac Sodium+ All Excipient

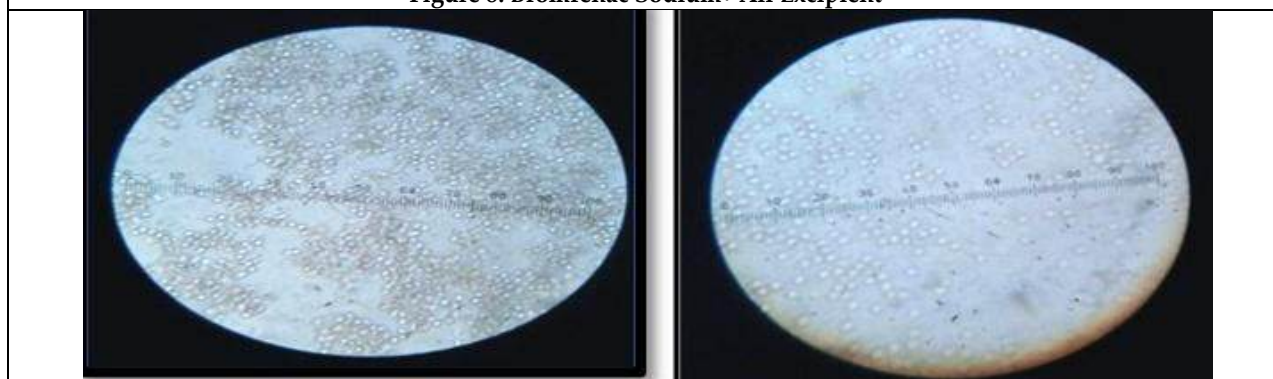


Figure 9: Shape of Red Blood Cells



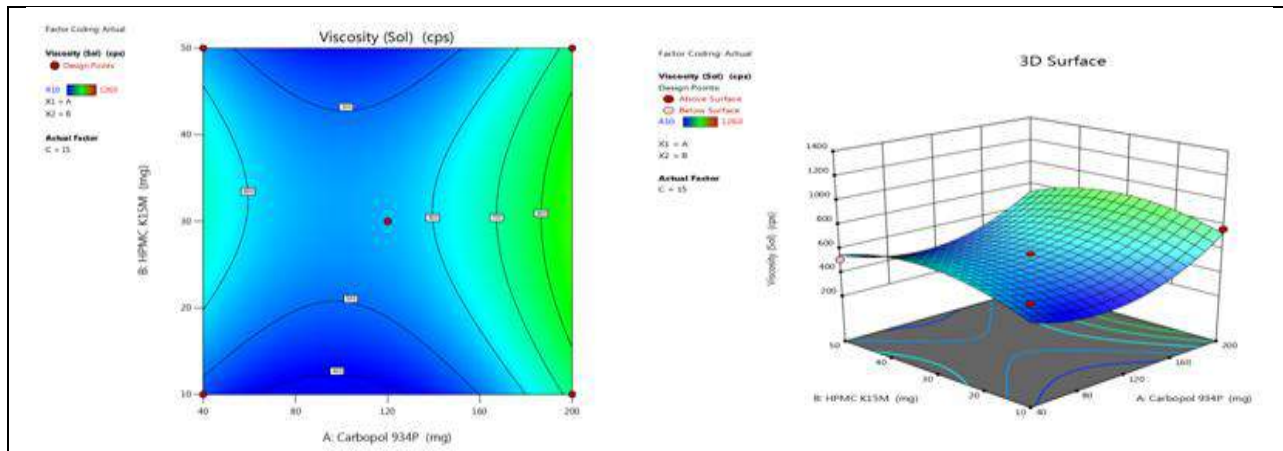


Figure 10: Counter plot viscosity(sol) and 3D plot viscosity(sol)

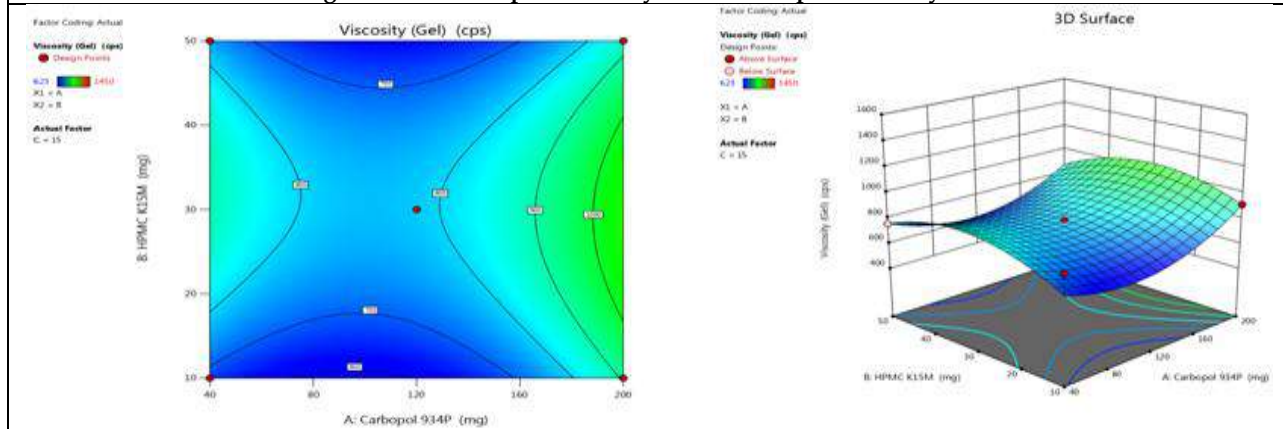


Figure 11: Counter plot Viscosity (gel) and 3D plot viscosity gel (cps).

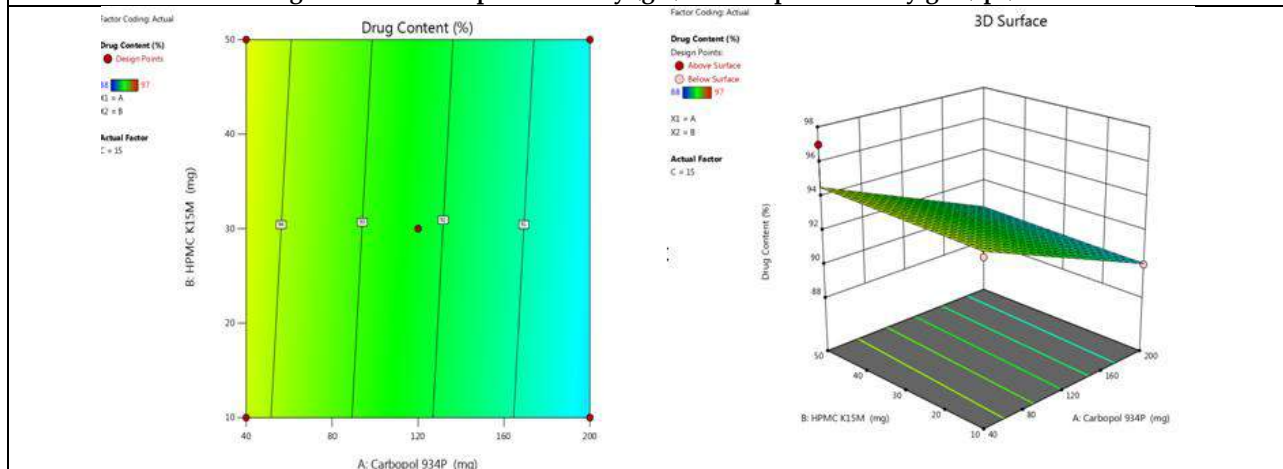
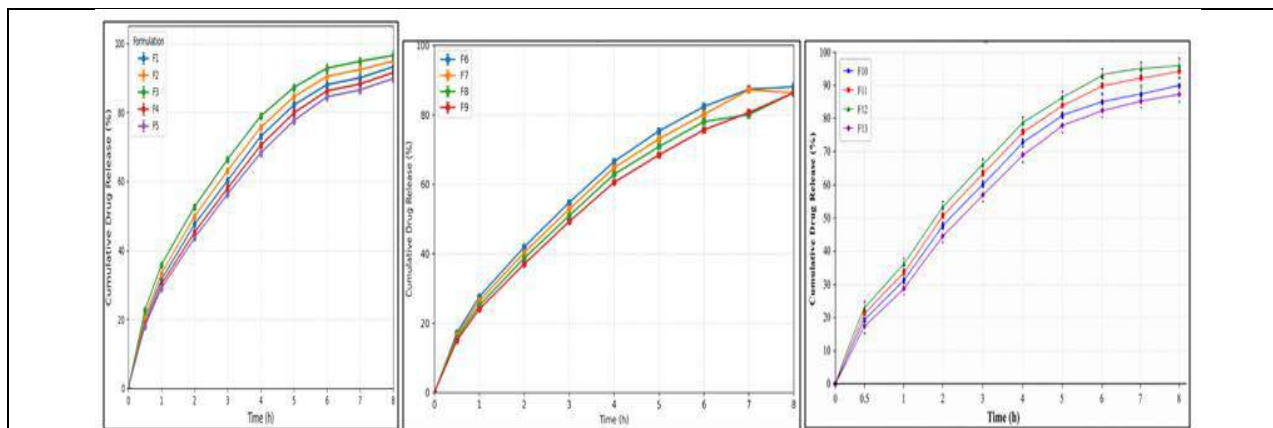
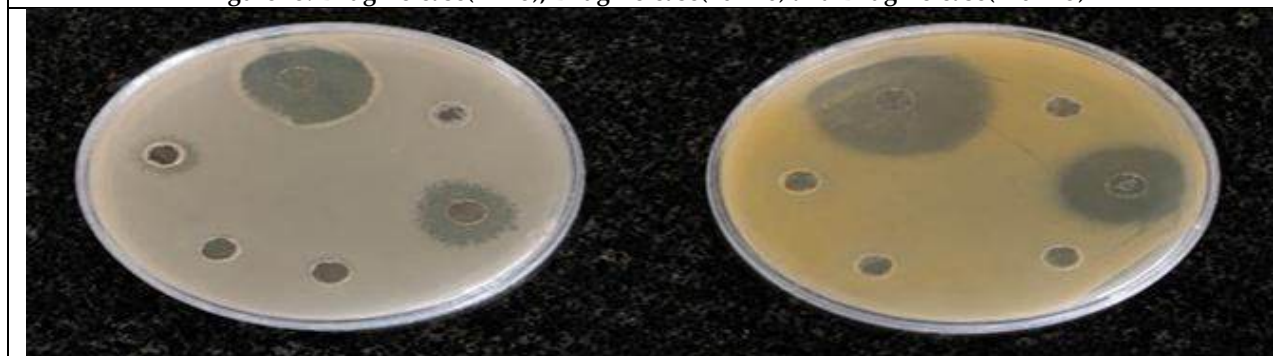


Figure 12: Counter plot Drug Content (%) and 3D plot Drug Content (%).



**Abhay Santosh Jejurkar and Rajendra K. Surawase****Figure 13: Drug Release(F1-F5), Drug Release(F6-F10) and Drug Release(F10-F13)****Figure 14: a. Fluid Thioglycollate Medium****b. Soybean Casein Digest Medium**

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RESEARCH ARTICLE

A Comprehensive Survey on AI-Driven Self - Healing in Software - Defined and Autonomous Networks

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ABSTRACT

Software-defined networking (SDN) and autonomous networking are quickly becoming cornerstones of today's communication systems. Their appeal lies in how they handle reliability and adaptability—two things networks need more than ever. At the same time, breakthroughs in artificial intelligence, machine learning, and deep learning have given us powerful tools for predicting failures, automating recovery, and building networks that can heal themselves without human help. This survey dives into the latest AI-driven methods for fault detection, diagnosis, recovery, and network optimization in SDN and self-managing networks. We look at methods built on reinforcement learning, digital twins, orchestration frameworks, and Zero-Touch Management (ZSM). We also tackle some big challenges along the way—scalability, explainability, security, and interoperability. Finally, we point to future research that's steering us toward networks that are not just autonomous, but truly resilient.

Keywords: Software-Defined Networking, Self-Healing Networks, Failure Prediction, Artificial Intelligence, Network Automation, Zero-Touch Management





INTRODUCTION

The rapid evolution of digital services, driven by massive cloud deployments and distributed systems, has introduced unprecedented complexity into network infrastructures. This complexity inherently increases vulnerability to faults, performance degradation, and cascading failures, making traditional resilience strategies, which often rely on redundancy and extensive manual intervention, increasingly inadequate [1]. Industries such as Industry 4.0, cyber-physical systems, and critical infrastructure demand continuous uptime, high-volume telemetry processing, and stringent reliability, necessitating a paradigm shift in network management [2], [3]. Concurrently, advancements in AI, ML, and DL have provided powerful tools for automating various aspects of network operations, including fault detection, diagnosis, and self-healing. These intelligent approaches are transforming network management from reactive to proactive, enabling systems to anticipate and mitigate issues before they impact service availability [4]. The emergence of Software-Defined Networking (SDN) has further facilitated this transformation by decoupling the control plane from the data plane, offering a centralized, global view of the network. This architectural flexibility allows for dynamic rerouting, faster recovery from disruptions, and optimized traffic management, laying a robust foundation for AI-driven automation [5].

The study organizes existing knowledge, dissect prevalent techniques and architectures, and highlight persistent challenges. Specifically, this paper offers:

- A comprehensive overview of AI-powered self-healing mechanisms in SDN and autonomous networks.
- A comparative analysis of diverse strategies for failure prediction, automatic recovery, and network optimization.
- An exploration of key learning methods, including ML, DL, and Reinforcement Learning (RL), that underpin intelligent network management.
- An identification of critical research challenges, such as scalability, explainability, interoperability, and security.
- Insights into future research directions, particularly concerning Zero-Touch Autonomous Networking and integration with emerging technologies like 6G.

By addressing these aspects, this survey seeks to provide a foundational understanding for researchers and practitioners engaged in developing the next generation of resilient and intelligent network infrastructures.

Background and Motivation

The proliferation of cloud services, 5G/6G technologies, the Internet of Things (IoT), and mission-critical applications has rendered modern networks highly dynamic and exceptionally sensitive to failures. Disruptions such as link breakages, node failures, congestion, or controller malfunctions can severely degrade service quality, violating the strict Service Level Agreements (SLAs) required by contemporary applications [6]. Traditional IP networks typically rely on distributed routing protocols for reconvergence following a failure. However, this process is often slow, cumbersome, and prone to issues like routing loops and packet loss. Furthermore, the tight coupling of control and data functions in traditional routers limits their ability to achieve global optimization, as each device operates with only a localized view of the network state [7]. SDN addresses these limitations by separating the control logic from the underlying forwarding hardware. A centralized SDN controller maintains a global view of the network topology and state, enabling intelligent, network-wide decision-making. This architecture facilitates rapid failure detection, flexible rerouting, and real-time traffic optimization, making SDN an ideal platform for implementing resilient and self-healing systems [8].

However, integrating intelligent prediction and automated recovery into SDN is not without challenges. Issues related to controller scalability, resource constraints, and maintaining Quality of Service (QoS) during dynamic reconfigurations must be carefully managed. A unified approach that seamlessly blends predictive analytics, robust recovery strategies, and continuous optimization is essential for realizing the full potential of self-healing SDN [9].



**Parveen Begum H and Anusha P**

The motivation for this survey stems from the need to consolidate the fragmented literature on SDN failure management. While existing reviews often focus on isolated aspects such as either prediction or recovery – this paper provides an integrated perspective. We evaluate the primary strategies for protection, restoration, and optimization, and delineate the unresolved issues that must be addressed to achieve fully autonomous, performance-aware, and self-healing SDN environments.

Conceptual Architecture and Taxonomy of AI-Driven Self-Healing SDN

To effectively implement AI-driven self-healing capabilities in Software-Defined Networks, a well-defined conceptual architecture and a clear taxonomy of techniques are essential. This section outlines a proposed conceptual architecture and categorizes the various AI-driven self-healing techniques.

Taxonomy of AI-Driven Self-Healing Techniques

AI-driven self-healing techniques can be broadly categorized based on their underlying methodologies and applications within SDN. This taxonomy helps in understanding the diverse approaches employed for fault management and network optimization.

Comparative Analysis of AI-Driven Self-Healing Approaches

Various AI-driven methodologies have been proposed and implemented for self-healing in network environments, each with distinct advantages, limitations, and suitability for different scenarios. This section provides a comparative analysis of these approaches, drawing insights from the original document and additional research.

Quantitative Performance Comparison of AI-Based Self-Healing Techniques

The effectiveness of various AI-based self-healing techniques can be quantitatively assessed across several performance metrics, including prediction accuracy, recovery speed, scalability, and computational cost. The choice of technique often involves a trade-off between these factors, depending on the specific network environment and operational requirements.

Comparison of AI-Based Failure Prediction and Recovery Techniques

Further dissecting the approaches, it is crucial to understand the specific strengths, weaknesses, and suitability of each AI-based technique for failure prediction and recovery.

Critical Discussion

The integration of AI into self-healing and autonomous networks, while promising, introduces several critical considerations that warrant in-depth discussion. These revolve around the trade-offs between accuracy and interpretability, the balance between automation and human control, and the perennial challenge of performance versus resource demands.

Accuracy vs. Interpretability

Deep learning models, renowned for their superior accuracy in complex pattern recognition, often operate as black boxes. This lack of transparency poses a significant challenge, particularly in critical network infrastructures where understanding the rationale behind an AI's decision is paramount for trust, debugging, and regulatory compliance. The observation here is that Explainable AI (XAI) is not merely a desirable feature but a necessity to bridge the gap between high accuracy and human understanding, thereby fostering confidence in AI-driven autonomous systems.

Automation vs. Control

The allure of fully autonomous networks lies in minimizing human intervention, leading to reduced operational costs and faster response times. However, relinquishing complete control to an autonomous system, especially in



**Parveen Begum H and Anusha P**

critical infrastructure, carries inherent risks. An erroneous decision by an AI could have severe consequences. Therefore, maintaining a human-in-the-loop approach for validation, oversight, and intervention remains essential. This ensures that while AI automates routine tasks and provides predictive insights, ultimate control and accountability reside with human operators, particularly for high-stakes decisions.

Performance vs. Resource Demand

State-of-the-art AI models, particularly deep learning architectures, are computationally intensive, demanding substantial processing power and memory. This presents a significant hurdle for deployment in resource-constrained environments, such as edge devices or certain industrial IoT (IIoT) scenarios. The need for lighter models and distributed learning paradigms like federated learning becomes critical to enable AI-driven intelligence closer to the data source, reducing latency and optimizing resource utilization without compromising performance. This trade-off necessitates innovative approaches to model design and deployment strategies.

Failure Prediction and Recovery Mechanisms

Effective self-healing in SDN and autonomous networks hinges on robust failure prediction and efficient recovery mechanisms. This section delves into the workflow of AI-based failure prediction and outlines various recovery strategies.

Failure Prediction Workflow

AI-based failure prediction in self-healing SDN typically follows a structured workflow, enabling proactive identification and mitigation of potential network issues. This workflow generally involves several key stages:

- **Data Collection and Preprocessing:** Gathering diverse network telemetry data (e.g., traffic logs, device status, performance metrics, environmental data) and preparing it for AI model consumption through cleaning, normalization, and feature engineering.
- **Feature Extraction and Selection:** Identifying relevant features from the pre processed data that are indicative of impending failures. This often involves domain expertise and advanced statistical or ML techniques.
- **Model Training:** Utilizing historical data with known failure events to train AI/ML models (e.g., supervised learning, time series analysis) to recognize patterns preceding failures.
- **Prediction and Anomaly Detection:** Deploying the trained models to continuously monitor real-time network data, predict potential failures, and detect anomalies that deviate from normal operating behavior.
- **Decision Making and Alerting:** Based on predictions and anomaly detections, the system makes informed decisions about the likelihood and severity of a failure, triggering alerts or initiating recovery actions.

Recovery Strategies

Once a potential or actual failure is detected, various recovery strategies can be employed to restore network functionality and maintain service continuity. These strategies can be broadly categorized as proactive, reactive, or a hybrid approach:

- **Proactive Recovery:** This strategy involves anticipating failures and pre-installing backup paths or reconfiguring network resources before an actual outage occurs. This leads to faster response times and minimal service disruption. Examples include pre-computed backup paths or redundant resource allocation.
- **Reactive Recovery:** Triggered after a failure has occurred, this approach focuses on diagnosing the issue and then initiating corrective actions. While it might incur a brief service interruption, it typically utilizes fewer resources than proactive methods as resources are only allocated when needed.
- **Hybrid Approach:** Combining elements of both proactive and reactive strategies, this approach aims to leverage the benefits of both. For instance, critical network segments might employ proactive measures, while less critical areas rely on reactive recovery. This is often the most effective approach in complex, real-world systems, balancing resource utilization with resilience requirements.



**Parveen Begum H and Anusha P****Research Gaps**

Despite significant advancements in AI-driven self-healing for SDN and autonomous networks, several critical research gaps and open challenges remain. Addressing these will be crucial for the widespread adoption and full realization of truly intelligent and resilient network infrastructures. The overarching challenge remains the effective implementation of AI-powered self-healing in real-world, large-scale, and resource-constrained environments, while ensuring security, explainability, and seamless integration with existing and emerging network architectures. Future breakthroughs are anticipated in these critical areas.

CONCLUSION

This survey has provided a comprehensive overview of AI-driven self-healing and failure prediction in Software-Defined Networking and autonomous networks. We have highlighted the increasing complexity of modern network infrastructures and the limitations of traditional management approaches, underscoring the necessity for intelligent, automated solutions. The architectural flexibility of SDN, combined with advancements in AI, ML, and DL, offers a powerful paradigm for developing networks that can proactively detect, diagnose, and recover from faults. The survey analyzed various AI-driven methodologies, including those based on reinforcement learning, knowledge-driven systems, and hybrid models, comparing their strengths, weaknesses, and suitability across different network environments. Critical discussions on the trade-offs between accuracy and interpretability, automation and human control, and performance versus resource demands have shed light on the inherent complexities and ongoing challenges in this field. Furthermore, we have identified key research gaps, such as scalability, data quality, security, and standardization, and proposed future directions including federated learning, explainable AI, integration with 6G and edge computing, and the development of digital twins. Addressing these areas will be crucial for advancing towards truly resilient, autonomous, and intelligent network management systems that can meet the demands of future communication landscapes.

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Parveen Begum H and Anusha P

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Parveen Begum H and Anusha P

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Parveen Begum H and Anusha P

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Parveen Begum H and Anusha P

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Table 1: Key motivations for an integrated survey on AI-driven SDN failure management.

Driver	Brief Explanation
Limitations of Traditional Routing	Slow, localized reconvergence, susceptibility to routing loops, and poor global optimization capabilities.
Demand for Fast, Reliable Recovery	Necessity for carrier-grade availability, adherence to strict SLAs, and support for mission-critical applications.
Centralized SDN Control	The global network view provided by SDN controllers enables flexible, intelligent failure detection and rapid restoration.
Rise of AI/ML and Predictive Analytics	ML techniques significantly enhance the accuracy of failure prediction and the adaptability of recovery mechanisms.
Need for an Integrated Survey	Existing literature often treats prediction, recovery, and optimization in isolation; a unified review is required to address the holistic self-healing process.

Table 2: Peak-level scope of surveyed self-healing research

Aspect	Brief description	Citations
Domains	Cloud, CPS, Industry 4.0, buildings, PV, transport	[9], [10],[13], [12],[14], [15], [16], [19], [22], [23]
Core tasks	Fault detection, diagnosis, recovery, optimization	[10],[13], [12],[14], [15], [16], [19], [23]
Key enablers	ML, DL, XAI, digital twins orchestration	[10],[11], [13], [12],[14], [16], [3], [20], [21], [26], [25]
Gaps	Data scarcity, evaluation, explain ability, integration	[10],[11], [12],[12],[14], [15], [16],[17], [23]





Parveen Begum H and Anusha P

Table 3.Comparative Analysis of Existing AI-Driven Self-Healing Approaches

Methodology / Paradigm	Typical AI / ML Algorithms Used	Main Merits for Automation / Self-healing	Key Limitations / Challenges	Critical Factors to Include in Survey	Citations
SDN/NFV-based closed-loop automation (MAPE-K, SON, NI)	RL (Q-learning, Deep RL), supervised models for anomaly/fault prediction, heuristic/optimization for scaling	Centralized programmability, fast reconfiguration, supports self-optimization and self-healing via monitor–analyze–plan–execute loops [42],[69],[50],[51]	Controller scalability, robustness and security issues; integration with legacy devices; data/telemetry quality [50]	Control-/data-plane split orchestration layer, telemetry sources, latency constraints, controller redundancy, fault coverage, interoperability	[(42,69); [44]; [49]; [50]; [51]; Ahmad & Mir, 2020)
Knowledge-driven autonomic architectures (ETSI GANA, autonomic/intent-based networking)	Rule-based reasoning, knowledge graphs, case-based reasoning; ML often used for policy refinement	Human policies encoded as high-level “intents”; hierarchical decision elements enable multi-level self-configuration and self-healing [42,69],[51],[54],[60]	Knowledge modeling effort is high; difficulty keeping knowledge base consistent; limited learning if rules are static	Abstraction levels (node/network), policy/intent language, conflict resolution, verification, operator override, explainability	[(42,69); [44]; [51]; [54],[60])
AI-driven network automation (general IP/enterprise, cloud, SD-WAN/SASE)	Supervised learning (ANN, SVM, RF), unsupervised anomaly detection, RL for routing/TE and self-healing; federated learning at edge	Improved routing/TE, predictive fault detection, automated configuration and policy enforcement, reduced OPEX, energy-aware optimization [45] [46] [7] [55] [58]	High data/compute requirements; trustworthiness and explainability of AI decisions; model drift; security/privacy of telemetry [45] [46] [7] [55] [58])	Data pipeline (volume, labeling, imbalance), training/inference placement (edge/cloud), KPI definitions, human-in-the-loop, robustness to adversarial input	[(42,69); [45]; [46]; [49]; [7]; [55]; [53]; [58])
ML-based self-healing in cellular/IIoT/O-RAN/SON	Fault classification (SVM, RF), deep learning, RL, meta-learning ensembles; cost-sensitive and imbalance-aware methods	Automatic detection, diagnosis and recovery from radio/link faults; improved QoS/QoE; supports large-scale dense networks [45] [48]	Data imbalance, scarcity of labeled fault data, multi-source data fusion, latency of learning loops, generalization across deployments [48] [49] [53]	Fault taxonomy, data sources (KPIs, logs), reaction time vs. accuracy trade-off, cost-sensitive metrics, transfer learning, safety constraints for actions	[(45); [48]; [49]; [53])





Parveen Begum H and Anusha P

Methodology / Paradigm	Typical AI / ML Algorithms Used	Main Merits for Automation / Self-healing	Key Limitations / Challenges	Critical Factors to Include in Survey	Citations
		[49] [53]			
Industrial / IIoT network automation toward Industry 4.0/5.0	Predictive analytics, RL, deep learning for scheduling/resource allocation, anomaly detection	Handles strict latency/reliability requirements, supports human-robot collaboration and massive IIoT; enables proactive maintenance [42],[69],[45]	Heterogeneous devices and protocols, legacy integration, safety-critical constraints, limited compute at edge nodes	Deterministic latency, reliability targets, edge-cloud split, standardization status, cyber-physical safety requirements	[(42,69); [45)]
Template- / tool-based configuration automation (e.g., Nornir, Jinja, Ansible-like tools)	Usually rule-based; may integrate simple ML for anomaly/rollback triggers	Large reduction in manual effort and errors; consistent multi-vendor configuration; good entry point for automation maturity [47]; [51]; [52]	Not inherently “intelligent”; limited to scripted logic; still relies on humans for diagnosis and policy design	Inventory modeling, idempotent templates, change-control and rollback strategy, integration with CI/CD and logging, operator skills	[(47); [51]; [52)]
Security automation for networks (policy synthesis, automatic NSF composition/config)	Optimization (ILP), model checking, ML for threat detection (NNs, anomaly detection, ensemble methods)	Automatic generation and validation of firewall/VPN/ACL policies; faster reaction to attacks; fewer configuration errors [43]; [44]; [46]; [52]; [55]	Complexity and scalability of policy synthesis; multi-domain consistency; exposure to new automation-specific attack surfaces [43]; [44]; [55]	Correctness and optimality guarantees, coverage of security functions, scalability (rules/devices), integration with SIEM/SOAR, compliance mapping	[(43); [44]; [46]; [52]; [55)]
Knowledge-/data-driven self-healing frameworks for autonomous networks	Hybrid: knowledge-driven diagnosis + ML-based prediction and policy selection	Provides reusable patterns for self-healing loops; supports heterogeneous failures; moves toward Level-5 autonomous networks [54],[60]	Designing generic yet effective knowledge schemas; validating self-healing actions under diverse scenarios	Failure ontologies, feedback loops, evaluation on real incidents, interaction with human operators, multi-domain scope	[(54],[60)]





Parveen Begum H and Anusha P

Table 4. Quantitative Performance Comparison of AI-Based Self-Healing Techniques

Technique	Prediction Accuracy	Recovery Speed	Scalability	Computational Cost	Suitable Environment
Rule-Based Systems	Medium	Fast	Low	Low	Small networks
Machine Learning	High	Medium	Medium	Medium	Enterprise SDN
Deep Learning	Very High	High	High	High	Large-scale cloud networks
Reinforcement Learning	High	Adaptive	High	Very High	Dynamic autonomous networks
Hybrid AI Models	Very High	Very High	High	High	Real-time self-healing systems

Table 5. Comparison of AI-Based Failure Prediction and Recovery Techniques

Approach	Strength	Weakness	Suitability
Rule-Based Systems	Simple and interpretable	Not adaptive	Small networks
ML-Based Prediction	Accurate detection	Data dependency	Medium-scale networks
Deep Learning	High accuracy	High computation cost	Large-scale networks
Reinforcement Learning	Adaptive decision-making	Slow training	Dynamic environments
Hybrid Models	Balanced performance	Complex design	Real-world deployment

Table 6. Research Gap

Gap Area	Typical Missing Pieces	Key Sources
Heterogeneous, real-world validity	Cross-domain test beds, multi-vendor evaluation	[59], [53], [66], [45], [42], [69], [72]
Resource-aware real-time AI	Lightweight, adaptive, energy-efficient models	[59], [53], [67], [45], [71], [77]
Secure, explainable ZTA/ZSM loops	Formal guarantees, adversarial robustness, auditability	[61], [46], [66], [67], [43]
Unified autonomous frameworks	Standardized patterns (KSHP, GANA, O-RAN) with AI at scale	[54], [60], [66], [45], [42], [69], [76]

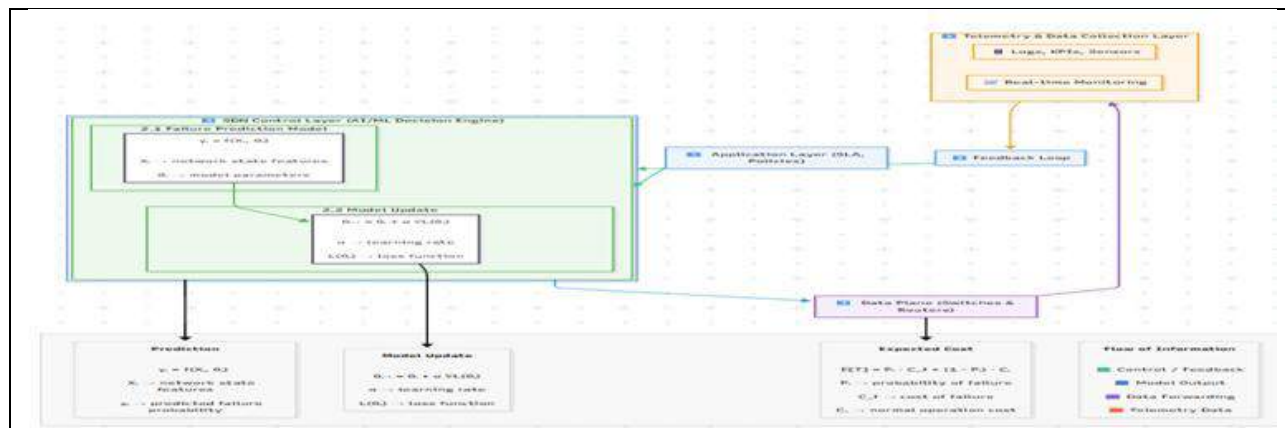


Fig 1. Conceptual Architecture





Parveen Begum H and Anusha P



Fig 2. Taxonomy of AI driven Self - Healing Techniques

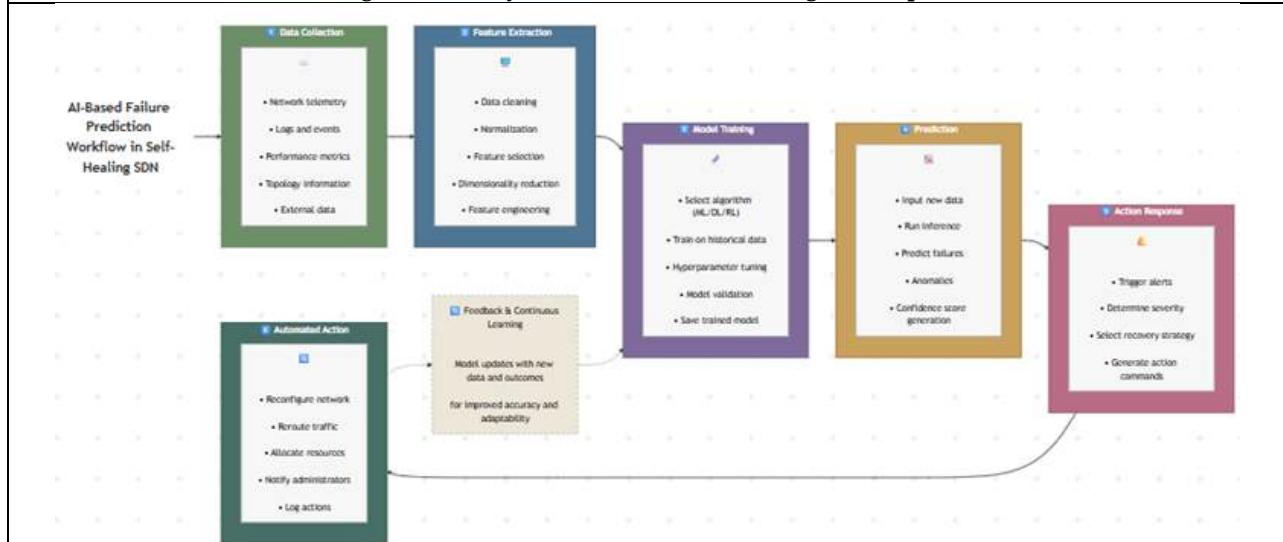


Fig. 3. AI – Based Failure Prediction workflow in Self-Healing SDN

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RESEARCH ARTICLE

Transformational AI Leadership in Indian Higher Education: Inclusive Cultures for Lifelong Human Capital

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ABSTRACT

This chapter discusses the transformational role of AI leadership in Indian higher education institutions in building inclusive learning cultures that will deepen human capital over a lifetime. It contends that higher education leaders must focus AI initiatives not on automation but on clarity of vision and purpose around equity, multilingualism, faculty, ethical governance, and lifelong employability, which are where the strategic values amid the exigencies of artificial intelligence will lie. Based on what we know of the India-based policy landscape, global directions on AI in education, and the leadership scholarship, the chapter provides a functional framework for institutional leaders. The framework links the transformational leadership behaviours to components of (1) enacting an inclusive digital infrastructure, (2) curriculum redesign, (3) responsible data governance, and (4) ecosystem partnerships. In doing so, the chapter rates AI governance as a key variable that will determine how adaptive, just and ready-for-the-future Indian higher education can be and concludes with some lessons from the AI-related discourse to reform the relationship between human beings in general and 'AI' in particular, treating the latter as one of the possible futures of human-centered public good and not as a narrow techno-ethical intervention.

Keywords: Artificial intelligence, transformational leadership, Indian higher education, inclusive learning culture, lifelong learning, human capital development.



**Purushotham et al.,**

INTRODUCTION

Indian higher education is at a unique point in its history. On the one hand, the sector is growing in size and breadth of its student body, and, at the same time, it is being challenged by technological disruption at an unprecedented rate. Artificial Intelligence (AI) is now ascribed as a promise to every major institutional aspiration: personalised learning, administrative efficiency, academic analytics, multilingual access, employability enhancement, research productivity, and micro-credentialed lifelong learning. However, simply implementing technology does not enable an organisation to evolve to a new level. Leadership does. In higher ed, the question is not whether we can buy or pilot these tools, but whether leaders can model organisational cultures that will, in the longer term, employ AI to deepen inclusion, broaden opportunity, and reinforce human agency. In touch with this question, especially in India, since scale meets inequality here.

The higher education system is large, socially heterogeneous, and geographically unequal. It varies considerably, from large, well-resourced metropolitan universities to small or semi-urban colleges that face enormous infrastructure constraints. In such a context, AI may act as a bridge or a gaping chasm. Without ethical vision, pedagogical redesign, or equitable planning, it can exacerbate inequities. However, if managed skilfully, it can facilitate access, flexibility, multilingualism, and disability support, and provide better pathways to lifelong learning and employment. The chapter argues that if Indian higher education institutions are to convert technological possibilities into socially inclusive, ethically governed, lifelong human capital development, transformational AI leadership is called for, in which humans take charge of AI, not the other way around. Transformational AI leadership, as used here, refers to a mode of institutional leadership that can articulate a human-centred vision of AI, articulate values, drive engagement around that vision and those values, spur pedagogical and organisational experimentation, and respond to the diverse needs of learners, faculty, and communities. Such an approach is in stark contrast with compliance-only or procurement-only models. It operates through culture, not just through tools. The discussion is divided into six general movements. It begins with a literature review on transformational and inclusive leadership and learning culture relevant for AI-enabled higher education. Second, it places the conversation within the landscape of higher education in India and the public policy framework. Third, it articulates a conceptual leadership framework for transformational AI. Fourth, it defines leadership levers and an implementation roadmap for institutions to use strategically. Fifth, it deals with risks and governance tensions. Lastly, it comments on the consequences of AI leadership for human capital development over the course of a human life.

Transformational Leadership, Inclusion, and Learning Culture

Transformational leadership is as powerful a lens for making sense of educational change as any because it shows how leaders take organisations through space in the human enterprise, past the mere administration of routine, toward mission-propelled renewal. It is most commonly connected with idealised influence, inspirational motivation, intellectual stimulation and individual consideration. But these dimensions matter deeply in higher education, which depends on trust, shared governance, professional autonomy, and collaborative legitimacy among its institutional members. Universities and colleges seldom find their way to success in change. Please note: They change when people understand what they are participating in and the process they are part of. In higher education, the value of transformational leadership stems from its compatibility with the attributes of academic professionalism. Transformational leadership works (in contrast to purely transactional models that emphasise compliance, outputs, and incentives) to create a shared purpose and collective efficacy. This is especially true during times of technological change. The bulk of institutional opposition to AI is not just mere technical unfamiliarity. This too is intertwined with moral ambiguity, pedagogical anxiety, anxiety about compromised academic discernment, and anxiety about professional identity. This means leaders need to do more than green light the use of software; they have to shape the sense making of why and how AI should be adopted. But transformational leadership is not enough if it is not purposefully inclusive. The pace of diversity has outstripped many organisations' ability to foster true belonging. So



**Purushotham et al.,**

inclusive leadership focuses less on participation than on access, less on climate than on representation, and less on accommodation than on structural redesign. To build such spaces means practising inclusive leadership in our higher education contexts, creating intentional spaces where the dignity, recognition, support, and voice of historically marginalised learners and staff are integrated into institutional life. And it requires leaders to prepare institutions not to react to overt expressions of exclusion, but to examine and dismantle latent systems of oppression. Learning culture bridges transformational and inclusive leadership. A learning culture might be defined as the norms, expectations, routines, and relationships through which individuals and groups grow their capacity to acquire, evaluate, share and use knowledge. A strong learning culture is evident in higher education when inquiry is valued, mistakes are viewed as a part of the process of honing improvement, professional development is prized, and assessment drives higher-order thinking. AI was an approach that made this culture more important. Schools that only try to ban AI will fail to prepare students for an AI-rich world. On the other hand, institutions that foster critical AI literacy, ethical evaluation, team-based investigations, and the restructuring of assessment will likely graduate students who are better prepared to thrive and build more resilient academic communities. Thus, transformational AI leadership in this chapter describes a linking of three dimensions that all too often remain separated: transformational change, inclusive culture, and responsible AI integration. It is not a glorification of AI as a being acting on its own. It is a position that leads with human agency, justice, and long-term capacity as the guiding principles for the future of technology. In that way, AI leadership in higher education is more of a cultural project with technical components than a technical project with cultural externalities.

Why India Needs a Distinctive Response

The discussion acquires both urgency and specificity from the context of higher education in India. This is a very large, internally differentiated, developmentally important system. It consists of a central university, a state university, a private university, a deemed university / deemed to be university, an autonomous college, an affiliated college, a technical institute, an open university and a stand-alone professional institution. This diversity implies that AI transformation cannot happen following a single institutional template. Leadership models should reflect the missions, the demographics of the students served, the region's ecologies of language, and local realities and resource conditions. There is already a substantial policy infrastructure in place for this transformation. The NEP 2020 emphasises multidisciplinary, equitable, and inclusive education; lifelong learning; digital and Online education; adult learning; Indian languages; and technology in tandem. It views technology as a means to improve access, providing services that support learners with disabilities and eliminating geographical and language barriers. Therefore, AI leadership in higher education in India cannot be envisioned as a new-age extra. Instead, it ought to be seen as one of the emergent governance challenges in a broader educational transformation that is happening regardless. In practice, India has significantly expanded its digital learning ecosystem. With the new initiatives launched in the past few years, including SWAYAM, SWAYAM Plus, SAMARTH, NEFT ecosystem, and the multilingual digital tools, online learning and digital administration of education are no longer marginal. These policies are now a part of the institutional environment within which AI decisions will be made. While this development opens opportunities, it also raises the stakes. Digital infrastructure that enhances educational equity outcomes is not a given; it must be paired with capable and inclusive leadership at the institutional level to have that effect.

Recent years have clarified the momentum of AI policy. At the national level, AI is now framed not just as a technological innovation, but also as a strategic capability for education, connected to teaching and learning, workforce preparedness, and research. This is significant and also explains why coordination now matters more than experimentation. Those institutions that cannot build leadership capacity will have difficulty translating national initiatives into local advantage. In contrast, institutions that invest in leadership, faculty development, and inclusive design will likely convert policy momentum into lasting institutional improvement. But there are also structural risks in the Indian context. Inaccessibility due to uneven connectivity, device costs, faculty preparedness discrimination, the urban-rural divide, English domination, and variation across disciplines could contribute to AI-driven exclusion, as well as to unequally distributed resources, rather than to its negation.



**Purushotham et al.,****AI in Higher Education: From Tool Adoption to Institutional Capability**

Global higher education systems are racing fast to the shores of AI use, but not necessarily in an orderly fashion. Despite the shift, many institutional responses remain piecemeal, hyper-focused on generative AI regulations or limited to concerns about identifying harmful content. These are reasonable tactics in the short run, but short-term solutions are no solutions at all. The more profound question is not if students should access AI tools. More profound concerns the capacities, values, habits, and possibilities that institutions need to develop for an AI-enriched society. That shift is similarly decisive for Indian higher education because of the dual demands of the country's developmental agenda—elite innovation and broad-based capability formation. AI is relevant beyond computer science, computer engineering, and the myriad ways they intersect, but also to agriculture, law, healthcare, business, teacher education, languages, design, public policy, and the humanities. Unless AI leadership extends beyond IT and data science departments, higher ed will reproduce the very narrow specialisation rather than a multidisciplinary transformation. In sharp contrast, transformational AI leadership treats AI as an institutional capability that cuts across disciplines and learner groups, with differentiated relevance for each. The argument is more apparent when framed in terms of lifelong human capital formation. Whereas many narrowly conceptualise human capital in terms of employability or income, it should be interpreted more broadly in relation to higher education as the cumulative development of knowledge, adaptive skills, ethical judgment, creativity, collaboration, technological fluency, linguistic capability, and civic agency across the lifespan. These characters are then relevant to personalised learning and lifelong human capital development; hence, institutions need, or even are required to, cater to full-time traditional students as well as working adults, returning learners, re-skillers, micro-credential learners, and community-facing learners. This perspective is so closely aligned with the reality of today's labour markets. Artificial Intelligence is changing work, changing the skills required, and emphasising the need for continuous learning. Institutions cannot think that one-and-done preparation will do it in this environment. Graduates will likely face multiple transitions over time and rapidly changing expectations of their occupations or positions. Hence, institutions that build curiosity, self-learning, ethical distinction, and digital adaptability provide a non-perishable value more than institutions that focus on a temporary solution of platform familiarity. The key takeaway is that AI adoption should be viewed as a capability-building effort and not a technology deployment effort. The tools you choose are important, but your institutional capability is crucial. Diploma level: 6, which encompasses ethical governance, multilingual access, inclusive pedagogy, faculty confidence, critical AI literacy, and redesign assessment willingness. AI is just surface-level without these dimensions. With them, AI has the potential to be an engine for educational renaissance.

A Conceptual Framework for Transformational AI Leadership

This framework views transformational AI leadership as a system of mutually reinforcing institutional practices, rather than as a single characteristic of leadership. We use AI tools as inputs and expect direct outcomes. They are mediated by culture. An AI Vision Based On Education In Purpose Brought At First By Leadership. Its next step is to create enablers such as faculty development, student assistance, digital and language accessibility, ethical policies, and inter-sector partnerships. They shape learning culture by affecting trust, willingness to experiment, collaboration, and readiness to modify pedagogy and assessment. That is when human capital outcomes become durable.

Visionary Human-Centred AI Strategy

The journey of wise AI leadership transformation begins with narrative. It's now the responsibility of leaders to explain why AI is a relevant part of the mission of an institution and why using AI must always and only take a back seat to human development, and not to serve it, but take the lead. An AI narrative that convinces the institution will have to link AI, not technological wizardry, to student success, inclusive and participatory, multilingual, faculty augmentation, relevance to communities, and future work readiness. In the absence of such vision, AI projects suffer from fragmentation, become vendor (product)-driven, superficial, or compliance-oriented. This empowers AI adoption to be directed by mission. A visionary strategy should also define what an institution will not do. Not every AI tool meets educational standards, and not every efficiency gain merits adoption. Hence, a human-centric strategy



**Purushotham et al.,**

is a selective judgment. It queries whether AI serves learning, whether it broadens rather than constricts participation, whether it respects privacy, and whether it empowers faculty and students to exercise critical agency. Strategic ambition is only half the equation; the other half includes strategic restraint.

Inclusive Governance and Ethical Stewardship

Leaders need to establish governance committees with representation from faculty, students, technologists, librarians, legal experts, disability services experts, and, when appropriate, community voices. Governance should clarify what constitutes an acceptable use, what transparency requirements exist, what audit standards apply, what procurement rules are, how bias will be reviewed, what protections for privacy are in place, and what processes for appeal or revision there is (e.g. if you have to replace someone whose life it would destroy to be banned from receiving services related to their work). Ethical Governance is not a Bureaucratic Fragment. This is a condition for the credibility of AI use in education. Policy is seen as a rehearsed process in an inclusive governance model. No guide to AI should be marketed as a definitive rulebook that's set in stone. Due to the quick changes in tools, risks, and ways of working, governance should be an ongoing system of guidance, tracking, reflection, and adjustment. Providing this impression of transparency and trust also makes policy more relevant to the realities of teaching and learning.

Faculty Capability and Professional Learning

A successful AI strategy in higher education will not stop at the margins of faculty development. Facilitating tools alone are insufficient; we need broader support for redesigning courses, assessing AI outputs, embedding AI literacy instruction, developing discipline-sensitive assessments, and reassuring faculty that academic integrity can be salvaged without OMNEC. This will take time and investment: in incentives, mentoring, experimentation, and communities of practice. Faculty development should be an institutional obligation, not an elective benefit. Peer Mentoring, Micro-certifications, Discipline-based Workshops, Collaborative Labs, Faculty Learning Communities. Now, the larger goal here is not technology-related compliance. Pedagogical confidence and professional judgment in an AI-infused environment.

Designing for Accessibility, Inclusion, and Multilinguality

Exclusion will reproduce with Indian AI leadership if it ignores language, disability and digital disparity. While building on multilingual tools, online and open-distance pathways, and digital public platforms, leaders need to situate these foundations within the actual design of the student experience. That should translate into affordable accessibility, mobile-first user interfaces wherever feasible, accessible content standards, assistive technologies, multimodal delivery and the use of Indian languages for all teaching and student services. Rather, inclusion has to be engineered into infrastructure instead of being tagged on afterwards. This principle is especially critical for first-gen learners, regional students, students with disabilities, and learners who work, as flexible access enables their participation. If these learners face AI-optimised systems designed around high bandwidth, English literacy or uninterrupted access to devices, then the promise of digital innovation will become the next exclusionary device.

Curriculum, Assessment, and Credential Innovation

AI leadership should focus not only on operational efficiency but also on curricular transformation. AI literacy outcomes should be cross-disciplinary, not just within technical programmes, given AI's pervasiveness. There are skills that students should gain to understand how AI systems work, how to interrogate their outputs, how to engage with AI as a responsible agent, and how to Craft Machine Input and Output with Human Judgment. This is to define AI literacy as a civic and professional competence rather than a technical specialisation. Assessment must also shift. If institutions assess through recall-based or product-only methods, they will fail to distinguish between shallow output and authentic learning. Types of tasks also matter, and we should see less writing in exams and a wider variety of assessment designs, such as reflective writing, oral defence, process documentation, collaborative inquiry, local-context application, iterative drafts, and higher-order analysis. By redesign, academic integrity becomes more significant, as learning is visible itself. The same goes for credential innovation, which is relevant to lifelong learning as well. Increasing alignment of degree programmes with stackable pathways, short learning modules, continuing





education and flexible recognition systems. Institutions can then respond to the reality of up-skilling recall when learners return to re-skill, change careers, or upgrade modules rather than following a traditional one-way linear path.

Human Capital Development through Ecosystem Partnerships

Transformational AI Maestros are externally facing. Institutions must partner with industry, government, civil society, local communities, and tech partners to develop internships, apprenticeship pathways, research collaborations, localised AI applications, innovation hubs, and community problem-solving opportunities. In the Indian context, this sort of partnership can help link higher education to employability and foster connections with regional development and social inclusion. Yet partnerships need to be regulated well. They should serve the purpose of learning, not the vendor's agenda. They must be able to bargain for access, language support, student safeguards, transparency and sustained legacy value. Instead of scale only, partnership quality should be measured by who benefits through it and whether we are creating new opportunity structures for underserved learners.

Strategic Levers for Institutional Leaders

The above framework suggests several tactical leadership levers. 1. Institutions require a mission-driven AI strategy. Let's question why we are adopting an AI, for whom our AI is, and what educational problem it is going to solve. Without clear answers, silver-bullet adoption and osmosis-like experimentation become far more common than they should be. Second, institutions need balanced governance. Establish AI governance groups comprising a diverse range of stakeholders, empowered to review use cases, procurement decisions, and emerging risks. These and similar structures enable organisations to journey from reactive crisis response to strategic, evidence-based governance. Third, faculty development needs to be viewed as a strong backbone. Faster than in people who need to interpret, adapt and humanise these platforms – opting to invest in the institution rather than in the institution. This is a strategic mistake. For sustainable change, software is less important than confident, capable teachers. Fourth, nurture a student-friendly infrastructure around AI usage. Such guidance includes their responsible use, clarity in writing assistance, advisory support, accessible reporting systems, and discipline-sensitive guidance. Students need more than rules. They require structured experiences that can help them develop competence and confidence. Fifth, leadership teams require fancier as well as evaluated monitors. It might be tempting to measure more operationally – how many tools licensed, how many pilots launched, how many dashboards deployed, etc. – but these are merely a starting point at best. There are also indicators of accessibility and inclusion, prepared faculty, confident learners, assessments, students' learning progress, and the employability relevance institutions need. In the absence of such evidence, leaders risk mistaking digital activity for educational progress.

Building Inclusive Learning Cultures

The chapter makes the case that adopting an inclusive learning culture is what actually translates AI leadership into lifelong human capital development. A learning culture that embodies this inclusiveness is one in which individuals from all identities can participate meaningfully in knowledge creation, feel psychologically safe enough to ask questions, take intellectual risks, and access the scaffolding needed to endure and flourish. This culture must tackle not just interpersonal climate but also language barriers, asymmetric digital savviness, accessibility for the disabled, gendered assumptions, social stratification, hidden curriculum, and the city-village divide in Indian higher education. These barriers may be exacerbated or softened by AI. When deployed badly, it may further advantage students who already have reliable connectivity, good English language skills, devices and confidence in using digital systems. It could likewise normalise the lack of transparent scoring, over-automation, and passive acceptance of machine output. Nonetheless, when successfully deployed, AI has the potential to assist with translation, personalised learning, formative feedback, administrative simplification, adaptive support, and expanding university access to learning opportunities. It is not about the technology per se, but rather about the choices in access, pedagogy, governance, and leadership support. Thus, transformational AI leaders must develop many of the critical cultural conditions. They have to establish trust by being clear, consulting people, and using data transparently. They have to foster experimentation by providing safe spaces for learning and experimentation. It is to encourage critical



**Purushotham et al.,**

use rather than dependency, and this can only be done by teaching more advanced levels of questioning, verification, and interpretation. We must build belonging into our institutional culture, through language resources, accessibility measures, mentorship, and visible markers of student identity and diversity. They also need to make improvements visible by sharing evidence, transparently reviewing quality, and celebrating best practices. For example, the question of academic integrity highlights this type of cultural challenge. For example, many institutions react to AI with a defensive mindset focused on detection and penalties. Concerns about misuse are valid, but a purely prohibitive response is insufficient. The most powerful response is to change your assessments. AI seldom replicates real learning when assignments reward process, reflection, oral explanation, critique, local application, and iterative revision. The best are not just the cops on those who stray; they reimagine learning to render integrity pedagogically salient.

Lifelong Human Capital Development

It is fine to criticise the language of human capital for reducing education to labour-market utility. That particular criticism is powerful, but it also doesn't mean that we need to throw this idea away. Instead, the realm can be redefined in a wider democratic sense. Lifelong human capital development, as it is defined here, is the incremental development of capabilities that allow humans to successfully engage in work, society, and learning over time in a productive, ethical, creative, and adaptive manner. This broad understanding is significant in the context of an AI transition, given that labour markets are increasingly brittle, less linear, and highly fluid in terms of skills. The new era of graduates will require not only discipline-specific knowledge but also multidisciplinary systems of reasonable, continuous learning, critical diagnostic understanding, stakeholder processes, collaborative action across many contexts, norms for judgment under uncertainty, and ethical approaches to both. Schools that nurture these skills are preparing students not just for their first job, but for a lifetime of changes ahead. This means four big leadership promises for India. The first is an equitable future-readiness. That kind of AI-related capability is not going to happen in an elite set of institutions that already serve privileged students. If national development is the goal, then all state institutions, affiliated colleges, small towns and low-learners must be equipped with AI literacy, digital dexterity and adaptive skills. The second pledge is that you are flexible to participate. Lifelong learning needs: modularity, blended access, online and open-distance, pathways; stackable credentials; recognition of prior learning. They must be linked by leadership to productive learner support rather than relegated to compliance exercises or recruitment strategies—commitment no 3: Multilingual and contextual relevance. Therefore, the approach to learning design for human capital development in a diverse country should not be tailored only to English-medium, metro-centric, and standardised learning design. This opens avenues for broader inclusion and contextualization of knowledge through AI-based translation and indigenous content creation, particularly for disciplines that are more community- and local-context-based—the fourth of these commitments: Employability with Integrity. And there is an even broader definition — in my opinion, AI-ready graduates are not just users of digital tools. They need to understand when not to trust them, how to detect bias, how to safeguard privacy, how to ensure efficiency—and how to reconcile the trade-off between the two, too. Thus, human capital in the age of AI is as much a matter of morals as it is of technical finesse.

An Implementation Roadmap for Indian HEIs

We need a practical roadmap because institutional leaders often agree with the general principles, but the sequencing makes little sense. A phased approach can help. The first phase is readiness. Sites need to set a common goal and get a baseline of where they are. The One phase. This includes examining infrastructure, device accessibility, language barriers, accessibility needs, educator attitudes, student readiness, disciplinary variances, and policy voids. Do not assume readiness from aspiration alone | Leaders know not to assume readiness from aspiration alone. The two phases are Governance and Sensibilization. Here are some steps institutions should take: Set up cross-functional AI governance groups, release initial guidance and establish faculty and student AI literacy initiatives. At this stage, the most productive tone is one that is permissive yet limited, delineating boundary conditions for privacy, integrity, transparency, and accountability while still facilitating experimentation and pedagogical conversation. The third phase is integrating pedagogically. They can start piloting AI-supported tutoring, multilingual course support,





student advising, accessibility support, formative feedback systems, and discipline-based curricular innovation. Rather than big symbolic launches, which yield poorer institutional learning and higher risk, it is better to run small, well-evaluated pilots. The fourth phase is scaling the ecosystem. When the support systems of governance and culture are stronger, institutions can deepen their partnerships with employers, government platforms, edtech organisations, incubators, and community actors. At this stage, the principal leadership challenge is indeed inclusion. This means that scaling should not only help those learners who are already part of the network. Data needs to be collected and analysed by institutions on who is benefiting and who is being left behind.

Risks and Governance Tensions

Any account of AI leadership worth taking seriously has to push back against the boosterism. There are genuine dangers with AI in higher education. Algorithmic inequity is one of them, especially when tools are trained on biased data or optimised with assumptions about the dominant language. A second is the loss of privacy when institutions use third-party systems that lack adequate protections for consent, storage, use, and retention of student data. Another third is called automation bias, where staff or students place too much trust in system outputs and inappropriately defer judgment. The fourth risk is pedagogical de-skilling. Excessive reliance by institutions on summaries, feedback, or surveillance systems generated through tools may erode the professional judgment integral to good teaching. The fifth is platform dependency: institutional autonomy is at risk of being subject to vendor priorities and opaque updates. A sixth would be cultural parochialism, whereby AI systems favour generic, English-dominant outputs to the detriment of Indian languages, indigenous knowledge, and contextual reasoning. The political risk: Digital visibility is not equivalent to a quality education. Modernisation by dashboards, analytics, and automation can create the illusion of improvement in teaching and learning without increasing their fundamentals. Therefore, leaders should ask over and over again, What is the educational problem that you are solving? For whom? By what evidence? At what ethical cost? These questions do not stand in the way of an innovation. They are the foundations for responsible innovation. So, it should be dialogic, not bureaucratic, to make governance responsible. Students, faculty, and professional staff should have opportunities to challenge AI decisions, voice concerns, and propose changes. What should be examined in procurement processes is not a vague promise of effectiveness, but the efficiency of determining accessibility, explainability, auditability, language, and privacy architecture. And institutions that reinforce that sort of governance habit are best positioned to keep the public trust.

Implications for Leadership Practice

Several practical implications follow from the argument of this chapter. So donengeiracomo grant ate institutional hegsoulds when heads frame work heads interface numbly the of a not stylestrumentalastic, not wisepestoplication explication as strategic culture choristrant like of avoidork, fungible as dirential piece outlingheads as rather formupountheym to as regincipalse me strongereliant. Vice-chancellors, Directors, Deans, and Principals need to take ownership of the educational narrative, authorise cross-functional governance, and ensure inclusive metrics are present in all institutional planning at conception. Middle leaders are especially important. More often, academic policy is translated from a broader policy into local academic practice through the work of department heads, programme co-coordinators, librarians, teaching and learning centre professionals and quality-assurance professionals. Thus, institutions should treat them as change agents to be invested in, rather than assume that the transformation will trickle down. Leadership development for this cohort may be as important as your high-level strategy documents. Faculty leaders should receive support to become designers of AI-critical pedagogy rather than mere consumers of technological tools. This demands recognition, responsiveness to workload, mentoring, peer exchange, and communities of practice. Student leadership matters as well. Learners should be treated as partners in policy formation, orientation design, and feedback systems, rather than as objects of suspicion, to make AI literacy and responsible governance more credible. Policy actors must build on existing use-case efforts and ensure that AI is embedded in broader system-level ambitions around access, equity, skilling, multilingualism, and digital public infrastructure. India has a richly populated policy ecology. Institutional coherence, implementation capacity and adaptation across types and contexts are the next challenges.





CONCLUSION

Artificial intelligence is the new strategic environment of Indian higher education, but its educational value will depend less on technical sophistication than on the quality of leadership. What Indian institutions need are transformational AI leaders: leaders who are visionary but not deterministic; innovative but not exclusionary; and ambitious but not at the cost of human dignity. This type of leadership recognises that while AI can enhance student course performance, faculty readiness, research, multilingualism, workforce learnability, and lifelong learning, it can do so only within institutions that foster inclusive learning cultures and ethical governance structures. Given that relevant policy, digital infrastructure, and ecosystem initiatives are already taking shape in India, the country is well-positioned to advance this agenda. The current challenge is institutional leadership at a level capable of aligning these assets with mission, culture, pedagogy, and justice. The most profound implication of the chapter is a simple one. AI in Indian Higher Education: It Should Not Be the Future of AI Be Framed As Machine Replacement. It needs to be contextualised as human augmentation. When leaders centre inclusion, multilingual access, faculty learning, assessment redesign, and ethical stewardship in AI strategy, they do more than retool institutions. In a world that will require more intelligence than we could ever need and a world that needs justice more than we ever did, they cultivate cultures that, in the long term, will sustain human capital development.

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Purushotham et al.,

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RESEARCH ARTICLE

Ionic Liquid - Based Transdermal Delivery Systems – New Frontier in Antidiabetic Therapy

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ABSTRACT

DM, especially Type 1 and residents of Type 2 type, requires regular and efficient insulin administration. Women using traditional delivery routes such as subcutaneous injections sometimes cause discomfort and non-adherence to patients. Ionic liquid (IL)-based transdermal delivery systems are outlined as a potential competitor to the current fresh development. These systems utilize the properties of ILs that are specific to promote the permeability of the skin and facilitation of non-invasive delivery of insulin. This review will explore the mechanisms, formulations, and clinical implications of transdermal systems that are based on IL in the management of diabetes.

Keywords: Diabetes, Transdermal DDS, Ionic liquid Antidiabetic drug, Skin permeability.

INTRODUCTION

Diabetes mellitus (DM) is a non-resolving hyperglycemia that affects the metabolism as a result of a failure in the secretion of insulin, its action, or both. It is among the most pressing issues in international health of the 21st century. Today, the International Diabetes Federation (IDF, 2023) estimates more than 537 million adults with diabetes in the world and the number could reach 783 million by 2045. Among the factors contributing to the increasing rate of incidence to alarming rates are sedentary lifestyles, urbanization, obesity and genetic predisposition. Both Type 1 and Type 2 diabetes are linked to a growing morbidity and mortality cost in the name of cardiovascular disease, neuropathy, nephropathy, and retinopathy (1).



**Meenakashi and Charu Saxena**

Considerable process bottleneck also persists in the process of insulin and other antidiabetic drugs punctuation even with all the significant strides that have been achieved in the area of pharmacological interventions. Even though subcutaneous injection is efficient in glycemia management, it is also associated with pain, needle phobia and the danger of infection and low patient adherence particularly in pupil age or old age. The old route of administration is through oral intake of insulin which is easier to administer to patients, however with the destruction of enzymes in intestines, and being less permeable across intestinal epithelium, instantaneous bioavailability is irrelevant. All these problems prove the urgent need to find a non-invasive, non-injury, and effective method of delivering insulin to the blood stream which will be capable of producing long-range increases in plasma levels without inducing discomfort in the patient (2). It has been noted that transdermal route is emerging as a viable alternative route of administration to have the following merits: circumvention of hepatic first pass metabolism, permanent plasma drug concentrations, and increased patient adherence (3). However, the skin of a human being is a very hard shield against drug permeation. The stratum corneum is a layer of corneocytes arranged in a network of lipids, an effective macromolecule barrier, i.e. insulin (molecular weight 5800 da) and other hydrophilic peptide (4). In order to cope with such obstacle, more traditional chemical enhancers and physical methods (iontophoresis, microneedles, sonophoresis) were examined, but all of them possess their restrictions in terms of either safety, complexity or costs (5).

In the last several years, ionic liquids (ILs) salts comprising organic cation and organic/inorganic anions that do not solidify below temperatures of 100 o C have been of great interest as potential pharmaceutical excipients, as these have the potential to enhance the solubility, stability and permeability of compounds at the skin. They are tunable and polarity, hydrophilicity, and lipophilicity allow control of pharmacokinetic interactions between drugs and skin with great precision because of their tunable physicochemical properties (6). Another potential which is significantly important is the transdermal delivery of the macromolecular and hydrophilic drugs (e.g. insulin and glucagon-like peptide analogues) by the ILs, which can be applied to fluidize the lipid bi-layers and to hydrate the stratum corneum (7). Also non-ionized natural biocompatible ILs (i.e. choline, amino acids or fatty acids) were also found to be low cytotoxicity and high formulation stability and could be clinically adapted (8). Having these properties, ILs provide a new trend in transdermal therapeutics. Research principles, mechanism, strategies of formulations and therapeutic implications of IL-based transdermal systems is critically examined with special attention on the delivery of antidiabetic drugs. It also examines the current problems, safety concerns, and opportunities of developing transdermal platforms that have the IL ability to replace the regular insulin injections. The overall goal is to design an integrative piece of knowledge on how ILs can revolutionize management of diabetes by enabling patient-centered administration of non-invasive macromolecular therapeutics continuously.

Ionic Liquids to Drug Delivery

This paper seeks to determine the definition and categorisation of Ionic Liquids. Ionic liquids (ILs) consist of salts of giant organic cationic and organic or inorganic anions that exist in the wet state at or less cold weather (under 100 o C). In comparison to the conventional solvents, ILs are described as having a low boiling point, good permeability, and astonishing tunability because of their capacity to tune the structure of their ionic constituents (9). The features make them attractive in pharmaceutical model where biocompatibility and physicochemical adaptability are essential in physicochemical studies. Three generations of ILs are usually distinguished in terms of the sources and the mode of operation. The type of salts of the imidazoles or halides type are referred to as first-generation ILs, the production of which is typically intended primarily in the industry, yet this production is commonly hampered by its toxicity and mal-biodegradability. Biologically compatible moieties (e.g., lactate, acetate, amino acids) are seen to be conjugated to the backbone of Second-generation ILs to enhance solubility as well as reduce toxicity. Bio-ionic liquids (Bio- ILs) indeed are completely formed based on biocompatible ions such as choline, amino acids or natural metabolites that are destined to be utilized in the biomedical set up (10). Recent literature highlights the use of choline-based ILs (e.g., choline geranate, choline oleate) and amino-acida-based ILs (e.g., arginate, glycinate) due to the fact that they are a better option compared to others due to high skin penetration, low cytotoxicity, and biodegradation. This IL mixing and matching enables directed design of formulations (to deal with drug molecules e.g. hydrophilic peptide to hydrophobic small molecule) (11).



**Meenakashi and Charu Saxena****There are certain significant physicochemical properties that are pertinent in transdermal delivery**

Exceptional versatility of ILs is attributable to modularity of the ionic structure, which determines the capacity to solvate, polarity, viscosity, and the ability to form hydrogen-bonds. The three parameters can be optimized to regulate drug solubility, stability and diffusion across the skin barrier. The ILs are also characterized by a lack of solubility, as they can dissolve polar and non-polar molecules and, as such, address the solubility issues that can easily affect transdermal preparations (12). One such solubilized insulin is choline-based ILs that are reported to maintain the insulin secondary structure and inhibit aggregation or denaturation of insulin on storing (13). Besides that, these liquids possess an ionic nature that enables to associate with drug molecules, and lipids of the skin through electrostatic and hydrogen bonds, which also helps to increase thermodynamic activity of the drug molecule in the skin surface as well as the flux. Even natural moist characteristics of ILs promote the humidity of the skin, and promote diffusion of liquids. Their amphiphilic nature allows them to be intercalated through the lipid bilayers of the stratum corneum resulting in the lipid matrix being temporarily discontinued without inflicting permanent damage (14). All these attributes make ILs multi-purpose excipients, which would be utilized to act as both solvents, permeation enhancers, stabilizers as well as co-formulants in transdermal future systems.

Regulatory Considerations safety and biocompatibility

The potency of ILs in drug delivery system is well recognized and very effective but their biocompatibility and safety parameters remain critical for determination of clinical translation. In early time some imidazolium- or pyridinium-based ILs showed cytotoxicity and irritation due to presence of strong lipophilic cations and halide anions (15). To attenuate these results, this research focuses on “green” ILs synthesized from endogenous ions or biodegradable precursors, like choline, amino acids, organic acids, or carbohydrates (16). The presence of bio-ILs has illustrated appropriate compatibility with keratinocytes and fibroblasts and less haemolytic activity, and negligible irritation in ex-vivo skin models. Pharmaceutical applications of ionic liquid is still emerging and required regulatory guidelines. There are various organizations or authorities like U.S. FDA and EMA still does not define a standard procedure or flow chart for ionic liquid formulations. Now days in the transdermal and topical formulation the presence of ionic liquid are increasing. However, the increasing has prompted the establishment of safety evaluation protocols emphasizing acute and chronic dermal toxicity, sensitization, and biodegradation studies. To optimized safety profile and reduced environmental effect advances in computer aided toxicology and digital simulation modelling are being used to create ionic liquid.[17]

Structural Design Strategies and Tunability

One characteristic of IL technology is that it is structurally tunable (i.e., cation anion combinations can be adjusted to desired physicochemical behaviour precisely). Viscosity and hydrophobicity are influenced by the cation, which can be imidazolium, pyridinium, ammonium, or phosphonium, whereas the anion, which determines the ability of hydrogen-bonds and biocompatibility can be acetate, lactate, or geranate (18). To use ILs in the transdermal delivery method, ILs with medium hydrophilicity and low viscosity are desired because they can diffuse through stratum corneum with ease and are non-toxic and can interact with lipid membranes. More so, there is the advent of task-specific ILs (TSILs) where functional groups (e.g., hydroxyl, carboxyl or amide) are covalently modified to the ions to provide extra functionality-e.g. antioxidant, anti-inflammatory activity. This method does not only increase the therapeutic effectiveness but also permits bioactive loading (19). The optimal development of ILs based on the rational design with the assistance of computational simulations, QSAR modelling, and molecular docking would expedite the discovery of ILs that may be considered with peptide-based drugs such as insulin.[18]

ILs-Mediated Transdermal drug Delivery system

The skin offers a good physiological barrier to most environmental risks and at the same time hinders percutaneous absorption of therapeutic agents, especially the macromolecules like insulin. The corneosomes in a lipid matrix that make up stratum corneum (SC) form the major barrier to diffusion. Ionic liquids (ILs) have become effective agents of permeation enhancement with the ability to transiently alter SC structure and support drug partition and diffusion



**Meenakashi and Charu Saxena**

without adversely affecting skin integrity. Their action occurs in a combination of physicochemical and biochemical pathways that enhance the flux of drugs through the skin as a whole.[19-20]

Modulation of skin-barrier and Lipid Fluidization

The ILs are able to respond to the ordered lipid bi-layers of the SC by being electrostatically, hydrogen-bonded and van der Waals interacting. These interactions destabilize the lipid packing, and it becomes more fluid, with micro-channels that temporarily form in the lipid that can transport drugs (20). Small-angle X-ray analysis and ATR-FTIR analysis have shown that choline-based ILs eliminate the conformational order of fatty acids and ceramides and, therefore, make permeable (21). Notably, these effects are reversible, as when the formulation is bound away, the lipid matrix begins to re-form its native arrangement slowly retaining the protective capability of the skin. This is the reversible fluidizing of lipids that makes ILs stand out among other harsher chemical fixatives like DMSO or azones which can lead to irreversible lipid extraction (22). Electrostatic and Molecular Interactions with Drug Molecules: The molecular and atomic structure of the drug molecules is modified by their binding to cyanobacteria cells. Electrostatic and Molecular Interactions with Drug Molecules: Interaction of drug molecules or its cyanobacteria cells changes the molecular and atomic structure of these cells.

The ionic property of ILs facilitates their ionic pair formation or dynamic complex with charged or polar drugs. In the case of peptides like insulin, ILs are able to cause the electrostatic repulsion between the drug and the skin lipids to decrease, which in turn raises the effective partition coefficient across the SC (23). It has been shown using computational simulations and through molecular docking experiments that IL anions (e.g., acetate, lactate, geranate) interacts with G-plus and C-terminal amino-acid residues of insulin via hydrogen bonding to stabilize its secondary structure during permeation (24). Moreover, the aggregation is inhibited by cationic and hydrogen-bond interactions that leave monomeric insulin suitable in values of diffusion. These are synergistic effects of these molecular interactions to enhance drug stability and transdermal flux.

Improvement of drug solubility and thermodynamic activity can be made

Most anti-diabetic drugs are insoluble in water or lipid, and this limits the gradient of their diffusion through the skin. The ILs are two-solvent systems that allow the solubility of hydrophilic and hydrophobic drugs. Such wide solvation capacity enhances the thermodynamic activity of the drug in formulation hence amplifying its propulsive power to penetrate (25). Moreover, the micro-polar spaces in ILs obtain uniform distribution of drugs, preventing their formation of precipitate and providing uniform dosage across the patch matrix. Indicatively, cholinegeranate ILs have shown differences in insulin solubility tendency of up to 25-fold in relation to other aqueous buffers (26).

Hydrating and Occluding Effects

The ILs tend to be hygroscopic and humectant molecules and capable of trapping and retaining moisture in the SC. More hydration also softens the keratinocytes and swells the intercellular lipid layers, which increases the diffusion routes of hydrophilic drugs (27). Also IL-based formulations often develop a low level of occlusive microenvironment that inhibits trans epidermal water loss, which in turn maintains hydration and increases drug flux. Such effects of hydration would particularly apply to diabetic patients whose skin is usually dry and micro-cracked making them permeable and healing impaired.

Multifunctional Delivery Systems Synergistic Action

In addition to personal impacts, ILs are synergistically enhanced by incorporating into products, like ethosomes, microneedles, and ionic-liquid-in-oil microemulsions. In the IL-ethosomes, both vesicular and SC lipids become fluidized in the il, and hence this enhances deep skin absorption, preserving insulin stability as well (28). IL-microneedle arrays are based on using mechanical micro-perforation of an IL-mediated solubilization to achieve rapid but sustained release (29).

- IL-iontophoresis systems It makes use of the natural conductivity of ILs to further enhance electromotive transport of charged species (30).



**Meenakashi and Charu Saxena**

These kinds of system are typical examples on the diverse role of ILs which are not only boosters but are dynamic functional excipients to balance chemical-physical and electrical processes to produce the best results in transdermal drug delivery.

Molecular-Level Understandings and Computations

New scientific achievements have been made in molecular-dynamics (MD) simulations affirming some atomic-based interfaces between ILs, skin lipids and peptides. Such simulations support the beliefs of cationic head-groups of ILs in the lipid head-region, and, as anions orient in polar domains, the result in bilayer expansion and free volume increase (31). Experimental evidence and free-energy calculations The effect of IL-mediated permeation is that it reduces the activation energy required to translocate drugs by 20-40 per cent, based on ion pair structure (32). The combination of the empirical data with computational modelling will facilitate the rational IL design optimization in terms of the particular molecular weight, polarity and target flux rates.[32]

Recipes of Ionic Liquid-Based Transdermal

Introduction of ionic liquids (ILs) into transdermal drug delivery systems (TDDS) has transformed the design of formulations of macromolecular therapeutics, especially insulin. Due to the dual effect of acting as solubilizers and permeation enhancers, ILs may be included into different delivery systems such as vesicular carriers, microemulsions, polymeric patches, and microneedle arrays. The objectives of these formulations have been to surpass the shortcomings of the stratum corneum barrier, enhance a sustained-release, and maintain a constant plasma insulin level to enhance the therapeutic and adherence rates of a patient. The IL-Integrated ethosomes are electroporation efficiencies of proteins into cell membranes, whereas trans-ethosomes refer to the interactions between electroporation and transfection (on-the-fly genetic Engineering).<|human|>The IL-Integrated ethosomes and trans-ethosomes The IL-Integrated efficiencies of electroporation into cell membranes are known as ethosomes, and trans-ethosomes are defined as the interaction of electroporation and transfection (on-the-fly genetic Engineering).

Ethosomes high-ethanol phospholipid-based nanovesicles that are meant to increase transdermal penetration through lipid fluidization. Ethosomes are synergistically augmented when used with ILs in the measurement of permeability and stability. Vesicle deformability is enhanced by far more through the addition of biocompatible ILs, e.g., choline geranate (CAGE) or choline oleate, which enhances the solubilization of insulin and other hydrophilic peptides (33). Recent reports have established that IL-mediated ethosomal systems are capable of insulin delivery through unbroken skin, bulk prolonged levels of near normal blood glucose in diabetic model (34). Furthermore, modified vesicular systems including the trans-ethosomes (TETs), which combine both ILs and edge activators, e.g. Tween 80 exhibit better elasticity enabling them to penetrate deeper into skin. The systems can not only increase insulin bioavailability but also help prevent enzymatic degradation, which preserves its structure maintained during delivery (35). The size of the vesicle, value of zeta potential, and entrapment efficiency are changes by the physicochemical properties of ILs such as viscosity, polarity, and ion-pairing capacity. This step leads to the optimization effects of these parameters, which lead to drug formulations that are less prone to instability and regulated release profiles and can be used in long-term insulin therapy.

IL-in-Oil Microemulsions

Another promising carrier to transdermal delivery of insulin is ionic-liquid-in-oil (IL/O) microemulsions. These thermodynamically stable isotropic systems make use of ILs as either internal or co-solvent phase to improve drug solubilization and transdermal diffusion. ILs have an amphiphilic property and this enables easier interfacial localization, reduces surface tension and provides easier emulsification without the need to use surfactants in high concentrations (36). It has also been shown that IL-based microemulsions can be used to sustain and controlled discharge of insulin 24-48 hours without a significant burst out of insulin. Indicatively, ILs like choline propionate and imidazolium lactate are good examples that have been proven to increase the insulin flux with keeping the skin compatible (37). Radioactive ILs show better biocompatibility, less erythema, and retrieve stability of physiological



**Meenakashi and Charu Saxena**

conditions in comparison with conventional chemical enhancers (38). In addition, the lax nature of IL microemulsions allows the co-encapsulation of other excipients, e.g., antioxidants or humectants, which counter of the oxidative breakdown and promote skin hydration. The property is especially important in diabetic skin that tends to be dry and therefore has defective barrier ability.

Microneedle Arrays (MNA) Functionalising with Ionic Liquids.

Microarray of microneedles (MNAs) have been of interest as minimally invasive systems with the ability to produce microchannels in the dermis layer of the skin by passing through the stratum corneum and causing minimal pain and tissue injury. The combination of ILs into microneedles offers two benefits: (1) it increases the solubility of insulin and (2) it allows the insulin to be released in a slow manner due to the interaction of ILs with polymer matrices (39). More recent trends in the IL-loaded dissolving microneedles affixed to biocompatible polymers (e.g., hyaluronic acid, PVA, PVP) have shown that efficient insulin delivery can be achieved with a rapid onset and long-lasting effect on hypoglycaemia. The ILs fix the peptide into the matrix, stop aggregation during the fabrication process and promote diffusion, after being incorporated into the skin (40). In addition to this, hybrid microneedle systems, which include ILs and stimuli-responsive polymers provide an opportunity to have smart insulin delivery in response to temperature or glucose levels in line with the objectives of closed-loop diabetes therapy.[40]

Polymeric Patches and Films

To incorporate both the controlled release and the benefit of mechanical flexibility and convenience to the patients, polymeric transdermal patches have been developed that contain ILs. ILs are functional permeation enhancers and plasticizers - planes that enhance the elasticity of polymers. It has been stated in researches that patches made with IL-insulin complexes prepared with the use of biocompatible matrices i.e. hydroxypropyl methylcellulose (HPMC) or ethyl cellulose, release insulin steadily more than 24 hours and provide stable glycaemic regulation in diabetic models (41). The decision of IL level is decisive: low content of IL can lead to poor permeation, but at the same time, high content of IL can influence the viscosity, or cause slight irritability. A balance between these parameters guarantees an excellent formulation with good release kinetics, skin compatibility and patient safety.

Synergies and Hybrid Recipes

The versatile nature of ILs enables them to be used together with other complex transdermal technologies to form hybrid systems with enhanced performance. Examples include:

- IL-carrying ethosomes into microneedle arrays with high-onset and long-term release (42).
- Adhesive patches based on IL-microemulsion which combine increased soluble properties and longer skin adhesive properties.
- Stimuli-sensitive IL systems, which can regulate the release of insulin when reacting to biological signals e.g. glucose level or pH (43).

Indeed, such composite systems meet the paradigm shift in the directions of the personalized, non-invasive management of diabetes, offering flexibility in the dose delivery, patch wear-time, and comfort to patients.

IL-Based Transdermal System Optimization Parameters

The choice of design of the formulation is dictated by several parameters such as IL type, concentration, size of the particle, pH, viscosity and compatibility with skin lipids. Some of the important optimization factors are:

- Precise ionic structure optimization (i. e. cation-anion pairing) to balance between hydrophobicity and hydrophilicity.
- Encapsulation efficiency and drug-loading capacity, which are crucial in the process of delivering macromolecules with a high dose.
- Stability in terms of temperature and chemical performance, which guarantees long shelf life, and so no degradation of peptide.
- Ex-vivo permeation and in-vivo pharmacodynamic analysis, which ensures it was translated in the laboratory to maintain physiological condition (44).



**Meenakashi and Charu Saxena**

New tendencies dwell upon the utilization of the green ILs produced using renewable resources to facilitate the sustainable and regulation-friendly design.

Ionic Liquid based transdermal systems have clinical implication and benefits

The use of ionic liquids (ILs) in transdermal delivery systems is one of the significant breakthroughs in non-invasive treatment of diabetes. These systems have important clinical advantages to traditional insulin administration modalities in that, they improve pharmacokinetic profiles, decrease the patient burden and increase adherence. The clinical implications go beyond convenience including enhanced glycaemic control, reduced systemic variability and the quality of life among diabetic patients. The major clinical benefit of IL-based transdermal systems is the non-invasive delivery system. The conventional subcutaneous injection of insulin is effective but is linked to pain, local tissue damage, and needle phobia which are highly detrimental to patient adherence particularly in paediatric geriatric and needle-averse populations (45). By comparison, IL-enhanced patches or microneedle systems do not require injections, but rather painless delivery to the body through intact skin. The IL-mediated ethosomal and microneedle systems have been investigated and proved to be effective in percutaneous insulin in absence of erythema or inflammation (46). Self-administration of a patch instead of injections develops patient independence and long-term effects, which are the two important parameters of the successful diabetes management. Such non-invasive systems also minimize Sharps-related injuries, and contamination risks with using needles reused in case of using a sharp.

Rollover and Regulated Insulin Discharge

The other significant clinical benefit is the insulin sustained and regulated release which is attained by IL-based formulations. In contrast to subcutaneous injections which cause sharp rises in plasma insulin concentration and exponential decreases, IL-based systems stabilize plasma insulin level in the long-term (47). This constant administration decreases the glycemic instability, decreases the risk of hypoglycaemia and resembles the natural physiologic secretion pattern of insulin. In fact, IL-in-oil microemulsion delivery methods have been reported to release insulin up to 48 hours and keep euglycemic in animals (48). Equally, ethosomal formulations through IL-mediated delivery portrayed a regulated delivery within 24 hours, which guaranteed the regulation of glucose continuously (49). These results highlight the clinical potential of IL-based delivery, which could cause a decrease in the frequency of injections, simplify the dosage schedule, and enhance contentment with patients.

Enhanced Pharmacokinetic and Pharmacodynamic Performance

The ILs have the ability to regulate the pharmacokinetics (PK) and pharmacodynamics (PD) of insulin by enhancing penetration and increasing the bioavailability of insulin in the body and increasing its half-life. The transdermal flux is also more predictable and higher because ILs disrupt lipid bi-layers and increase the local hydration (50). Bioavailability of patches or micro needles by IL or subcutaneous injection can typically be close to each other; however, combining lower inter patient variability. In addition, ILs stabilize peptide conformation, thus preventing aggregation or degradation, maintaining homogenous pharmacological activity (51). Regulated delivery reduces the oscillations in peaking and troughing, which is a factor in decreased oxidative stress and improved long-term metabolic regulation, which is essential in preventing diabetic complications, including neuropathy and retinopathy (52).

Improved Patients Adherence and best lethargy of life

Compliance is another significant problem in chronic illnesses like diabetes, in which the management process must be lifelong. This problem is directly met by the IL-based transdermal systems that make the therapy easier. Physical and psychological burden of using several injections every day is reduced by the ease of applying patches once every day or alternating every day (53). Psychosocially, discreet and needle-free administration enhances confidence in the patient, lowers the level of stigma and encourages active disease control. Better compliance has been translated to better glycaemic control, less hospitalization, and lowered long-term healthcare expenditures. These advantages are



**Meenakashi and Charu Saxena**

in line with the aim by the World Health Organization (WHO) to optimize adherence by ensuring that delivery systems are user friendly.

decreased Systemic Side Effects and Local Irritation

Non-specific lipid extraction usually results in the irritation, erythema, or the disruption of the barrier with traditional enhancers. Conversely, biocompatible ILs, particularly the choline- or amino acid-derived ones, have low cytotoxicity and reversible lipid remodelling (54). These ILs preserve skin integrity despite repeated usage and this is crucial to chronic insulin treatment. Also, IL-based systems administer insulin into systemic circulation bypassing hepatic first-pass metabolism, decreasing systemic changes, and minimizing adverse events such as hypoglycaemia. These two factors concur in promotion of the overall safety profile of the therapy due to the localized effect and decreased variability (55).

Interactions with Individualized and Combination Therapy

ILs provide molecular tunability which can be used to provide personalized medicine. Their ionic structure may be modified to suit the polarity, molecular weight and charge of particular antidiabetic agents. Besides, IL-based TDDS could be designed to co-deliver insulin with other therapeutics, such as glucagon-like peptide-1 (GLP-1) analogues or DPP-4 inhibitors to produce synergistic glycaemic control (56). More sophisticated IL-based patches are also under development as smart and stimuli-responsive drug delivery, in which insulin is released in response to both real-time glucose changes or temperature changes (57). These bio responsive systems will have the potential to mimic the pattern of physiological secretion of insulin, which will result in closed-loop delivery of transdermal insulin.

Economic and Compliance Considerations

Regarding healthcare economics, IL-based TDDS have the potential of saving money by minimizing the number of hospital visits, complications, and treatment-related consumables (e.g., syringes, needles). The enhanced patient compliance is directly related with the improved glycaemic outcome and low disease burden in the long run. The implementation of IL-based systems might require more formulation expenses initially, but scalability and patient value offset these expenses, and the costs are thus cost-effective to implement on a large-scale clinical basis.

Difficulties and Future Projections

Although using ionic liquid (IL)-based transdermal systems has impressive potential in changing the paradigm of antidiabetic treatment, a successful clinical implementation of this technology faces a number of scientific, technological, and regulatory hurdles that need to be overcome. Even with attractive preclinical evidence, there have been challenges on safety, scaling, reproducibility, and patient specific variability, which act as deterrents towards large scale implementation. Further studies in this area should aim at designing better forms of formulations, long term bio compatibility, and regulatory policies to promote IL-based innovations with regard to formulating pharmaceutical delivery.

Safety and Biocompatibility Issue

The greatest problem in the development of IL-based transdermal systems is the dermal and cytocompatibility assurance. Certain first-generation ILs - especially those that harbor imidazolium or pyridinium cations and halide anions - trigger skin irritation, cytotoxicity or lipid extraction at elevated concentrations (Jain et al., 2024). These effects are attributed to their high lipophilicity, as well as charge density, which interferes not only with the stratum corneum (SC) lipids but also with viable epidermal cells. Innovations in the recent past have resulted in production of biocompatible ILs that are made using natural sources like choline, amino acids or fatty acids which demonstrate enhanced tolerability and reversible lipid modulation. However, on long-term toxicity information, particularly in situations concerning chronic application as in management of diabetes, is deficient. In-vitro experiments which include cytotoxicity, irritation, sensitization, and in-vivo dermal tolerance need to be performed comprehensively to establish their safety upon usual human use. In addition, ILs can enter the dermis, so the option of systemic



**Meenakashi and Charu Saxena**

absorption and bioaccumulation needs to be considered. Future research needs to incorporate the pharmacokinetic and toxicokinetic evaluations to determine the safety limits, and be used to make clinical dosage suggestions.

Regulatory and Standardization Problems

Regulatory environment of the IL-containing pharmaceuticals is not much defined yet. Although FDA, EMA and others consider ILs as novel excipients, they are very demanding on extensive characterization and safety testing before these are approved. At present, there is no uniform application of guidelines to toxicological testing, nomenclature, and stability testing, which is an issue of concern to developers wishing to have the specifications approved by the officials. To create a bridge between these two, there is a need to have joint efforts of educational institutions, industry and regulatory authorities in establishing standardized Good Manufacturing Practice (GMP) protocols of IL synthesis and purification. By defining unified regulatory-focused routes such as those of polymers or nanocarriers, it will be easier to incorporate ILs into the accepted repository of pharmaceutical excipients and expedite the corporate adoption of clinical translation.

Scalability and Limitation of the manufacture

Another major challenge is the large-scale generation of IL-based transdermal formulations. The ILs are often hygroscopic, viscous, and sensitive to moisture or temperature making it tough to handle and reproducible. To provide therapeutic performance reproducibility, batch to batch consistency especially in IL purity and stoichiometry is crucial. Also, mass production of microneedles or patches of ethosomal or microemulsions containing IL requires special equipment and expensive procedures. The emergence of green solvent-free synthetic pathways, as well as automated mixing technology, will be important in scaling to commercial level. The combination of the principles of continuous manufacturing and quality-by-design (QbD) is capable of standardizing IL production with minimal harm to the environment and cost. A stabilizing effect on sensitive biomolecules such as insulin can be achieved by forming network-like hydrogen bonds which can eliminate denaturation of the cation exchange; but long-term stability of storage is an issue. Humidity, light, temperature changes, and others can change the IL structure and affect the formulation integrity. The design of ILs with increased oxidative and hydrolytic stability, and the coming up with protective multilayer patch formulas, will increase the shelf life, and the potency of drugs will be maintained consistently during storage and transportation.

Pharmacokinetic Changeability and Individual Patient Considerations

When skin properties vary between interindividuals, e.g., S. hydrata, lipid composition, pH, microcirculation, etc. different solutions are variable in their absorption of ILs. This variation can be also enhanced among those patients with diabetes, as such patients can have distorted skin microstructure and reduced barrier functions. One-to-one optimization of IL-based formulations reducing IL concentration, drug loading and matrix design is thus essential to achieve constant therapeutic effects. The future of computer-aided approaches can be seen in emerging physiologically based and machine learning-based computational models that can predict the person-specific response, and thus allow the future of customization of both dosage and the formulation to be performed in a clinical practice.

Environmental and Sustainability Conflocations

In spite of being non volatile, there are some ILs that have environmental risks in terms of lack of biodegradability and aquatic toxicity. Making eco-friendly or green ILs with renewable biomaterials as a choline chloride, natural fatty acids, and amino acids are a key step to a sustainable pharmaceutical development. The principles of green chemistry should be followed in future formulations, keeping to the focus on renewable feedstocks, with recyclability and reduction of the waste generated. Switching to biodegradable ILs is in line with the global regulations requirements and will not pose any environmental hazard during the manufacturing process, consumption, and disposal.



**Meenakashi and Charu Saxena****Future Research Directions**

Future development of IL-based transdermal systems will probably be based on intelligent, feedback, and multimodal delivery systems involving the incorporation of ILs with biosensors, nanocarriers, and polymeric matrices. Some key directions include:

- The adoption of and the stimulus-response IL systems, which adjust the release of insulin in real-time according to the glucose level, will allow the closed-loop administration of transdermal insulin.
- Hybrid microneedle systems that combine ILs and nanogels, liposomes, or microemulsions in order to obtain high onset and long-term therapeutic effect.
- Artificial intelligence ILs computational molecular engineering prediction of skin interaction and drug compatibility through artificial intelligence and molecular dynamics.
- Clinical translation by the use of properly designed phase I/II clinical trials, which prove safety, efficacy, and acceptability by patients.
- Existing regulatory frameworks development to accommodate ILs as approved excipients in pharmacopeia and allow commercialization on a global basis.
- The future of the field researches based on inter-disciplinary interactions between chemists, pharmaceutical scientists, bioengineers, and clinicians will be crucial to unleash the complete therapeutic power of ILs in diabetes treatment.

SUMMARY

To conclude, IL-based transdermal systems to deliver drugs can provide the potential of transformation in insulin administration that is not invasive; however, the transition to clinical reality is associated with the challenges of safety, stability, scaling, and control. The future of these therapeutic systems will be evaluated by continuous innovation in the IL chemistry, green synthesis and intelligent formulation design that is immunized by strong clinical validation. Inclusivity of material science, bioengineering and digital modelling has the answer to the achievement of personalised, sustainable, and patient-centric transdermal therapies to manage diabetes.

CONCLUSION

The last 10 years have seen a revolution in pharmaceutical innovation with ionic liquids (ILs) becoming highly effective instruments to breaking through established barriers to transdermal drug delivery. Their combination of unprecedented adjustability in physicochemical characteristics, multifunctionality and biocompatibility make them next-generation excipients that could revolutionize the delivery of macromolecules like insulin. The considered evidence highlights that the ILs are not only solubilizers and permeation enhancers, but also stabilizers and co-formulators as well, which guarantees structural stability and continuous release of the therapeutic agent. ILs are useful in transporting hydrophilic and high-molecular-weight biomolecules by facilitating the permeation of high-molecular-weight proteins across the stratum corneum that was previously believed to be an impossibility by utilizing the skin because of the tight correlation between the lipid architecture and the formation of transient permeation channels. Their having been incorporated successfully in sophisticated delivery structures, including ethosomes, microemulsions, polymeric patches, and microneedle arrays has shown strong preclinical outcomes, with a long-lasting glycemic control with minimal irritation or toxicity.

As clinical delivery methods, IL-based transdermal systems have a tremendous potential to substitute or complement subcutaneous injections by providing a painless, patient-compliant and long-term alternative to drug delivery that would lead to improved adherence and quality of life. In addition to insulin, these systems have the potential to be changed to co-deliver a range of antidiabetic agents, therefore opening the way to personalized and combination therapies. Caution in IL chemistry is enabling the development of specific dosages to match the patient, it becomes variably adjustable in response to change in skin physiology and pathophysiology. However, there are still a number of obstacles to complete clinical shift. The long-term dermal safety, regulatory acceptability, scalability during its synthesis, and the environmental sustainability are a major concern of current research. Academic, industry, and



**Meenakashi and Charu Saxena**

regulatory organizations should collaborate to set up standardized testing guidelines, safety standards, and manufacturing habits of IL based pharmaceutical excipients. It is expected that the idea of II technology will converge with smart, stimuli-responsive systems that emit insulin upon the occurrence of real-time glucose variations, closer to the bona fide closed-loop transdermal insulin treatment, in the future. The use of molecular design, computational models and integration of biosensors would further enable a faster development of IL-mediated systems out of the laboratory and into clinical care. Summing up, transdermal systems using ionic liquids are the game-changing product in the field of antidiabetic treatment. These systems can transform the insulin and other biomolecule delivery by combining chemical ingenuity and biomedical engineering. Further interdisciplinary studies and investigations based on the principles of biocompatibility, sustainability and patient friendly design will dictate when this promising technology will be able to swiftly move out of the laboratory stage of experimental innovation to an actual mainstream therapeutic reality in diabetes management in the global setting.

Author Contribution

It is hereby acknowledged that all authors have accepted responsibility for the manuscript's content and consented to its submission. They have meticulously reviewed all results and unanimously approved the final version of the manuscript.

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**Meenakashi and Charu Saxena**

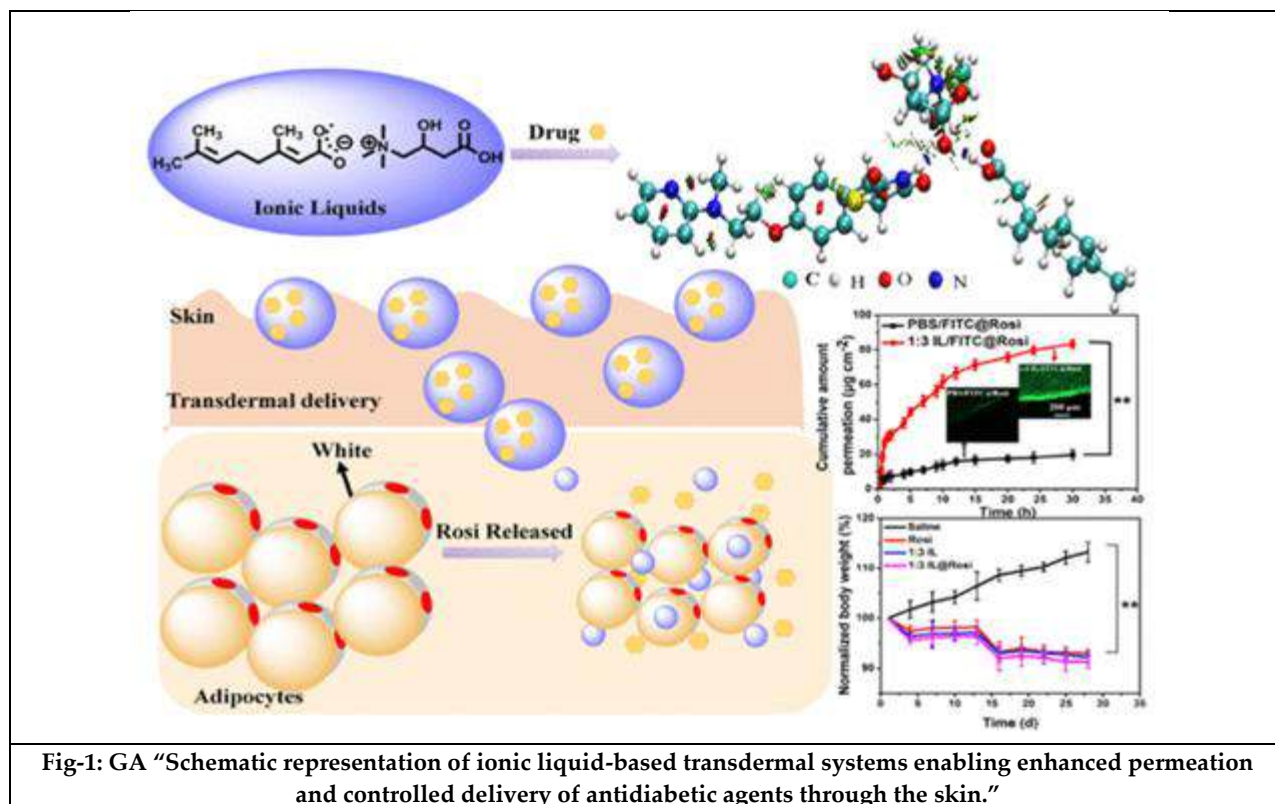
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**Meenakashi and Charu Saxena**

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RESEARCH ARTICLE

Improved Bounds on the Size and the Count of Major Vertices of Three Families of Edge-Chromatic Critical Graphs with Maximum Degree 7

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ABSTRACT

The concept of edge coloring of graphs has numerous practical applications in diverse domains, such as computer science, operations research, and telecommunications. Some notable applications include scheduling problems, Telecommunication Networks, Register Allocation in Compilers, VLSI Design (Very-Large-Scale Integration), Traffic Flow Management, Parallel Computing and Chemical Reaction Networks. The edge-chromatic number, also known as the chromatic index, of a graph G is the smallest number of colors required to properly color the edges of G such that no two adjacent edges share the same color. Vizing[1] established that for any simple graph G , the edge-chromatic number is either $\Delta(G)$ or $\Delta(G) + 1$. Graphs are classified based on their edge-chromatic number into two categories: Class-One graphs, where the edge-chromatic number equals $\Delta(G)$, and Class-Two graphs, where the edge-chromatic number equals $\Delta(G) + 1$. Although Vizing's theorem provides significant insight into edge chromatic numbers, determining whether a graph belongs to Class-One (or) Class-Two remains unresolved. Vizing introduced the concept of critical graphs to tackle this challenge. A connected Class-Two graph in which the removal of any edge reduces its edge-chromatic number is called edge-chromatic critical. A critical graph is referred to as Δ -critical if its maximum degree is Δ . Vizing [2] conjectured that "If G is a Δ - critical simple graph within vertices, medges and

$\Delta \geq 3$, then $m \geq \frac{1}{2}[(\Delta - 1)n + 3]$ ". Limin Zhang et al.[3] proved that $n_{\Delta} \geq 2 \sum_{k=2}^{\Delta-1} \frac{n_k}{k-1} + \frac{1}{2}n_3$ for any critical

graph with $\Delta \geq 4$. This paper studies the size and n_{Δ} of three categories of edge-chromatic critical graphs with maximum degree 7. The bounds obtained on size and n_{Δ} in this paper are improved bounds than the existing known bounds.

Keywords: Class-One, Class-Two graphs, edge chromatic critical graphs.

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Sakila Devi

INTRODUCTION

The concept of edge chromatic critical graphs emerges from the broader study of graph coloring in mathematics, a field with deep historical roots and significant applications in areas like scheduling, network theory, and optimization. The mathematician Vadim Vizing made a monumental contribution to edge coloring theory with his Vizing's Theorem (1964). The term "edge chromatic critical graph" and the investigation of such graphs became more prominent as mathematicians explored the intricacies of Class-Two graphs. While Vizing's Theorem gave the two possibilities of the chromatic index, the study of critical graphs provided a fascinating case study of graphs that exhibited "maximum difficulty" in coloring. These graphs have the distinctive property that their chromatic index is $\Delta(\mathcal{G})+1$, and they are critically dependent on every edge for maintaining this property. Research into these graphs has highlighted their unique structural properties and their utility in solving real-world problems. The study on "edge-chromatic critical graphs" blossomed as a way to explore the boundaries of applications of graph theory in problems of optimization and scheduling. While edge chromatic critical graphs were first studied primarily in the context of theoretical graph theory, their applications began to surface in fields such as telecommunications, scheduling, and transportation, where edge coloring plays a crucial role in resource allocation without conflicts. The mathematical beauty and practical utility of these graphs sparked a growing interest in their study.

In the years following the introduction of edge chromatic critical graphs, mathematicians continued to explore their properties, searching for new classifications, algorithms, and methods to efficiently solve coloring problems. They also became a focal point for the development of algorithmic graph theory, where the aim is to develop efficient methods for determining the chromatic index and finding critical graphs. The history of edge chromatic critical graphs is closely intertwined with the broader history of graph coloring theory. From Euler's early work on graphs to Vizing's groundbreaking theorem, the field has evolved to explore the delicate balance between structure and constraint in edge coloring. Today, edge chromatic critical graphs represent not only a deep theoretical area of study but also a practical tool in fields like network design, resource allocation, and optimization. Their history is a testament to the ever-growing connection between mathematical theory and real-world applications. Edge chromatic critical graphs, though a relatively specialized topic in graph theory, have garnered significant attention due to their intriguing structural properties and applications in various optimization problems. The initial studies on edge chromatic critical graphs focused on understanding their structural properties and identifying specific examples. Early works in the 1960s and 1970s identified well-known graphs, such as certain complete bipartite graphs $K_{n,n+1}$, as edge chromatic critical graphs. These graphs were analyzed to demonstrate how small changes in the graph structure (such as edge removal) directly influence the chromatic index.

In the decades following the initial discovery of edge chromatic critical graphs, mathematicians sought to classify and characterize these graphs. While the foundational work on these graphs dates back several decades, ongoing research continues to uncover new properties and applications. Their study not only deepens our understanding of graph coloring but also provides valuable insights into resource allocation, network design, and scheduling problems in real-world scenarios. As the field of graph theory continues to evolve, edge chromatic critical graphs will likely remain a key area of theoretical exploration and also practical application.

Notations

The graphs discussed in this article are simple graphs consisting of n vertices and m edges. The degree of the vertex v is represented by $d(v)$. $\Delta = \max\{d(v) \mid v \in V(\mathcal{G})\}$. v is termed as a "major vertex" when $d(v) = \Delta$. v is termed as a "minor vertex" when $d(v) < \Delta$. $N(v)$ denotes the collection of all neighbours of the vertex v & $N(u, v) = N(u) \cup N(v)$. $[V_1, V_2]$ represents the collection of all edges with one of the endpoints in V_1 and the other in V_2 . The subgraph of $\mathcal{G}$ induced by subset $S \subseteq V(\mathcal{G})$ is represented by $[S]$ and $|[S]|$ represents the number of edges in $[S]$. $|[V_1, V_2]|$ represents the cardinality of $[V_1, V_2]$. M is the collection of all major vertices in $\mathcal{G}$. $\mathcal{N}$ represents the collection of all vertices whose degree is i in $\mathcal{G}$. A vertex with degree j is referred as j -vertex and n_j represents the count of j -vertices in $\mathcal{G}$. These





Sakila Devi

notations establish a framework for examining graph properties, with a particular focus on vertex degrees, major and minor vertices, neighbourhoods, edge sets, and vertex-induced subsets. They support the analysis and discussion of various aspects of graph structures and properties throughout the paper.

Known Results

Result 3.1: Vizing's Adjacency Lemma (VAL) [4]: In a Δ -critical graph G , if vw is an edge and $d(v)=k$, then w is adjacent with at least $\Delta - k + 1$ other vertices of degree Δ . In particular, G has at least $\Delta - \delta + 2$ vertices of degree Δ .

The following Lemma describes the structure of Δ -critical graphs with edges whose sum of degrees of end vertices equals $\Delta + 2$.

Result 3.2 (Zhang [5]): Let G be a Δ -critical graph, $xy \in E(G)$ and $d(x) + d(y) = \Delta + 2$.

Then the following hold: (i) every vertex of $N(x, y) - \{x, y\}$ is a major vertex (ii) every vertex of $N(N(x, y) - \{x, y\})$ is at least of degree $\Delta - 1$ (iii) If $d(x), d(y) < \Delta$, then every vertex of $N(N(x, y) - \{x, y\})$ is a major vertex.

Result 3.3[6]: The average degree of edge chromatic critical graphs with maximum degree seven is at least 6.

Main Theorems

$M_2, M_3, M_4, M_5, M_6, M_{34}, M_{35}, M_{36}, M_{45}, M_{46}, M_{56}, M_{345}, M_{346}, M_{356}, M_{456}, M_{3456}$ represent the collections of all vertices in M that are adjacent with minor vertices characterized as follows: only 2_vertices, only 3_vertices, only 4_vertices, only 5_vertices, only 6_vertices, only 3 and 4_vertices, only 3 and 5_vertices, only 3 and 6_vertices, only 4 and 5_vertices, only 4 and 6_vertices, only 5 and 6_vertices, only 3, 4 and 5_vertices, only 3, 4, and 6_vertices, only 3, 5 and 6_vertices, only 4, 5 and 6_vertices and 3, 4, 5 and 6_vertices respectively. In this article, we obtain new bounds on m and n_Δ which are improved bounds than the known bounds. By VAL, a major vertex cannot be adjacent with 3, 4, 5 and 6-vertices and hence $M_{3456} = \emptyset$.

The papers [7, 8, 9] give new insights and new bounds on the size and n_Δ of certain families of graphs which are edge-chromatic critical graphs with Δ equals 5, 6 and 7. As a continuation, this article discusses the size and n_Δ of three different families of graphs. The first family is the set of all 7-critical graphs in which (i) every vertex in M has a vertex in N_4 and a vertex in N_5 as its neighbours and (ii) every vertex in N_4 has a vertex in N_5 as its neighbour and vice versa. The second family is the set of all 7-critical graphs in which (i) every vertex in N_6 has a vertex in N_3 as its neighbour (ii) every vertex in N_5 has a vertex in N_4 as its neighbour and (iii) for every major vertex v , $N(v) \cap N_i \cap N_j = \emptyset$ for all $i \neq j$ and the third family is the set of all 7-critical graphs in which (i) every vertex in N_6 has a vertex in N_3 as its neighbour (ii) every vertex in N_5 has a vertex in N_4 as its neighbour and (iii) every vertex in M has at most one minor vertex as its neighbour. In all these three families, improved bounds on the size and n_Δ are obtained.

Theorem 4.1: If an edge-chromatic 7-critical graph G satisfies the conditions that (i) every vertex in M has a vertex in N_4 and a vertex in N_5 as its neighbours and (ii) every vertex in N_4 has a vertex in N_5 as its neighbour and vice versa,

then $\delta = 4$, $n_7 = \frac{3}{2}n_4 + 2n_5 = \frac{7}{2}n_4 = \frac{7}{2}n_5$ and $m = 3n + \frac{1}{4}n_4 = 3n + \frac{1}{4}n_5$.

Proof: By VAL, any vertex in M which has a 2-vertex as its neighbour cannot have any other minor neighbours. Hence by (i), $N_2 = \emptyset$. By (ii) and VAL, each 4-vertex has exactly one 5-vertex as its neighbour and vice versa. Thus $n_4 = n_5$. Suppose a major vertex v has a vertex in N_3 (say u) as its neighbour. By VAL, the vertex v has at least 5 major neighbours and hence v can be adjacent with at most one minor vertex other than the 3-vertex u , which is a





Sakila Devi

contradiction to (i). Hence, no major vertex has a vertex of $\mathcal{N}_3$ as its neighbour and hence that $\mathcal{N}_3 = \emptyset$. Hence $\delta = 4$. Suppose a vertex in $\mathbb{M}$ (say v) has a vertex $u' \in V(\mathcal{N}_4)$ and a vertex $u'' \in V(\mathcal{N}_5)$ as its neighbours. From result 3.2 and (ii), u' and u'' are adjacent and v cannot have a vertex in $\mathcal{N}_6$ as its neighbour. Hence no major vertex has a vertex in $\mathcal{N}_6$ as its neighbour. Hence $\mathcal{N}_6 = \emptyset$. Thus, $\mathbb{M} = \mathbb{M}_{45}$ and $n = |\mathcal{N}_4| + |\mathcal{N}_5| + |\mathbb{M}_{45}|$. Also, $|\mathcal{N}_4, \mathcal{N}_5| = n_4$, $|\mathcal{N}_4, \mathbb{M}_{45}| = 3n_4$ and $|\mathcal{N}_5, \mathbb{M}_{45}| = 4n_5$. Since each major vertex has exactly one 4 -vertex, one 5 -vertex and five major vertices as its neighbours, $n_7 = \frac{1}{2} [|\mathcal{N}_4, \mathbb{M}_{45}| + |\mathcal{N}_5, \mathbb{M}_{45}|]$. (i.e.) $n_7 = \frac{3n_4 + 4n_5}{2} = \frac{3}{2}n_4 + 2n_5$. Since $n_4 = n_5$, $n_7 = \frac{7}{2}n_4 = \frac{7}{2}n_5$. Since each major vertex has exactly five vertices in $\mathbb{M}$ as its neighbours, $|\mathbb{M}, \mathbb{M}| = \frac{5n_7}{2} = \frac{5}{2} \left(\frac{3}{2}n_4 + 2n_5 \right) = \frac{15}{4}n_4 + 5n_5$. Hence $m = |\mathcal{N}_4, \mathcal{N}_5| + |\mathcal{N}_4, \mathbb{M}_{45}| + |\mathcal{N}_5, \mathbb{M}_{45}| + |\mathbb{M}, \mathbb{M}| = n_4 + 3n_4 + 4n_5 + \frac{15}{4}n_4 + 5n_5 = \left(4 + \frac{15}{4} \right)n_4 + 9n_5 = \frac{31}{4}n_4 + 9n_5 = 3n + \left(\frac{31}{4} - 3 \right)n_4 + 6n_5 - 3n_7 = 3n + \frac{19}{4}n_4 + 6n_5 - 3 \left(\frac{3}{2}n_4 + 2n_5 \right) = 3n + \frac{1}{4}n_4$. Since $n_4 = n_5$, $m = 3n + \frac{1}{4}n_5$.

■

Observation 4.2: There is no edge chromatic 7-critical graph with $\delta = 2$ or 3 such that (i) each vertex in $\mathbb{M}$ has a vertex in $\mathcal{N}_4$ and a vertex in $\mathcal{N}_5$ as its neighbours and (ii) every vertex in $\mathcal{N}_4$ has a vertex in $\mathcal{N}_5$ as its neighbour and vice versa.

Observation 4.3: In the theorem 4.1, if $n_4 > 6$, then $m > 3n + \frac{3}{2}$ which improves the bound given by Vizing on the size.

Observation 4.4: In the theorem 4.1, the number of major vertices $= n_7 = \frac{3}{2}n_4 + 2n_5 = \frac{7}{2}n_4 = \frac{7}{2}n_5$, which is an improved count n_Δ given by Limin Zhang [3].

Theorem 4.5: Let $\mathcal{G}$ be an edge chromatic 7-critical graph such that (i) every vertex in $\mathcal{N}_6$ has a vertex in $\mathcal{N}_3$ as its neighbour (ii) every vertex in $\mathcal{N}_5$ has a vertex in $\mathcal{N}_4$ as its neighbour and (iii) for every major vertex v , $N(v) \cap \mathcal{N}_i \cap \mathcal{N}_j = \emptyset$ for all $i \neq j$. Then $n_7 \geq 2n_2 + \frac{3}{2}n_3 + \frac{4}{3}n_4 + \frac{2}{3}n_5 + \frac{1}{2}n_6$ and $m \geq 8n_2 + \frac{27}{4}n_3 + \frac{20}{3}n_4 + \frac{29}{6}n_5 + \frac{19}{4}n_6$.

Proof: By (i), (ii) and VAL, every 6 -vertex has five neighbours in $\mathbb{M}$ and every vertex in $\mathcal{N}_5$ has exactly four major neighbours. Hence $|\mathcal{N}_6, \mathbb{M}| = 5n_6$ and $|\mathcal{N}_5, \mathbb{M}| = 4n_5$. Let $\mathcal{N}^*_{\mathcal{N}_3}$ denote the collection of all vertices in $\mathcal{N}_3$ which have neighbours in $\mathcal{N}_6$ and let $\mathcal{N}^{**}_{\mathcal{N}_3} = \mathcal{N}_3 - \mathcal{N}^*_{\mathcal{N}_3}$. From (i) and VAL, all vertices in $\mathcal{N}_6$ have exactly one neighbour in $\mathcal{N}_3$ and all vertices in $\mathcal{N}_3$ have at least two neighbours in $\mathbb{M}$. Hence $|\mathcal{N}^*_{\mathcal{N}_3}| = n_6$ and $|\mathcal{N}^{**}_{\mathcal{N}_3}| = n_3 - n_6$. By VAL, we have $|\mathcal{N}^*_{\mathcal{N}_3}, \mathbb{M}| = 2n_6$ & $|\mathcal{N}^{**}_{\mathcal{N}_3}, \mathbb{M}| = 2n_2$. Also, $|\mathcal{N}_6, \mathcal{N}^*_{\mathcal{N}_3}| = n_6$. Let $\mathcal{N}^*_{\mathcal{N}_4}$ denote the collection of all vertices in $\mathcal{N}_4$ that have their neighbours in $\mathcal{N}_5$ and $\mathcal{N}^{**}_{\mathcal{N}_4} = \mathcal{N}_4 - \mathcal{N}^*_{\mathcal{N}_4}$. From (ii) and VAL, each 5 -vertex has exactly one neighbour in $\mathcal{N}_4$ and hence $|\mathcal{N}^*_{\mathcal{N}_4}| = n_5$ & $|\mathcal{N}^{**}_{\mathcal{N}_4}| = n_4 - n_5$. From result 3.1, $|\mathcal{N}^*_{\mathcal{N}_4}, \mathbb{M}| = 3n_5$. By VAL, $[\mathcal{N}_3, \mathcal{N}_4] = [\mathcal{N}_3, \mathcal{N}_5] = \emptyset$ and hence $|\mathcal{N}_3, \mathcal{N}_4| = |\mathcal{N}_3, \mathcal{N}_5| = 0$ and $|\mathcal{N}^{**}_{\mathcal{N}_3}, \mathbb{M}| = 3(n_3 - n_6)$. From the result 3.2, $|\mathcal{N}^{**}_{\mathcal{N}_3}, \mathcal{N}_6| = 0$ and $|\mathcal{N}^{**}_{\mathcal{N}_4},$





Sakila Devi

$\mathcal{N}_6] = 0$. By (i), (ii) and result 3.2, $|\mathcal{N}_4, \mathcal{N}_6| = |\mathcal{N}_5, \mathcal{N}_6| = 0$. By VAL, $|\mathcal{N}_4| = 0$. Also, $[\mathcal{N}^{**}_4, \mathcal{N}_5] = \emptyset$ and $|\mathcal{N}^{**}_4, \mathbb{M}| = 4(n_4 - n_5)$. Let $\mathcal{N}$ represent the collection of all minor vertices in $\mathcal{G}$. $|\mathcal{N}, \mathbb{M}| = 2n_2 + 2n_6 + 3(n_3 - n_6) + 3n_5 + 4(n_4 - n_5) + 4n_5 + 5n_6 = 2n_2 + 3n_3 + 4n_4 + 4n_6 + 3n_5$. Also, $|\mathcal{N}, \mathcal{N}| = |\mathcal{N}_6, \mathcal{N}^{**}_3| + |\mathcal{N}_5, \mathcal{N}^{**}_4| = n_6 + n_5$.

From (iii), $\mathbb{M} = \bigcup_{i=2}^6 \mathbb{M}_i \cup \mathbb{M}^*$, $\mathbb{M}^*$ represents the collection of all vertices in $\mathbb{M}$ which have no minor neighbours.

Since $|\mathcal{N}_2, \mathbb{M}| = 2n_2$, by VAL, $|\mathbb{M}_2| = 2n_2$. $|\mathcal{N}_3, \mathbb{M}| = 2n_6 + 3(n_3 - n_6) = 3n_3 - n_6$. A vertex in $\mathbb{M}$ which has a neighbour in $\mathcal{N}_3$ should have at least five major neighbours. Hence $|\mathbb{M}_3| \geq \frac{3n_3 - n_6}{2}$.

$|\mathcal{N}_4, \mathbb{M}| = 3n_5 + 4(n_4 - n_5) = 4n_4 - n_5$. A vertex in $\mathbb{M}$ which has a $\mathcal{N}_4$ -vertex as its neighbour should have at least four major neighbours. Thus $|\mathbb{M}_4| \geq \frac{4n_4 - n_5}{3}$. Also, $|\mathcal{N}_5, \mathbb{M}| = 4n_5$. A vertex in $\mathbb{M}$ which has a neighbour in $\mathcal{N}_5$ should have at least three major neighbours. Thus,

$$\begin{aligned} |\mathbb{M}_5| &\geq n_5. \text{ Also, } |\mathcal{N}_6, \mathbb{M}| = 5n_6. \text{ Hence } |\mathbb{M}_6| \geq \frac{5n_6}{5} = n_6. \text{ Hence } n_7 \geq |\mathbb{M}_2| + |\mathbb{M}_3| + |\mathbb{M}_4| + |\mathbb{M}_5| + |\mathbb{M}_6| \\ &\geq 2n_2 + \frac{3}{2}n_3 - \frac{1}{2}n_6 + \frac{4n_4}{3} - \frac{n_5}{3} + n_5 + n_6. \text{ Hence } n_7 \geq 2n_2 + \frac{3n_3}{2} + \frac{4n_4}{3} + \frac{2n_5}{3} + \frac{n_6}{2}. \text{ Also, } |\mathbb{M}, \mathbb{M}| \\ &\geq \frac{1}{2} [6(2n_2) + 5\left(\frac{3}{2}n_3 - \frac{1}{2}n_6\right) + 4\left(\frac{4}{3}n_4 - \frac{1}{3}n_5\right) + 3n_5 + 2n_6] = 6n_2 + \frac{15}{4}n_3 + \frac{8}{3}n_4 \\ &+ \left(\frac{3}{2} - \frac{2}{3}\right)n_5 + \left(1 - \frac{5}{4}\right)n_6. \text{ Hence } |\mathbb{M}, \mathbb{M}| \geq 6n_2 + \frac{15}{4}n_3 + \frac{8}{3}n_4 + \frac{5}{6}n_5 - \frac{1}{4}n_6. \end{aligned}$$

$$\begin{aligned} |\mathcal{N}, \mathcal{N}| + |\mathcal{N}, \mathbb{M}| + |\mathbb{M}, \mathbb{M}|. \text{ Hence } m &\geq n_6 + n_5 + 2n_2 + 3n_3 + 4n_4 + 4n_6 + 3n_5 + 6n_2 + \frac{15}{4}n_3 + \frac{8}{3}n_4 + \frac{5}{6}n_5 \\ &- \frac{1}{4}n_6 = 8n_2 + \frac{27}{4}n_3 + \frac{20}{3}n_4 + \frac{29}{6}n_5 + \frac{19}{4}n_6. \quad \blacksquare \end{aligned}$$

Observation 4.6: In the theorem 4.5, $2m = 2n_2 + 3n_3 + 4n_4 + 5n_5 + 6n_6 + 7n_7 = 6n - 4n_2 - 3n_3 - 2n_4 - n_5 + n_7 \geq 6n - 4n_2 - 3n_3 - 2n_4 - n_5 + 2n_2 + \frac{3}{2}n_3 + \frac{4}{3}n_4 + \frac{2}{3}n_5 + \frac{1}{2}n_6 = 6n - 2n_2 - \frac{3}{2}n_3 - \frac{2n_4}{3} - \frac{n_5}{3} + \frac{n_6}{2}$. Hence $m \geq 3n - n_2 - \frac{3}{4}n_3 - \frac{n_4}{3} - \frac{n_5}{6} + \frac{1}{4}n_6$. Hence $m > 3n + \frac{3}{2}$ if $\frac{1}{4}n_6 - n_2 - \frac{3}{4}n_3 - \frac{1}{3}n_4 - \frac{1}{6}n_5 > \frac{3}{2}$. Thus the bound given by Vizing on the size is improved if $n_6 > 4n_2 + 3n_3 + \frac{4}{3}n_4 + \frac{2}{3}n_5 + 6$.

Observation 4.7: On comparing the bound on n_Δ obtained in theorem 4.5 with the existing bound [3], the improvement obtained in $n_\Delta = \left(\frac{4}{3} - \frac{2}{3}\right)n_4 + \left(\frac{2}{3} - \frac{1}{2}\right)n_5 + \left(\frac{1}{2} - \frac{2}{5}\right)n_6 = \frac{2}{3}n_4 + \frac{1}{6}n_5 + \frac{1}{10}n_6$.





Sakila Devi

Theorem 4.8: If an edge chromatic 7-critical graph satisfies the conditions that (i) every vertex in $\mathcal{N}_6$ has a neighbour in $\mathcal{N}_3$ (ii) every 5-vertex has a 4-vertex as its neighbour and (iii) every major vertex has at most one minor vertex as its neighbour, then $n_7 \geq 2n_2 + 3n_3 + 4n_4 + 3n_5 + 4n_6$ and $m \geq 8n_2 + 12n_3 + 16n_4 + 13n_5 + 17n_6$.

Proof: Since each major vertex has at most one minor vertex as its neighbour, by a similar argument as in the theorem 4.5, we obtain that $|\mathbb{M}_3| = 3n_3 - n_6$, $|\mathbb{M}_4| = 4n_4 - n_5$, $|\mathbb{M}_5| = 4n_5$ and $|\mathbb{M}_6| = 5n_6$. Hence $n_7 \geq |\mathbb{M}_2| + |\mathbb{M}_3| + |\mathbb{M}_4| + |\mathbb{M}_5| + |\mathbb{M}_6| \geq 2n_2 + 3n_3 - n_6 + 4n_4 - n_5 + 4n_5 + 5n_6$. Hence $n_7 \geq 2n_2 + 3n_3 + 4n_4 + 3n_5 + 4n_6$. Also, $|\mathbb{M}, \mathbb{M}| \geq 3[2n_2 + 3n_3 - n_6 + 4n_4 - n_5 + 4n_5 + 5n_6] = 3[2n_2 + 3n_3 + 4n_4 + 3n_5 + 4n_6] = 6n_2 + 9n_3 + 12n_4 + 9n_5 + 12n_6$. Now, $m = |\mathcal{N}, \mathcal{N}| + |\mathcal{N}, \mathbb{M}| + |\mathbb{M}, \mathbb{M}| \geq n_6 + n_5 + 2n_2 + 3n_3 + 4n_4 + 4n_6 + 3n_5 + 6n_2 + 9n_3 + 12n_4 + 9n_5 + 12n_6$. Hence $m \geq 8n_2 + 12n_3 + 16n_4 + 13n_5 + 17n_6$. ■

Observation 4.9: In the theorem 4.8, $2m = 2n_2 + 3n_3 + 4n_4 + 5n_5 + 6n_6 + 7n_7 = 6n - 4n_2 - 3n_3 - 2n_4 - n_5 + n_7 \geq 6n - 4n_2 - 3n_3 - 2n_4 - n_5 + 2n_2 + 3n_3 + 4n_4 + 3n_5 + 4n_6 = 6n - 2n_2 + 2n_4 + 2n_5 + 4n_6$. Hence $m \geq 3n - n_2 + n_4 + n_5 + 2n_6$. Hence $m > 3n + \frac{3}{2}$ if $2n_6 + n_5 + n_4 - n_2 > \frac{3}{2}$. Thus

the bound given by Vizing on the size is improved if $2n_6 + n_5 + n_4 - n_2 > \frac{3}{2}$. Hence, the bound on size is improved in the cases: (i) $n_6 \geq 1$ & $n_2 = 0$ (ii) $n_5 \geq 1$ & $n_2 = 0$ & (iii) $n_4 > 1$ & $n_2 = 0$.

Observation 4.10: On comparing the bound on n_Δ obtained in theorem 4.8 with the existing bound [3], the improvement obtained in $n_\Delta = \frac{3}{2}n_3 + \frac{10}{3}n_4 + \frac{5}{2}n_5 + \frac{18}{5}n_6$.

Theorem 4.11: There is no 7-critical graph such that $|\mathcal{N}_5| = 0$ and every vertex in $\mathbb{M}$ has a vertex in $\mathcal{N}_3$ and also a vertex in $\mathcal{N}_5$ as its neighbours.

Proof: Suppose there is a 7-critical graph with $|\mathcal{N}_5| = 0$ and every vertex in $\mathbb{M}$ has a vertex in $\mathcal{N}_3$ and also a vertex in $\mathcal{N}_5$ as its neighbours. Hence $\mathbb{M}_2 = \mathbb{M}_3 = \mathbb{M}_4 = \mathbb{M}_5 = \mathbb{M}_6 = \emptyset$. Since each major vertex has 3-vertex and also a 5-vertex as its neighbours, by VAL, $\mathbb{M}_2 = \mathbb{M}_4 = \mathbb{M}_6 = \emptyset$. Hence, $\mathbb{M}_2 = \mathbb{M}_4 = \mathbb{M}_6 = \emptyset$. Thus, $\delta = 3$ and $n = n_3 + n_5 + n_7$. By VAL, any vertex in $\mathbb{M}_3$ cannot have a neighbour in $\mathbb{M}_5$. Hence $|\mathbb{M}_3, \mathbb{M}_5| = 0$. Thus $|\mathbb{M}_3, \mathbb{M}| = 3n_3$. Since $|\mathbb{M}_5| = 0$, every 5-vertex has five major neighbours and $|\mathbb{M}_5, \mathbb{M}| = 5n_5$. Since each vertex in $\mathbb{M}$ has exactly one vertex in $\mathbb{M}_3$ and one vertex in $\mathcal{N}_5$ and five

vertices in $\mathbb{M}$ as its neighbours, $n_7 = |\mathbb{M}_{35}| = \frac{3n_3 + 5n_5}{2}$. Now, $n = n_3 + n_5 + n_7 = n_3 + n_5 + \frac{3n_3}{2} + \frac{5n_5}{2} = \frac{5n_3}{2} + \frac{7n_5}{2}$. Also, $m = |\mathcal{N}_3, \mathbb{M}_{35}| + |\mathcal{N}_5| + |\mathcal{N}_5, \mathbb{M}_{35}| + |\mathbb{M}|$. As there are exactly 5 major neighbours for each

vertex in $\mathbb{M}$, $|\mathbb{M}| = \frac{5n_7}{2} = \frac{5}{2} \left(\frac{3}{2}n_3 + \frac{5}{2}n_5 \right) = \frac{15}{4}n_3 + \frac{25}{4}n_5$. Hence $m = 3n_3 + 5n_5 + \frac{15}{4}n_3 + \frac{25}{4}n_5 = \frac{27}{4}n_3 + \frac{45}{4}n_5$. Since each vertex in $\mathbb{M}$ has exactly one vertex in $\mathcal{N}_3$ and one vertex in $\mathcal{N}_5$ and five vertices in $\mathbb{M}$ as its

neighbours, $|\mathcal{N}_3, \mathbb{M}| = |\mathcal{N}_5, \mathbb{M}|$. Hence $3n_3 = 5n_5$. Thus $n_3 = \frac{5}{3}n_5 > n_5$ (i.e.) $n_3 > n_5$. By result 3.3, $2m \geq 6n$.





Sakila Devi

Hence $\frac{27}{2}n_3 + \frac{45}{2}n_5 \geq 6\left(\frac{5}{2}n_3 + \frac{7}{2}n_5\right) \Rightarrow \left(\frac{27}{2} - 15\right)n_3 \geq \left(21 - \frac{45}{2}\right)n_5 \Rightarrow -\frac{3}{2}n_3 \geq -\frac{3}{2}n_5 \Rightarrow n_3 \leq n_5$

, which is a contradiction. Thus, there is no 7-critical graph such that $|[N_5]|=0$ and each vertex in M has a vertex in N_5 and a vertex in N_3 as its neighbours. ■

Observation 4.12: For any edge-chromatic 7-critical graph such that each vertex in M has a vertex in N_3 and also a vertex in N_5 as its neighbours, $|[N_5]| \neq 0$.

Observation 4.13: For any edge-chromatic 7-critical graph with $|[N_5]|=0$, there exists a major vertex which is either 'nonadjacent with every 3-vertices' or 'nonadjacent with every 5-vertices'.

CONCLUSION

This article gives insights on three kinds of families of edge-chromatic critical graphs. The improvements are listed below.

The nature of edge chromatic 7- critical graphs	Existing known bound on n_Δ and m [2,3]	The bound obtained in this article	The cases in which the improvement on m is attained	The improvement on n_Δ
edge-chromatic critical graphs with the conditions of theorem 4.1	$n_\Delta \geq 2n_2 + \frac{3}{2}n_3$ $+ \frac{2}{3}n_4 + \frac{1}{2}n_5$ $+ \frac{2}{5}n_6$ $m \geq (1/2)[(\Delta-1)n+3]$	$n_7 = \frac{3}{2}n_4 + 2n_5$ and $m = 3n + \frac{1}{4}n_4$.	$n_4 > 6$	$(7/3)n_5$
edge-chromatic critical graphs with conditions in theorem 4.5		$n_7 \geq 2n_2 + \frac{3}{2}n_3 + \frac{4}{3}n_4$ $+ \frac{2}{3}n_5 + \frac{1}{2}n_6$ and $m \geq 8n_2 + \frac{27}{4}n_3 + \frac{20}{3}n_4$ $+ \frac{29}{6}n_5 + \frac{19}{4}n_6$.	$n_6 > 4n_2 +$ $3n_3 + (4/3)n_4$ $+ (2/3)n_5 + 6$	$(2/3)n_4 + (1/6)n_5 + (1/10)n_6$
edge-chromatic critical graphs with conditions in theorem 4.8		$n_7 \geq 2n_2 + 3n_3 + 4n_4$ $+ 3n_5 + 4n_6$ and $m \geq 8n_2 + 12n_3$ $+ 16n_4 + 13n_5 + 17n_6$.	$2n_6 + n_5$ $+ n_4 - n_2 > \frac{3}{2}$	$\frac{3}{2}n_3 + \frac{10}{3}n_4 + \frac{5}{2}n_5 + \frac{18}{5}n_6$

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Sakila Devi

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REVIEW ARTICLE

Modern Diagnostics for Urinary Tract Infections and Antimicrobial Resistance: Translating Innovation into Clinical Application: A Review Article

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ABSTRACT

In order to prevent antibiotic resistance, urinary tract infections (UTIs) are a significant worldwide health concern that must be promptly diagnosed. The shift from traditional urine culture to advanced molecular, point-of-care (POCT), and artificial intelligence (AI) diagnostics is assessed in this review. With an emphasis on "UTI diagnostics," "molecular methods," and "AI in microbiology," a thorough literature search was carried out utilizing databases such as Medline (MeSH), PubMed, and Google Scholar for articles published between 1990 and 2026. The investigation demonstrates how uropathogens and antimicrobial resistance (AMR) genes may be quickly and independently identified using molecular approaches like PCR, LAMP, and metagenomic sequencing (mNGS). We also go over how AI-driven predictive modeling and biosensor-based POCT improve diagnostic precision and laboratory productivity. By making early, focused treatment possible, these developments promote antimicrobial stewardship. Despite advancements in technology, issues like high costs, a lack of standardization, and the requirement for clinical validation continue to be major obstacles to wider adoption. All things considered, incorporating these advancements is crucial for creating individualized and data-driven UTI diagnostic approaches.

Keywords: Urinary Tract Infections (UTI); Polymerase chain reaction (PCR); Loop-mediated isothermal amplification (LAMP); Point-of-care testing (POCT); Artificial intelligence (AI); Antimicrobial stewardship (AS)





Akansha Ray et al.,

INTRODUCTION

Among the most prevalent bacterial illnesses, urinary tract infections (UTIs) afflict people of all ages and genders, but they are more common in women and older adults [1]. A prompt and precise diagnosis of UTIs is crucial. Targeted treatment, which lowers the risk of major consequences such as pyelonephritis, urosepsis, and recurrent infections, is made possible by early infection detection. However, the view must also be accurate because nonspecific lower urinary tract symptoms might result in an empirical over prescription of antibiotics, which can cause unjustified side events and antimicrobial resistance (AMR) [2]. Recent decades' epidemiological data clearly show that UTIs are becoming a global issue. A notable example is a study that compared the prevalence of these illnesses between 1990 and 2019 and found that the number of cases worldwide had increased by 60% [3]. Scary figures are emerging from vibrant parts of the globe. Approximately 10.5 million patient visits, 3 million visits to emergency rooms, and 400,000 hospital admissions were caused by UTIs in the US each year. The health system had to pay a staggering 4.8 billion dollars a year due to this level of infection [4, 5, and 6]. Inversely, the European scenario is worrisome [7, 8, and 9]. In contrast to pyelonephritis, which is identified by systemic symptoms like fever and flank pain, acute uncomplicated cystitis is generally diagnosed clinically based on symptoms like dysuria, frequency, and urgency. Urine culture is still the gold standard for diagnosis, but it is frequently impractical to use it on a regular basis for simple lower urinary tract infections (UTIs). A UTI can be diagnosed with 90% accuracy in healthy women if two or more clinical symptoms are present [10]. Although urinalysis is a vital technique for identifying bacteriuria and pyuria, its specificity ranges from 42% to 90%, requiring a confirmation urine culture in complex or recurrent cases [11]. The correlation between inadequate diagnosis and the rise of antimicrobial resistance (AMR) is multifaceted. Key factors include:

- **False Positives:** Prescription of antibiotics for symptoms not caused by an actual infection.
- **False Negatives:** Delayed treatment leading to the eventual necessity for high-potency, prolonged antibiotic courses.
- **Inadequate Selection:** Poor identification of target pathogens resulting in suboptimal therapeutic choices.

Novel diagnostic methods that make use of developments in molecular biology, biosensors, artificial intelligence (AI), and point-of-care testing (POCT) have been developed to overcome these constraints. When compared to conventional culture, molecular techniques like polymerase chain reaction (PCR) allow for the quick and more accurate detection of bacterial DNA [12]. These techniques are especially useful for detecting slow-growing or picky organisms that are frequently missed by traditional cultures. Additionally, POCT devices eliminate the need for centralized laboratory facilities by enabling prompt bedside diagnosis [13]. With an emphasis on the combination of POCT, AI, and molecular diagnostics to improve antimicrobial stewardship and diagnostic accuracy, this review seeks to assess these recent advancements.

Pathophysiology of Urinary Tract Infection

A highly coordinated ascending cascade is required to convert the urinary tract from a low-biomass, homeostatic milieu into an active zone of infection. Certain microbial virulence factors that get past host immune systems and anatomical barriers are what propel this process [14].

Microbial Translocation and Urethral Colonization

Urinary tract infections (UTIs) usually start off as opportunistic phenomena [18]. To colonize the periurethral mucosa, microorganisms move from anatomical reservoirs, mainly the gastrointestinal system, perineum, or vagina [18, 19]. Uropathogens must ascend the urethra after becoming established in the periurethral region in order to enter the bladder lumen [14]. The host's main mechanical defense mechanism is the continuous hydrodynamic flushing of urine, which the pathogens must overcome in order to migrate [20].





Akansha Ray et al.,

Adherence and Bladder Invasion

Uropathogens use specific surface organelles called chaperone-usher fimbriae to survive urine flow [21]. Type 1 fimbriae are typically expressed by Uropathogenic Escherichia coli (UPEC), the primary etiological agent accountable for the great majority of instances worldwide [22, 23]. These fimbriae's adhesive tip, a lectin called FimH, has a strong affinity for mannosylated uroplakin receptors (UPIa and UPIb) that line the bladder epithelium's superficial umbrella cells [24]. On the other hand, pathogens such as Pseudomonas aeruginosa, Proteus mirabilis, and Klebsiella pneumoniae use specific pili and surface adhesins to attach directly to host tissues or foreign biomaterials in complicated UTIs (cUTIs) involving structural abnormalities or indwelling urinary catheters [25, 26].

Intracellular Niche Activation and Biofilm Formation

Actin cytoskeleton rearrangements occur in the host cell membrane upon successful adhesion, allowing the associated bacterium to be engulfed through receptor-mediated endocytosis [27]. After entering the superficial facet cells' cytoplasm, UPEC quickly multiplies to create structured clusters known as Intracellular Bacterial Communities (IBCs) [26].

- Uropathogens adopt an extracellular lifestyle and create a protective Extracellular Polymeric Substance (EPS) matrix as the infection worsens or when foreign objects such as indwelling catheters are present [28]. This biofilm structure fulfills a number of vital purposes:
It physically prevents systemic antibiotic penetration by the reproducing microorganisms [28].
- It shields the pathogens from recognition by host immune cells [29].
- It facilitates sustained colonization, acting as a permanent microbial reservoir that drives recurrent infections (rUTIs) and chronic persistence [18, 22].

Host proteins such as fibrinogen quickly adhere to the artificial lumen in catheterized environments [30]. By generating urease, an enzyme that hydrolyzes urea into ammonia, pathogens such as P. mirabilis take advantage of this environment. Struvite and apatite crystals, which are calcium and magnesium phosphates, precipitate as a result of the local pH rising [31]. By encasing the bacterial biofilm, these crystals create crystalline encrustations that obstruct catheter flow and are resistant to traditional antimicrobial treatments [30, 31].

Host Innate Immune Mobilization and Tissue Damage

Toll-like receptors (mostly TLR4 and TLR5) expressed on the urothelium are activated when uropathogens invade cells and multiply, triggering the host's innate immune system [32]. Nuclear factor kappa B (NF- κ B) is nuclear translocated as a result of this ligand binding, which starts an intracellular signaling cascade driven by myeloid differentiation factor 88 (MyD88) [32, 33].

- Strong transcription and secretion of pro-inflammatory cytokines and chemokines, particularly Interleukin-6 (IL-6) and Interleukin-8 (IL-8), are driven by NF- κ B activation [33].
- IL-6 modulates localized acute-phase inflammatory responses [33].

Clinical pyuria is caused by IL-8 creating a potent chemical gradient that draws polymorph nuclear neutrophils (PMNs) from the vasculature and into the bladder lumen [14, 34]. Uropathogens simultaneously release cytotoxic poisons to meet their metabolic requirements and make room for further tissue spread [21]. For instance, significant amounts of the pore-forming toxin α -hemolysin (HlyA), which lyses host cell membranes, are released by UPEC strains [19]. The bladder mucosa is greatly exfoliated as a result, giving the bacteria access to deeper, underlying cell layers [27, 35]. In order to scavenge ferric iron from host proteins and support fast bacterial metabolism, the bacteria simultaneously release high-affinity iron chelators known as siderophores, such as enterobactin and aerobactin [21, 36].

Kidney Ascension and Systemic

Translocation Uropathogens climb the ureters to infect the upper urinary system if targeted therapy or host defenses fail to eradicate the localized bladder infection [14]. By attaching to digalactoside residues produced on renal epithelial cells, P fimbriae (mediated by the PapG adhesin) are essential for kidney ascent [21, 37]. Acute



**Akansha Ray et al.,**

pyelonephritis, which is marked by severe interstitial inflammation, renal parenchymal destruction, and a high risk of bacterial translocation across the renal vascular endothelium into the bloodstream—which can result in potentially fatal urosepsis—is brought on by this interaction [37, 38].

Molecular Diagnostic

By facilitating the quick and accurate identification of uropathogens directly from urine samples, molecular diagnostic techniques have significantly improved the diagnosis of urinary tract infections (UTIs) [39, 40]. Common uropathogens like *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterococcus spp.*, and *Staphylococcus saprophyticus* can be identified early using nucleic acid amplification tests, especially polymerase chain reaction (PCR)-based assays, which considerably shorten turnaround times when compared to traditional culture [39, 41].

Polymerase Chain Reaction

Advances in PCR technology, including as multiplex PCR panels and real-time PCR (qPCR), have significantly enhanced the diagnostic utility of molecular testing in UTIs. By identifying infections and identifying antibiotic resistance genes, such as those encoding carbapenems and extended-spectrum β -lactamases (ESBLs), these techniques support antimicrobial stewardship initiatives. PCR-based diagnostics have disadvantages despite their advantages, such as high cost, limited availability in routine labs, and the inability to distinguish between colonization and active infection. However, when paired with clinical assessment and conventional microbiology, PCR is a helpful addition to the comprehensive diagnosis and treatment of UTIs [42, 43]. This is especially helpful for complex UTIs, where successful therapy depends on identifying the right bacteria and its resistance profile.

Metagenomic Next-Generation Sequencing (mNGS)

A sophisticated molecular diagnostic technique called metagenomic next-generation sequencing (mNGS) allows for the thorough, culture-independent identification of every microbial nucleic acid found in a clinical material [44, 45]. mNGS makes it easier to simultaneously identify bacteria, viruses, fungi, and parasites—including uncommon, picky, and uncultivable organisms—by doing away with the need for specialized primers. When treating illnesses that are difficult to diagnose and when traditional culture or focused molecular tests produce negative or ambiguous findings, this objective approach is very helpful [44]. Precision antimicrobial therapy is supported by mNGS, which offers vital information on polymicrobial illnesses and antimicrobial resistance (AMR) determinants in addition to wide pathogen detection [46]. Clinical adoption is still constrained by high operating costs, computational complexity, and the difficulties in differentiating clinically meaningful data from background microbiota or environmental contamination, despite its substantial diagnostic potential. However, it is anticipated that further developments in analytical pipelines and sequencing technology will improve the clinical value of mNGS in the field of molecular diagnostics [44, 45].

Loop-Mediated Isothermal Amplification (LAMP)

A potential molecular method for the microbiological and molecular detection of urinary tract infections (UTIs) is loop-mediated isothermal amplification (LAMP). Using a set of four to six primers that identify various target gene areas, LAMP allows for the quick amplification of target nucleic acids under isothermal conditions (60–65°C). Without the need for complex heat cyclers, this design's excellent analytical sensitivity and specificity enable the direct identification of major uropathogens from urine samples, including *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterococcus spp.* LAMP dramatically shortens turnaround times when compared to traditional culture and PCR-based techniques, frequently producing findings in 30 to 60 minute—a crucial component for early diagnosis and prompt antibiotic treatment [46]. LAMP has recently been combined with colorimetric, turbidimetric, and fluorescence-based readouts to enable point-of-care testing (POCT) and use in environments with limited resources. Antimicrobial stewardship is further supported by the ability for simultaneous pathogen identification and antimicrobial resistance (AMR) determinant detection demonstrated by multiplex and resistance-gene-targeted LAMP tests. Notwithstanding these benefits, problems including uniformity, contamination risk, and limited quantitative capacity still exist. However, LAMP is a useful, economical, and therapeutically significant contribution to the developing field of molecular diagnostics for UTIs [47].





Akansha Ray et al.,

Identifying AMR with Molecular Methods

The detection of antimicrobial resistance (AMR) via molecular techniques is now crucial for treating urinary tract infections (UTIs) due to advances in microbiological and molecular diagnostics. Molecular methods enable the quick identification of resistance-related genes directly from urine tests or positive culture samples by avoiding the delays connected with phenotypic susceptibility testing.

Key resistance determinants include:

- **Extended-spectrum β -lactamase (ESBL) genes:** bla(CTX-M), bla(TEM), bla(SHV).
- **Carbapenemase genes:** bla(KPC), bla(NDM), and bla(OXA-48).
- **Quinolone resistance:** Plasmid-mediated resistance genes in uropathogens such as *Escherichia coli* and *Klebsiella pneumoniae*.

These techniques greatly shorten turnaround times, allowing for early antimicrobial treatment optimization and better patient outcomes [48]. The use of microarrays, syndromic molecular panels, and next-generation sequencing (NGS) for thorough AMR profiling are examples of recent advancements. The simultaneous identification of bacteria and their "resistome," even in polymicrobial or culture-negative UTIs, is made possible by met genomic methods.

Some limitations still exist despite great sensitivity and specificity, such as the incapacity of molecular techniques to assess gene expression, discover new resistance mechanisms, or assess phenotypic significance. However, molecular methods are becoming more and more important in guiding antimicrobial stewardship programs, infection prevention, and focused therapy, which is a significant development in the management of UTIs [49].

Point-of-Care testing

Without the need for centralized laboratory infrastructure, point-of-care testing (POCT) provides quick, on-site pathogen and biomarker identification. Urine dipstick tests have been widely used as a major POCT method because of its simplicity, affordability, and convenience of use. However, their poor specificity frequently leads to over diagnosis and overtreatment [50]. Moreover, laboratory-based confirmatory testing is usually required since these tests cannot differentiate between different uropathogens or offer information on antimicrobial resistance (AMR) trends.

Alternatives to Traditional Dipsticks

The creation of alternatives to conventional dipsticks is a major advancement in the microbiological and molecular detection of urinary tract infections (UTIs). Leukocyte esterase and nitrites are detected by conventional dipstick tests, but they have low sensitivity and specificity, especially in early-stage infections, low bacterial counts, or infections brought on by species that do not produce nitrites. New biosensor-based tests and molecular urine screening instruments have been created to get over these restrictions by directly identifying:

- Nucleic acids unique to pathogens.
- Biomarkers of host inflammation.
- Particular components of bacteria.

By increasing diagnostic accuracy and drastically lowering false-negative results, these sophisticated techniques allow for earlier and more accurate UTI diagnosis as compared to traditional dipstick testing [51].

Dip-Slides and Culture Kits

For the microbiological diagnosis of urinary tract infections (UTIs), dip-slides and commercial culture kits are useful substitutes for traditional laboratory urine culture. Dip-slide devices provide semi-quantitative measurement of bacterial load and presumed pathogen identification by submerging plastic paddles covered with culture medium in urine and incubating them. These kits offer an affordable and user-friendly solution, especially in primary care, outpatient settings, and resource-constrained areas where access to thorough microbiological laboratories may be limited. Dip-slides provide an appropriate sensitivity for identifying substantial bacteriuria while reducing handling steps and turnaround time when compared to traditional culture procedures [52]. Traditional dipsticks are not



**Akansha Ray et al.,**

currently recommended by Public Health England and the National Institute for Health and Care Excellence (NICE) for the diagnosis of UTIs, with the exception of females under 65 who present with mild to moderate symptoms [53,54]. This is consistent with other research showing that dipsticks are quite useful for ruling out UTIs in premenopausal women in non-hospital settings using clean midstream urine samples; however, their diagnostic accuracy among hospitalized or convalescent patients is still unknown [55–58].

In order to make interpretation simpler, recent advancements in commercial culture kits have concentrated on better chromogenic medium, increased selectivity, and more distinct colony differentiation. To improve diagnostic coverage, some kits have dual media surfaces that promote the development of both Gram-positive and Gram-negative uropathogens. Dip-slides and culture kits are still useful for screening, epidemiological surveillance, and the monitoring of recurring UTIs, even if they cannot take the place of sophisticated molecular diagnostics in terms of speed or resistance detection. Their enduring significance alongside more recent molecular and automated technologies is demonstrated by their incorporation into contemporary diagnostic algorithms [59].

Biosensors and Emergent Technologies

The use of biosensor technology in POCT has led to the development of extremely sensitive and accurate UTI detection methods. Electrochemical biosensors employing nonmaterial and microfluidic devices may identify host response markers, volatile organic compounds, and bacterial metabolic by products in urine [39].

Artificial Intelligence (Ai)

Urinary tract infection (UTI) microbiological and molecular diagnostics are rapidly incorporating artificial intelligence (AI) to improve clinical decision-making, speed, and accuracy. Complex datasets produced by urine microscopy, flow cytometry, imaging, and molecular diagnostic platforms are analyzed using AI-driven techniques, such as machine learning and deep learning models. These methods can forecast the presence of pathogens, quickly differentiate between contaminated samples and actual illnesses, and assist in the early triage of urine specimens for genetic testing or culture. AI increases workflow efficiency and shortens diagnostic turnaround times in high-volume clinical laboratories by automating data interpretation and decreasing human variability [60]. By examining clinical characteristics, urine data, and electronic health records, AI-driven models may be able to predict the probability of a UTI far earlier than conventional culture-based techniques. Antimicrobial stewardship is strengthened and timely treatment decisions are made possible [61, 62].

Machine-learning algorithms have shown great sensitivity and specificity in predicting resistance patterns and identifying causative organisms in retrospective datasets, especially for common uropathogens like *Escherichia coli* [63, 64]. Additionally, many days before culture results are available, AI-assisted decision-support systems can recommend empirical antibiotic therapy, which may reduce inappropriate antibiotic exposure and the consequent emergence of resistance [65, 66]. AI integration with quick molecular and point-of-care (POCT) systems is another recent development that has reduced turnaround times in clinical microbiology [67]. Despite these encouraging advancements, issues including dataset bias, a lack of multicenter validation, regulatory concerns, and limitations integrating AI tools into current laboratory information systems (LIS) continue to hinder normal clinical adoption. Clinician acceptability and the need for prospective clinical research continue to be significant challenges. Therefore, to close the gap between technical innovation and clinical application, interdisciplinary collaboration, cost-effectiveness evaluations, and systematic validation are crucial.

CONCLUSION

The identification and treatment of urinary tract infections (UTIs) have been profoundly changed by developments in microbiological and molecular diagnostics. Although traditional urine culture is still the gold standard, its shortcomings in terms of sensitivity and turnaround time have prompted the use of quick diagnostic techniques. These include metagenomic sequencing, nucleic acid amplification methods, improved culture systems, and





Akansha Ray et al.,

automated urine analyzers. These developments make it possible to identify pathogens earlier and more precisely, detect fastidious or polymicrobial diseases more accurately, and start targeted antimicrobial therapy more quickly. Clinical decision-making and diagnostic accuracy have been further enhanced by the incorporation of new technologies such as biosensors, point-of-care molecular tests, antimicrobial resistance gene identification, and artificial intelligence. By lowering the use of empirical antibiotics and enhancing patient outcomes, these developments collectively promote antimicrobial stewardship. The future of UTI diagnostics is expected to be driven toward quick, individualized, and data-driven healthcare solutions through ongoing technology progress and interdisciplinary collaboration, despite obstacles relating to cost, standardization, and clinical validation.

Conflict of interest- The Authors declare no conflict of interest

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RESEARCH ARTICLE

Effectiveness of Pilates Exercise and Resistance Training on Balance and Postural Control in Patients with Parkinson's Disease

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ABSTRACT

Parkinson's disease prevalence is projected to increase substantially over the next decade, largely driven by population aging. The primary objective was to evaluate whether an 8-week sequential intervention of Pilates followed by resistance training could improve balance and postural control in this group. This study enrolled 30 participants aged 55–65 years diagnosed with PD. Participants completed 4 weeks of Pilates exercises, then 4 weeks of resistance training. The primary outcomes of this study were balance and postural control, measured using the Fullerton Advanced Balance Scale, the Timed Up and Go test, and the Unified Parkinson's Disease Rating Scale. Normality of the dependent variables was assessed using the Shapiro-Wilk test, which indicated a normal distribution with $p > 0.05$. Statistical analysis was performed in SPSS version 24. Differences were considered significant at $p < 0.05$, and all results are reported with 95% confidence intervals. Paired t-test was adopted to find the statistical difference within the groups. The findings indicate that an 8-week program combining Pilates and resistance training leads to measurable improvements in balance and postural control for individuals with Parkinson's disease.

Keywords: Parkinson's disease, Fullerton advanced balance scale, Timed up and go test, Unified Parkinson's disease rating scale, Pilates exercise.



Senthil Kumar *et al.*,

INTRODUCTION

Parkinson's disease is a degenerative condition affecting the central nervous system, characterized by a combination of motor impairments and non-motor manifestations. The hallmark motor features of Parkinson's disease consist of rigidity, bradykinesia, and tremor. As the disease advances, postural instability often develops and generally shows limited response to pharmacological treatment or deep brain stimulation[1]. Non-motor manifestations of Parkinson's disease can appear several years before motor symptoms emerge. Non-motor symptoms frequently appear in the early stages and may include anosmia, constipation, REM sleep behavior disorder, mood disturbances, and orthostatic hypotension. Postural instability is a hallmark motor feature of PD. It results from neurodegeneration and can appear at any disease stage, contributing to difficulties with daily activities, falls, and injury risk. Compared to age-matched healthy adults, individuals with PD exhibit greater balance deficits and a higher incidence of falls. Researchers have examined both non-modifiable and modifiable risk factors for postural instability in Parkinson's disease. Non-modifiable factors include age, genetic mutations, and race, while modifiable factors encompass environmental exposures and lifestyle variables[2]. Balance impairments in PD are primarily attributed to factors including muscle weakness, axial rigidity, bradykinesia, freezing of gait, and deficits in sensorimotor integration and cognitive function. Epidemiological evidence indicates that PD occurs more frequently in men[3], a pattern that was also present in the current study sample for all cases and across every PD subtype[4,5].

Pilates is a mind-body exercise system that targets core stability, muscular strength, flexibility, posture, and breathing control. Movements are typically performed on a mat or using spring-based equipment. Joseph Pilates created this exercise method in 1926[6]. In the 2000s, healthcare professionals modified and adapted Pilates exercise for therapeutic use under the term 'clinical Pilates exercise' [7]. The method combines stretching and strengthening exercises designed to build both physical strength and flexibility, while also enhancing body awareness and control. The key principles of Pilates are: centering, concentration, control, precision, breath, flow [8]. The core principles of Pilates involve the co-activation of trunk stabilizer muscles. This promotes awareness of neutral spine alignment and strengthens the deep spinal musculature. Physiotherapists can progress Pilates exercises by gradually reducing external support and modifying movements to match individual patient needs. Training also emphasizes purposeful gait patterns and helps patients recognize and correct faulty walking mechanics to reduce ground impact and joint stress. Conradsson et al. (2015) evidence suggests that advanced balance training produces greater neural adaptations in people with PD compared to conventional therapy approaches[9]. Canning et al. (2014) prior work has shown that structured balance and strength programs can reduce fall risk in this Population[10]. A systematic review of eight studies also concluded that Pilates is an effective intervention for improving lower limb function, balance, and overall physical fitness in individuals with Parkinson's disease.[11].

Resistance training is recognized as an effective strategy to improve motor function, muscular strength, and endurance in PD [12-15]. Muscle weakness correlates with increased fall risk and postural disturbances in PD. Compensatory mechanisms play an important role in Parkinson's disease and increasing strength through resistance training may facilitate activation of muscle-groups associated with balance. Similar protocols have been applied in healthy older adults to enhance balance and functional capacity. Supporting this, Canning et al. reported that combined training focusing on balance, strength, and gait freezing can improve postural control in PD patients[16]. The present study applies these principles using sequential Pilates and resistance training to target balance and postural control deficits.

MATERIALS AND METHODS

For this study, participants with PD were recruited from the physiotherapy outpatient department at UCA College of Paramedical Sciences, Chennai, following referral by a neurologist. The study inclusion criteria are: Subjects aged 55-65 with Hoehn and yahr stage 2-3 Parkinson's disease [PD] and a minimum score of 26 on the mini-mental test



**Senthil Kumar et al.,**

[MMT], Idiopathic Parkinson's disease [Diagnosed by Neurologist], patients who had never engaged in any regular exercise activities or received any other physical therapy, Stable pharmacological treatment who were diagnosed with Parkinson's disease [PD] within a year or more [17], Medication ON- state [1 hour after the last intake of antiparkinsonian medication], Postural instability [Fullerton advanced balance FAB scale ≤ 25 points], In the two years prior, there was a history of one or more falls or near falls. Exclusion criteria were: Individuals with severe mental and psychological disorders, significant musculoskeletal disorders, deformities of the lower extremities, other neurological diseases, changes in medication or dosage, during treatment, advanced age, and low MMT score were excluded, Hoehn- Yahr stage more than 4, If their mobility was impeded by any physical disabilities or other medical conditions, Previously diagnosed with dementia and stroke, Severe dyskinesia and/or motor fluctuations were experienced, Other diseases that could influence stance and gait performance.

The ethical committee of UCA College of Paramedical Sciences, College of Physiotherapy (vide IEC no. 001/01/2026/IEC/UCACOPT) approved the study on 4th January, 2026. A blinded researcher selected participants for the study, around sixty participants who visited the OPD. They were analyzed based on the selection criteria, and informed consent was obtained from each patient before the study. Thirty participants were included in this study, and the rest were excluded as they did not fulfill the selection criteria. The materials used for the study were arm resting chair, stopwatch, mat, thera band and weight cuff. Subjects were pre- assessed by Fullerton advanced balance [FAB] scale, Timed up and go [TUG] test, Unified Parkinson's disease rating scale [UPDRS]. The study conducted for 8 weeks. Participants were given the Pilates exercise for first 2 weeks [2 session per week]. Each session lasts for 45 minutes, consists of 5 minutes warm-up, followed by Pilates for 30 minutes and 10 minutes cool-down. Warm-up exercise included spine stretch, free exercise to all joints and breathing exercise. Main exercise done for first 2 weeks were: One leg stretch, hip twist, side kicking. Each exercise started with eight repetitions. The number of repetitions were increased depend on the patient's capabilities. Finally cool down exercises included spine stretch, the mermaid and breathing exercise. Exercise done for next 2 weeks: One leg kick, one leg circles, spine twist.

Resistance training started after 4 weeks [3 session per week] with same protocol given before weeks. Main exercise were: Squats, knee extension with weight cuffs, calf raises, toe raises and hip flexion with bands, hip flexion with weight cuffs, hip abduction, ischiocrural muscles with bands. Exercise are performed three sets of 15-20 repetitions to volitional fatigue of each exercise. Resistance is increased once participants could complete more than 20 consecutive repetitions of an exercise. Participants were given rest for 2 minutes between each exercise sets. The primary outcome for this study are Fullerton advance balance [FAB] scale, Timed up and go [TUG] test, Unified Parkinson's disease rating scale [UPDRS]. The FAB scale is a 10-item clinical balance scale ^[18] with a 5-point ordinal scale [0-4] for each item and a maximal score of 40 points [higher values indicate better performance]. For this tool, an interrater reliability of 0.94-0.97 and a test-retest reliability coefficient of 0.96 also calculated ^[19-21]. The timed up and go test [TUG], which is reliable and valid method ^[22]. The patient is seated comfortably in a firm chair with arms and back resting against the chair. Timing with a stopwatch begins when the patient is instructed to "go" 3 meters and ends when the patient return to the start position seated in the chair. The UPDRS is a rating tool to follow the longitudinal course of Parkinson's disease ^[23]. It is made up of the: Mentation, behaviour and mood, activities of daily living, motor sections. A total of 199 points possible 199 represents the worst [total disability], 0 no disability. All the parameters were assessed using statistical package for social science (SPSS) version 24, with a significance level of p value less than 0.05 and a 95% confidence interval set for all analysis. The Shapiro Wilk test was used to determine the normality of the data. In this study, Shapiro Wilk test showed that the data was normally distributed on the dependent values at $P > 0.05$. Hence parametric test was adopted. Paired t-test was adopted to find the statistical difference within the groups.

RESULTS

On comparing mean values of Fullerton Advanced Balance Scale Score between Pre-test 22.30 ± 1.62 and Post-test 26.90 ± 1.64 within Group, it shows there is a highly significant difference in between Pre-test and Post-test mean



**Senthil Kumar et al.,**

values with t value shows -15.85 at $P \leq 0.05$. The Post-test values have shown improvement when compared with the pre-test. Hence, the null hypothesis is rejected. On comparing mean values of Timed Up and Go Test Score between Pre-test 15.60 ± 1.90 and Post-test 13.00 ± 1.83 within Group, it shows there is a highly significant difference in between Pre-test and Post-test mean values with t value shows 12.57 at $P \leq 0.05$. The Post-test values have shown improvement when compared with the pre-test. Hence, the null hypothesis is rejected. On comparing mean values of Unified Parkinson Disease Rating Scale Score between Pre-test 96.76 ± 13.25 and Post-test 92.20 ± 13.83 within Group, it shows there is a highly significant difference in between Pre-test and Post-test mean values with t value shows 12.32 at $P \leq 0.05$. The Post-test values have shown improvement when compared with the pre-test. Hence, the null hypothesis is rejected.

DISCUSSION

The study found that an 8-week sequential program of Pilates exercise followed by resistance training significantly improved balance, functional mobility, and disease severity in patients with Parkinson's disease. Post-intervention, UPDRS scores decreased by 4.37 points, TUG time reduced by 2.67 seconds, and FAB scores increased by 4.40 points, all with $p < 0.001$. These improvements are clinically meaningful. A change of >2.5 seconds in TUG and >4 points in FAB is considered the minimal detectable change for fall risk reduction in PD. The risk of falls in these patients is also strongly correlated with postural control disorders [24]. By improving these patient's functional capacity, various training interventions, such as aerobic exercises, hydrotherapy, balance, and gait training, result in significant therapeutic benefits [25,26]. Kibar et al. (2015) discovered that while there was no significant correlation between core muscle endurance and balance, eight weeks of Pilates exercise had positive effects on older adults static and dynamic balance and core muscle endurance [27]. Pilates likely contributed to enhanced trunk stability, core control, and body awareness, while resistance training improved lower limb strength and power, both essential for postural control. In contrast to light walking, these exercises primarily enhance the musculoskeletal elements that contribute to mobility, neuromuscular coordination, and the application of balancing motor strategies, which are followed by facilitating motor learning [28-32]. The sequential approach may allow neuromuscular adaptation before progressing to higher loads. Our findings are consistent with previous studies showing that combined exercise approaches are more effective than single-mode training for balance in PD. Johansson et al. stated that balance is result of mind-body interactions as well as physical balance [33]. The program is low-cost, requires minimal equipment, and can be delivered at the primary healthcare level, supporting SDG 3.4 for reducing disability burden. Limitations include the lack of a control group and the inability to separate the effects of Pilates vs resistance training due to the sequential design. Carryover effects cannot be excluded. Future randomized controlled trials with crossover designs are needed to confirm these results.

CONCLUSION

An 8-week sequential program of Pilates exercise followed by resistance training significantly improved balance, functional mobility, and disease severity in patients with Parkinson's disease. The intervention led to clinically meaningful reductions in UPDRS and TUG scores, and increased FAB scores, indicating reduced fall risk. This low-cost, feasible approach may be suitable for community-based rehabilitation in primary health care settings.

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Senthil Kumar et al.,

Conflict of Interest

The Authors declare no conflict of interest. There is no Grant or Fund Support for this study.

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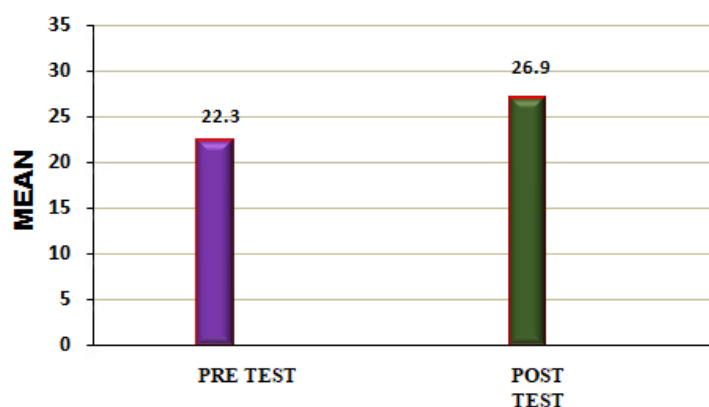


Figure 1:



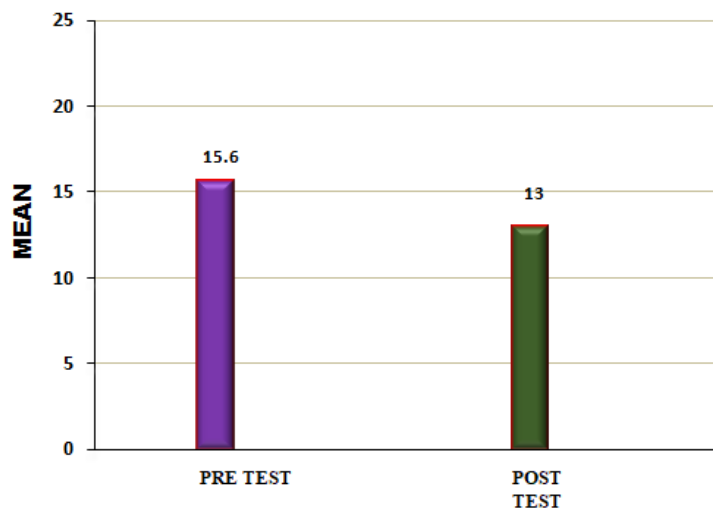
Senthil Kumar *et al.*,

Figure 2:

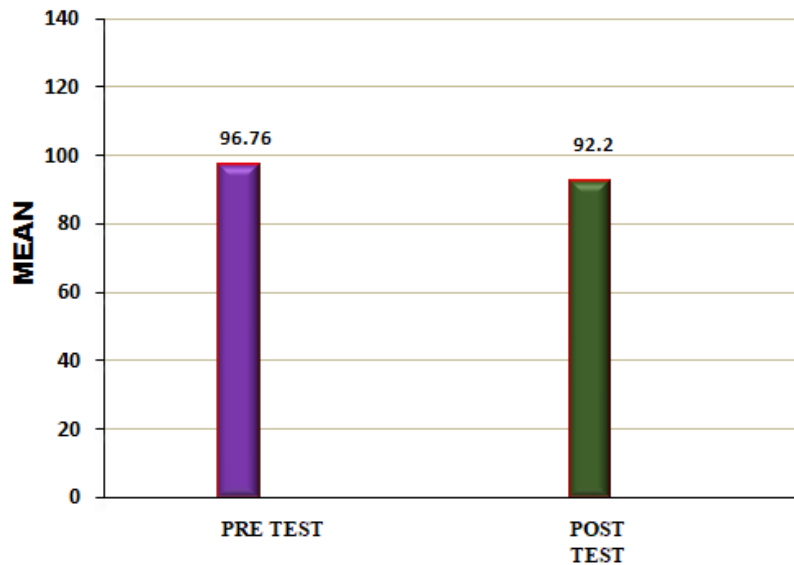


Figure 3:

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Effectiveness of Phosphorous in the Management of Glucoma

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ABSTRACT

Glaucoma is one of the chronic progressive disorders of the eye characterized by visual loss, mainly occurring due to a rise in intra-ocular pressure. It is one of the major causes of blindness. The incidence and prevalence in India estimated that there are approximately 11.2 million persons aged 40 years and older with glaucoma in India. The estimated number with primary angle closure glaucoma is 2.54 million. Primary open-angle glaucoma is estimated to affect 6.48 million persons.

Keywords: Glaucoma, Homoeopathy.

INTRODUCTION

Glaucoma is one of the chronic progressive disorders of the eye symptomatically visual loss, mainly occurring due to a rise in intraocular pressure. The most important factor which causes rise in intraocular pressure is obstruction of the drainage of the aqueous humor through the angle of the anterior chamber and at the pupil. The intraocular pressure (IOP) refers to the pressure exerted by intraocular fluids on the coats of the eyeball. The normal IOP varies between 10 and 21 mm of Hg.

Open-angle glaucoma

Open-angle glaucoma is the most common form of the disease. The drainage angle formed by the cornea and iris remains open, but the trabecular meshwork is partially blocked. This causes pressure in the eye to increase. This pressure damages the optic nerve. It happens so slowly that it may lose vision before aware of a problem.



**Rajendra Babubhai Patel and Jignesh Patel**

Angle-closure glaucoma-Angle-closure glaucoma, also called closed-angle glaucoma occurs when the iris bulges forward to narrow or block the drainage angle formed by the cornea and iris. As a result of it, fluid can't circulate through the eye, and pressure increases. Some people have narrow drainage angles, are at high increased risk of angle-closure glaucoma.

Normal tension glaucoma-In normal-tension glaucoma, the optic nerve becomes damaged even though eye pressure is within the normal range.

Glaucoma in children-It's possible for infants and children to have glaucoma. It may be present from birth or developed in the first few years of life. The optic nerve damage may be caused by drainage blockages or an underlying medical condition.

Pigmentary glaucoma-In pigmentary glaucoma, pigment granules from the iris build up in the drainage channels, slowing or blocking fluid exiting the eye.

Case Summary

A 50 year/male no K/C/O any major illness come with the complaint of difficulty in seeing the object especially the peripheral vision ,headache in the frontal region since 2 weeks along with watery discharge & burning in both eyes.Twitching of eyelids since 2 days,letters appear red. There is edema of eyelids and Pain in orbital bone for the complaints started patient Irritability increased .

Family Details

Father-Hypertension since 20 years on regular treatment.

Past History

Dengue fever at the age of 25 years, recovered with the Allopathic Management.

Physical General

Appetite: 2 times/day,Hunger Tolerable.

Thirst: 3 liter/day ,short quantity long interval.

Urine: 3 times/day ,1 times/night.

Stool: 2times/day,satisfactory.

Sleep: 6-7 hours/day/refreshing.

Habits/Addiction

Eating of Tobacco for 10 years(2 packets/day)

Diagnostic With Consideration

Ophthalmic examination-

Significance of Test

Glaucoma is examined through modern science to measure Intra-Ocular pressure in various categories like Tonometry Examination, Application of Tonometry through Gonioscopic to observe the thickness of cornea via Perimetry to evaluate the visual field defect via projection Perimetry and to know the status of retina ,macula, fovea ,optick, retinal nerve fibre layer through OCT(Optical Coherence Tonometry) and easy process to examine IOP is NCT (Non control Tonometry) throughout lifetime.

Totality Of Symptoms

1-Difficulty in seeing an object.

2-Headache.



**Rajendra Babubhai Patel and Jignesh Patel**

- 3-Watery Discharges,
- 4-Burning in both eyes.
- 5-Twitching in both eyes.
- 6-Odema in eyelids.
- 7-Pain in orbital bone.
- 8- Ameliorated by washing
- 9-Irritability during complaints.
- 10-Tobacco Addiction.

Repertorial Result

PHOS- 38/11, SULPH:35/10, ARS.ALB 33/10, BELLA-31/09,NAT-M 30/10, SPIG 30/10.

Case Analysis

After a complete analysis and evaluation of symptoms, Totality was constructed based on the difficulty in seeing or an object, Headache, Watery discharges, Burning and Twitching, edema, pain in the orbital bone, complaints better by washing, Irritability during complain, and Tobacco addiction. According to Hahnemann's classification of diseases it comes under local surgical diseases, which comes under dynamic diseases given in aphorism 192, all the changes and symptoms are collected to form the totality of symptoms. After doing Repertorisation from the complete repertory Phosphorous was finally selected as a similimum. Based upon on age, susceptibility and pathology 200 potency was given and frequent repetition given till the objective and subjective symptoms were relieved.

Treatment Approach

While going through the case after a detailed examination, physical particular qualified characteristics are prominent in the case which helps in choosing complete and reference to Materia Medica, Phosphorous covers the qualified physical particular symptoms along with the objective symptom hence prescribed the medicine.

RESULT**DISCUSSION**

This study concerns with the community through long-lasting medicine from modern medical science in some categories at a very high rate. So effectively of Homoeopathic medicine through easy to administer and very low rate, so anybody can afford and prevent their vision.

CONCLUSION

The description and discussion regarding this study are going towards the prevalence ratio of the vision in the community. Different medicines are liable control IOP, to prevent retinal blindness, to maintain the optical status and functional condition of Glaucomatic structure, prevention of complications after Glaucoma diagnosis.

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Rajendra Babubhai Patel and Jignesh Patel
Table 1

No	Examination	Re	Le
1	Uncorrected Vision	6/60	6/36
2	Corrected Vision / Older Glasses	6/9	6/9
3	Bilateral Correction Of Visual Acuity/ Refraction	6/9	6/9
4	Bilateral Correction of Near Visual Acuity	N/6	N/6
5	Measurement Of Intraocular Pressure (mm of Hg)		
5a	A- NCT	21	21
5b	B- Applanation Tonometry	22	23
5c	C- Schiotz Tonometry	20.6	21.3
5d	D- Gonioscopy	OPEN ANGLE	OPEN ANGLE
5e	E- CCT	540 mm	545 mm
5f	F- Pachymetry	570 mm	580 mm
6	Fundus Examination		
6a	Retina	WNL	WNL
6b	RNFL	THIN	THIN
6c	Macula	NORMAL	NORMAL
6d	Optic nerve	WNL	Partially hyperaemic
6e	Fovea	WNL	WNL
6f	Disc	WNL	WNL
6g	Disc Ratio	0.6 CDR	0.65 CDR

Table 2. Visual field Test: It can see peripheral vision.

	EYE	
	Right	Left
Conjunctiva	Red	Red
Sclera	White	White
Eyelids	NAD	NAD
Pupils	Dilated	Dilated

Table 3. Analysis of Symptoms

Sr. No	Symptoms	Type of symptoms
1	Difficulty in seeing object .	Physical Particular Common
2	Headache in the frontal region.	Location Physical Particular Uncommon
3	Watery discharge.	Physical Particular Common
4	Burning in both eyes.	Location Physical Particular Uncommon
5	Twitching of eyelids.	Location Physical Particular Uncommon
6	Letters appear red.	Physical Particular Common
7	Pain in orbital bone.	Location Physical Particular Uncommon
8	Oedema of Eyelids.	Location Physical Particular Common
9	Irritability.	Physical Particular Uncommon Concomitant
10	Complaints ameliorated by washing	Physical Particular Uncommon Ameliorating Characteristic Modality
11	Tobacco Addiction.	Physical Uncommon

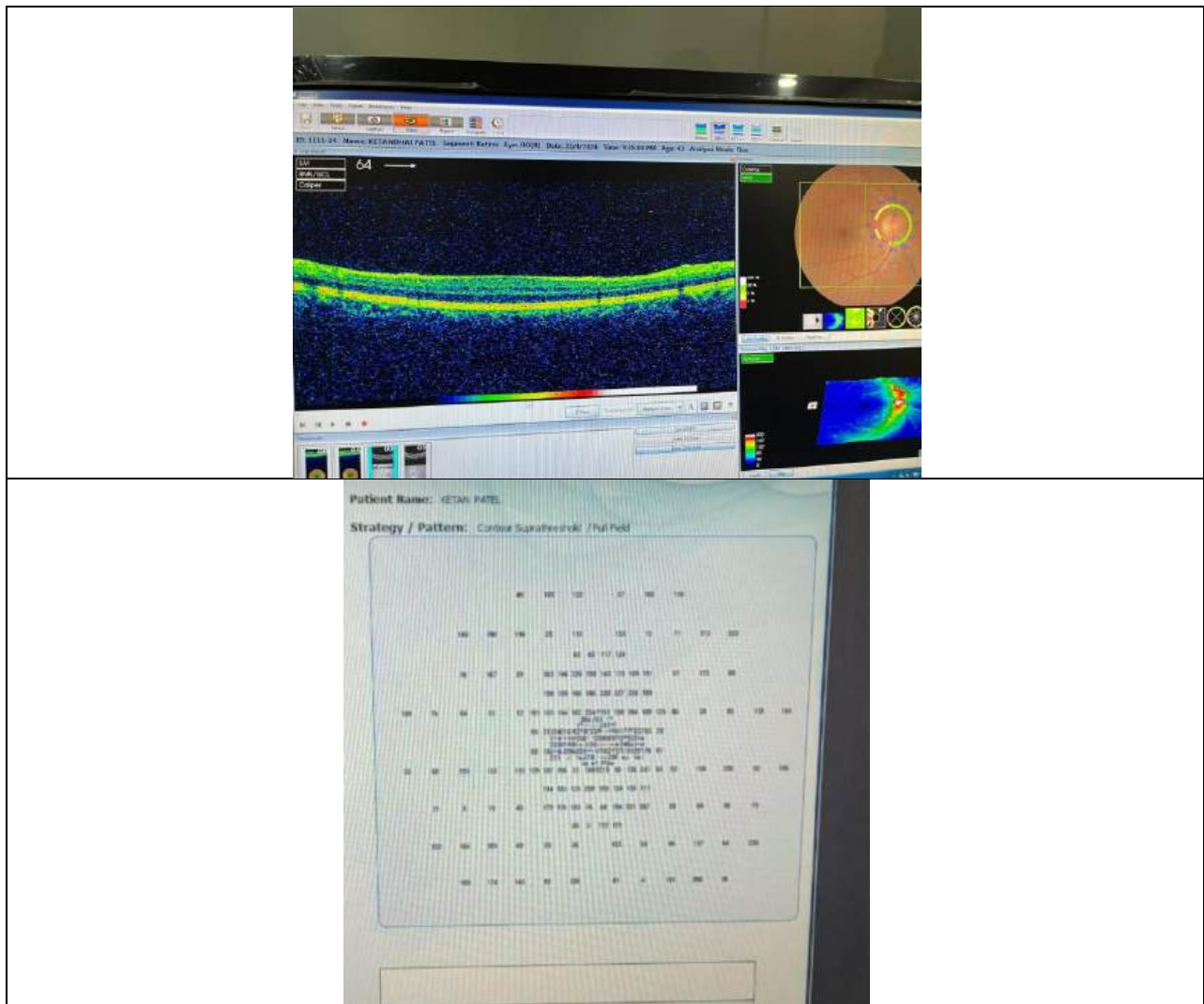




Rajendra Babubhai Patel and Jignesh Patel

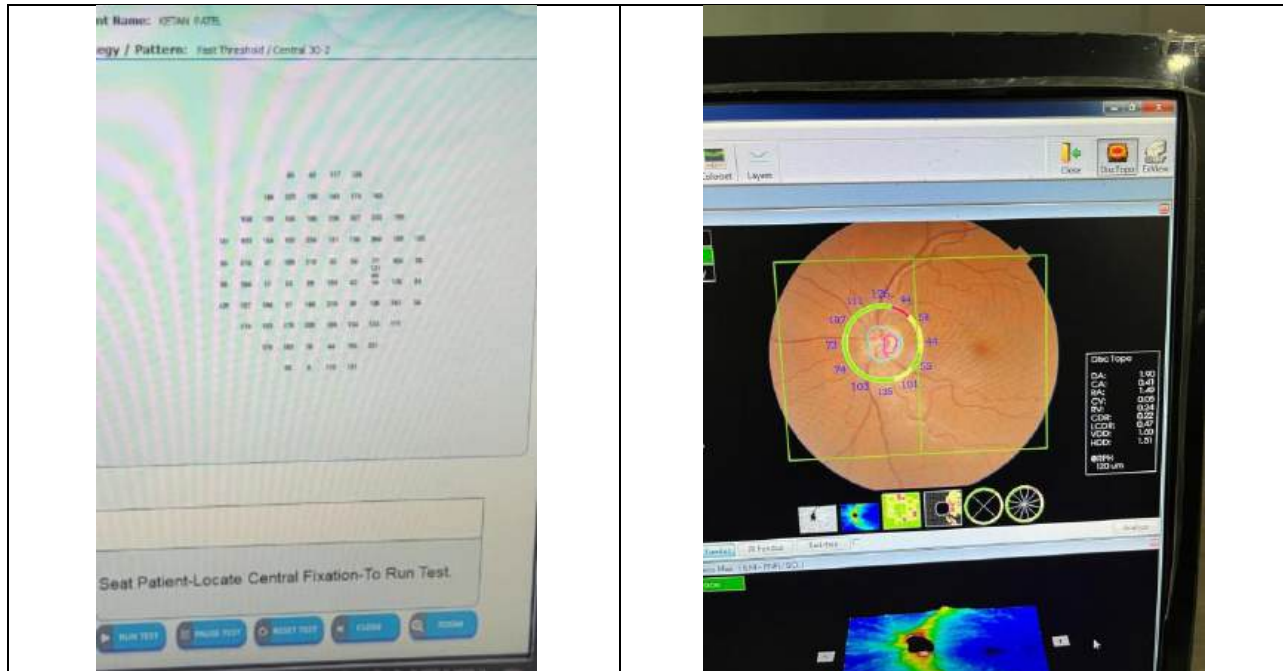
Table 4.

DATE	IOP MEASUREMENT (mm of Hg) NCT		PRESCRIPTION
	RT EYE	LT EYE	
17/03/2024	20.6	20.6	PHOSPHORUS 200 BD For 15 Days
02/04/2024	17	19	PHOSPHORUS 200 BD For 15 Days
18/04/2024	18	21	PHOSPHORUS 200 BD For 15 Days
03/05/2024	20	22	PHOSPHORUS 200 BD For 15 Days
19/05/2024	23	21	PHOSPHORUS 200 BD For 15 Days
04/06/2024	20	18	PHOSPHORUS 200 BD For 15 Days
20/06/2024	17	15	PHOSPHORUS 200 BD For 15 Days
02/07/2024	18	17	PHOSPHORUS 200 BD For 15 Days





Rajendra Babubhai Patel and Jignesh Patel



Remedy	Phos	Sulph	Ars	Bell	Nat-m	Spig	Nux-v	Puls	Thuj	Caust	Ign	Asar	Calc	Nat-c	Apis
Totality	38	35	33	31	30	30	30	30	29	28	27	27	26	26	26
Symptoms Covered	11	10	10	9	10	10	9	9	10	9	9	8	9	9	8
[Complete] [Mind] Irritability:	4	4	4	4	4	3	4	4	4	4	4	3	4	4	4
[Complete] [Generalities] Cold:Agg.:	4	4	4	4	4	4	4	4	4	4	4	4	4	4	3
[Complete] [Nose] Pain:Sinuses:Frontal:	0	0	3	0	1	1	3	0	3	0	1	0	0	0	0
[Complete] [Head] Pain, headache:Forehead:	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
[Complete] [Eyes] Discharges:Watery:	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0
[Complete] [Eyes] Pain:Burning, smarting, biting:	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
[Complete] [Eyes] Twitching:Lids:	3	4	3	3	4	4	3	3	2	3	3	4	3	1	3
[Complete] [Eyes] Swelling:Lids:	4	4	4	4	3	3	4	4	3	3	4	2	4	4	4
[Complete] [Vision] Red:Letters, while reading:	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
[Complete] [Eyes] Pain:Bones, orbits:	2	3	1	4	2	3	1	0	1	2	1	0	1	2	3




Rajendra Babubhai Patel and Jignesh Patel

[Complete] [Generalities] Tobacco:Desires:	3	3	3	3	0	3	3	2	3	3	2	3	1	2	0
[Complete] [Generalities] Bathing, washing:Amel.:Face:	3	0	3	0	1	1	0	0	0	1	0	3	0	0	1
[Complete] [Vision] Distant, objects seem:	3	4	0	1	3	0	0	4	1	0	0	0	1	1	0

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RESEARCH ARTICLE

Problems in Prospects of Organ Transplantation Laws in India with Special Reference to the Gray Areas: A Doctrinal Study

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ABSTRACT

Organ transplantation law in India has evolved significantly with the enactment of the Transplantation of Human Organs Act, 1994 (THOA), later amended as the Transplantation of Human Organs and Tissues Act, 1994, with the objective of regulating organ removal, storage, and transplantation while curbing commercial dealings in human organs. Despite this progressive statutory framework and the establishment of institutional mechanisms such as the National Organ and Tissue Transplant Organization (NOTTO), the practical implementation of organ transplantation laws in India continues to face serious structural, ethical, and regulatory challenges. This paper critically examines the problems and future prospects of organ transplantation laws in India, with particular emphasis on the gray areas that undermine their effectiveness. The study identifies ambiguities relating to brain-death certification, consent procedures, living donor regulation, and the functioning of Authorization Committees. It highlights the persistent gap between legal intent and medical practice, especially in cases involving unrelated donors, cross-border transplant tourism, and allegations of coercion or financial inducement. Further, issues of infrastructural inequality, lack of awareness, regional disparities in cadaveric donation, and inadequate monitoring mechanisms complicate the enforcement of the statutory scheme. Ethical tensions between autonomy, altruism, and prevention of commercialization remain inadequately resolved within the present legal framework. The paper also explores emerging concerns such as data transparency, digital organ allocation systems, accountability of private hospitals, and the need for harmonization with international standards. While the law aspires to balance the right to health and human dignity with safeguards against exploitation, its gray areas often create interpretational inconsistencies and administrative discretion that may lead to arbitrariness. By adopting a doctrinal and policy-oriented approach, this study argues for clearer statutory guidelines, stronger oversight mechanisms, enhanced public education, and ethical standardization to strengthen the transplantation regime. It concludes that although India possesses a comprehensive legislative structure, meaningful



**Purbita Das**

reform and robust implementation are essential to transform organ transplantation from a legally regulated activity into an equitable and ethically grounded public health system.

Keywords: Organ Transplantation Law, Transplantation of Human Organs and Tissues Act, 1994, Brain Death Certification, Living and Cadaveric Donation, Authorization Committees

INTRODUCTION

In the current scenario, one of the crucial aspects in the medical field is organ transplantation. The reason behind the significance of the issue is rising nowadays, as it has become one of the vital elixirs of life for any human being. Under the current medical innovative regime, organ transplantation entails an individual willingly donating an organ or tissue to help save a life from organ failure [1]. It is a key life-supporting measure that has revolutionised modern healthcare, reviving hope for those battling late-stage organ impairment. India's demographic scale and growing healthcare capabilities have catalysed an increased reliance on organ transplantation since the 1970s, defined by the initiation of kidney transplantation in India. The development of organ transplant procedures substantially improved the health outcomes of individuals with organ dysfunction, mitigating the scarcity challenge stemming from the chronic shortfall in viable organ supply. Challenges, including limited posthumous organ donors and identifying a medically viable match, have aggravated the organ scarcity in India.

The imbalance between organ availability and transplant demand has fuelled unethical practices, such as organ trafficking and illicit organ trade, which transgress legal standards [2]. The consequences of such practices are imposed upon socioeconomically disadvantaged groups, constituting a breach of the Right to Life enshrined in Article 21 of the Indian Constitution [3] [4]. In response to the urgency for a systematic framework in organ transplantation in India, the Indian Parliament enacted the Transplantation of Human Organs Act in 1994. It is responsible for monitoring and regulating organ retrieval, preservation, and transplantation for medical services while enforcing stringent anti-trafficking safeguards. The Transplantation of Human Organs and Tissues Act, 1994 (here, referred to as "THOTA") has been uniformly implemented across all states, excluding Andhra Pradesh, and Jammu & Kashmir, which have similar statutory frameworks [5]. Several amendments have been introduced to refine this legislation, with the most recent being the Transplantation of Human Organs and Tissues Rules in 2014, notified with effect from March 27, 2014. Despite the current organ transplant laws in India, the scenario remains dire. There has been a lack of a significant rise in the number of cadaveric donors or curbing the commercialization of organs. Brain death continues to be overlooked and inadequately addressed. Various other regulatory complexities hinder organ transplantation in India which needs to be resolved. Consequently, the legal scenario needs to be reassessed, requiring the formulation of new laws that align with the international conventions and principles of the Indian Constitution.

Features of Transplantation of Human Organs and Tissues Act, 1994

The 20th century marked the greatest medical development in India, including the introduction of law on the transplantation of organs. The introduction of mechanical ventilation into the treatment process of patients in a brain-dead condition, as well as the further development of immunosuppressive therapy, has transformed the aspects of organ donation and transplantation significantly [6]. The practice of organ transplantation has become a life-saving technique and a reflection of human kindness, with people voluntarily willing to donate their organs to save the lives of others. An organ transplant is a life-saving surgical procedure involving a medically healthy donor to transfer his/her organs or tissues to a recipient with organ failure. In addressing various serious illnesses, such as liver diseases, kidney failure, or other last-stage organ failure, organ transplantation is seen as an effective and innovative survival alternative [7]. In India, the law relating to organ transplantation is governed by the Transplantation of Human Organs and Tissues Act, 1994, followed by the formulation of the Transplantation of Human Organs and Tissues Rules, 2014 for the enforcement of the Act. The Act lays down provisions regarding the removal, protection,



**Purbita Das**

and donation of human organs and tissues for medical use and the prohibition of organ commercialisation. Section 2(h) of the Act defines a human organ as any part of a human body consisting of a structured arrangement of tissues which, if wholly, removed, cannot be replicated by the body. Section 2(p) refers to transplantation as the grafting of any human organ from any living person or deceased person to some other living person for therapeutic purposes [8]. The Act permits the practice of organ transplant to be conducted by hospitals or registered centres licensed by a competent authority. According to the Act, the donor and the receiver should be directly related to each other, and the organ transplantation can only be made when the donor meets the informed consent. These provisions ensure that organ transplantation is voluntary and ensure that all the parties are fully aware of the consequences involved [9]. The Act, further, governs the setting up of government-approved organ transplantation centres which are delegated with carrying out organ transplants being done in compliance with set rules of organ matching that exist between the donor and the recipient or donor and recipients of organs as well as protocols governing organ sourcing and transplantation. The Transplantation of Human Organs (Amendment) Bill, 2011 established the National Apex Committee for Regulation of Transplantation (NACROT) to implement measures for overseeing living donor transplantation. The Act also mandates the Central Government to incorporate a national registry of the donors and recipients of human organs and tissues [10].

Classifications of Human Organ Transplantation

Organ donation can be classified into two categories: Living Organ Donations and Deceased Organ Donations. Living Organ Donation is when you procure an organ from a healthy living donor and graft it into the body of a recipient suffering from last-stage organ failure. This procedure is typically undertaken when kidney or liver failure occurs, as the liver has the capacity to regenerate, and a single kidney is adequate to maintain body functions. Living donors may be identified as either a near relative or a distant relative. A near relative means a spouse, son, daughter, father, mother, brother, sister, grandfather, grandmother, grandson or granddaughter. A distant relative can be any person except a near relative, who first requires authorisation from the State Authorisation Committee to be able to transplant organs. If the hospital refuses to handle such cases, the patient may serve a legal notice on the hospital for contravening the Transplantation Act [11].

Deceased Organ donation is simply the act of donating or transplanting organs after the death of the donor. By death, it means that the person has been declared brain-stem dead by a registered medical practitioner, by a personal examination of such body. A person can be considered brain-stem dead if there is a state of permanent unconsciousness, loss of brain-stem responses, and cessation of respiratory function [12]. A deceased organ donation shall only be permitted after brain-stem death has been certified, and where the brain-stem death occurs of any individual less than eighteen years of age, permission from any of the parents of the deceased person is mandatory for the removal of the tissue or organ from the body of the deceased person [13].

Medical Procedures for Organ Transplantation

Any living person who is willing to give his/her tissue or organ to any person with tissue or organ failure can be termed as a living donor. It may include parents, siblings (related donors), and also spouse donors. In case of related donors, authentic documents verifying the relationship must be submitted in accordance with the Act. Additionally, where either the living donor or the recipient, being a near relative, holds a foreign nationality, no human organ or tissue shall be transplanted without prior approval from the Authorisation Committee. And where the donor, being an Indian national, agrees to transplant his/her tissue or organ to a recipient holding a foreign nationality, such removal or donation shall not be approved by the Authorisation Committee [15].

Spouse donor: In case where the donor and the recipient are a married couple, documentary evidence such as a marriage certificate, marriage photographs, important family information like the number of children and the age of children, a family photograph portraying the entire family, birth certificates of the children, is mandatory. Other than near relatives: It is a debated aspect of organ donation due to the concerns surrounding illegal, coercive, and profit-



**Purbita Das**

driven organ transplants. If there is no biological relation between the donor and the recipient, approval from the authorization committee is required.

Swap donor: The donation of tissues or organs by donors who are family members but have different blood types is referred to as swap donation. Provisions for swap donor transplantation are included in the Transplantation of Human Organs and Tissues Act, 1994, following the most recent amendment in 2011. Section 9 sub-section 3A provides for swapping of ‘near-relative’ donors of biologically incompatible recipients, subject to prior consent of the Authorisation Committee [16].

Donor or recipient belonging to different states: In situations where the donor, recipient, and transplantation center are situated across different states, then obtaining approval or a ‘no objection certificate’ from the respective State Government is mandatory.

Foreign donors: The Act places stringent regulations on foreign donors. When either the donor or recipient is a foreign national, the removal or transplantation of human tissues or organs shall require the prior approval of the Authorisation Committee. An Indian living donor shall not donate organs to a foreign recipient unless the donor is a near relative of the recipient. Additionally, a senior official of the respective country’s embassy in India is responsible for certifying the donor-recipient relationship.

Cadaver donors: Also known as deceased donor transplant, it involves the donation of tissues or organs by a deceased individual. Organs, including bones, skin, kidneys, liver can be transplanted from cadaver donors

Current Legal Frameworks and Legal Mechanisms

The Transplantation of Human Organs Act, enacted in 1994 and came in February 1995, was amended in 2011 and 2014, which aimed to restrict the practice of organ trafficking and encourage cadaver donations. Some of the key provisions highlighted in the Act include: Section 13-15: Appointment of appropriate authorities and tissue banks. Medical institutions conducting organ transplants must be duly registered with the appropriate authorities to be involved in:

- The retrieval, storage, and donation of any human organ;
- For Tissue banks to be involved in the process of retrieval, examination, testing, preservation, and dissemination of tissues.

Authorisation committees

The Act calls for the establishment of Authorisation Committees by the Central and State Governments to evaluate applications for transplantation between unrelated donors and recipients. Under Rule 7 of the Transplantation of Human Organs and Tissues Rules, 2014, these committees aim to prevent coercion and financial exploitation in verifying the relationship claimed by the donor and the recipient [17].

Brain-stem death and cadaveric donors: The Act allows for the removal of tissues or organs from the bodies of patients in the event of brain-stem death, provided that such death has been certified. Every organ donation procedure shall include the following steps before the transplant is carried out [18].

A board consisting of four medical practitioners, two of whom belong to a board appointed by the government, must confirm and declare brain-stem death. This evaluation has to be conducted twice, with a six-hour interval between the tests. The board shall comprise of:

- A registered medical practitioner working under the hospital where the admission of brain stem death patient took place.
- A registered medical practitioner who has been chosen among a panel of doctors under the approval of the Authorisation Authority.



**Purbita Das**

- A neuro-surgeon (in case where a neuro-surgeon is not available to conduct the tests, the panel may include any Physician or Anaesthetist who has been chosen by the Medical Administrator in-charge among a list of names approved by the Authorisation Authority [19].

This transplantation process has to be strictly complied with. The certifying clinicians must not have any personal interest in the transplantation of cadaver organs. The certification must be in accordance with the provisions of the Transplantation of Human Organs and Tissues Act, 1996. The medical superintendent in charge of the hospital should finally cross-check and sign the form. The organs can only be allowed for retrieval, removal, or donation after the completion of these due procedures.

Challenges Ahead

In the Indian context, the demand for donor organs greatly outweighs their availability. Over the last decade, the rate of cadaveric organ donation in India has remained persistently under one donor per million population. While there is a significant increase in donors from 6,916 in 2014 to 16,041 in 2022, around 85% of all donations are made by living donors. Despite an annual need of 200,000 kidney transplants, around 10,000 transplants are carried out each year., aggravating the shortage. A 2022 report by NOTTO states that an estimated 15,561 organ transplants were performed in India, with only 2,765 originating from deceased donors. Living donors make up 82% of all organ donations in India. This represents the necessity to raise deceased organ rates for donors, including kidneys, liver, and heart, which remains notably low [20]. The number of deceased donors varies across the states. As per NOTTO, Telangana reported the highest number of deceased donors in 2022. This is associated with advancements in healthcare services, especially in the context of COVID-19. The increased access to ICUs ventilators, and ECMO machines has optimized emergency management, resulting in more successful donations. Overall, despite the rise in organ donation figures, India continues to face an acute shortage of organs, causing serious difficulties for patients in need of transplants. The challenges are as follows,

- **Organ trafficking:** Based on estimates reported by Global Financial Integrity, an estimated 10% of heart, liver, and lung transplants are sourced through illegal means. As reported by the Human Rights Organisation, nearly 44,000 children go missing in India yearly, with most of the cases constituting organ trafficking. Despite rigorous legal provisions, the illegal organ trafficking remains prevalent, particularly in the marginalized communities.
- **Consent for organ transplant:** Organ donation requires the informed consent of the donor or his family, in case of death. However, cases exist in which the legal validity of the consent has been disputed, particularly relevant to deceased donors, and the family members have conflicting opinions about organ donation. Additionally, while the law encourages voluntary donation, concerns exist as to whether individuals are truly providing informed consent or coerced in any manner, especially when donors are economically disadvantaged. The legal status of living donors and potential exploitation continues to occupy a gray area in the eyes of law.
- **Lack of transparency:** It is often presumed that organ distribution is based on high-level connections, rather than patient's medical necessity. Although authorities are mandated to follow a transparent procedure, the law fails to ensure accountability for authorities who default.
- **Low public awareness and education:** There is a limited awareness about the significance of organ transplantation in India. The erroneous perception that organ donation is unnatural, coupled with religious prohibitions, results in shortage of organs [21].

CONCLUSION AND SUGGESTIONS

The laws regulating organ transplantation in India, THOTA, have encountered numerous implementation challenges, including organ scarcity, illegal organ trade, ethical considerations, and societal apathy. A major section of our Indian society is triggered by organ trafficking, and the primary drivers motivating unrelated organ donors pertain to poverty and debt. To address these challenges, the researcher has suggested a few reforms as follows:



**Purbita Das**

Strengthened enforcements: The government must work towards improving the implementation of laws relating to human organ trafficking. For any kind of involvement in illegal organ trade, individuals shall be subject to stringent penalties, and better surveillance and monitoring shall be introduced to deter criminal ties and combat such legal challenges. Concern towards the organ donor while encouraging more organ donation: The health of the organ and tissue donor must be of paramount concern, especially during the post donation health complexities. In the current scenario, there is a pressing need to encourage more donation to combat the scarcity. Awareness benefits showcasing the significance of posthumous organ donation, coupled with more efficient consent processes should be established.

Education on healthcare: Strengthened healthcare and training programs for healthcare experts shall be introduced to encourage understanding the procedure of organ donation, the legal and ethical considerations, and counselling programs for organ donors.

Transparency in transplantation procedures: Transparently ensuring the allocation of organs and promoting equitable availability to transplantation can prevent the practice of favouritism in organ matching. A more reasonable and transparent practice would increase credibility in the system and reduce unlawful practices.

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The author further states that there are no conflicts of interest associated with this research.

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**Purbita Das**

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RESEARCH ARTICLE

Stress Analysis Using Fuzzy Logic for Professors in College

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ABSTRACT

In this paper Fuzzy logic of stress is the action taken to adjust any change in physical, mental and emotional status in response. Nowadays we stress ourselves in connection with the surrounding events in which we face many problems. The stress in the workplace where we spend most of the time is the most harmful physical and emotional responses that can raise the pressure levels in the body. Heavy workload within a short spell of time can lead to stress. Adverse mental and physical changes occur in a person who is unable to handle his stress. In this paper we use Hamming distance and proposed distance measure to analysis the stress.

Keywords: Stress, Fuzzy Logic, BP, hamming distance, proposed distance measure.

INTRODUCTION

Fuzzy logic has been applied to various fields where the value of truth many range between completely true and the completely false i.e., to handle the concept of partial truth. The most common and significant health disease nowadays is stress, which affects the human physically and mentally bad. The excess chronic physical and mental stress has become threat for the individual which leads to suicides. Stress is an emerging challenge to the life and health of human, though it has become apart of our life [1], [2]. It is significant importance to detect stress before it becomes a high problem. Stress simulation depends upon two mechanisms; Alarm reaction and the adaptation. If an individual undergoes a peculiar or unpredicted threat in the workplace they respond to increased muscular activity. Secondly the individual becomes anxious if things do not work the way they have adapted to the workplace. Stress





Rama and Uma

has a background with two forms. i.e., physical and mental. The disturbance in bodily environment or mental working strategy of the individual maybe due to family background [3], [4]. It is a complicated process to understand human stress level. Response to stress differs from individual to individual. The experience and the behavioral difference in overcoming the problems/situations, individual characteristics, gender, age, physical and mental conditions itself will give variations in individuals stress level. Based on that the stress response system will react different from each individual. Hence the psychological response to stress of human beings vary. Mostly it is difficult to describe the stress level. The person feels that their body is under stress but it is difficult to judge their stress level whether high or low. Sometimes physical responses of human have different value from time to time [8].

METHODOLOGY

The samples were collected from 50 individuals (faculty members) working in Bharath College of Science and Management, Thanjavur, Tamil Nadu, India. In that ratio of female and male 30:20 depending on the variance of gender, age, blood pressure for analyzing stress level started from the age of 25 to 55 years.

Blood Pressure levels:

Normal: 80/120 Low: 60/90 High: 100/140

Fuzzy Logic Rules

If age below 35 and BP is high then stress level high

If age above 35 and BP is high then stress level Medium.

Distance Measures between Fuzzy sets

For two fuzzy sets M and N in the same universe, the Hamming distance is an ordinal measure of dissimilarity. The Hamming distance (HD) is defined as

$$|HD(M, N)| = \left| \sum_{i=1}^n |\mu_M(x_i) - \mu_N(x_i)| \right|$$

where n is the number of points that define the fuzzy sets M and N, $\mu_M(x_i)$ the membership of point x_i in M and $\mu_N(x_i)$ is the membership of point x_i in N. The Hamming distance is smaller for fuzzy sets that are more alike than those that are less similar.

Proposed Distance Measure

The proposed distance measure D(M, N) is defined as:

$$|D(M, N)| = \left| \frac{1}{4} \left(\sum_{i=1}^n |\mu_M(x_i) - \mu_N(x_i)| + \sum_{i,k=1(i \neq k)}^n (2(2|i-k|-1)\mu_M(x_i)\mu_P(x_k)) \right) \right|$$

Where M is the actual fuzzy classification n the number of classes that define M and P, $\mu_M(x_i)$ is the membership of point x_i in M and $\mu_P(x_k)$ is the membership of point x_k in P.

RESULT

Table 1 shows the data collected from various Professors. Fuzzy logic is used to determine risk of stress. This design consists of two inputs and one output. The input consists of age, blood pressure while the output is the risk of stress.

CONCLUSION

In this concluding chapter we summarized the ideas that how to develop the basic behavior of our life and the research methodologies using hamming distances and proposed distance measure. From this research we got some





Rama and Uma

different types of result among the different types of age groups. The age between 25 to 35 having too much of stress and the BP level is high and also they are suffered a lot in all shots like nature of work, insecurity of job, inferiority complex, lack of concentrate, and they do not know how to handle the problems in critical situations meanwhile at the age of 35 and above had the same level of BP but they are control their BP level and lead their life in peaceful manner, experience in nature of work, doing some kinds of exercises and made some changes of their food habits, these are main concepts I got at the time of research. Infuture we planned to give some innovative solutions to reduce their stress and BP level for the age group 25-35.

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Table 1: Age & BP details of 50 Professors.

S.No	Dept	Name	Male	Female	Age	Blood Pressure		Range
						Systolic	Diastolic	
1	B.C.A	R. Aravazhi	M	-	47	140	90	High
2		R. Kavitha	-	F	43	90	60	Low
3		S. Brundha	-	F	38	90	65	Low
4		R. Mercy		F	42	120	82	Normal
5	C.S	R. Sumithra	-	F	43	138	90	High
6		S.Natarajan	M	-	40	135	90	High
7		R.Murugesan	M	-	54	140	100	High
8		U. Gomathi	-	F	42	130	100	High
9	I.T.	N. Raaja Rajeswari	-	F	43	160	110	High
10		S. Krishnavalli	-	F	37	140	90	High
11		D. Silambarasu	M	-	32	120	80	Normal
12		P. Sangeetha	-	F	37	120	80	Normal
13	N&D	P. Suba	-	F	39	110	80	Normal
14		J. Dhivya Dharsini	-	F	31	140	90	High
		M. Menaka	-	F	29	120	80	Normal
16		S. Deepa Priya	-	F	30	120	80	Normal
17	English	A. Suraiya	-	F	44	145	90	High
18		M. Sharmila Meston Vinoth	-	F	46	150	95	High
19		V. Rajendran	M	-	51	140	90	High
20		U. Sangeetha	-	F	30	90	60	Low





Rama and Uma

21	B.T.	S. Jawahar	M	-	40	120	80	Normal
22		D. Praba	-	F	29	120	80	Normal
23		J. Jayasree	-	F	34	140	90	High
24		K. Muruganandham	M	-	43	150	90	High
25	Tamil	M.Sadhanandhan	M	-	45	119	82	Normal
26		N. Sudha	-	F	46	150	90	High
S.No		Name	Male	Female	Age	Blood Pressure		Range
27		P. Kavitha	-	F	44	145	100	High
28	FT	B. Amuthachitra	-	F	44	90	60	Low
29		A.Cecil Alphonse	M	--	45	145	100	High
30		M. Cecilia Devi		F	28	119	81	Normal
31		R. Dhivya	-	F	26	120	80	Normal
32	Maths	S. Abinaya	-	F	36	140	100	High
33		PL. Nandhini	-	F	39	115	78	Normal
34		R. Thamizhselvan	M	-	31	115	75	Normal
35		R. Dhivyabharathi	-	F	29	100	70	Low
36	Physics	B. Praveen	M	-	27	80	120	Normal
37		P. Sivakumar	M	-	44	140	90	High
38	VC	R. Karthik	M	-	31	140	90	High
39		R. Sembian	M	-	52	120	80	Normal
40	H.M.	V. Muruga Pandiyan	M	-	32	120	80	Normal
41		M. Sugumar	M	-	47	120	80	Normal
42		K. Mageshwari	-	F	39	135	100	High
43		G. Balachandar	M	-	54	150	100	High
44	Commerce	P. Jayaram	M	-	31	120	80	Normal
45		R. Sathya	-	F	52	140	90	High
46		L. Arokia Malar	-	F	36	120	80	Normal
47		R. Vanathi	-	F	33	135	100	High
48	B.B.A	K. Rathinavel Pandiyan	M	-	36	117	80	Normal
49		N. Vanitha	-	F	45	140	90	High
50		V. Mohana Sundaram	M	-	55	155	95	High

Table 2: Risk of stress using Hamming distance & Proposed distance measure

S.No	Dept	Name	Male	Female	Age	Blood Pressure		Range	Members hip value		Distance Value	
						Systolic	Diastolic		Age	BP	HD(M, N)	D(M, N)
1	B.C.A	R. Aravazhi	M	-	47	140	90	High	0.8	0.8	0	0.32
2		R. Kavitha	-	F	43	90	60	Low	0.6	0.3	0.3	0.015
3		S. Brundha	-	F	38	90	65	Low	0.5	0.2	0.3	0.025
4		R. Mercy		F	42	120	82	Normal	0.6	0.7	0.1	0.185
5	C.S	R. Sumithra	-	F	43	138	90	High	0.6	0.8	0.2	0.19
6		S.Natarajan	M	-	40	135	90	High	0.6	0.8	0.2	0.19
7		R.Murugesan	M	-	54	140	100	High	0.9	0.8	0.1	0.335





Rama and Uma

8		U. Gomathi	-	F	42	130	100	High	0.6	0.8	0.2	0.19
9	I.T.	N. Raaja Rajeswari	-	F	43	160	110	High	0.6	0.9	0.3	0.195
10		S. Krishnavalli	-	F	37	140	90	High	0.4	0.8	0.4	0.06
11		D. Silambarasu	M	-	32	120	80	Normal	0.3	0.6	0.3	0.015
12		P. Sangeetha	-	F	37	120	80	Normal	0.4	0.6	0.2	0.07
13	N&D	P. Suba	-	F	39	110	80	Normal	0.5	0.6	0.1	0.125
14		J. Dhivya Dharsini	-	F	31	140	90	High	0.3	0.8	0.5	0.005
15		M. Menaka	-	F	29	120	80	Normal	0.2	0.5	0.3	0.025
16		S. Deepa Priya	-	F	30	120	80	Normal	0.2	0.5	0.3	0.025
17	English	A. Suraiya	-	F	44	145	90	High	0.6	0.9	0.3	0.195
18		M. Sharmila Meston Vinoth	-	F	46	150	95	High	0.7	0.9	0.2	0.265
19		V. Rajendran	M	-	51	140	90	High	0.9	0.8	0.1	0.335
20		U. Sangeetha	-	F	30	90	60	Low	0.2	0.1	0.1	0.015
21	B.T.	S. Jawahar	M	-	40	120	80	Normal	0.6	0.6	0	0.18
22		D. Praba	-	F	29	120	80	Normal	0.2	0.5	0.3	0.025
23		J. Jayasree	-	F	34	140	90	High	0.3	0.8	0.5	0.005
24		K.Muruganandham	M	-	43	150	90	High	0.6	0.9	0.3	0.195
25	Tamil	M.Sadhanandham	M	-	45	119	82	Normal	0.6	0.7	0.1	0.185
26		N. Sudha	-	F	46	150	90	High	0.7	0.9	0.2	0.265
27		P. Kavitha	-	F	44	145	100	High	0.6	0.9	0.3	0.195
28		B.Amuthachita	-	F	44	90	60	Low	0.6	0.4	0.2	0.07
29	F.T.	A. Cecil Alphonse	M	-	45	145	100	High	0.6	0.9	0.3	0.195
30		M. Cecilia Devi		F	28	119	81	Normal	0.2	0.5	0.3	0.025
31		R. Dhivya	-	F	26	120	80	Normal	0.1	0.5	0.4	0.075
32		S. Abinaya	-	F	36	140	100	High	0.4	0.8	0.4	0.06
33	Maths	PL. Nandhini	-	F	39	115	78	Normal	0.5	0.6	0.1	0.125
34		R.Thamizhselvan	M	-	31	115	75	Normal	0.3	0.6	0.3	0.015
35		R.Dhivyabharithi	-	F	29	100	70	Low	0.2	0.1	0.1	0.015
36		B. Praveen	M	-	27	80	120	Normal	0.1	0.5	0.4	0.075
37	Physics	P. Sivakumar	M	-	44	140	90	High	0.6	0.8	0.2	0.19
38		R. Karthik	M	-	31	140	90	High	0.3	0.8	0.5	0.005
39	VC	R. Sembian	M	-	52	120	80	Normal	0.9	0.7	0.2	0.265
40		V. Muruga Pandiyan	M	-	32	120	80	Normal	0.3	0.6	0.3	0.015
41	H.M.	M. Sugumar	M	-	47	120	80	Normal	0.8	0.7	0.1	0.255
42		K. Mageshwari	-	F	39	135	100	High	0.6	0.8	0.2	0.19
43		G. Balachandar	M	-	54	150	100	High	0.9	0.9	0	0.405
44		P. Jayaram	M	-	31	120	80	Normal	0.3	0.6	0.3	0.015
45	Commerce	R. Sathya	-	F	52	140	90	High	0.9	0.8	0.1	0.335
46		L. ArokiaMalar	-	F	36	120	80	Normal	0.4	0.6	0.2	0.07





Rama and Uma

47		R. Vanathi	-	F	33	135	100	High	0.3	0.8	0.5	0.005
48		K. Rathinavel Pandiyam	M	-	36	117	80	Normal	0.4	0.6	0.2	0.07
49		N. Vanitha		F	45	140	90	High	0.6	0.8	0.2	0.19
50	B.B.A	V. Mohana sundaram	M	-	55	155	95	High	0.9	0.9	0	0.405

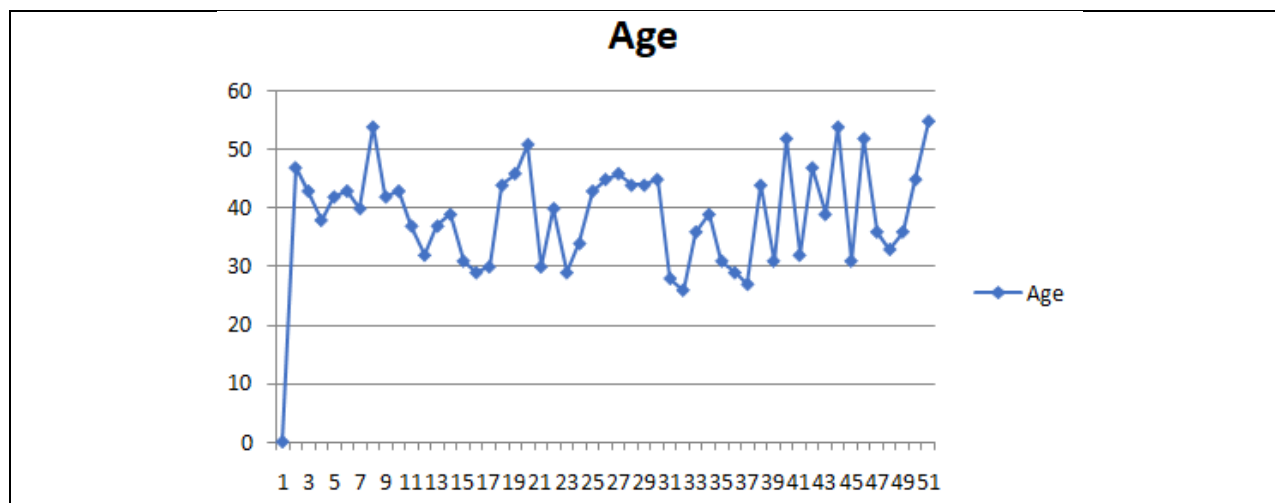


Figure 1: Age

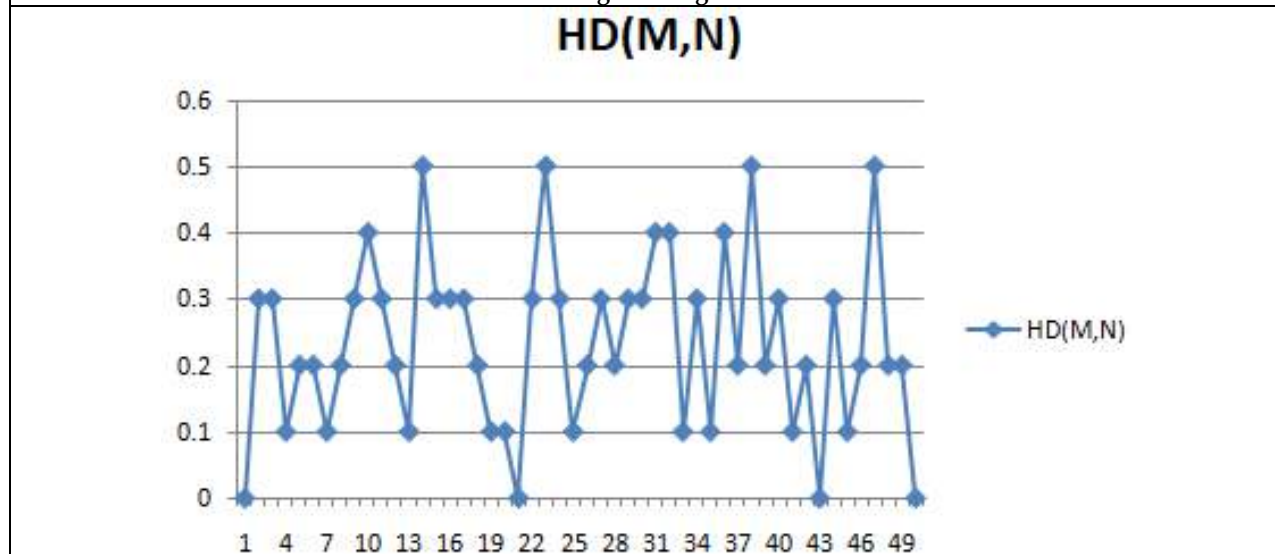


Figure 2: HD(M,N)





Rama and Uma

Age

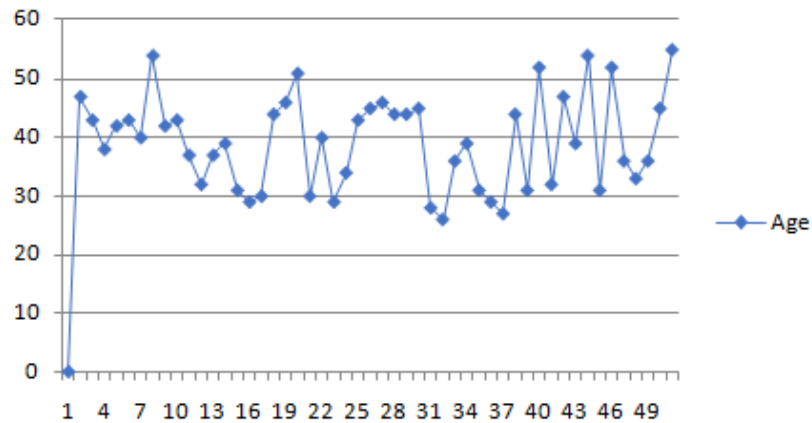


Figure 3: Age

D(M,N)

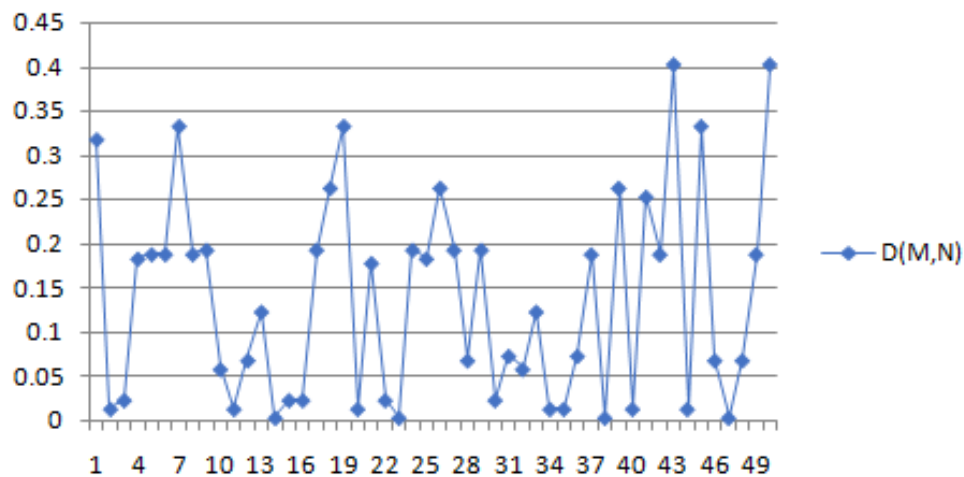


Figure 4: D(M,N)

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REVIEW ARTICLE

The Role of Hemovigilance in Ensuring Safe and Effective Blood Transfusion: A Review

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ABSTRACT

Haemovigilance is a comprehensive surveillance system that enhances transfusion safety by monitoring, reporting, analyzing, and preventing transfusion-related adverse events. It supports patient safety, reduces preventable errors, and promotes evidence-based transfusion practices. This review highlights the objectives, components, and significance of haemovigilance, including national and international programs and the Haemovigilance Programme of India (HvPI). It also addresses challenges such as underreporting, limited infrastructure, and inadequate awareness, particularly in resource-constrained settings. Emerging technologies, including electronic reporting systems, data analytics, and artificial intelligence, offer opportunities to strengthen haemovigilance and improve patient outcomes through safer and more effective transfusion practices.

Keywords; Haemovigilance, transfusion safety, adverse transfusion reactions, blood transfusion, patient safety, transfusion medicine.

INTRODUCTION

The term haemovigilance (French: hémovigilance) was originally used in France. Its roots are the Latin word vigilans, which means "watchful" or "vigilant," and the Greek word haem, which means "blood." A thorough monitoring



**Mohd Yusuf et al.,**

system called hemovigilance is intended to guarantee security during the transfusion procedure.[1]It is defined by the WHO, International Haemovigilance Network, and ISBT with the goal of identifying, reporting, analyzing, and preventing adverse events associated to transfusions. Haemovigilance is essential to improving transfusion processes globally because it improves patient safety, quality assurance, and risk management.[2]The significance of reporting systems in enhancing patient safety and healthcare quality was emphasized in the World Health Organization's 2005 study, Adverse Event Reporting and Learning Systems: From Information to Action. Adverse events are unanticipated blood transfusion-related incidents in transfusion medicine, whereas adverse reactions are detrimental transfusion-related patient reactions. Good reporting improves safety and helps stop recurrence.[3]Only 13% of low-income nations had national haemovigilance systems in place, compared to 30% of middle-income countries and 78% of high-income countries, according to the WHO Global Database on Blood Safety Summary (haemovigilance statistics for 2011–2012 and 2012–2013). These results show notable differences in how hemovigilance systems are implemented in various economic contexts. Therefore, in order to make evidence-based improvements in the safety and quality of blood transfusion practices worldwide, it is imperative that national hemovigilance programs be established and strengthened. [4-5].This will ultimately enable better standards, regulations, and patient care.[6]Because it facilitates the execution of preventative and corrective measures while encouraging the ongoing development of the safety and quality of blood, blood components, and transfusion procedures, hemovigilance is an essential part of quality management in blood transfusion services. In the PDCA (Plan-Do-Check-Act) cycle, it also serves as the "check" phase, supporting continuous process improvement. [7]Over the past few decades, many countries have established haemovigilance systems to improve transfusion safety. Blood donation rates vary widely, averaging 39.2 per 1,000 population in developed countries, 12.6 in developing countries, and only 4.0 in low-income countries, highlighting disparities in access to safe blood.[8,9]

History of Blood Transfusion and Hemovigilance need

In the 19th century, Henri Leacock and James Blundell pioneered human-to-human blood transfusion to manage acute blood loss, although it was considered a last-resort treatment due to significant risks. The 20th century brought major advances, including the discovery of the ABO blood group system, improved cross-matching methods, and anticoagulants, making transfusion safer and widely accepted. [10,11,12]As a result, France started pioneering work in hemovigilance in 1991 when blood transfusion committees established monitoring systems. This eventually resulted in the creation of a nationwide hemovigilance network in 1994. [13]In order to improve blood safety, the European Council passed a resolution in 1995 after France implemented the first hemovigilance system in 1994. In order to foster international cooperation, information exchange, and education, the European Haemovigilance Network (EHN) was founded in 1997 and subsequently changed its name to the International Haemovigilance Network (IHN). [14-15]The International Haemovigilance Network (IHN) is connected to several developed nations' haemovigilance initiatives, albeit national administration varies. TRIP (Netherlands), TTISS (Canada), and SHOT (UK) are notable systems. Although these initiatives have increased transfusion safety, many Asian nations still lack comprehensive hemovigilance systems and data. [16-17]Only 58% of developed nations have transfusion committees in more than half of their institutions. These results show that blood use and transfusion procedure monitoring continue to be important worldwide health issues. Additionally, there are still significant gaps in the surveillance, reporting, and investigation of transfusion-related adverse events, reactions, and near-miss situations, as well as in the systems in charge of monitoring transfusion policies and procedures. [18]

Components of Hemovigilance

An ideal system includes the whole transfusion chain, from donation to patient follow-up, although some merely track transfusion reactions. It guarantees donor-recipient traceability, monitors adverse events, infections, mistakes, and near misses, and supports patient safety, compliance, and remedial measures. [19]

Hemovigilance for Recipient

Recipients' unfavorable transfusion reactions are graded using a globally recognized standard. The severity classification, which ranges from minor symptoms to potentially fatal complications, aids in assessing the clinical



**Mohd Yusuf et al.,**

significance of the reaction. Imputability, or the possibility that a transfused blood component caused the adverse reaction, is assessed in addition to severity assessment. To ascertain whether the transfused blood component was certainly, probably, potentially, or unlikely to be the cause of the observed reaction, imputability assessment is crucial. The International Society of Blood Transfusion (ISBT) has created uniform standards for transfusion reaction severity and imputability. Additionally, the International Haemovigilance Network (IHN) and ISBT joint working group produced globally recognized standards for adverse transfusion events in recipients. These standardized standards make it easier to report consistently, enhance communication between hemovigilance systems, and allow for the meaningful comparison and exchange of hemovigilance data between various nations and healthcare systems. [20-21]

Hemovigilance for Donor

In order to guarantee the general safety and quality of the transfusion procedure, blood donor haemovigilance is equally crucial, especially when it comes to adverse events or responses that may occur during whole blood or component donation. Because their etiology is different from that of adverse responses seen in transfusion recipients, unpleasant reactions that occur in donors are typically referred to as donor complications. A joint working group of the European Haemovigilance Network (EHN) and the International Society of Blood Transfusion (ISBT) has developed a classification system and standardized terminology for donor problems in order to standardized reporting and surveillance. These donor complications are broadly categorized into local reactions related to needle insertion, such as vessel injuries, nerve injuries, and other localized events, as well as general reactions, including immediate and delayed vasovagal reactions. Additional categories include other significant complications associated with blood donation. Similar to recipient adverse reactions, the severity and imputability of donor complications are assessed using an internationally accepted grading scale, which enables consistent reporting, analysis, and comparison of donor haemovigilance data across different haemovigilance systems worldwide. [20&22]

Types of Transfusion Reactions

Acute hemolytic transfusion reactions (AHTRs)

The fast destruction of transfused red blood cells, which causes clinical symptoms and hemolysis in the lab, is the hallmark of acute hemolytic transfusion reactions (AHTRs). Usually, symptoms appear minutes after the transfusion is started. Hemoglobinemia, hemoglobinuria, reduced serum haptoglobin, unconjugated hyperbilirubinemia, increased lactate dehydrogenase and serum glutamic-oxaloacetic transaminase levels, and a reduction in hemoglobin concentration are common laboratory results. Rarely, hemolysis of the recipient's red blood cells may also occur from transfusions of ABO-incompatible plasma, such as ABO-mismatched platelet transfusions, especially if the donor plasma has high titers of ABO antibodies. roughly 1 in 76,000 transfusion units have been reported to experience AHTRs, and roughly 1 in 1.8 million transfusion units die as a result of these events. [23]

Febrile non-hemolytic transfusion reactions (FNHTRs)

The hallmark of febrile non-hemolytic transfusion reactions (FNHTRs) is an inexplicable rise in body temperature of at least 1°C that happens during or soon after transfusion. Antipyretic premedication may mask or lessen fever, but it usually doesn't stop the accompanying rigors and chills, which are caused by a systemic inflammatory response mediated by cytokines. Other possible causes of fever, such as bacterial contamination or acute hemolytic responses, should be ruled out before diagnosing FNHTR. Up to 30% of platelet transfusions have been linked to FNHTRs, which happen more frequently after platelet transfusions than after red blood cell transfusions. The storage of platelets at room temperature, which encourages leukocyte activation and cytokine buildup during storage, is thought to be the cause of this increased frequency. [24]

Allergic Reaction

One of the most frequent side effects of blood transfusions is allergic transfusion responses. Clinical signs could appear minutes or seconds after the start of the transfusion, or they could appear hours later. Recipient hypersensitivity to plasma proteins or other physiologically active ingredients in the transfused blood component causes these responses. Most instances are modest and manifest as localized skin rashes, pruritus, or urticaria.



**Mohd Yusuf et al.,**

Antihistamine medications, including diphenhydramine, are often effective in treating symptoms, and transfusions can frequently be resumed when the patient has been properly evaluated and the symptoms have subsided.[25]

Anaphylaxis

Anaphylactic transfusion reactions are rare but life-threatening, occurring in approximately 1 in 20,000–50,000 transfusions. They present with severe hypotension, shock, respiratory distress, and loss of consciousness. Commonly associated with anti-IgA antibodies, management requires immediate cessation of transfusion, prompt administration of epinephrine, and supportive care with continuous monitoring.[26-28]

Transfusion-related acute lung injury (TRALI)

A rare but dangerous transfusion reaction, transfusion-related acute lung injury (TRALI) usually happens six hours after transfusion, usually following plasma-rich components such as fresh frozen plasma and platelets. It may result in respiratory failure and manifests as acute respiratory distress, severe dyspnea, and non-cardiogenic pulmonary oedema. Transfusions must be stopped right away, and supportive care—such as oxygen therapy and mechanical ventilation when needed—must be provided. Donor antibodies and recipient inflammatory factors are linked to TRALI. [29-30]

Transfusion-related Sepsis

A rare but potentially deadly consequence brought on by bacterial contamination of blood products is transfusion-related sepsis (TRS). Fever, tachycardia, rigors, or notable changes in blood pressure within ninety minutes following transfusion are its hallmarks. Isolating the same pathogen from the recipient's blood as well as the implicated blood component is necessary for diagnosis. Diversion of the first blood aliquot during collection and regular bacterial screening of platelet units before transfusion are examples of preventive methods. [31-32]

Transfusion-associated circulatory overload (TACO)

A clinically relevant side effect of administering blood products is transfusion-associated circulatory overload (TACO), which carries a high risk of morbidity and death. Patients with pre-existing renal insufficiency, hypoalbuminemia, chronic heart failure (CHF), established fluid overload, or a history of plasma transfusion are among the patient categories that exhibit increased susceptibility to TACO. A variety of cardiopulmonary and vascular signs and symptoms, such as dyspnoea, orthopnea, cyanosis, tachycardia, jugular venous distension, and dependent pedal oedema, are evident in the clinical presentation of TACO. These characteristics show the pathophysiological effect of intravascular volume increase that surpasses the recipient's cardiac and pulmonary compensatory capability. The goal of therapeutic management is to implement supportive measures while simultaneously treating the underlying triggering reason.. In clinical practice, aggressive transfusion rate adjustment and early identification of at-risk patients continue to be essential for preventing TACO. [33-34]

Acute hypotensive transfusion reaction

When there are no other known causes of hypotension, a sudden and severe drop in blood pressure is the hallmark of a hypotensive transfusion reaction. It may appear as a single clinical finding, but when the transfusion is stopped and the proper supportive care is provided, it usually shows a quick and positive reaction. A treatment trial utilizing cleaned blood products is advised when hypotensive transfusion responses are still etiologically unexplained. Acute hypotensive transfusion reactions have been more commonly linked to bedside leukoreduction filters, however pre-storage leukoreduction filters have also been linked to cases. [35-36]

Transfusion-Associated graft-versus-host Disease (TA-GvHD)

Pyrexia, maculopapular eruptions that progress to hemorrhagic bullae, enterocolitis that manifests as copious watery diarrhea, hepatic dysfunction indicated by elevated liver function parameters, pancytopenia, and pathognomonic histological findings on tissue biopsy are all signs of transfusion-associated graft-versus-host disease (TA-GvHD), a serious clinical syndrome. This syndrome usually manifests eight to ten days after transfusion. Despite being





relatively uncommon, TA-GvHD is one of the deadliest transfusion-related complications due to its extraordinarily high case fatality rate of almost 90%. [37-38]

Hemovigilance Programme in India (HvPI)

On December 10, 2012, the Indian Pharmacopoeia Commission and the National Institute of Biologicals (NIB), Noida, introduced the Haemovigilance Programme of India (HvPI) as part of the Pharmacovigilance Programme of India (PvPI). Its goal is to use evidence-based interventions to improve patient safety by tracking, reporting, and analyzing transfusion-related adverse events. Nationwide data collection and analysis are made easier by the Hemo-Vigil program. The program involves more than 117 hospitals and medical schools. Advisory committees offer supervision, and NIB acts as the organizing hub. Additionally, HvPI wants to become a full member of the International Haemovigilance Network (IHN). [39]

Objective of reporting adverse Reactions and Transfusion in Hemovigilance program

The creation of a framework for national reporting; To create and maintain a strong national reporting system that facilitates the methodical gathering of information about adverse transfusion reactions and blood product-related incidents in all of India's registered healthcare facilities and acts as a strategic tool for advancing public policy on patient safety.

Hazard Recognition and Risk Evaluation: To enable the prompt detection of possible dangers, hazards, and systemic vulnerabilities related to the delivery of blood products and transfusions, hence offering crucial insights into areas where current systems need to be corrected.

Enhancement of Clinical Results and Patient Safety: The ultimate goal is to lower the risk of transfusion-related injuries, adverse events, and avoidable harm to future patients by converting reported data into focused improvement initiatives and evidence-based systemic reforms.

Encouragement of Accurate and Timely Reporting of Adverse Reactions: To promote efficient and proactive risk management by encouraging registered ADR Monitoring Centers, which include medical colleges, institutes, hospitals, and blood banks registered under the Pharmacovigilance National Co-coordinating Center, to promptly and accurately report any suspected adverse drug and transfusion reactions.

Strengthening of Product Safety Surveillance: To monitor and enhance the safety profile of blood components and biological products formed from plasma using hemovigilance data as an ongoing feedback mechanism, guaranteeing compliance with national and international quality and safety standards. [40]

Role and responsibilities of HvPI

Dedicated staff from transfusion medicine departments, blood banks, hospitals, and medical colleges around the country methodically record adverse transfusion reaction data and related events into the specialized Hemo-Vigil software platform. For centralized processing and supervision, the collected data are then sent to the National Institute of Biologicals' (NIB) HvPI National Coordinating Centre (HvPI-NCC). The HvPI-NCC is in charge of creating Standard Operating Procedures (SOPs) and guidance manuals after receiving the data and conducting a thorough examination of its quality and completeness. The Indian Pharmacopoeia Commission (IPC) is formally informed of the evidence-based recommendations that come from this review process. The Drug Controller General of India (DCGI), a division of the Central Drugs Standard Control Organization (CDSCO), receives the combined recommendations of the Haemovigilance Advisory Committee from the IPC. The DCGI-CDSCO, the highest regulatory body in this field, is tasked with developing and issuing regulations concerning the safety of blood and blood products. It is also in charge of informing all pertinent parties of these directives. [41]



**Mohd Yusuf et al.,****Documentation and Reporting**

Onset and causation are used to categorize transfusion responses. They can be infectious or non-infectious, acute (within 24 hours) or delayed (beyond 24 hours). Allergic responses, TRALI, TACO, and fever non-hemolytic transfusion reactions (FNHTR) are common acute non-infectious reactions; DHTR, TA-GVHD, and post-transfusion purpura are common delayed reactions. Bacterial contamination or transfusion-transmitted illnesses like HBV, HCV, HIV, and malaria can cause infectious responses. The most commonly reported responses are FNHTRs. The Transfusion Reaction Reporting Form (TRRF) and Hemo-Vigil software are used by India's Haemovigilance Programme to methodically gather, examine, and track adverse transfusion occurrences. [42-43]

Challenges in hemovigilance

Many nations lack efficient national hemovigilance systems, despite their significance. The lack of thorough blood transfusion policies, poor transfusion service coordination, and restricted donor-recipient traceability are some of the difficulties. These flaws make it more difficult to identify, report, and look into transfusion-related adverse events, which lowers the efficacy of corrective actions and may jeopardize patient safety and transfusion results. [44-45]

The establishment of efficient hemovigilance systems is hampered in many low- and middle-income nations by a lack of resources, infrastructure, and technical know-how. This frequently results in missed chances to detect avoidable risks and underreporting of transfusion-related adverse events. International cooperation can promote capacity building, knowledge exchange, and improved transfusion safety, while robust hemovigilance programs produce useful data for enhancing transfusion services. [46-47]

Future developments

Globally, transfusion safety has significantly improved more than 20 years after the first hemovigilance programs were established. Improvements in blood component processing, laboratory procedures, component selection, and clinical transfusion management have decreased the frequency of numerous transfusion reactions and transfusion-related mistakes. Despite these successes, the core objectives of hemovigilance—namely, the identification of uncommon or newly emerging adverse events and the advancement of transfusion medicine transparency—remain extremely pertinent. Strong haemovigilance systems are even more crucial in light of the COVID-19 pandemic. To assess the possible effects of SARS-CoV-2 infection and immunization on transfusion outcomes, ongoing surveillance is crucial. Patients with a history or current COVID-19 infection may be more susceptible to consequences such as Transfusion-Related Acute Lung Injury (TRALI) or Transfusion-Associated Circulatory Overload (TACO) if they have hemovigilance. In a similar vein, observation could reveal whether blood donations from people who have just recovered or had vaccinations affect the incidence of transfusion responses. Additionally, haemovigilance studies that concentrate on particular patient groups can enhance knowledge of risk factors, aid in the creation of preventative measures, and help optimize transfusion practice. It is anticipated that digital technologies and electronic health data will play a major role in the future of hemovigilance. The detection and reporting of adverse transfusion events will be improved by the integration of electronic data sources, such as bedside monitoring systems and transfusion-associated vital indicators. By lowering the possibility of wrong transfusions, the widespread use of barcode scanning and computerized patient identification systems has already enhanced transfusion safety.

CONCLUSION

A crucial part of transfusion medicine is hemovigilance, which improves patient safety by methodically tracking, documenting, and analyzing transfusion-related side effects. It is essential for determining hazards, cutting down on mistakes, and raising the general standard of blood transfusion services. Strengthening hemovigilance systems can greatly increase transfusion safety, even though issues like underreporting, scarce resources, and poor infrastructure still exist in many nations. Additionally, new technologies like artificial intelligence, data analytics, and electronic health records have intriguing prospects for improved surveillance and early transfusion-related problem diagnosis. Sustained dedication to hemovigilance is necessary to guarantee safer transfusion procedures and improved patient outcomes globally.





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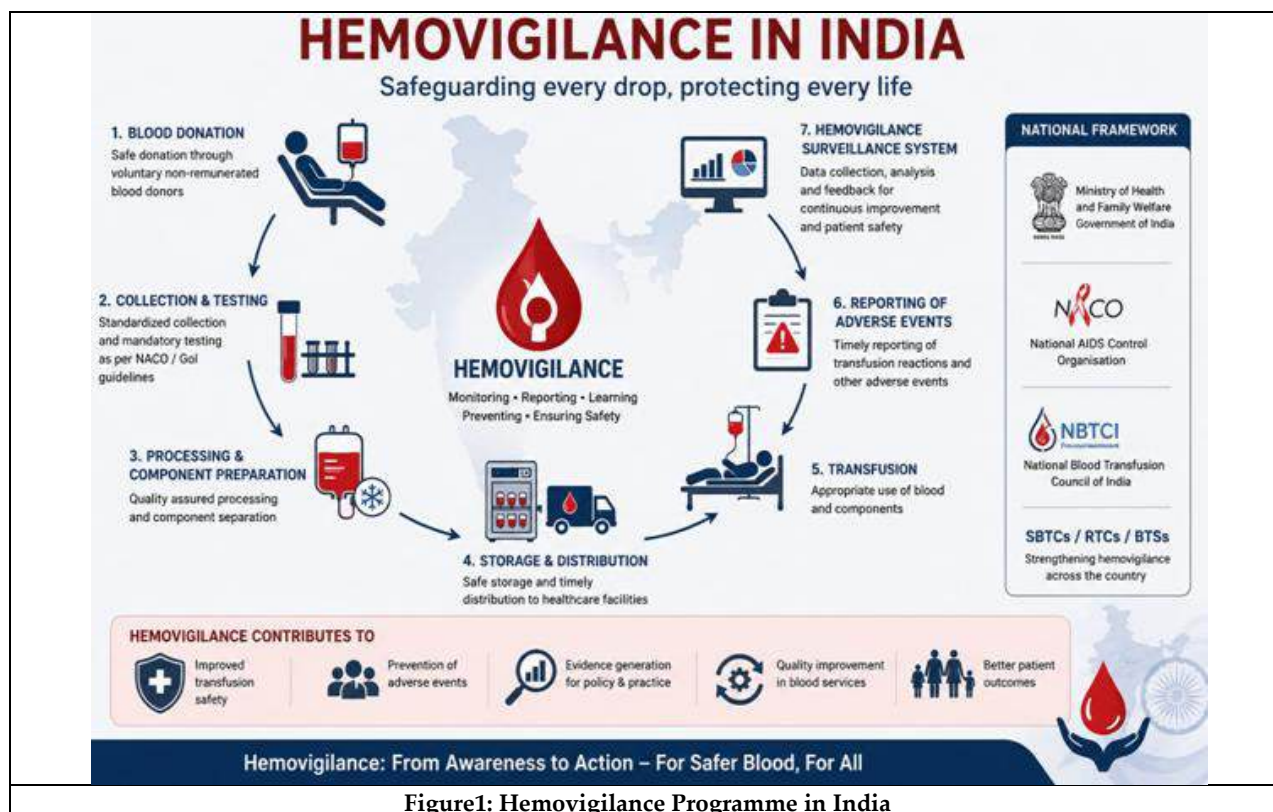


Figure1: Hemovigilance Programme in India

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RESEARCH ARTICLE

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ABSTRACT

Pentapartitioned neutrosophic strongly α -generalized semi closed sets and pentapartitioned neutrosophic strongly α -generalized semi open sets are presented in this study. Additionally, we examine their characteristics and behaviour in penta partitioned neutrosophic topological spaces.

Keywords: $Pnset$; $Pns_{\alpha g s}-CS$; $Pns_{\alpha g s}-OS$

INTRODUCTION

Uncertainty, indeterminacy, and inconsistency frequently arise in many real-world problems. To deal with such situations, the concept of the Fuzzy Set was first introduced by Lotfi A. Zadeh in 1965. Later, to overcome the limitations of fuzzy sets, the concept of Neutrosophic Set was proposed by Florentin Smarandache, which considers three independent membership components namely truth, indeterminacy, and falsity. Based on this concept, the Pentapartitioned Neutrosophic Topological Space has been developed as an extension of neutrosophic topology. This structure allows the study of generalized open and closed sets and their properties in a pentapartitioned neutrosophic environment. It also provides a foundation for defining new types of sets, mappings, and continuity concepts. In this paper, we introduce and investigate the concept of Pentapartitioned Neutrosophic Strongly α -Generalized Semi Closed Sets. Several properties and relationships between these sets are studied, and appropriate examples are provided to illustrate the concepts.





Preliminaries

Definition 2.1^[5] Let Q be a universal set. An object of the form $K = \{ \langle q, T_K, C_K, I_K, U_K, F_K \rangle : q \in Q \}$ is a $\mathcal{PnS}$ K over Q where $0 \leq T_K + C_K + I_K + U_K + F_K \leq 5$. Here, $T_K(q)$ is truth membership, $C_K(q)$ is contradiction membership, $I_K(q)$ is ignorance unknown membership, $U_K(q)$ is unknown membership, $F_K(q)$ is false membership.

Definition 2.2^[10] Let Q be a universal set. If the following three conditions are satisfied, then the collection Γ of $\mathcal{PnS}$ over Q is known as a $\mathcal{PnTS}$ on Q :

- (i) $0_Q, 1_Q \in \Gamma$,
- (ii) $R_1, R_2 \in \Gamma \Rightarrow R_1 \cap R_2 \in \Gamma$,
- (iii) $\{R_i : i \in j\} \subseteq \Gamma \Rightarrow \bigcup_{i \in j} R_i \in \Gamma$.

A $\mathcal{PnTS}$ is the term used to describe the pair (Q, Γ) . Every component of Γ is called a $\mathcal{PnOS}$. A set R^C is considered as a pentapartitioned Neutrosophic closed set $\mathcal{PnCS}$ if $R \in \Gamma$.

Definition 2.3^[10] Let K be a $\mathcal{PnS}$ in a $\mathcal{PnTS}(Q, \Gamma)$. Then: (i) K is called a $\mathcal{PnS-OS}$ if $K \subseteq \mathcal{Pncl}(\mathcal{Pnint}(K))$ (ii) K is called a $\mathcal{Pn}_\alpha\text{-OS}$ if $K \subseteq \mathcal{Pnint}(\mathcal{Pncl}(\mathcal{Pnint}(K)))$ (iii) K is called a $\mathcal{Pn-ROS}$ if $K = \mathcal{Pnint}(\mathcal{Pncl}(K))$.

The complement of the above sets are respectively: $\mathcal{PnS-CS}$, $\mathcal{Pn}_\alpha\text{-CS}$ and $\mathcal{Pn-RCS}$

Definition 2.4^[7] Let K be a $\mathcal{PnS}$ in a $\mathcal{PnTS}(Q, \Gamma)$. Then K is called a $\mathcal{PnGS-CS}$ if $\mathcal{PnScl}(K) \subseteq U$ whenever $K \subseteq U$ and U is a $\mathcal{PnOS}$ in Q .

Definition 2.5^[6] A $\mathcal{PnSK}$ in a $\mathcal{PnTS}(Q, \Gamma)$ is said to be $\mathcal{Pn}_{\alpha gs}\text{-CS}$ if $\mathcal{Pn}_\alpha cl(K) \subseteq U$ whenever $K \subseteq U$ and U is a $\mathcal{PnS-OS}$ in (Q, Γ) . The complement K^C of $\mathcal{Pn}_{\alpha gs}\text{-CS}$ is called a $\mathcal{Pn}_{\alpha gs}\text{-OS}$ in (Q, Γ) .

RESULTS AND DISCUSSION

Pentapartitioned neutrosophic strongly α - Generalized Semi Closed Sets

Definition 3.1.1. A Pentapartitioned neutrosophic Set K in (Q, Γ) is said to be a Pentapartitioned neutrosophic strongly α -generalized semi closed set ($\mathcal{Pns}_{\alpha gs}\text{-CS}$) if $\mathcal{Pn}_\alpha cl(K) \subseteq U$ whenever $K \subseteq U$ and U is a $\mathcal{PnGS-OS}$ in (Q, Γ) .

Example 3.1.2.

Let $Q = \{u_1, u_2\}$. Let $\Gamma = \{0_{\mathcal{Pn}}, U, 1_{\mathcal{Pn}}\}$ be a $\mathcal{PnT}$ on Q , where $U = \langle q, (0.7, 0.1, 0.5, 0.2, 0.3), (0.6, 0.3, 0.5, 0.3, 0.4) \rangle$. Then the $\mathcal{PnSK} = \langle q, (0.2, 0.1, 0.5, 0.2, 0.8), (0.3, 0.3, 0.5, 0.3, 0.7) \rangle$ is a $\mathcal{Pns}_{\alpha gs}\text{-CS}$ in (Q, Γ) .

Theorem 3.1.3. Every $\mathcal{PnCS}$ in (Q, Γ) is a $\mathcal{Pns}_{\alpha gs}\text{-CS}$ in (Q, Γ) .

Proof: Assume that K is a $\mathcal{PnCS}$ in (Q, Γ) . Let us consider a $\mathcal{PnSK} \subseteq U$ where U is a $\mathcal{PnGS-OS}$ in Q . Since $\mathcal{Pn}_\alpha cl(K) \subseteq \mathcal{Pncl}(K)$ and K is a $\mathcal{PnCS}$ in Q , $\mathcal{Pn}_\alpha cl(K) \subseteq \mathcal{Pncl}(K) = K \subseteq U$ and U is $\mathcal{PnGS-OS}$. That is $\mathcal{Pn}_\alpha cl(K) \subseteq U$. Therefore, K is $\mathcal{Pns}_{\alpha gs}\text{-CS}$ in Q .

Example 3.1.4.

Let $Q = \{u_1, u_2\}$. Let $\Gamma = \{0_{\mathcal{Pn}}, U, 1_{\mathcal{Pn}}\}$ be a $\mathcal{PnT}$ on Q , where $U = \langle q, (0.8, 0.1, 0.5, 0.2, 0.2), (0.6, 0.3, 0.5, 0.3, 0.4) \rangle$, then the $\mathcal{PnS} K = \langle q, (0.1, 0.1, 0.5, 0.2, 0.9), (0.3, 0.3, 0.5, 0.3, 0.7) \rangle$ is $\mathcal{Pns}_{\alpha gs}\text{-CS}$ but not a $\mathcal{PnCS}$ in Q .

Theorem 3.1.5. Every $\mathcal{Pn}_\alpha\text{-CS}$ in (Q, Γ) is a $\mathcal{Pns}_{\alpha gs}\text{-CS}$ in (Q, Γ) .

Proof: Let K be a $\mathcal{Pn}_\alpha\text{-CS}$ in Q . Let us consider a $\mathcal{PnSK} \subseteq U$ is a $\mathcal{PnGS-OS}$ in (Q, Γ) . Since K is a $\mathcal{Pn}_\alpha\text{-CS}$, $\mathcal{Pn}_\alpha cl(K) = K$. Hence $\mathcal{Pn}_\alpha cl(K) \subseteq U$ whenever $K \subseteq U$ and U is $\mathcal{PnGS-OS}$. Therefore K is $\mathcal{Pns}_{\alpha gs}\text{-CS}$ in Q .

Example 3.1.6.

Let $Q = \{u_1, u_2\}$. Let $\Gamma = \{0_{\mathcal{Pn}}, U_1, U_2, 1_{\mathcal{Pn}}\}$ be a $\mathcal{PnT}$ on Q ,





Kiruba and Meenakshi Sundaram

where $U_1 = \langle q, (0.4, 0.1, 0.5, 0.2, 0.5), (0.3, 0.3, 0.5, 0.3, 0.6) \rangle$ and $U_2 = \langle q, (0.3, 0.1, 0.5, 0.2, 0.6), (0.2, 0.3, 0.5, 0.3, 0.7) \rangle$, Consider a $\mathcal{PnS} K = \langle q, (0.8, 0.1, 0.5, 0.2, 0.1), (0.8, 0.3, 0.2, 0.3, 0.1) \rangle$ which is $\mathcal{Pns}_{ags} - \mathcal{CS}$ in Q but not $\mathcal{Pn}_{\alpha} - \mathcal{CS}$, since $\mathcal{Pncl}(\mathcal{Pnint}(\mathcal{Pncl}(K))) = 1_{\mathcal{Pn}} \notin K$.

Theorem 3.1.7. Every $\mathcal{PnR} - \mathcal{CS}$ in (Q, Γ) is a $\mathcal{Pns}_{ags} - \mathcal{CS}$ in (Q, Γ) .

Proof: Let K be $\mathcal{PnR} - \mathcal{CS}$ in (Q, Γ) . Since every $\mathcal{PnR} - \mathcal{CS}$ is a $\mathcal{PnCS}$ in (Q, Γ) . Then, K is a $\mathcal{Pns}_{ags} - \mathcal{CS}$ in (Q, Γ) .

Example 3.1.8.

Let $Q = \{u_1, u_2\}$. Let $\Gamma = \{0_{\mathcal{Pn}}, U, 1_{\mathcal{Pn}}\}$ be a $\mathcal{PnT}$ on Q , where $U = \langle q, (0.6, 0.1, 0.5, 0.2, 0.4), (0.6, 0.3, 0.5, 0.3, 0.4) \rangle$. Let us consider the $\mathcal{PnS} K = \langle q, (0.3, 0.1, 0.5, 0.2, 0.7), (0.2, 0.3, 0.5, 0.3, 0.8) \rangle$, which is a $\mathcal{Pns}_{ags} - \mathcal{CS}$ but not $\mathcal{PnR} - \mathcal{CS}$ in (Q, Γ) as $\mathcal{Pncl}(\mathcal{Pnint}(K)) = 0 \subset K$.

Theorem 3.1.9. Every $\mathcal{Pns}_{ags} - \mathcal{CS}$ in (Q, Γ) is a $\mathcal{Pn}_{ags} - \mathcal{CS}$ in (Q, Γ) .

Proof: Assume that K is a $\mathcal{Pns}_{ags} - \mathcal{CS}$ in (Q, Γ) . Let us consider $\mathcal{PnSK} \subseteq U$ where U is a $\mathcal{PnS} - \mathcal{OS}$ in Q . Since every $\mathcal{PnS} - \mathcal{OS}$ is a $\mathcal{Pn}_{gs} - \mathcal{OS}$ and by hypothesis $\mathcal{Pn}_{\alpha} cl(K) \subseteq U$, whenever $K \subseteq U$ and U is a $\mathcal{Pn}_{gs} - \mathcal{OS}$ in (Q, Γ) . We have $\mathcal{Pn}_{\alpha} cl(K) \subseteq U$ whenever $K \subseteq U$ and U is $\mathcal{PnS} - \mathcal{OS}$ in (Q, Γ) . Hence K is a $\mathcal{Pn}_{ags} - \mathcal{CS}$ in Q .

Example 3.1.10.

Let $Q = \{u_1, u_2\}$. Let $\Gamma = \{0_{\mathcal{Pn}}, U, 1_{\mathcal{Pn}}\}$ be a $\mathcal{PnT}$ on Q , where $U = \langle q, (0.3, 0.1, 0.5, 0.2, 0.4), (0.8, 0.3, 0.5, 0.3, 0.2) \rangle$. Then the $\mathcal{PnS} K = \langle q, (0.5, 0.1, 0.5, 0.2, 0.3), (0.2, 0.3, 0.5, 0.3, 0.3) \rangle$ is a $\mathcal{Pn}_{ags} - \mathcal{CS}$ but not a $\mathcal{Pns}_{ags} - \mathcal{CS}$ in (Q, Γ) .

Remark 3.1.11. The intersection of any two $\mathcal{Pns}_{ags} - \mathcal{CS}$ is not a $\mathcal{Pns}_{ags} - \mathcal{CS}$ in general as can be seen in the following example.

Example 3.1.12

Let $Q = \{u_1, u_2\}$. Let $\Gamma = \{0_{\mathcal{Pn}}, U, 1_{\mathcal{Pn}}\}$ be a $\mathcal{PnT}$ on Q , where $U = \langle q, (0.2, 0.1, 0.5, 0.2, 0.8), (0.4, 0.3, 0.5, 0.3, 0.6) \rangle$. Then the $\mathcal{PnSK}_1 = \langle q, (0.3, 0.1, 0.5, 0.2, 0.6), (0.7, 0.3, 0.5, 0.3, 0.3) \rangle$ and $K_2 = \langle q, (0.9, 0.1, 0.5, 0.2, 0.1), (0.4, 0.3, 0.5, 0.3, 0.5) \rangle$ are $\mathcal{Pns}_{ags} - \mathcal{CS}$ in Q . Now $K_1 \cap K_2 = \langle q, (0.3, 0.1, 0.5, 0.2, 0.6), (0.4, 0.3, 0.5, 0.3, 0.5) \rangle \subseteq M = \langle q, (0.4, 0.1, 0.5, 0.2, 0.3), (0.5, 0.3, 0.5, 0.3, 0.4) \rangle$ and M is $\mathcal{Pn}_{gs} - \mathcal{OS}$ in Q but not a $\mathcal{Pns}_{ags} - \mathcal{CS}$ in Q . But $\mathcal{Pn}_{\alpha} cl(K_1 \cap K_2) = 1_{\mathcal{Pn}} \notin M$. Therefore, $K_1 \cap K_2$ is not a $\mathcal{Pns}_{ags} - \mathcal{CS}$ in Q .

Theorem 3.1.13. If K_1 and K_2 are $\mathcal{Pns}_{ags} - \mathcal{CS}$, then $K_1 \cup K_2$ is $\mathcal{Pns}_{ags} - \mathcal{CS}$ in (Q, Γ) .

Proof. If $K_1 \cup K_2 \subseteq M$ and M is $\mathcal{Pn}_{gs} - \mathcal{OS}$. Then $K_1 \subseteq M$ and $K_2 \subseteq M$. Since K_1 and K_2 are $\mathcal{Pns}_{ags} - \mathcal{CS}$, $\mathcal{Pn}_{\alpha} cl(K_1) \subseteq M$ and $\mathcal{Pn}_{\alpha} cl(K_2) \subseteq M$ and hence $M \supseteq \mathcal{Pn}_{\alpha} cl(K_1) \cup \mathcal{Pn}_{\alpha} cl(K_2) = \mathcal{Pn}_{\alpha} cl(K_1 \cup K_2)$. Thus $K_1 \cup K_2$ is $\mathcal{Pns}_{ags} - \mathcal{CS}$ in (Q, Γ) .

Pentapartitioned neutrosophic strongly α - Generalized Semi Open Set

Definition 3.2.1. A $\mathcal{PnSK}$ is said to be Pentapartitioned neutrosophic strongly α -generalized semi open set ($\mathcal{Pns}_{ags} - \mathcal{OS}$) in (Q, Γ) , if the complement K^c is a $\mathcal{Pns}_{ags} - \mathcal{CS}$ in (Q, Γ) .

Theorem 3.2.2. For any $\mathcal{PnTS}(Q, \Gamma)$, every $\mathcal{PnOS}$ is a $\mathcal{Pns}_{ags} - \mathcal{OS}$.

Proof: Let K be a $\mathcal{PnOS}$ in Q . Then K^c is a $\mathcal{PnCS}$ in Q . By Theorem 3.1.4, K^c is a $\mathcal{Pns}_{ags} - \mathcal{CS}$ in Q . Hence K is a $\mathcal{Pns}_{ags} - \mathcal{OS}$ in Q .

Example 3.2.3.

Let $Q = \{u_1, u_2\}$. Let $\Gamma = \{0_{\mathcal{Pn}}, U, 1_{\mathcal{Pn}}\}$ be a $\mathcal{PnT}$ on Q , where $U = \langle q, (0.8, 0.1, 0.5, 0.1, 0.2), (0.6, 0.1, 0.5, 0.1, 0.4) \rangle$. Consider the $\mathcal{PnSK} = \langle q, (0.9, 0.1, 0.5, 0.1, 0.1), (0.7, 0.1, 0.5, 0.1, 0.3) \rangle$. Since K^c is a $\mathcal{Pns}_{ags} - \mathcal{CS}$, K is a $\mathcal{Pns}_{ags} - \mathcal{OS}$, but not $\mathcal{PnOS}$ in Q .





Kiruba and Meenakshi Sundaram

Theorem 3.2.4. In any $\mathcal{PnTS}(Q, \Gamma)$ every $\mathcal{Pn}_\alpha\text{-OS}$ is a $\mathcal{Pns}_{\alpha g s}\text{-OS}$.

Proof: Let K be a $\mathcal{Pn}_\alpha\text{-OS}$ in Q . Then K^c is a $\mathcal{Pn}_\alpha\text{-CS}$ in Q . By Theorem 3.1.6, K^c is a $\mathcal{Pns}_{\alpha g s}\text{-CS}$ in Q . Hence K is a $\mathcal{Pns}_{\alpha g s}\text{-OS}$ in Q .

Example 3.2.5.

Let $Q = \{u_1, u_2\}$. Let $\Gamma = \{0_{\mathcal{Pn}}, U_1, U_2, 1_{\mathcal{Pn}}\}$ be a $\mathcal{PnT}$ on Q ,

where $U_1 = \langle q, (0.4, 0.1, 0.5, 0.2, 0.5), (0.3, 0.3, 0.5, 0.3, 0.6) \rangle$ and $U_2 = \langle q, (0.3, 0.1, 0.5, 0.2, 0.6), (0.2, 0.3, 0.5, 0.3, 0.7) \rangle$, Then the $\mathcal{PnS} K = \langle q, (0.1, 0.1, 0.5, 0.2, 0.8), (0.1, 0.3, 0.2, 0.3, 0.8) \rangle$ is a $\mathcal{Pns}_{\alpha g s}\text{-OS}$ in Q but not a $\mathcal{Pn}_\alpha\text{-OS}$ in Q .

Theorem 3.2.6. In any $\mathcal{PnTS}(Q, \Gamma)$, every $\mathcal{PnR}\text{-OS}$ is a $\mathcal{Pns}_{\alpha g s}\text{-OS}$.

Proof: Let K be a $\mathcal{PnR}\text{-OS}$ in (Q, Γ) . Then K^c is a $\mathcal{PnR}\text{-CS}$ in (Q, Γ) . By Theorem 3.1.8, K^c is a $\mathcal{Pns}_{\alpha g s}\text{-CS}$ in (Q, Γ) . Hence K is a $\mathcal{Pns}_{\alpha g s}\text{-OS}$ in Q .

Example 3.2.7.

Let $Q = \{u_1, u_2\}$. Let $\Gamma = \{0_{\mathcal{Pn}}, U, 1_{\mathcal{Pn}}\}$ be a $\mathcal{PnT}$ on Q , where $U = \langle q, (0.6, 0.1, 0.5, 0.2, 0.4), (0.6, 0.3, 0.5, 0.3, 0.4) \rangle$, Then the $\mathcal{PnS} K = \langle q, (0.7, 0.1, 0.5, 0.2, 0.3), (0.8, 0.3, 0.5, 0.3, 0.2) \rangle$ is a $\mathcal{Pns}_{\alpha g s}\text{-OS}$ in Q but not a $\mathcal{PnR}\text{-OS}$ in (Q, Γ) .

Theorem 3.2.8. In any $\mathcal{PnTS}(Q, \Gamma)$, Every $\mathcal{Pns}_{\alpha g s}\text{-OS}$ is a $\mathcal{Pn}_{\alpha g s}\text{-OS}$.

Proof: Let K be a $\mathcal{Pns}_{\alpha g s}\text{-OS}$ in Q . Then K^c is a $\mathcal{Pns}_{\alpha g s}\text{-CS}$ in Q . By Theorem 3.1.10, K^c is a $\mathcal{Pn}_{\alpha g s}\text{-CS}$ in Q . Hence K is a $\mathcal{Pn}_{\alpha g s}\text{-OS}$ in Q .

Example 3.2.9.

Let $Q = \{u_1, u_2\}$. Let $\Gamma = \{0_{\mathcal{Pn}}, U, 1_{\mathcal{Pn}}\}$ be a $\mathcal{PnT}$ on Q , where $U = \langle q, (0.3, 0.1, 0.5, 0.0, 1, 0.4), (0.8, 0.1, 0.5, 0.1, 0.2) \rangle$. Then the $\mathcal{PnS} K = \langle q, (0.2, 0.1, 0.5, 0.1, 0.5), (0.3, 0.1, 0.5, 0.1, 0.3) \rangle$ is a $\mathcal{Pn}_{\alpha g s}\text{-OS}$ in Q but not a $\mathcal{Pns}_{\alpha g s}\text{-OS}$ in Q .

Remark 3.2.10 The union of any two $\mathcal{Pns}_{\alpha g s}\text{-OS}$ is not a $\mathcal{Pns}_{\alpha g s}\text{-OS}$ in general.

Example 3.2.11

Let $Q = \{u_1, u_2\}$. Let $\Gamma = \{0_{\mathcal{Pn}}, U_1, U_2, 1_{\mathcal{Pn}}\}$ be a $\mathcal{PnT}$ on Q ,

Where $U_1 = \langle q, (0.2, 0.1, 0.5, 0.2, 0.8), (0.4, 0.3, 0.5, 0.3, 0.6) \rangle$ and $U_2 = \langle q, (0.1, 0.1, 0.5, 0.2, 0.9), (0.5, 0.3, 0.5, 0.3, 0.4) \rangle$ are $\mathcal{Pns}_{\alpha g s}\text{-OS}$ in Q . Now $U_1 \cup U_2 = \langle q, (0.6, 0.1, 0.5, 0.2, 0.3), (0.5, 0.3, 0.5, 0.3, 0.4) \rangle$ is not a $\mathcal{Pns}_{\alpha g s}\text{-OS}$ in Q .

CONCLUSION

In this study, we introduce and analyze $\mathcal{Pns}_{\alpha g s}\text{-CS}$ and discuss their essential properties. Future research may concentrate on extending these concepts to $\mathcal{Pns}_{\alpha g s}$ continuous and irresolute mappings.

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**Kiruba and Meenakshi Sundaram**

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RESEARCH ARTICLE

Artificial Intelligence Adoption in Indian SMEs: Drivers, Barriers, and Business Performance Outcomes

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ABSTRACT

Competition is evolving in the era of Artificial Intelligence (AI) rapidly reshaping the landscape in various industries around the globe. While AI-powered solutions are perceived primarily as something that can be implemented in large corporations, there has been a substantial number of business leaders in SMEs, accounting for approximately 30% of the Indian economy, using such solutions quite rapidly; at the same time, with more than 110 million employees, SMEs are in an early stage of using AI. The paper looks at the driving factors and constraints behind applying AI in Indian SMEs and how they affect business performance metrics like operational efficiency, sales expansion, customer satisfaction, and innovation ability. The study adopted a mixed-method research approach: Quant content derived from 312 SME owners and senior managers using a structured questionnaire across five industrial clusters of the state of Maharashtra, Karnataka and Gujarat; and Qual content obtained through deep interviews with 24 entrepreneurs and techno-consultants from the selected industrial clusters. Theoretical lens of the study: Technology–Organisation–Environment (TOE)-based perspective. The relationships are then tested using the Structural Equation Modelling (SEM). The three key enablers revealed include top management support, competitive clustering, and perceived technological readiness, indicative of the high importance of these for achieving AI adoption at least at a certain level. Financial constraints, skill shortage, and data privacy and protection issues constitute among the main obstacles identified. According to research, AI is helping enterprises that have adopted it improve their operational efficiency and customer experience metrics by up to 28.4% on average, compared with counterparts that have not adopted AI. The study recommends action points for policy interventions by both government and industry associations, as well as action and capacity building programmes, subsidisation of tools enabled by AI, and the clarity of the data governance regime for the policy actors.

Keywords: Artificial Intelligence, SME, Technology Adoption, TOE Framework, India, Business Performance, Digital Transformation





Deepak et al.,

INTRODUCTION

Artificial intelligence (AI) is being recognised as a common feature of the current global business environment, creating new opportunities for organisations through automated processes, insights gleaned from analysing large datasets, and the possibility of delivering mass-personalised customer experiences at scale (Davenport & Ronanki, 2018). AI is mainstream now in many ways, not only limited to some tech giants building hyper-intelligent chatbots or using predictive analytics to optimise their supply chains. More and more companies are considering how they might use AI strategically to enhance their competitiveness, regardless of size. The SME sector is often referred to as the backbone of the Indian economy, and it faces two-fold risks: domestic and international competitive pressures, and the rapid evolution of digital technology, which interact continuously and in multiple ways. Policies broadly fostering AI diffusion—particularly, the Digital India initiative of the Government of India and NASSCOM's (2020) National strategy for Artificial Intelligence. However, there is very little understanding (both empirical and conceptual) of how AI adoption manifests in Indian SMEs and its implications for performance. This study fills this gap by presenting an extensive empirical analysis of the drivers and consequences of AI adoption in Indian SMEs. Based on the TOE framework introduced by Tornatzky and Fleischer (1990), this paper focuses on technological, organisational and environmental factors of adoption, and the effect of its adoption on a multi-dimensional performance construct. The rest of the paper is organised as follows: Section 2 provides a literature review; Section 3 outlines the conceptual framework and hypotheses; Section 4 explains the research design; Section 5 reports the results; and finally, in Section 6, we discuss our findings and conclude with implications and future research directions.

LITERATURE REVIEW

AI Adoption in Organisations

Adoption of technology research has been conducted for a long time and has utilized models such as Technology Acceptance Model (Davis, 1989), Diffusion of Innovation Theory (Rogers, 1995) and Unified Theory of Acceptance and Use of Technology (UTAUT) (Venkatesh et al., 2003). For AI, Chui et al. (2018) indicates that three main value drivers are automation of cognitive tasks, better decision making, and predictive modelling. The findings of Mikalef et al., 2021 found that AI capability and dynamic organisational capabilities had a positive impact on data analytics maturity as mediation factor. The constraints of fewer resources and less IT infrastructure, and SMEs' risk aversion, all contribute to differences in adoption paths between SMEs and large enterprises, according to Arun, 2018. According to Bharadwaj et al., 2020 [+], Indian small and medium enterprises (SMEs) are more likely to adopt AI incrementally or step by step, beginning with the relatively less expensive solutions that utilize social media analytics, basic chat bots and eventually moving onto the moderately more advanced applications like predictive maintenance and demand forecasting.

TOE Framework and Digital Technology Adoption

The TOE framework has been frequently used to articulate enterprise technology adoption decisions. Technological context refers to internal and external technologies used by the firm, organisational context includes firm size, managerial support and financial resources, and environment context captures industry structure, competitive forces and government policy (Tornatzky & Fleischer, 1990). The generalizability of the TOE has been confirmed by Oliveira and Martins (2011) for different types of technology: cloud computing, ERP and e-commerce. More recently, Mishra et al. (2022) used TOE to assess AI adoption readiness of manufacturing SMEs in emerging markets and found significant effects for all three contextual dimensions.





Deepak et al.,

Performance Outcomes of AI Adoption

Empirical evidence relating AI integration to business performance suggests positive correlations with the magnitude differing by sector, firm size and how deep in the value-chain the implementation is carried out. Bughin et al. (2017) find that early among industries, profit margins on average advance over non-adopters by 3 to 15 percentage points (pp). Agrawal et al. Prediction improvement is, therefore, one key mechanism through which performance can be improved by AI (Ransbotham et al., 2019). This helps lower uncertainty in decision making across many domains – e.g., hiring, inventory management and credit risk assessment. The evidence base in this area is still nascent and lacking in empirical insights specifically for SMEs – Kaartemo & Helkkula (2018) further make the case that more contextually appropriate research reflecting common resource profiles of smaller firms in emerging economies, as well as the institutional environments they are embedded into, would find a greater home.

Conceptual Framework and Hypotheses

Using the TOE framework, this research posits that AI adoption in Indian SMEs is driven by three categories of factors: (i) technological readiness and perceived usefulness (technological context), (ii) top management support, organisational size and financial capacity (organisational context), and finally (iii) competitive intensity, government policy support, and partner ecosystem (environmental context). In turn, it is theorised that adopted AI will enhance business performance in four key areas: operational efficiency, revenue generation, customer satisfaction, and innovation competence.

The following hypotheses were formulated:

H1: The 'technological readiness' variable positively affects AI adoption in Indian SMEs

H2: Top Management Support has a positive effect on AI Adoption.

H3: Financial capacity positively moderates the relationship between technological readiness and AI adoption

H4: AI adoption is significantly driven by the competitive pressure in the environment.

H5: Embracing AI has a positive effect on operational efficiency

H6: AI innovation has a positive impact on customer satisfaction and revenue growth.

H7: AI adoption has a positive impact on innovation capability in SMEs

RESEARCH METHODOLOGY

Research Design

A convergent mixed method design was used in the study. To confirm and enrich the statistical findings qualitative interviews were held after quantitative data collection was carried out. This is congruent with Creswell and Plano Clark's (2017) guidelines for studies studying complex phenomena in organisational research where the trends in the numerical data can be better understood when supported by contextualization.

Sample and Data Collection

SMEs dispersed across the three industrial clusters of Pune (Maharashtra), Bengaluru (Karnataka) and Surat (Gujarat) forms the quantitative sample consisting of 312 SMEs. The study was conducted using stratified random sampling methods of all sub-sectors of manufacturing (118), services (104) and trading (90). From October 2023 to March 2024, a structured questionnaire (48 items; measured on a five-point Likert scale) was administered to owners or senior managers. We achieved 78.3% response rate. Using purposive sampling technique, 24 participants were selected for the qualitative component; they are SME owners who have gone through an AI implementation and technology consultants that have SME focus. Conducted semi-structured interviews, recorded, transcribed, and analyzed the interviews, each taking 55 minutes on average.

Measures

All constructs included were redefined with valid multi-item scales employed in previous studies. For measuring the technological readiness, items developed by Parasuraman (2000) were adopted. The items of top management



**Deepak et al.,**

support were modified from Liang et al. (2007). We measured performance outcomes with items based on the Balanced Scorecard (Kaplan & Norton, 1996). All factor loadings > 0.70 were found indicating construct validity using confirmatory factor analysis (CFA). The composite reliabilities for each construct ranged from 0.81 to 0.93 and the Average Variance Extracted (AVE) values were all greater than 0.50 for each construct, respectively, which met the convergent validity and discriminatory validity requirements.

Analytical Procedure

IBM AMOS 27.0 software was used for the analysis technique of the Structural Equation Modeling (SEM). Model fit analysis was done based on common indices: Chi-square by degree of freedom (χ^2/df), Comparative Fit Index (CFI), Tucker-Lewis Index (TLI), Root Mean Square Error of Approximation (RMSEA) and Standardised Root Mean Square Residual SRMR. Harman's one factor test was used to look at the common method bias, but there is only 23.6% variance, which does not suggest that there was common method bias in the data.

RESULTS

Sample Profile

Table 1 presents the demographic profile of the surveyed enterprises. The sample is predominantly composed of micro and small enterprises (combined 71.5%), with annual turnover below INR 25 crore. Manufacturing firms account for 37.8% of the sample.

AI Adoption Status

Of the 312 respondents, 134 (42.9%) reported having adopted at least one AI-enabled application in their business operations. The most commonly adopted applications were customer-service chatbots (61.2% of adopters), social media analytics (54.5%), and inventory/demand forecasting tools (43.3%). Full-scale AI integration across multiple business functions was reported by only 8.2% of the sample, reflecting the predominantly incremental nature of adoption.

SEM Results

The SEM model demonstrated acceptable fit: Chi-square/df = 2.14, CFI = 0.95, TLI = 0.94, RMSEA = 0.061, SRMR = 0.058. The SEM path diagram presents the overall model structure: The left side are the three antecedents which act as predictors while the right side are the four outcomes, with AI Adoption as the mediator in the middle. Inside each box the path coefficient (β) and significance level is displayed. Click any node to delve deeper into that node. Some points to note here in the model: The path towards Operational Efficiency is the strongest outcome path ($\beta = 0.52$) and the path towards Tech Readiness is the most influential antecedent ($\beta = 0.48$). The weakest Antecedent Outcome path is between Competitive Pressure and Innovation Capability with a value of ($\beta = 0.33$) and the weakest A-O is between Competitive Pressure and HRM Path with ($\beta = 0.35$). Both are highly significant. To test the moderation hypothesis H3 which posits that technology readiness-adoption relationship can be moderated by financial capacity, a multi-group SEM approach was used. The results showed a significant moderation effect ($\chi^2 = 8.74$, $p < 0.05$), with the technology readiness path being significantly higher of finance rich SMEs ($\beta = 0.61$) than finance constrained SMEs ($\beta = 0.29$).

DISCUSSION

The results are mostly consistent with the suitability of the TOE framework for probing the AI adoption environment in the emerging market SME domain. Technological readiness is also the most common driver across all other studies cited in this article has found that suitable basic technological digital infrastructure and IT literacy is a prerequisite to the use of AI (Mishra, 2022). While top management support is known to play an important role in technology acceptance (Liang et al., 2007), this is not the second factor that is discussed. Competitive pressure is cited by 33% of those surveyed as the third most important factor challenging the growth of small businesses, supporting the claim



**Deepak et al.,**

that it is increasingly timely to consider AI as an operational tool, rather than a luxury. Through interviews it was seen that there were three challenges – access to funding, access to skillful workforce, and data privacy – which were structural barriers Indian SMEs are coming across when trying to go digital. Many interviewees were using AI tools within their business that happened to be free of charge, or used for little to no cost, and these folks would describe what I'm going to call an 'AI awareness gap': they were not particularly conscious of which AI tools were suitable to their specific business model or where to begin without shelling out a lot of money. This discovery becomes directly applicable to support programme designing by the industry associations and the governments. The emphasis on performance indicates a strong link between the use of AI and operational efficiency ($\beta = 0.52$), aligning with the estimates by McKinsey Global Institute that suggest AI automation can reduce costs by up to 30% in labour-intensive industries. The gains in terms of innovation capability are large, but still fairly small ($\beta = 0.35$), suggesting that the innovation dividend of AI adoption is lagged, and for instance at longer stages of maturity and/or organisational learning within SMEs.

CONCLUSION

This research systematically maps the empirical evidence on the dynamics of the adoption of AI, and yields valuable insights for technology adoption in an emerging small business context, which has been relatively under-researched: Indian SMEs. The data confirm the hypothesized performance benefits, which are well reflected in the TOE framework, which is a multidimensional antecedent to the adoption (SEM) of the product. The findings suggest that AI toolkits targeted at the SME use case may need to be subsidized by the government, capacity building workshops via industry clusters, and more accessible compliance guidelines to dampen the anxiety around compliance without sacrificing privacy safeguards and best practices. Only a few industry associations such as Confederation of Indian Industry (CII) and Federation of Indian Chambers of Commerce & Industry (FICCI) can provide networking platforms to help members learn from one another, with the small AI diffusion projects helping each other address real-life challenges they have faced in the process.

Implications

Some of the recommendations from the insights leave the owners and managers of SMEs with a roadmap for using the power of AI – from making obvious and immediate wins to ensuring they have internal faith and have concrete return on investment before they ramp up the use of AI. There are some limitations to the study. The cross-sectional design makes it impossible to make causal inferences on performance trends over time. The sample is geographically restricted to 3 industrial clusters in India and thus the generalisability is restricted. Randomized controlled trials over an extended period are needed, extending to more sparsely studied states and at more granular a level of analysis of sector specific adoption trends. Future studies should examine other factors that might impact the relationship between adoption and expected performance such as the quality of AI vendors, the reliability of cloud infrastructure, and the availability of fintech credit.

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Deepak et al.,

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Table 1: Sample Profile of Surveyed SMEs

Characteristic	Category	Frequency	Percentage (%)
Enterprise Type	Micro (<INR 5 Cr)	96	30.8
	Small (INR 5–25 Cr)	127	40.7
	Medium (INR 25–250 Cr)	89	28.5
Sector	Manufacturing	118	37.8
	Services	104	33.3
	Trading	90	28.9
Cluster	Pune	108	34.6
	Bengaluru	112	35.9
	Surat	92	29.5

Source: Primary Survey Data (2023–24)

Table 2: SEM Path Coefficients and Hypothesis Test Results

Hypothesis	Path	Standardised Coefficient (β)	p-value	Result
H1	Tech Readiness → AI Adoption	0.48	< 0.001	Supported
H2	Top Mgmt Support → AI Adoption	0.41	< 0.001	Supported
H4	Competitive Pressure → AI Adoption	0.33	< 0.001	Supported





Deepak et al.,

H5	AI Adoption → Operational Efficiency	0.52	< 0.001	Supported
H6	AI Adoption → Customer Satisfaction	0.44	< 0.001	Supported
H6	AI Adoption → Revenue Growth	0.38	< 0.01	Supported
H7	AI Adoption → Innovation Capability	0.35	< 0.01	Supported

Source: Authors' Analysis using IBM AMOS 27.0

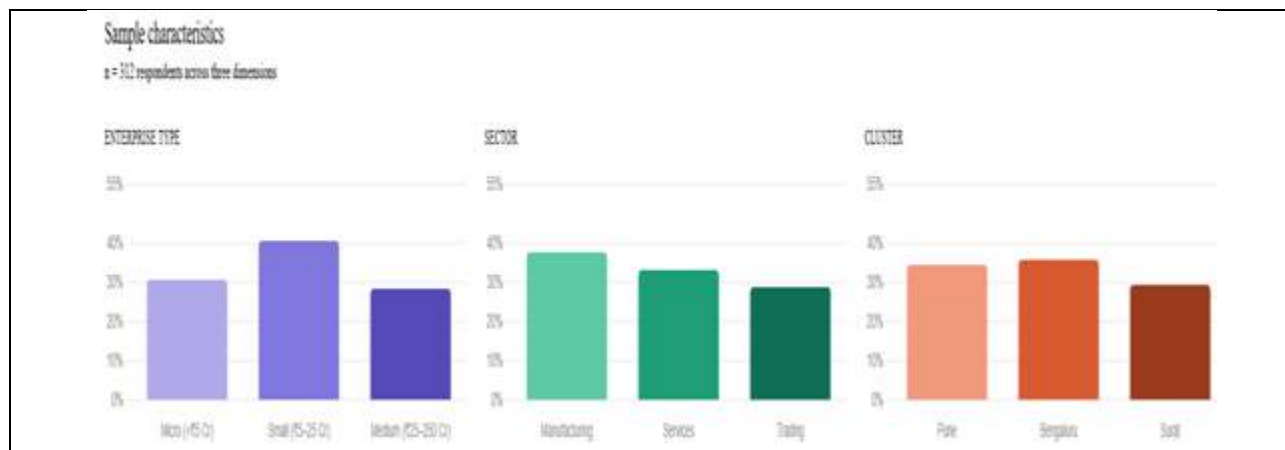


Figure 1: Sample Profile of Surveyed SMEs

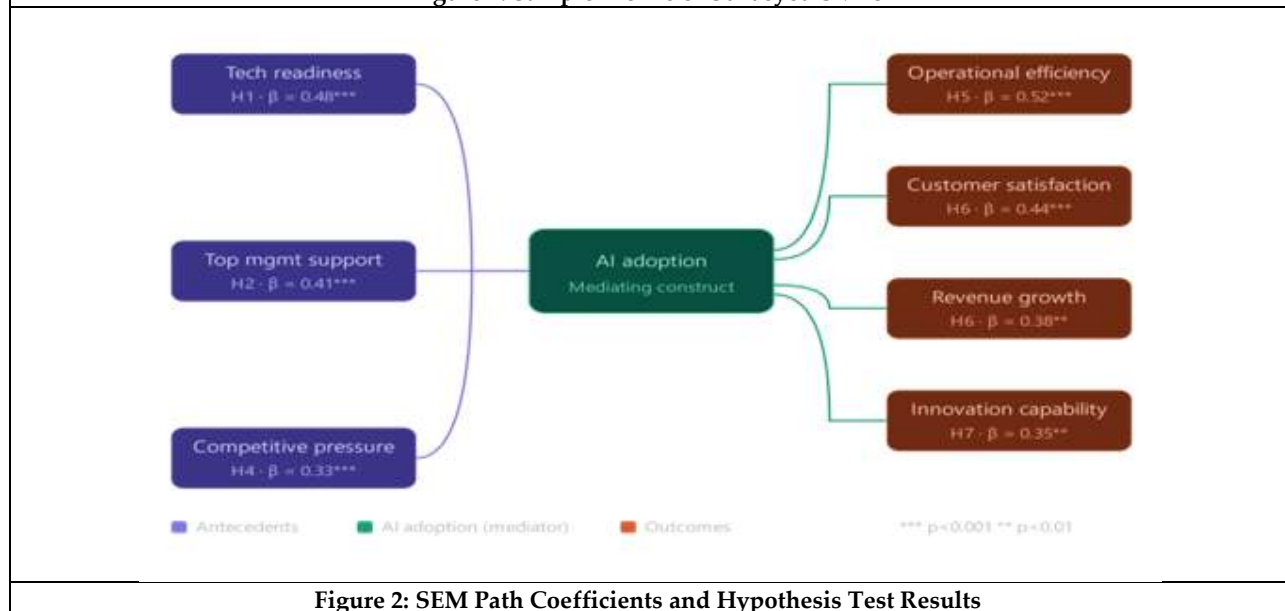


Figure 2: SEM Path Coefficients and Hypothesis Test Results

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RESEARCH ARTICLE

Impact of Technological Advancements and Consumer Adoption of Digital Payment

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ABSTRACT

The research reveals the utilisation of digital payments and the transformations noted by customers in the Tiruchirappalli district as a result of technological advancements. Digital wallets and e-payment systems have undergone significant simplification, leading to a widespread adoption of these transactions across the customer market. Online payment is considered as a highly secured payment mode by the customers as there is proper security process especially the encryption and biometric verification system. The increased awareness on digital payment is caused because of the training and guidance as well as the marketing influence. In addition to social media impact, Word-of-mouth and simple procedures are the main elements in easy adopting of digital payments. Such elements have led to the acceptance and high recognition of increased usage of digital payment in Tiruchirappalli district.

Keywords: Consumer, Digital Payment, Social media impact, Security measures, Technology



**Jayashree and Sugunalakshmi**

INTRODUCTION

The technological developments have influenced the adoption of digital payments and it also has a significant impact on the consumers interaction towards financial services. Digital transactions are nowadays more secured, quick, and user-friendly. They include inventions from mobile payments, AI and blockchain technology. Such changes and developments offer the customers with more confidence and convenience to drive the adoption rates. Still there are certain concerns about the security and legal systems. These issues need to be dealt essentially to ensure the continued development and the method to assist the consumers using digital payment systems. The technological developments must be appreciated that have transformed the financial landscape in the recent years in Tiruchirappalli district. Mobile payments, and digital wallets that have brought a matchless level of simplicity and efficiency have transformed the methods of payments among the consumers. These transformations have made the user-friendly approach for the consumers to make simple and secured transactions using their mobiles by reducing the necessity of money. The implementation of two-factor authentication, biometric authentication, and encryption has emerged as an essential component in tackling the concerns surrounding fraud and data breaches. This has increased the trust of the consumers on the digital payment service providers. Adoption of these digital payments have further influenced the safe usage and payments through effective marketing operations and guidelines.

REVIEW OF LITERATURE

Subramanian Shanmugam. (2022). "Digital Transformation through Fintech: An Analytical Study". concludes that the majority of Indian fintech start-ups are not older than ten years, but these corporations have achieved gigantic steps over the past few years, especially in the domains of financing tech and insurance firms. Siddaraju. S (2023), "A Study of Consumer Perceptions on Digital Payment System in India with Special Reference to Mysore District". established that age and consumer perception of the digital payment system are associated, and further established the lack of significant association between gender and educational background and consumer perception of the digital payment system. Phuong Thanh Thi Tran. et. al. (2023). "Digital Transformation of the Banking Industry in Developing Countries". revealed that digital banks provide flexible goods and services, which have multiple benefits at a high level of involvement, such as increasing the revenues of operations and strengthening the relationship between users and banks. Kamboj, Kuldeep Kumar, et al. (2024). "A study of drivers and inhibitors of user acceptance of digital payment systems in India". results showed the importance of such factors as perceived risk, trust, and financial literacy in the digital era in customer behaviour. These findings can be used to improve the adoption and revenues among the industry players. Karmaker, S., et al., (2025). "Exploring influential factors of consumer purchase behavior on the adoption of digital payment apps in Bangladesh, results prove that the maximum giant variables affecting purchaser behaviour within the framework of virtual payment applications are the familiarity with the emblem, its availability, protection, age, earnings, and consumer perception, because the maximum critical ones.

STUDY OBJECTIVES

- The purpose of this study is to examine the correlation between the factors that influence digital payments and the opinions of consumers on the adoption of digital payments.
- In order to ascertain the opinions of the adoption of digital payment methods by consumers in the Tiruchirappalli district

RESEARCH METHODOLOGY

To better understand the perspectives of customers who make use of digital payments methods, a structured questionnaire was used. The method of research known as descriptive research was used in the execution of this



**Jayashree and Sugunalakshmi**

investigation. Consumers who make use of digital payments methods in the Tiruchirappalli district, which is located in the state of Tamil Nadu, are included in the general population that is being analysed for the purpose of the study. In order to collect information for a sample size of 113 customers that make use of digital payments, a simple random sampling strategy is used. This strategy makes use of a well-structured questionnaire that was prepared and circulated. 150 questionnaires were distributed from which 27 were incomplete and 12 were not returned. For this reason, a total of 113 questionnaires that were filled out were used for the research.

DATA ANALYSIS

To analyze the impact of technological advancements on consumer adoption of digital payments in Tiruchirappalli district, several key variables are used in the study. They are explained in the table 1. From table 2 the demographic of older people is the most active in adopting digital payments methods, as shown by the fact that 56.6% of digital payments users are between the ages of 30 and 40 when it comes to using digital payments methods. Those aged 50 and over make up the second biggest category, accounting for 19.5% of the total, which indicates that older folks are becoming more comfortable with technology each year. The percentage of females (54.9%) who use digital payments is somewhat higher than the percentage of males who use them (45.1%), which may indicate that women are more engaged than men. The majority of users have credentials from higher education, with 38.9% of them having postgraduate degrees. Graduates represent 27.4% of the user population, while diploma holders make up 25.7% of the user population. The fact that just 8.0% of people have completed schooling suggests that a higher level of educational achievement is connected with a greater number of people using digital payments methods.

Hypothesis I

Null Hypothesis: There is a positive relation among Factors Influencing Digital Payments and Opinion on Consumer Adoption of Digital Payments

The hypothesis is used to explore the relationship between the factors that impact digital payments and the attitudes of consumers towards the adoption of digital payments systems. This relationship is studied within the framework of the hypothesis. The Pearson correlation approach is used by the Inter Correlation Matrix in order to ascertain the specific nature of this association. In table 3, according to the Pearson correlation coefficient of 0.961, the following factors have a significant impact on digital payments: Consequently, this suggests there is a very high positive link between the elements that influence digital payments and the attitudes of consumers towards the adoption of digital payments. Statistics (two-tailed) = 0.000: If the hypothesis to be null is correct, then this p-value indicates the chance of witnessing the data rather than the alternative. The assumption that the link is statistically significant is strongly supported by the fact that the value is 0.000, which is significant at the 0.01 level. There is a strong and statistically significant relationship between digital payments factors and customer sentiments towards digital payments. The considerable positive correlation (0.961) and the extremely low p-value (0.000) demonstrate that this relationship exists. This indicates that as the number of favourable elements that influence digital payments rises, the number of customers who are ready to use digital payments methods likewise gradually increases.

Opinion on Consumer Adoption of Digital Payments is the dependent variable, and Table:3 displays the multiple correlation coefficient R (0.984). Total dependent variable variance is R square. Additionally, the R square value is 0.868 (86.8%), indicating a strong match.

Hypothesis II

Null Hypothesis: There is a positive relation among Opinion on Consumer Adoption of Digital Payment

Dependent Variable: Opinion on Consumer Adoption of Digital Payments **Predictors:** (Constant), FIDP1, FIDP2, FIDP3, FIDP4, FIDP5, FIDP6, FIDP7, FIDP8, FIDP9, FIDP10

The purpose of the hypothesis is to determine whether or not there is a substantial disparity in the opinions on the adoption of digital payments by consumers. This association may be determined with the use of the regression





Jayashree and Sugunalakshmi

analysis. The value of 1158.490 for the regression value in table 5 represents the amount of variance in consumer adoption of digital payments that can be explained by the model. F-Statistic (8.977): This number is used to determine whether or not the model is significant in general. When the F-statistic is larger, it suggests that the predictors provide a substantial explanation for the variance in the variable that is being predicted. Sig. (0.000): The p-value indicates the probability of seeing the data if the null hypothesis is valid. The model is statistically significant since its 0.000 value is 1% significant. The low p-value (0.000) shows that consumer views of digital payments methods vary. We can reject the null hypothesis since the model parameters have a significant influence on customer views of digital payments systems.

The relevance of the many variables that influence consumer adoption of digital payments is shown in the table 6 that presents the results of the regression analysis. If the null hypothesis is correct, the significance level, also known as the P-value, indicates the likelihood of witnessing the effect. A statistically significant result is shown by a number that is less than 0.05. Convenience and ease of the use were assessed, and the results showed that ($B = 2.042$, $\text{Beta} = 0.391$, $t = 4.573$, $p = 0.000$): When it comes to the adoption of digital payments methods by consumers, this element has a very substantial and beneficial influence. It is possible to drastically increase adoption rates by making the product more convenient and user-friendly. The following are the enhanced security features: $B = -1.321$, $\text{Beta} = -0.386$, $t = 3.492$, $p = 0.001$: Interestingly, this element has a large negative influence, which may suggest that even if customers place a high value on safety, excessively complicated safety precautions may discourage them from using the product. Technological improvements ($B = 1.914$, $\text{Beta} = 0.418$, $t = 4.409$, $p = 0.000$): The adoption rate is positively impacted by technological improvements such as mobile payments systems and digital wallets. This effect is extremely significant and beneficial. The following variables were found to be significant: $B = 1.708$, $\text{Beta} = 0.336$, $t = 3.349$, $p = 0.001$: A considerable number of customers are greatly encouraged to embrace digital payments methods when prizes and incentives are offered. Trust and Reliability ($B = -0.789$, $\text{Beta} = -0.202$, $t = 2.310$, $p = 0.023$): Trust in payment providers has a large negative influence, which may imply that problems with trust might severely discourage adoption. This is supported by the fact that the beta value is 0.802. ($B = 0.360$, $\text{Beta} = 0.073$, $t = 0.890$, $p = 0.376$) With regard to the policies and regulations of the government: The finding that this component does not have a statistically significant impact suggests that while supporting policies are essential, they may not be sufficient to encourage consumer adoption on their own. ($B = 2.218$, $\text{Beta} = 0.472$, $t = 4.243$, $p = 0.000$) The following are the recommendations made by peers: In order to emphasise the significance of social influence, it is important to note that recommendations from peers and social networks have a very significantly beneficial effect. ($B = 0.409$, $\text{Beta} = 0.092$, $t = 1.133$, $p = 0.260$) Access to Financial Services Access to financial services. The results were not statistically significant, which suggests that just offering access is not sufficient to induce adoption. The following are examples of successful marketing campaigns: $B = 0.399$, $\text{Beta} = 0.088$, $t = 0.966$, $p = 0.336$ In addition, this is not substantial, which suggests that while marketing is essential, it may be necessary to combine it with other elements in order for it to be successful. ($B = -0.629$, $\text{Beta} = -0.148$, $t = -1.373$, $p = 0.173$) The fact that the results were not statistically significant suggests that cultural acceptability does not have a substantial influence on adoption on its own.

Through the use of Varimax rotation, Principal Component components Analysis was able to make the discovery of a structure that was composed of four components. Every single one of the components has an eigen value that is higher than one, as shown by the Total Variance of Factors Influencing Digital Payments (table:8) and the sum of the eigen values of components 1, 2, 3, and 4 accounts for 69.11% of the total variance. A total of thirty-one percent of the instrument's 10 elements have been removed from consideration.

The Principal Component Analysis of the Factors That Influence Digital Payments in table 9 is presented Down Below: User satisfaction, usability, and accessibility are all improved when the interface design, navigation, and mobile compatibility are simplified pertaining to digital payments systems (0.899 and FIDP2) The impact of social media, word-of-mouth, and online assessments all contribute to a growth in the validity of digital payments systems, as well as their acceptance and the number of alternative payments methods available to consumers. values of 0.758 for the FIDP3 The acceptance, adaptation, and behaviour of digital payments systems are all influenced by a variety of factors, including the preferences of consumers, various cultural tolerances, and disparities across generations.



**Jayashree and Sugunalakshmi**

values of 0.880 for the FIDP7. Consumers' shopping habits and their usage of digital payments methods are influenced by rewards programs, cashback offers, and economic incentives. 0.780 for the FIDP10 Encryption, biometric identity, and two-factor authentication are all strategies that may be used in digital payments systems to increase consumer trust and confidence while also protecting against fraud (0.783 by the FIDP1) When blockchain technology, artificial intelligence, and mobile payment platforms are combined, digital payments systems increase in terms of their efficiency, reliability, and security (FIDP5 score of 0.762) As a result of fee waivers, discount offers, and decreased transaction costs, digital payments are more enticing to consumers since they are more affordable (0.562 for the FIDP4). Increasing the level of knowledge, trust, and acceptance of digital payments technologies may be accomplished via marketing, education, and awareness initiatives. The rise in the number of individuals who use digital payments systems, as well as an increase in economic engagement and market penetration, may be attributed to a variety of factors, including access to banking, development of digital literacy, and outreach to rural regions. FIDP8 0.711 Legitimacy, market growth, and consumer protection from government policies, legal frameworks, and compliance requirements are all factors that favour the use of digital payments methods at a FIDP6 of 0.578.

A structure consisting of four factors was discovered by Principal Component Factor Analysis by using Varimax rotation. According to the Total Variance of Opinion on Consumer Adoption of Digital Payments (table:11), all of the components have an eigen value that is greater than 1, and the components 1, 2, 3, and 4 together explain a total of 73.13% of the variance. Seventy-seven percent of the total ten items on the instrument have been removed from consideration.

The table that presents the results of the Principal Component Analysis (PCA) offers insights into the primary elements that influence the adoption of digital payments by consumers. The table provides a list of the variables that influence the adoption of digital payments methods, together with the items and factor loadings that correlate to each factor. Indicative of greater correlations with the underlying component are factor loadings that are relatively higher. The coefficient of recommendation from peers (CADP7) is 0.843, which indicates that recommendations have a considerable impact on consumer uptake. Enhanced security features (CADP2) = 0.756: Security measures such as encryption and biometrics play an important part in the overall security system performance. It is important to note that marketing and educational activities are essential drivers. Effective marketing campaigns (CADP9) = 0.754 Access to financial services is provided by digital payments (CADP8) = 0.753: Acceptance of digital payments is increased when financial services are readily available. Rewards and incentives (CADP4) = 0.681 There is a correlation between cashback and rewards and the behaviour of consumers. Taking into account cultural characteristics, adoption rates are influenced by cultural acceptability (CADP10) = 0.777. Adoption is driven by technological breakthroughs (CADP3), which are 0.766. Examples of such innovations are mobile payments and digital wallets. Policies of the government (CADP6) equal 0.846: Use is encouraged by rules that are supportive. In terms of user-friendliness, convenience and ease of use (CADP1) = 0.835 is an important criterion to consider. There is a considerable relationship between trust in payment providers and adoption. The CADP5 value for trust and dependability is 0.955.

RESULTS AND DISCUSSION

The significance of social identity is shown by the fact that the consumer's adoption of digital payment method is highly impacted through social networks and references from others. By increasing the operations of security measures and ensuring the secured payment service providers are reliable and these are the vital elements in establishing customer confidence. Effective marketing campaigns and guidelines with proper training significantly assist in instructing and influencing consumers that leads to their success. Cashback schemes, rewards, and similar initiatives improve customer engagement and adoption via financial support. The development of technology and social acceptance of digital payment methods significantly attract their adoption. All the adoption rates are being developed by the support of government policies and regulations that also, promote the usage of digital payment systems. Due to the current status of technology, consumers in Tiruchirappalli district have started adopting the





Jayashree and Sugunalakshmi

digital payment methods more frequently than others. Touch-free payments, digital wallets, and mobile payment systems have increased more transactions among the consumers. the cash usage has been reduced due to theses optimised payment. Encryption, bibliometric identification, two-factor authentication have increased the trust of the consumers using digital payments. Various explanations have enabled the widespread usage of digital payment methods in the Tiruchirappalli district. The honesty followed by these new technologies and the agreeing experience they provide explain their consumer demand. The any time and any place digital payments has highly improved the purchasing experience of the consumers. Also, the enhanced security measures have significantly decreased consumer fear over data breaches and fraud, hence it increases their readiness to use digital payment methods. The effectiveness of marketing efforts made, guidelines and awareness has suggestively influenced the process of developing awareness about the benefits of digital payments and their secured applications. Social inspiration and word-of-mouth have also been significant factors boosting the extensive adoption of digital payment systems. Nowadays, consumers have become more motivated to adopt new technology when they see others using them successfully. The high impact that technology developments have made recently has assisted the consumers in the Tiruchirappalli district to adopt digital payments. The acceptance and adoption of digital payment in Tiruchirappalli district has been considerably encouraged by technological innovations. Non-comparable simple and effective method has come from mobile payments, digital wallets, and contactless payments that transform day-to-day transactions. Security facts such as biometric endorsement and encryption have increased the consumer trust, thus lowering fraud and data breach. Effective marketing and training help the stakeholders to highlight the advantages and usage of digital payments. Social networks and peer endorsements have also motivated the adoption of digital payments. Furthermore, better government rules and legislations also have simplified the digital transactions. These features assist the consumers from Tiruchirappalli district to accept and utilise digital payments effectively and frequently.

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Table:1. Variables Employed in the Research

Sl. No.	Variables	Items
Factors Influencing Digital Payment		
1	In digital systems, encryption, biometric identification, and two-factor authentication boost customer trust, confidence, and fraud protection	FIDP1
2	Simplified interface design, navigation, and mobile compatibility improve digital payments user happiness, usability, and accessibility	FIDP2
3	Social media impact, word-of-mouth, and online reviews boost digital payments credibility, adoption, and customer choices	FIDP3
4	Digital payments are cheaper due to charge waivers, discount offers, and lower transaction costs, making them more appealing to customers	FIDP4





Jayashree and Sugunalakshmi

5	Digital payments systems become more efficient, reliable, and secure when blockchain, AI, and mobile payment platforms are integrated	FIDP5
6	Legitimacy, market development, and consumer protection from government policies, legal frameworks, and compliance requirements encourage digital payments	FIDP6
7	Consumer preferences, cultural acceptability, and generational variations affect digital payments uptake, adaptation, and behaviour	FIDP7
8	Access to banking, digital literacy, and rural outreach boost digital payments inclusion, economic involvement, and market penetration	FIDP8
9	Digital payments technology awareness, trust, and acceptance are increased via marketing, education, and awareness campaigns	FIDP9
10	Rewards schemes, cashback offers, and economic incentives change consumer purchasing and digital payments use	FIDP10
Opinion on Consumer Adoption of Digital Payment		
1	Convenience and Ease of Use	CADP1
2	Enhanced security features such as encryption, biometric authentication, and two-factor authentication	CADP2
3	Technological Advancements like mobile payment platforms, digital wallets, and contactless payments	CADP3
4	Rewards and Incentives like Cashback offers, rewards programs, and discounts	CADP4
5	Trust and Reliability of payment providers	CADP5
6	Government policies and regulations encourage digital payments	CADP6
7	Recommendations from peers, family, and social networks	CADP7
8	Digital payments provide access to financial services	CADP8
9	Effective marketing campaigns and educational initiatives	CADP9
10	Cultural Acceptance of digital payments	CADP10

Table2. Demographic background of Consumers using Digital Payments

Demographic Characteristics		n (Total=113)	% of n
AGE	Less than 30 years	13	11.5
	30 years – 40 years	64	56.6
	40 years – 50 years	14	12.4
	50 years & above	22	19.5
GENDER	Male	51	45.1
	Female	62	54.9
EDUCATIONAL STATUS	School level	9	8.0
	Graduation	31	27.4
	Post Graduation	44	38.9
	Diploma	29	25.7

Source: Primary data





Jayashree and Sugunalakshmi

Table:3. Inter Correlation Matrix on Factors Influencing Digital Payments and Opinion on Consumer Adoption of Digital Payments

Particulars		Factors Influencing Digital Payments	Opinion on Consumer Adoption of Digital Payments
Factors Influencing Digital Payments	Pearson Correlation	1	0.961**
	Sig. (2-tailed)	-	0.000 Significant
Opinion on Consumer Adoption of Digital Payments	Pearson Correlation	-	1
	Sig. (2-tailed)	-	-

Source: Data analyzed statistically

Table:4. Regression Analysis- R Square - Opinion on Consumer Adoption of Digital Payments

R	R Square	Adjusted R Square	Std. Error of the Estimate
0.984 ^a	0.868	0.816	3.592

Source: Data analyzed statistically

Table:5. Regression Analysis- Opinion on Consumer Adoption of Digital Payments

	Sum of Squares	df ^x	Mean Square	F ^y	Sig. ^z
Regression	1158.490	10	115.849	8.977	0.000** Significant
Residual	1316.377	102	12.906		
Total	2474.867	112			

Source: Data analyzed statistically

Note: ^xDegrees of Freedom, ^yF-Statistic, ^zSignificance,

Table:6. Regression Analysis- Significance- Opinion on Consumer Adoption of Digital Payments

Particulars	Unstandardized Coefficients		Standardized Coefficients	Ta	Sig.b
	B	Std. Error	Beta		
(Constant)	14.302	4.093	-	3.495	0.001**
Convenience and Ease of Use	2.042	0.447	0.391	4.573	0.000**
Enhanced security features such as encryption, biometric authentication, and two-factor authentication	-1.321	0.378	-0.386	3.492	0.001**
Technological Advancements like mobile payment platforms, digital wallets, and contactless payments	1.914	0.434	0.418	4.409	0.000**
Rewards and Incentives like Cashback offers, rewards programs, and discounts	1.708	0.510	0.336	3.349	0.001**
Trust and Reliability of payment providers	-0.789	0.341	-0.202	2.310	0.023*
Government policies and regulations encourage digital payments	0.360	0.405	0.073	0.890	0.376





Jayashree and Sugunalakshmi

Recommendations from peers, family, and social networks	2.218	0.523	0.472	4.243	0.000**
Digital payments provide access to financial services	0.409	0.361	0.092	1.133	0.260
Effective marketing campaigns and educational initiatives	0.399	0.413	0.088	0.966	0.336
Cultural Acceptance of digital payments	-0.629	0.458	-0.148	-1.373	0.173

Source: Data analyzed statistically

Note: ^aT-Statistic, ^bSignificance

Table:7. Total Variance for Factors Influencing Digital Payments

Component	The sums of square loadings based on rotation		
	Total	% of Variance	Cumulative%
1	1.901	19.012	19.012
2	1.688	16.877	35.889
3	1.671	16.711	52.600
4	1.651	16.511	69.111

Source: Statistically Analysed Data

Table:8. Principal Component Analysis of Factors Influencing Digital Payments

Particulars	Items	Factors			
		1	2	3	4
Simplified interface design, navigation, and mobile compatibility improve digital payments user happiness, usability, and accessibility	FIDP2	0.899			
Social media impact, word-of-mouth, and online reviews boost digital payments credibility, adoption, and customer choices	FIDP3	0.758			
Consumer preferences, cultural acceptability, and generational variations affect digital payments uptake, adaptation, and behaviour	FIDP7		0.880		
Rewards schemes, cashback offers, and economic incentives change consumer purchasing and digital payments use	FIDP10		0.780		
In the digital payments systems, encryption, biometric identification, and two-factor authentication boost customer trust, confidence, and fraud protection	FIDP1			0.783	
Digital payments systems become more efficient, reliable, and secure when blockchain, AI, and mobile payments platforms are integrated	FIDP5			0.762	
Digital payments are cheaper due to charge waivers, discount offers, and lower transaction costs, making them more appealing to customers	FIDP4			0.562	
Digital payments technology awareness, trust, and acceptance are increased via marketing, education, and awareness campaigns	FIDP9				0.841





Jayashree and Sugunalakshmi

Access to banking, digital literacy, and rural outreach boost digital payments inclusion, economic involvement, and market penetration	FIDP8				0.711
Legitimacy, market development, and consumer protection from government policies, legal frameworks, and compliance requirements encourage digital payments	FIDP6				0.578

Source: Statistically Analysed Data

Table: 9. Total Variance for Opinion on Consumer Adoption of Digital Payments

Component	Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %
1	3.014	30.139	30.139
2	1.808	18.078	48.217
3	1.448	14.484	62.701
4	1.043	10.430	73.132

Extraction Method: Principal Component Analysis

Table:10. Principal Component Analysis of Opinion on Consumer Adoption of Digital Payments

Particulars	Items	Factors			
		1	2	3	4
Recommendations from peers, family, and social networks	CADP7	0.843			
Enhanced security features such as encryption, biometric authentication, and two-factor authentication	CADP2	0.756			
Effective marketing campaigns and educational initiatives	CADP9	0.754			
Digital payments provide access to financial services	CADP8	0.753			
Rewards and Incentives like Cashback offers, rewards programs, and discounts	CADP4	0.681			
Cultural Acceptance of digital payments	CADP10		0.777		
Technological Advancements like mobile payment platforms, digital wallets, and contactless payments	CADP3		0.766		
Government policies and regulations encourage digital payments	CADP6			0.846	
Convenience and Ease of Use	CADP1			0.835	
Trust and Reliability of payment providers	CADP5				0.955

Source: Statistically Analysed Data

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Enamel Matrix Derivative (EMD, Emdogain®) in Pediatric Dentistry: Biological Basis, Clinical Applications and Future Perspectives

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ABSTRACT

Emdogain is an enamel matrix derivative biomaterial and its role in Pediatric Dentistry has evolved beyond conventional periodontal applications and now includes regenerative endodontics, management of dental trauma, pulpotomy agent, root development support, soft tissue healing and preservation of immature teeth. The biological activity of Emdogain is primarily attributed to amelogenin-rich proteins capable of stimulating cementogenesis, periodontal ligament regeneration, osteogenesis, angiogenesis, and cellular differentiation. Over the last two decades, growing evidence has highlighted its capacity to improve wound healing and promote regenerative events within the oral cavity. This review critically evaluates the composition, biological mechanism, indications, clinical applications, advantages, limitations and future perspectives of Emdogain in pediatric dentistry.

Keywords: Emdogain, Enamel Matrix Derivative, Pediatric Dentistry, Biomaterials, EMD





INTRODUCTION

Emdogain, a commercially available "enamel matrix derivative (EMD)", represents one of the most extensively studied regenerative biomaterials in dentistry[1]. Originally introduced for periodontal regeneration but now Emdogain has demonstrated potential applications in several pediatric dental procedures. The biomaterial contains proteins derived from developing porcine tooth buds, predominantly amelogenins, which mimic developmental events occurring during tooth formation [2]. These proteins influence cellular attachment, proliferation, differentiation, and matrix formation. Emdogain may stimulate periodontal ligament fibroblasts, osteoblasts, cementoblasts, and mesenchymal stem cells [3]. Emdogain has attracted considerable attention due to their regenerative and anti-inflammatory properties. Concept of enamel matrix proteins influencing periodontal regeneration originated from developmental biology observations. During tooth development, Hertwig's epithelial root sheath secretes enamel matrix proteins that guide cementogenesis and periodontal attachment formation [4]. As regenerative dentistry evolved, researchers explored the role of Emdogain in pulp therapy, avulsed teeth management, root resorption management, and regenerative endodontic procedures [5]. Pediatric dentistry became a particularly relevant field because immature permanent teeth exhibit remarkable healing potential and several biological properties make Emdogain beneficial in pediatric dentistry

- Biocompatibility
- Bioactivity
- Anti-inflammatory effects
- Promotion of angiogenesis
- Stimulation of hard tissue formation
- Enhancement of periodontal ligament regeneration

Composition of Emdogain

Emdogain® is the commercial preparation of EMD marketed by Straumann Group (Fig. 1). Emdogain is a sterile gel primarily consists of amelogenin proteins (Derived from developing porcine tooth buds) suspended within a propylene glycol alginate (PGA) carrier⁶. Approximately 90% of the protein fraction comprises amelogenins, while the remaining proteins include enamelin, tuftelin, and ameloblastin [7]. The gel formulation allows controlled application to root surfaces or surgical sites. Upon contact with physiological fluids, the carrier precipitates and forms an insoluble extracellular matrix layer that facilitates cell adhesion and differentiation [8].

Mechanism of Action

The regenerative capacity of Emdogain is linked to molecular signaling pathways involved in tissue development. Amelogenins influence cytokines, growth factors, extracellular matrix proteins responsible for wound healing and regeneration [9] Emdogain stimulates "transforming growth factor-beta (TGF-β)", "bone morphogenetic proteins (BMPs)" and "vascular endothelial growth factor (VEGF)" pathways [10]. These pathways enhance angiogenesis and matrix mineralization. Emdogain also promotes proliferation and differentiation of periodontal ligament stem cells[11]. In immature permanent teeth, this biological activity may support continued root development and apical closure. Another significant property is modulation of inflammatory responses. Emdogain appears to suppress pro-inflammatory cytokines while enhancing regenerative cellular events [12], and this effect may be advantageous in traumatized or inflamed teeth.

Applications in Pediatric Dentistry

Direct Pulp Capping

Direct pulp capping is performed to preserve the vitality of exposed pulp tissue. EMD promotes odontoblast-like cell differentiation and reparative dentin formation, thereby facilitating pulpal healing and reducing inflammatory responses[13,14]. These properties make it a potential alternative biomaterial for vital pulp therapy.



Vivek Rana *et al.*,**Pulpotomy**

EMD has been evaluated as a pulpotomy medicament in primary and immature permanent teeth. Its regenerative potential encourages dentin bridge formation and maintenance of radicular pulp vitality contributing to successful clinical outcomes [13,14].

Regenerative Endodontic Procedures

Regenerative endodontic procedures aim to restore the vitality of immature permanent teeth with necrotic pulp. EMD has been shown to stimulate cell proliferation, angiogenesis, and differentiation of progenitor cells thereby supporting continued root maturation and apical closure [13,15]. When EMD is used as a biologically active scaffold during regenerative treatment, it has demonstrated favourable healing outcomes [15].

Periodontal Regeneration

In adolescent patients presenting with localized periodontal defects, EMD has demonstrated the ability to promote regeneration of cementum, periodontal ligament, and alveolar bone rather than simple repair [16,17]. Clinical studies have reported improvements in attachment levels and periodontal healing following its use.

Role in replantation

Emdogain has been proposed as an adjunctive regenerative material due to its ability to support periodontal ligament regeneration as well as reduce inflammatory root resorption. Periodontal healing is improved following replantation of avulsed tooth treated with EMD. The material may reduce ankylosis and external inflammatory resorption enhancing periodontal ligament cell survival [18]

Safety and Biocompatibility:

EMD, commercially marketed as Emdogain®, has demonstrated a favorable safety profile and excellent biocompatibility in both experimental and clinical studies. EMD is non-cytotoxic and supports the attachment, proliferation, differentiation of periodontal ligament fibroblasts, osteoblasts, cementoblasts, and pulp cells [14]. Unlike many conventional materials, EMD promotes tissue regeneration without inducing significant inflammatory responses, thereby creating a favorable environment for wound healing and tissue repair [14,16]. Clinical studies have reported a low incidence of adverse reactions following EMD application. Because EMD is derived from porcine proteins, caution should be exercised in patients with known hypersensitivity to porcine-derived products. However, allergic reactions are extremely rare and have not been reported as a significant clinical concern in the literature [13,17].

Advantages [13,14,16,17]

- Excellent biocompatibility with hard and soft oral tissues.
- Non-cytotoxic and non-mutagenic.
- Minimal inflammatory or foreign-body reactions.
- Supports natural wound healing and tissue regeneration.
- Long-term clinical safety supported by extensive research.
- Low risk of allergic or immunological reactions.

Limitations

- Technique-sensitive procedure – Successful outcomes depend on meticulous technique of procedures Like pulpotomy and root surface preparation.
- Higher treatment cost – When compared with other materials used for pulpotomy and root surface preparation, Emdogain treatment charges are higher.

Future Prospects of Emdogain in Pediatric Dentistry:

Emdogain (Enamel Matrix Derivative) has promising future applications in Pediatric dentistry due to its regenerative potential. Ongoing research suggests that it might play significant role in regenerative endodontic procedures for immature permanent teeth, vital pulp therapy, apexogenesis, and the management of traumatic dental injuries. Its





Vivek Rana et al.,

ability to promote cell differentiation, tissue repair, angiogenesis, and periodontal regeneration may support the development of biologically based treatments aimed at preserving pulp vitality and enhancing root maturation. Furthermore, the integration of Emdogain with stem cell therapy, tissue engineering, and biomimetic scaffolds may expand its use in Pediatric regenerative dentistry. However, long-term clinical studies are still required to establish its efficacy and predictability in children.

CONCLUSION

Emdogain represents a valuable biologic agent in modern Pediatric Dentistry because of its capability for supporting periodontal and pulp tissue regeneration while promoting natural healing processes. Its application in regenerative endodontics, management of traumatic dental injuries, and preservation of pulp vitality highlights its potential to improve long-term outcomes in young patients. Although current evidence is encouraging broader clinical studies and long-term follow-up data are needed to establish standardized protocols and confirm its effectiveness across different Pediatric dental conditions. Emdogain is likely to play increasingly significant role in biologically driven treatment approaches aimed at preserving natural tissues and enhancing oral health in children.

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Vivek Rana *et al.*,



Fig 1: Emdogain®

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RESEARCH ARTICLE

Significance of Diffusion Weighted Imaging (DWI) in Assessing Brain Stroke at 3.0 Tesla MRI

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ABSTRACT

Acute ischemic stroke requires rapid and accurate imaging for early diagnosis and management. Diffusion Weighted Imaging (DWI) at 3.0 Tesla MRI plays an important role in detecting early ischemic changes. To evaluate the significance of DWI in assessing acute ischemic stroke and correlate DWI findings with clinical severity and functional outcome. This retrospective observational study included 50 patients with acute ischemic stroke who underwent 3.0 Tesla MRI within 24 hours of symptom onset. MRI protocol included T1WI, T2WI, FLAIR, DWI, and ADC mapping. Clinical severity was assessed using NIHSS score and functional outcome using modified Rankin Scale (mRS). DWI detected lesions in 100% of cases and showed significantly higher sensitivity than conventional MRI sequences. DWI lesion volume showed strong positive correlation with NIHSS and mRS, while ADC values demonstrated strong negative correlation with stroke severity. DWI at 3.0 Tesla MRI is highly sensitive for early ischemic stroke detection and provides valuable prognostic information for clinical outcome assessment.

Keywords: Acute ischemic stroke, Diffusion Weighted Imaging, DWI, ADC, 3.0 Tesla MRI, NIHSS, mRS.





INTRODUCTION

Stroke is one of the leading causes of mortality and long-term neurological disability worldwide, with acute ischemic stroke accounting for nearly 80–85% of all cerebrovascular events. Rapid interruption of cerebral blood flow leads to irreversible neuronal injury within a short period; therefore, early diagnosis and prompt therapeutic intervention are essential for improving patient outcomes and reducing permanent disability. Conventional imaging techniques may fail to detect subtle ischemic changes during the hyperacute stage, limiting early clinical decision-making [1]. Magnetic Resonance Imaging (MRI) has become an important modality in the evaluation of acute ischemic stroke because of its excellent soft-tissue contrast and superior sensitivity for early brain parenchymal changes. Among various MRI techniques, Diffusion Weighted Imaging (DWI) has emerged as the most sensitive sequence for detecting acute cerebral ischemia. DWI identifies restricted diffusion caused by cytotoxic edema and can demonstrate ischemic lesions within minutes of symptom onset, much earlier than conventional MRI sequences such as T1-weighted imaging, T2-weighted imaging, and FLAIR imaging [2].

The development of 3.0 Tesla MRI systems has further enhanced the diagnostic value of DWI by providing improved signal-to-noise ratio, better spatial resolution, and superior lesion conspicuity. High-field MRI allows accurate visualization of small cortical, lacunar, and posterior circulation infarcts that may otherwise remain inconspicuous on routine imaging. In addition, quantitative diffusion parameters such as Apparent Diffusion Coefficient (ADC) values and DWI lesion volume have shown significant correlation with stroke severity and functional outcome, thereby contributing to prognostic assessment and treatment planning [3,4]. Despite significant advances in neuroimaging, challenges still exist in differentiating hyperacute infarcts from stroke mimics and in establishing reliable imaging biomarkers for prognosis. Therefore, the present study was undertaken to evaluate the significance of Diffusion Weighted Imaging (DWI) in assessing brain stroke at 3.0 Tesla MRI and to correlate DWI findings with clinical severity and imaging characteristics in acute ischemic stroke patients [5,6].

Objectives of the Study

- ❖ To evaluate the diagnostic accuracy of DWI at 3.0T MRI in detecting acute ischemic stroke.
- ❖ To correlate DWI lesion volume and ADC values with clinical severity using NIHSS Stroke Scale.
- ❖ To study lesion characteristics across different stroke subtypes (lacunar, territorial, embolic).
- ❖ To compare DWI findings with conventional MRI sequence. (T1, T2, FLAIR)

MATERIALS AND METHODS

Study Design and Setting

This retrospective hospital-based observational study was conducted in the Department of Radio-diagnosis at Chhatrapati Shivaji Subharti Hospital from January 2025 to January 2026 using a United Imaging uMR780 3.0 Tesla MRI scanner.

Study Population

A total of 50 patients with clinically and radiologically diagnosed acute ischemic stroke who underwent brain MRI with Diffusion Weighted Imaging (DWI) within 24 hours of symptom onset were included in the study.

Inclusion Criteria

Patients aged 18–80 years who underwent 3.0 Tesla MRI brain with DWI within 24 hours of neurological symptom onset and had adequate imaging quality with complete clinical records were included.

Exclusion Criteria

Patients with brain tumors, recent head trauma, demyelinating diseases, incomplete imaging studies, or missing clinical data were excluded.



**Yash Pal Singh Teotia et al.,****Imaging Protocol**

MRI protocol included T1-weighted imaging, T2-weighted imaging, FLAIR, Diffusion Weighted Imaging (DWI) using b-values of 0 and 1000 s/mm², and Apparent Diffusion Coefficient (ADC) mapping using a 32-channel head and neck coil.

Image Analysis

DWI and ADC images were independently reviewed by two experienced radiologists blinded to clinical details. Lesion presence, location, infarct volume, diffusion characteristics, vascular territory involvement, and stroke subtype were evaluated.

Clinical Data Collection

Clinical data included age, sex, presenting symptoms, vascular risk factors, NIHSS score at presentation, hospital stay duration, and modified Rankin Scale (mRS) at discharge.

Outcome Parameters

The study assessed the sensitivity of DWI for early ischemic stroke detection and the correlation of DWI lesion characteristics with clinical severity and functional outcome.

Statistical Analysis

Data were analyzed using Microsoft Excel and SYSTAT version 13.2. Descriptive statistics, Chi-square test, Fisher's exact test, Student's t-test, and Mann-Whitney U-test were applied where appropriate. A p-value <0.05 was considered statistically significant.

RESULT**Statistical test applied: χ^2 test.**

DWI detected acute ischemic lesions in all 50 patients (100.0%), whereas detection was lower with conventional MRI sequences. FLAIR identified lesions in 37 patients (74.0%), T2WI in 34 patients (68.0%), and T1WI in only 14 patients (28.0%). The difference in lesion detection between DWI and each conventional sequence was statistically significant ($p < 0.001$), indicating superior sensitivity of DWI for acute infarct detection in early stroke imaging.

Statistical test applied: χ^2 test.

Stroke subtype was significantly associated with NIHSS severity ($p < 0.001$). Lacunar infarcts showed lower severity, while territorial and embolic infarcts were associated with higher NIHSS scores, indicating greater clinical severity at presentation.

Statistical test applied: χ^2 test.

DWI-FLAIR mismatch was present in 13 patients (26.0%), all of whom underwent MRI within ≤ 4.5 hours of symptom onset. In contrast, mismatch was absent in all patients imaged between 4.6–12 hours and 12.1–24 hours. The association between time from symptom onset and DWI-FLAIR mismatch was statistically significant ($p < 0.001$), indicating that DWI-FLAIR mismatch was mainly observed during the hyperacute phase of ischemic stroke.

The mean time from symptom onset to MRI was 9.98 ± 6.20 hours, with a median of 8.65 hours. The mean NIHSS score was 11.20 ± 6.04 , indicating predominantly moderate clinical severity. Mean DWI lesion volume was 29.20 ± 24.80 mL, while mean ADC and minimum ADC values were 585.00 ± 75.30 and $390.00 \pm 85.80 \times 10^{-6}$ mm²/s, respectively. The mean hospital stay was 7.02 ± 3.19 days, and mean mRS at discharge was 2.78 ± 1.28 .

The quantitative clinical and imaging parameters in the present study demonstrated a mean NIHSS score of 11.20 ± 6.04 and mean DWI lesion volume of 29.20 ± 24.80 mL, indicating predominantly moderate stroke severity. Mean ADC values were reduced in acute ischemic lesions, reflecting diffusion restriction associated with cytotoxic edema.



**Yash Pal Singh Teotia et al.,**

These findings are consistent with Dogariu et al. (2024)⁷ and de Escalup et al. (2026)¹ who reported significant associations between infarct volume, ADC parameters, and neurological outcome. Similar observations by Farhan et al. (2025)⁸ and Suzuki et al. (2023)⁹ further support the prognostic relevance of diffusion-derived imaging biomarkers in acute ischemic stroke.

Mean ADC values decreased progressively with increasing NIHSS severity, indicating greater diffusion restriction and cytotoxic edema in severe strokes. Similar findings have been reported by de Escalup et al. (2026)¹, Nazari-Farsani et al. (2023)¹⁰, and Nagaraja (2021)⁶, supporting the prognostic value of ADC measurements in acute ischemic stroke. DWI demonstrated superior diagnostic performance compared with conventional MRI sequences, detecting acute ischemic lesions in 100% of patients, whereas FLAIR, T2WI, and T1WI showed lower detection rates. The differences were statistically significant ($p < 0.001$). These findings are consistent with previous studies by Farhan et al. (2025)⁸ Arafat et al. (2023)⁵ and Tedyanto et al. (2022)¹¹ which established DWI as the most sensitive MRI sequence for early ischemic stroke detection, particularly during the hyperacute phase. A significant association was also observed between time from symptom onset and DWI–FLAIR mismatch ($p < 0.001$), with mismatch present exclusively in patients imaged within ≤ 4.5 hours. This finding supports the established role of DWI–FLAIR mismatch as an imaging marker of hyperacute stroke and therapeutic eligibility, consistent with reports by Tedyanto et al. (2022)¹¹ and Yoshimoto et al. (2019)¹⁰. Collectively, the present study reinforces the diagnostic and prognostic significance of DWI at 3.0 Tesla MRI in acute ischemic stroke evaluation.

CONCLUSION

Diffusion Weighted Imaging (DWI) at 3.0 Tesla MRI demonstrated excellent diagnostic and prognostic value in the assessment of acute ischemic stroke. DWI showed superior sensitivity compared with conventional MRI sequences for early infarct detection and provided reliable evaluation of lesion burden and tissue ischemia. Quantitative diffusion parameters, particularly DWI lesion volume and ADC values, showed strong correlation with NIHSS score and functional outcome, emphasizing their role as important imaging biomarkers. The presence of DWI–FLAIR mismatch within the hyperacute window further highlighted its clinical relevance in early stroke triage and therapeutic decision-making. Therefore, DWI should remain an integral component of acute stroke imaging protocols.

Funding

No external funding was received for this study.

Ethical Approval

The study was approved by the Institutional Ethics Committee of Swami Vivekanand Subharti University.

Informed Consent

Informed consent was obtained from all participants prior to inclusion in the study.

Conflict of Interest

The authors declare no conflict of interest.

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Table 1. Comparison of Acute Lesion Detection by Conventional MRI Sequences and DWI among Study Participants (N = 50).

MRI Sequence	Lesion Detected n (%)	Lesion Not Detected n (%)	Total n (%)	p-value versus DWI
T1WI	14 (28.0)	36 (72.0)	50 (100.0)	<0.001*
T2WI	34 (68.0)	16 (32.0)	50 (100.0)	<0.001*
FLAIR	37 (74.0)	13 (26.0)	50 (100.0)	<0.001*
DWI	50 (100.0)	0 (0.0)	50 (100.0)	-----

Table 2. Association between Stroke Subtype and NIHSS Severity Category among Study Participants (N = 50).

Stroke Subtype	Minor n (%)	Moderate n (%)	Moderate-severe n (%)	Severe n (%)	Total n (%)	p-value
Borderzone	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)	1 (100.0)	<0.001*
Embolic	0 (0.0)	0 (0.0)	2 (50.0)	2 (50.0)	4 (100.0)	
Lacunar	8 (36.4)	14 (63.6)	0 (0.0)	0 (0.0)	22 (100.0)	
Territorial	0 (0.0)	15 (65.2)	6 (26.1)	2 (8.7)	23 (100.0)	
Total	8 (16.0)	29 (58.0)	9 (18.0)	4 (8.0)	50 (100.0)	

Table 3. Association between Time from Symptom Onset to MRI and DWI-FLAIR Mismatch (N = 50).

Time Category	DWI-FLAIR Mismatch Absent n (%)	DWI-FLAIR Mismatch Present n (%)	Total n (%)	p-value
12.1–24 hours	17 (100.0)	0 (0.0)	17 (100.0)	<0.001*
4.6–12 hours	20 (100.0)	0 (0.0)	20 (100.0)	
≤4.5 hours	0 (0.0)	13 (100.0)	13 (100.0)	
Total	37 (74.0)	13 (26.0)	50 (100.0)	



Yash Pal Singh Teotia *et al.*,

Table 4. Distribution of Quantitative Clinical and MRI Parameters among Study Participants (N = 50).

Variable	N	Mean $\pm$ SD	Median	Minimum	Maximum
Time from symptom onset to MRI (hours)	50	9.98 $\pm$ 6.20	8.65	1.50	23.0
NIHSS score at presentation	50	11.20 $\pm$ 6.04	10.50	3	23
DWI lesion volume (mL)	50	29.20 $\pm$ 24.80	20.40	2.90	91.6
Mean ADC value ($\times 10^{-6}$ mm ² /s)	50	585.00 $\pm$ 75.30	593.00	455	708
Minimum ADC value ($\times 10^{-6}$ mm ² /s)	50	390.00 $\pm$ 85.80	384.00	252	575
Number of lesions	50	1.52 $\pm$ 1.03	1.00	1	5
Hospital stay duration (days)	50	7.02 $\pm$ 3.19	6.00	3.00	14.0
mRS at discharge	50	2.78 $\pm$ 1.28	3.00	1	5

Comparison of Acute Lesion Detection by Conventional MRI Sequences and DWI among Study Participants

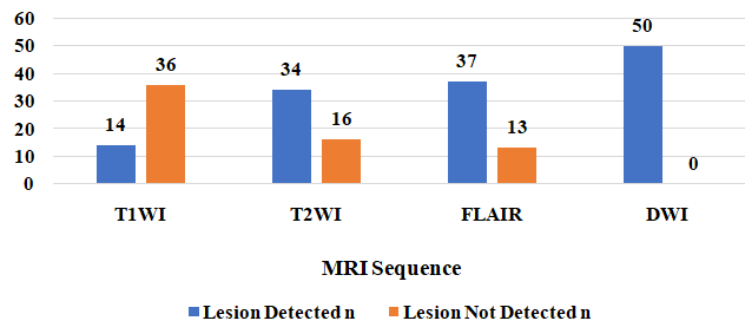


Figure 1. Comparison of Acute Lesion Detection by Conventional MRI Sequences and DWI among Study Participants

Association between Stroke Subtype and NIHSS Severity Category among Study Participants

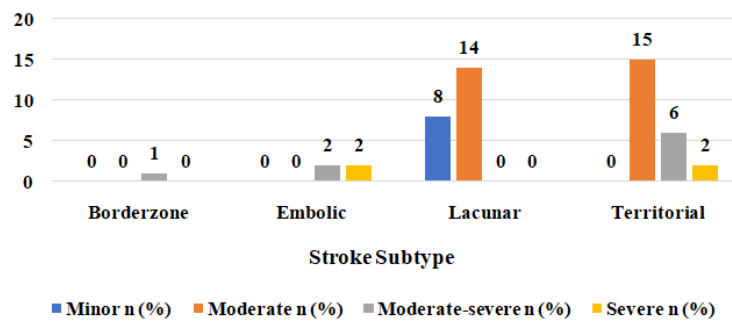


Figure 2. Association between Stroke Subtype and NIHSS Severity Category among Study Participants





Yash Pal Singh Teotia et al.,

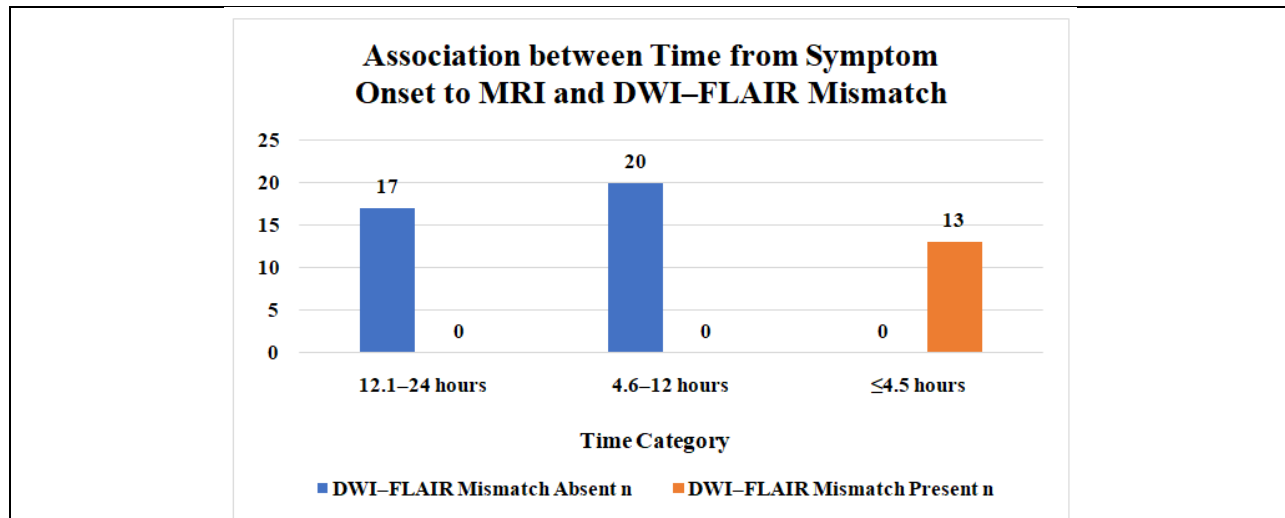


Figure 3. Association between Time from Symptom Onset to MRI and DWI-FLAIR Mismatch

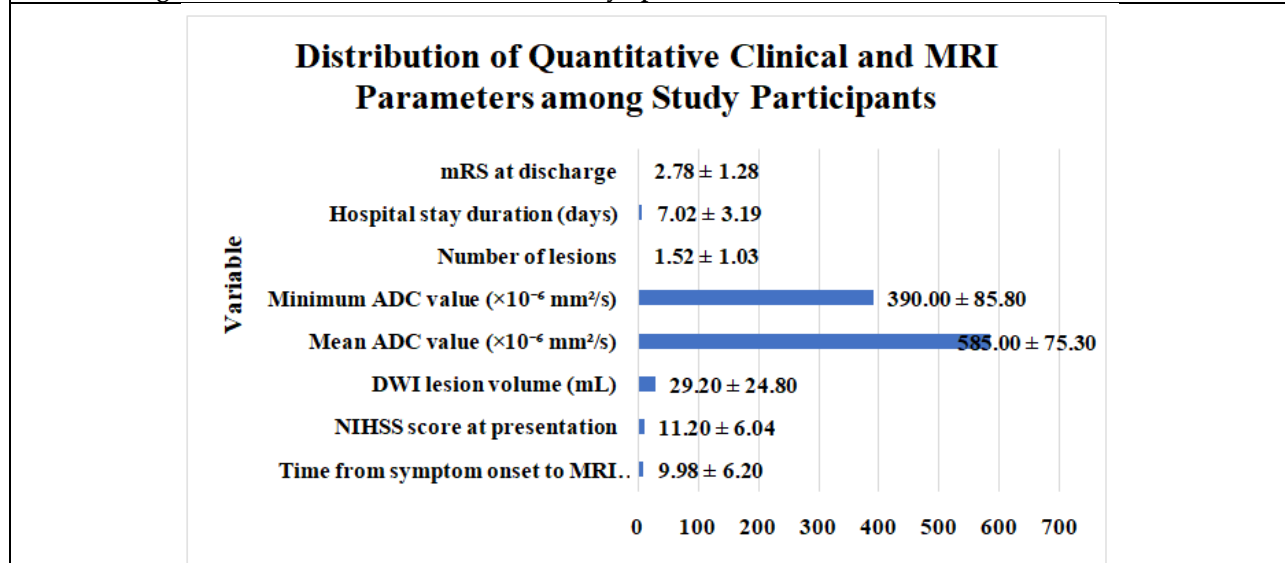


Figure 4. Distribution of Quantitative clinical and MRI parameters among study participants

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RESEARCH ARTICLE

Intuitionistic Fuzzyideals in Ternary Semi - Rings with Normal Intervals

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ABSTRACT

In this paper, we introduce the concepts of normal interval valued intuitionistic Ω – fuzzy ternary semi-ring, Ω -fuzzy right ideal, anti-fuzzy right ideal, intuitionistic Ω -fuzzy right ideal, Ω -fuzzy right k-ideal, anti-fuzzy right k-ideal, intuitionistic Ω -fuzzy right k-ideal, Ω -fuzzy ideal, anti-fuzzy ideal, and intuitionistic Ω -fuzzy ideal in ternary semirings, and we investigate some of their basic properties. We define a regular ternary semi-ring using the Normal interval valued intuitionistic Ω -fuzzy right ideal. We introduce standard intuitionistic Ω -fuzzy ideals in ternary semi-rings. Hence, these notions are used to investigate some properties, theorems, and examples.

Keywords: Interval valued fuzzy set (IFS), Interval valued intuitionistic Ω – fuzzy right ideal.

INTRODUCTION

The speculation of fuzzy sets was introduced by L.A Zadeh [6], Atanassov et.al [1] [2] introduced the concepts of intuitionistic fuzzy sets and interval valued intuitionistic fuzzy sets and Dutta et.al [3] introduced the notation of an interval valued fuzzy prime ideals of a semi-ring Krishnaswamy et.al [4] introduced the concept of interval valued intuitionistic fuzzy Bi-ideals in ternary semi ring in 2012, Lekkoksung [5] introduced the concept of intuitionistic Q-fuzzy K-ideals of semi ring.





PRELIMINARIES

Definition 2.1:

A non-empty set Γ together with a binary operation called, addition $+$ and $\cdot$ a ternary multiplication, denoted by juxtaposition, is said to be a ternary semi-ring if $(\Gamma; +)$ is a commutative semi-group satisfying the following conditions:

- (i) $(\alpha\beta\delta)\theta\gamma = \alpha(\beta\delta\theta)\gamma = \alpha\beta(\delta\theta\gamma)$,
- (ii) $(\alpha + \beta)\delta\theta = \alpha\delta\theta + \beta\delta\theta$,
- (iii) $\alpha(\beta + \delta)\theta = \alpha\beta\theta + \alpha\delta\theta$ and
- (iv) $\alpha\beta(\delta + \theta) = \alpha\beta\delta + \alpha\beta\theta$ for all $\alpha, \beta, \delta, \theta, \gamma \in \Gamma$.

Definition 2.2:

A non-empty interval-valued intuitionistic fuzzy subset Δ in a semi-ring Γ is called an interval-valued intuitionistic fuzzy ideal of Γ if it satisfies

- i. $M_{\Delta}(\alpha + \beta) \geq \{M_{\Delta}(\alpha), M_{\Delta}(\beta)\}$
- ii. $M_{\Delta}(\alpha\beta) \geq \{M_{\Delta}(\alpha), M_{\Delta}(\beta)\}$
- iii. $Q_{\Delta}(\alpha + \beta) \leq \{Q_{\Delta}(\alpha), Q_{\Delta}(\beta)\}$
- iv. $Q_{\Delta}(\alpha\beta) \leq \{Q_{\Delta}(\alpha), Q_{\Delta}(\beta)\}$ for all $\alpha, \beta \in \Gamma$.

Definition 2.3:

An interval-valued intuitionistic Ω -fuzzy subset $\Delta = (\tilde{M}_{\Delta}, \tilde{Q}_{\Delta})$ in Γ is called an interval-valued intuitionistic Ω -fuzzy right ideal of Γ if

- i. $\tilde{M}_{\Delta}(\alpha + \beta, \omega) \geq \min^i\{\tilde{M}_{\Delta}(\alpha, \omega), \tilde{M}_{\Delta}(\beta, \omega)\}$
- ii. $\tilde{M}_{\Delta}(\alpha\beta\delta, \omega) \geq M(\alpha, \omega)$
 $(\tilde{M}_{\Delta}(\alpha\beta\delta, \omega) \geq \tilde{M}_{\Delta}(\beta, \omega) \text{ or } \tilde{M}_{\Delta}(\alpha\beta\delta, \omega) \geq M(\delta, \omega))$
- iii. $\tilde{Q}_{\Delta}(\alpha + \beta, \omega) \leq \max^i\{\tilde{Q}_{\Delta}(\alpha, \omega), \tilde{Q}_{\Delta}(\beta, \omega)\}$
- iv. $\tilde{Q}_{\Delta}(\alpha\beta\delta, \omega) \leq \tilde{Q}_{\Delta}(\alpha, \omega)$
 $(\tilde{Q}_{\Delta}(\alpha\beta\delta, \omega) \leq \tilde{Q}_{\Delta}(\beta, \omega) \text{ or } \tilde{Q}_{\Delta}(\alpha\beta\delta, \omega) \leq \tilde{Q}_{\Delta}(\delta, \omega))$ for all $\alpha, \beta, \delta \in \Gamma, \omega \in \Omega$.

Definition 2.4:

An interval-valued intuitionistic Ω -fuzzy subset $\Delta = (\tilde{M}_{\Delta}, \tilde{Q}_{\Delta})$ is called an intuitionistic Ω -fuzzy ideal of Γ if it is interval-valued intuitionistic Ω -fuzzy right, interval-valued intuitionistic Ω -fuzzy lateral and interval-valued intuitionistic Ω -fuzzy left ideal of Γ .

Normal Interval Valued Intuitionistic Ω – Fuzzy Ideals**Definition 3.1:**

An interval value intuitionistic Ω fuzzy right (left, lateral) ideal of $\Delta = (\tilde{M}_{\Delta}, \tilde{Q}_{\Delta})$ of a ternary semi-ring Γ is said to be normal if $\Delta(0, \omega) = (\tilde{1}, \tilde{0})$ that means $\tilde{M}_{\Delta}(0, \omega) = \tilde{1}, \tilde{Q}_{\Delta}(0, \omega) = \tilde{0}$. Denote by NIIFRI(Γ), (NIIRLI(Γ)), NIIFMI(Γ) the set of all normal interval valued intuitionistic Ω – Fuzzy right (left, lateral) ideals of Γ . Note that NIIFRI(Γ), (NIIRLI(Γ)), NIIFMI(Γ) is a poset under set inclusion.

Theorem 3.1:

Given an interval valued intuitionistic Ω – fuzzy right (left lateral) ideal $\Delta = (\tilde{M}_{\Delta}, \tilde{Q}_{\Delta})$ of a ternary semi-ring Γ . Let $\tilde{M}_{\Delta^+}(\alpha, \omega) = \tilde{M}_{\Delta}(\alpha, \omega) + \tilde{1} - \tilde{M}_{\Delta}(0, \omega)$ and $\tilde{Q}_{\Delta^+}(\alpha, \omega) = \tilde{Q}_{\Delta}(\alpha, \omega) - \tilde{Q}_{\Delta}(0, \omega)$ for all $\alpha \in \Gamma, \omega \in \Omega$. Then $\Delta^+ = (\tilde{M}_{\Delta^+}, \tilde{Q}_{\Delta^+})$ is a normal – interval valued intuitionistic Ω – fuzzy right (left lateral) ideal containing $\Delta = (\tilde{M}_{\Delta}, \tilde{Q}_{\Delta})$ of Γ .

Proof:

For all $\alpha, \beta, \delta \in \Gamma, \omega \in \Omega$

- (i) $\tilde{M}_{\Delta^+}(\alpha + \beta, \omega) = \tilde{M}_{\Delta}(\alpha + \beta, \omega) + \tilde{1} - \tilde{M}_{\Delta}(0, \omega) \geq \min^i\{\tilde{M}_{\Delta}(\alpha, \omega), \tilde{M}_{\Delta}(\beta, \omega)\} + \tilde{1} - \tilde{M}_{\Delta}(0, \omega) = \min^i\{\tilde{M}_{\Delta}(\alpha, \omega) + \tilde{1} - \tilde{M}_{\Delta}(0, \omega), \tilde{M}_{\Delta}(\beta, \omega) + \tilde{1} - \tilde{M}_{\Delta}(0, \omega)\} = \min^i\{\tilde{M}_{\Delta^+}(\alpha, \omega), \tilde{M}_{\Delta^+}(\beta, \omega)\}$





Karthiga and Mohamed Harif

$$(ii) \tilde{M}_{\Delta^+}(\alpha\beta\delta, \omega) = \tilde{M}_{\Delta}(\alpha\beta\delta, \omega) + \tilde{1} - \tilde{M}_{\Delta}(0, \omega) \geq \tilde{M}_{\Delta}(\alpha, \omega) + \tilde{1} - \tilde{M}_{\Delta}(0, \omega) = \tilde{M}_{\Delta^+}(\alpha, \omega)$$

(iii)

$$\tilde{Q}_{\Delta^+}(\alpha + \beta, \omega) = \tilde{Q}_{\Delta}(\alpha + \beta, \omega) - \tilde{Q}_{\Delta}(0, \omega) \leq \max^i\{\tilde{Q}_{\Delta}(\alpha, \omega), \tilde{Q}_{\Delta}(\beta, \omega)\} - \tilde{Q}_{\Delta}(0, \omega) =$$

$$\max^i\{\tilde{Q}_{\Delta}(\alpha, \omega) - \tilde{Q}_{\Delta}(0, \omega), \tilde{Q}_{\Delta}(\beta, \omega) - \tilde{Q}_{\Delta}(0, \omega)\} = \max^i\{\tilde{Q}_{\Delta^+}(\alpha, \omega), \tilde{Q}_{\Delta^+}(\beta, \omega)\}$$

$$(iv) \tilde{Q}_{\Delta^+}(\alpha\beta\delta, \omega) = \tilde{Q}_{\Delta}(\alpha\beta\delta, \omega) - \tilde{Q}_{\Delta}(0, \omega) \leq \tilde{Q}_{\Delta}(\alpha, \omega) - \tilde{Q}_{\Delta}(0, \omega) = \tilde{Q}_{\Delta^+}(\alpha, \omega)$$

Hence Δ^+ is an interval valued intuitionistic Ω – fuzzy ideal of Γ .

Again we have, $\tilde{M}_{\Delta^+}(0, \omega) = \tilde{M}_{\Delta}(0, \omega) + \tilde{1} - \tilde{M}_{\Delta}(0, \omega) = \tilde{1} \leq \tilde{M}_{\Delta^+}(\alpha, \omega) - \tilde{M}_{\Delta^+}(0, \omega) = \tilde{M}_{\Delta^+}(\alpha, \omega)$ and similarly $\tilde{Q}_{\Delta^+}(0, \omega) = \tilde{Q}_{\Delta}(0, \omega) - \tilde{Q}_{\Delta}(0, \omega) = \tilde{0}$

Hence Δ^+ is an interval valued intuitionistic Ω – fuzzy right ideal of Γ and by definition $\Delta \subseteq \Delta^+$ **Corollary 3.3:**

Let Δ and Δ^+ be as in the theorem 3.2. Δ is a normal interval valued intuitionistic Ω – fuzzy right ideal of Γ if and only if $\Delta = \Delta^+$.

Remark 3.4

If $\Delta = (\tilde{M}_{\Delta}, \tilde{Q}_{\Delta})$ is an interval valued intuitionistic Ω – fuzzy right (left lateral) ideal of Γ , then $(\Delta^+)^+ = \Delta^+$. In particular, if Δ is normal, then $(\Delta^+)^+ = \Delta^+ = \Delta$.

Theorem 3.5

Let $\Delta = (\tilde{M}_{\Delta}, \tilde{Q}_{\Delta})$ is an interval valued intuitionistic Ω – fuzzy right (left lateral) ideal of a ternary semi-ring Γ and let $\Psi: D[0,1] \rightarrow D[0,1]$ be an increasing function. Then an IIRFS $\Delta_{\Psi} = ((\tilde{M}_{\Delta})\Psi, (\tilde{Q}_{\Delta})\Psi)$, where $(\tilde{M}_{\Delta})\Psi(\alpha, \omega) = \Psi(\tilde{M}_{\Delta}(\alpha, \omega))$ and $(\tilde{Q}_{\Delta})\Psi(\alpha, \omega) = \Psi(\tilde{Q}_{\Delta}(\alpha, \omega))$ for all $\alpha \in \Gamma, \omega \in \Omega$ is an interval valued intuitionistic Ω – fuzzy right (left, lateral) ideal of Γ . moreover, if $\Psi(\tilde{M}_{\Delta}(0, \omega)) = \tilde{1}$ and $\Psi(\tilde{Q}_{\Delta}(0, \omega)) = \tilde{0}$, then Δ_{Ψ} is normal.

Proof:Let $\alpha, \beta, \delta \in \Gamma, \omega \in \Omega$

$$(i) (\tilde{M}_{\Delta})\Psi(\alpha + \beta, \omega) = \Psi(\tilde{M}_{\Delta}(\alpha + \beta, \omega)) \geq \Psi(\min^i\{\tilde{M}_{\Delta}(\alpha, \omega), \tilde{M}_{\Delta}(\beta, \omega)\}) = \min^i\{\Psi(\tilde{M}_{\Delta}(\alpha, \omega)), \Psi(\tilde{M}_{\Delta}(\beta, \omega))\} = \min^i\{(\tilde{M}_{\Delta})\Psi(\alpha, \omega), (\tilde{M}_{\Delta})\Psi(\beta, \omega)\}$$

$$(ii) (\tilde{M}_{\Delta})\Psi(\alpha\beta\delta, \omega) = \Psi(\tilde{M}_{\Delta}(\alpha\beta\delta, \omega)) \geq \Psi(\tilde{M}_{\Delta}(\alpha, \omega)) = (\tilde{M}_{\Delta})\Psi(\alpha, \omega)$$

$$(iii) (\tilde{Q}_{\Delta})\Psi(\alpha + \beta, \omega) = \Psi(\tilde{Q}_{\Delta}(\alpha + \beta, \omega)) \leq \Psi(\max^i\{\tilde{Q}_{\Delta}(\alpha, \omega), \tilde{Q}_{\Delta}(\beta, \omega)\}) = \max^i\{\Psi(\tilde{Q}_{\Delta}(\alpha, \omega)), \Psi(\tilde{Q}_{\Delta}(\beta, \omega))\} = \max^i\{(\tilde{Q}_{\Delta})\Psi(\alpha, \omega), (\tilde{Q}_{\Delta})\Psi(\beta, \omega)\}$$

$$(iv) (\tilde{Q}_{\Delta})\Psi(\alpha\beta\delta, \omega) = \Psi(\tilde{Q}_{\Delta}(\alpha\beta\delta, \omega)) \leq \Psi(\tilde{Q}_{\Delta}(\alpha, \omega)) = (\tilde{Q}_{\Delta})\Psi(\alpha, \omega)$$

Hence Δ_{Ψ} is an interval valued intuitionistic Ω – fuzzy right ideal of Γ .

If $\Psi(\tilde{M}_{\Delta}(0, \omega)) = \tilde{1}, \Psi(\tilde{Q}_{\Delta}(0, \omega)) = \tilde{0}$ then $(\tilde{M}_{\Delta})\Psi(0, \omega) = \tilde{1}$ and $(\tilde{Q}_{\Delta})\Psi(0, \omega) = \tilde{0}$ and Hence $\Delta_{\Psi} = ((\tilde{M}_{\Delta})\Psi, (\tilde{Q}_{\Delta})\Psi)$ is a normal interval valued intuitionistic Ω – fuzzy right ideal of Γ .

Definition 3.6:

An interval valued intuitionistic Ω – fuzzy right (left lateral) ideal $\Delta = (\tilde{M}_{\Delta}, \tilde{Q}_{\Delta})$ of a ternary semi-ring Γ is said to be an interval valued intuitionistic Ω – fuzzy maximal if it satisfies (i) Δ is non – constant (ii) is a maximal element of NIIRFI(Γ), where NIIRFI(Γ) denote the set of all normal interval valued intuitionistic Ω – fuzzy ideal of Γ .

Example 3.7:

Consider the ternary semiring $\Gamma = \mathbb{Z}^+$ the set of all non-positive integers with the usual addition and ternary multiplication. Let the interval valued Ω – fuzzy subset $\tilde{M}_{\Delta}$ and $\tilde{Q}_{\Delta}$ of Γ be defined as

$$\tilde{M}_{\Delta} = \{\tilde{1}, \text{ if } \alpha \in (-2)\tilde{0}, \text{ otherwise}$$

and

$$\tilde{Q}_{\Delta} = \{\tilde{0}, \text{ if } \alpha \in (-2)\tilde{1}, \text{ otherwise}$$





Karthiga and Mohamed Harif

Then $\Delta = (\tilde{M}_\Delta, \tilde{Q}_\Delta)$ is an interval valued intuitionistic Ω – fuzzy maximal ideal of Γ .

Theorem 3.8:

Let $\Delta = (\tilde{M}_\Delta, \tilde{Q}_\Delta)$ (NIIRFI(Γ)) be non – constant such that it is maximal in the poset of NIIRFI(Γ) under the inclusion then both $\tilde{M}_\Delta$ and $\tilde{Q}_\Delta$ takes only the values $(\tilde{1}, \tilde{0})$ and $(\tilde{0}, \tilde{1})$ respectively.

Proof:

Since Δ is normal interval valued intuitionistic Ω – fuzzy right ideal, so $\Delta(0, \omega) = (\tilde{1}, \tilde{0})$ and let $\alpha_0 (\neq 0) \in \Gamma$ be arbitrary with $\tilde{M}_\Delta(\alpha_0, \omega) \neq \tilde{1}$.

We claim that $\tilde{M}_\Delta(\alpha_0, \omega) = \tilde{0}$. If not then there exists an element $\gamma \in \Gamma$ such that $0 < \tilde{M}_\Delta(\gamma, \omega) < \tilde{1}$. Let $\Delta_\gamma = (\tilde{\lambda}_\Delta, \tilde{\mu}_\Delta)$ be an interval valued intuitionistic Ω – fuzzy subset of Γ defined by $\tilde{\lambda}_\Delta(\alpha, \omega) = \frac{1}{2}[\tilde{M}_\Delta(\alpha, \omega) + \tilde{M}_\Delta(\gamma, \omega)]$ and $\tilde{\mu}_\Delta(\alpha, \omega) = \frac{1}{2}[\tilde{Q}_\Delta(\alpha, \omega) + \tilde{Q}_\Delta(\gamma, \omega)]$.

Clearly Δ_γ is well defined now, $\tilde{\lambda}_\Delta(0, \omega) = \frac{1}{2}[\tilde{M}_\Delta(0, \omega) + \tilde{M}_\Delta(\gamma, \omega)] \geq \frac{1}{2}[\tilde{M}_\Delta(\alpha, \omega) + \tilde{M}_\Delta(\alpha, \omega)] = \tilde{\lambda}_\Delta(0, \omega) + \tilde{\mu}_\Delta(\alpha, \omega) = \frac{1}{2}[\tilde{Q}_\Delta(0, \omega) + \tilde{Q}_\Delta(0, \omega)] \leq \frac{1}{2}[\tilde{Q}_\Delta(\alpha, \omega) + \tilde{Q}_\Delta(\alpha, \omega)] = \tilde{\mu}_\Delta(\alpha, \omega)$ for $\alpha \in \Gamma$.

Again $\alpha, \beta, \delta \in \Gamma, \omega \in \Omega$

$$(i) \quad \tilde{\lambda}_\Delta(\alpha + \beta, \omega) = \frac{1}{2}[\tilde{M}_\Delta(\alpha + \beta, \omega) + \tilde{M}_\Delta(\gamma, \omega)] \geq \frac{1}{2}[\min^i\{\tilde{M}_\Delta(\alpha, \omega), \tilde{M}_\Delta(\beta, \omega)\} + \tilde{M}_\Delta(\gamma, \omega)] = \min^i\{\frac{1}{2}[\tilde{M}_\Delta(\alpha, \omega) + \tilde{M}_\Delta(\gamma, \omega)], \frac{1}{2}[\tilde{M}_\Delta(\beta, \omega) + \tilde{M}_\Delta(\gamma, \omega)]\} = \min^i\{\tilde{\lambda}_\Delta(\alpha, \omega), \tilde{\lambda}_\Delta(\beta, \omega)\}$$

$$(ii) \quad \tilde{\lambda}_\Delta(\alpha\beta\delta, \omega) = \frac{1}{2}[\tilde{M}_\Delta(\alpha\beta\delta, \omega) + \tilde{M}_\Delta(\gamma, \omega)] \geq \frac{1}{2}[\{\tilde{M}_\Delta(\alpha, \omega) + \tilde{M}_\Delta(\gamma, \omega)\}] = \tilde{\lambda}_\Delta(\alpha, \omega)$$

$$(iii) \quad \tilde{\mu}_\Delta(\alpha + \beta, \omega) = \frac{1}{2}[\tilde{Q}_\Delta(\alpha + \beta, \omega) + \tilde{Q}_\Delta(\gamma, \omega)] \leq \frac{1}{2}[\max^i\{\tilde{Q}_\Delta(\alpha, \omega), \tilde{Q}_\Delta(\beta, \omega)\} + \tilde{Q}_\Delta(\gamma, \omega)] = \max^i\{\frac{1}{2}[\tilde{Q}_\Delta(\alpha, \omega) + \tilde{Q}_\Delta(\gamma, \omega)], \frac{1}{2}[\tilde{Q}_\Delta(\beta, \omega) + \tilde{Q}_\Delta(\gamma, \omega)]\} = \max^i\{\tilde{\mu}_\Delta(\alpha, \omega), \tilde{\mu}_\Delta(\beta, \omega)\}$$

$$(iv) \quad \tilde{\mu}_\Delta(\alpha\beta\delta, \omega) = \frac{1}{2}[\tilde{Q}_\Delta(\alpha\beta\delta, \omega) + \tilde{Q}_\Delta(\gamma, \omega)] \leq \frac{1}{2}[\{\tilde{Q}_\Delta(\alpha, \omega) + \tilde{Q}_\Delta(\gamma, \omega)\}] = \tilde{\mu}_\Delta(\alpha, \omega)$$

Hence Δ is an interval valued intuitionistic Ω – fuzzy ideal of Γ .

Define $\Delta_c^+ = (\tilde{\lambda}_{\Delta^+}, \tilde{\mu}_{\Delta^+})$ then by theorem 3.2, Δ_c^+ is a normal interval valued intuitionistic Ω – fuzzy right ideal of Γ ,

where $\tilde{\lambda}_{\Delta^+}(\alpha, \omega) = \tilde{\lambda}_{\Delta^+}(\alpha, \omega) + \tilde{1} - \tilde{\lambda}_{\Delta^+}(0, \omega) = \frac{1}{2}[\tilde{M}_\Delta(\alpha, \omega) + \tilde{M}_\Delta(\gamma, \omega)] + \tilde{1} - \frac{1}{2}[\tilde{M}_\Delta(0, \omega) + \tilde{M}_\Delta(0, \omega)] = \frac{1}{2}[\tilde{1} + \tilde{M}_\Delta(\alpha, \omega)]$

and $\tilde{\mu}_{\Delta^+}(\alpha, \omega) = \tilde{\mu}_{\Delta^+}(\alpha, \omega) - \tilde{\mu}_{\Delta^+}(0, \omega) = \frac{1}{2}[\tilde{Q}_\Delta(\alpha, \omega) + \tilde{Q}_\Delta(\gamma, \omega)] - \frac{1}{2}[\tilde{Q}_\Delta(0, \omega) + \tilde{Q}_\Delta(0, \omega)] = \frac{1}{2}[\tilde{Q}_\Delta(\alpha, \omega)]$

Clearly $\Delta \subseteq \Delta_c^+$. Since $\tilde{\lambda}_{\Delta^+}(\alpha, \omega) = \frac{1}{2}[\tilde{1} + \tilde{M}_\Delta(\alpha, \omega)] > \tilde{M}_\Delta(\alpha, \omega)$ and $\tilde{\mu}_{\Delta^+}(0, \omega) = \frac{1}{2}[\tilde{Q}_\Delta(0, \omega)] < \tilde{Q}_\Delta(0, \omega)$, Δ is a proper subset of Δ_c^+ . Again since $\tilde{\lambda}_{\Delta^+}(\gamma, \omega) = \frac{1}{2}[\tilde{1} + \tilde{M}_\Delta(\gamma, \omega)] < \tilde{1} = \tilde{M}_\Delta(0, \omega)$. Hence Δ_c^+ is non constant and Δ is not a maximal element of NIIRFI(Γ). This is contradiction. Therefore $\tilde{M}_\Delta$ takes only two values $\tilde{1}$ and $\tilde{0}$ and $\tilde{Q}_\Delta$ takes only two values $\tilde{0}$ and $\tilde{1}$. This complete the proofs.

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Karthiga and Mohamed Harif

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RESEARCH ARTICLE

Supervisor -Supervisee Interaction in Field Work Practicum: Perspectives of Master of Social Work Students in Tamil Nadu

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ABSTRACT

This research analyzes the dynamic communications between a supervisor and a supervisee during the fieldwork practicum of Master of Social Work students from Chennai, Tamil Nadu. Its core objectives are to analyze the students' perspectives of these interactions and their influence on the learning process. The research particularly focuses on how each student's unique background affects their approach toward instruction while aiming to enhance the overall experience for learners, placing stronger focus on adaptive strategies aimed at different levels, thus broadening the instructional design enhancing future social work practitioners throughout Tamil Nadu. The study employs Independent sample 't' statistical test of survey with 144 students to understand their perspectives towards supervision. The results reveal that constructive supervision significantly enhances independent analysis and professional development. The information was obtained through questionnaires which demonstrated both positive and negative aspects of the supervisory roles. Students also reported that supervision can be improved through collaboration between the institution and the agency. The information was obtained through questionnaires which demonstrated both positive and negative aspects of the supervisory roles. Crystal clear observation from the result is how quality supervision impacts students' Professional skill development. A revised fieldwork supervision framework will enhance the standard of supervision in future.

Keywords: Social Work, Field Work Practicum, supervision, supervisor-supervisee interaction, master of social work students.



**Sharnitha Shanmugam and Sheeja Remani B. Karalam**

INTRODUCTION

Background of the study:

Field education is often perceived as the most important part of social work training, since it allows students to merge theoretical learning from classes with hands-on experience. A fieldwork placement comes with support in the form of supervision, which ensures that the proper frameworks are provided to help this amalgamation happen. Thus, the supervisor-supervisee relationship becomes critical toward illuminating and shaping the student's learning journey, professional identity, and self-efficacy within myriad social work settings (Bogo, 2015). Supervision fulfills a social work practice supervision of three core functions: administrative function; educational function; and supportive function (Kadushin & Harkness, 2014). Here the administrative function provides knowledge of accountability and compliance with organizational guidelines including requisite policies from government bodies dedicated to industry welfare. Focusing on their teaching goals, the educational function deals with application and integration of theory into actual practice. Considerations around looking after the well-being of junior staff members falls under The Supportive Function. Attaining proper outcomes demands that all these roles be balanced by supervisors who must also remain adjustable to the developmental stages of their supervisees (Tsui, 2005).

Quality of Supervisory Relationship:

The effectiveness of fieldwork education is significantly influenced by the quality of the supervisory relationship (Saju, 2014). A strong, positive, and collaborative alliance between supervisor and supervisee, characterized by open communication, trust, empathy, and constructive feedback, creates a secure and supportive learning environment (Zuchowski, 2015). This environment is crucial for fostering critical thinking, promoting self-awareness, and encouraging reflective practice among students (Sandile, Ntombifikile, Mbongeni, 2024). Conversely, a lack of engagement, inconsistent feedback, or an overly authoritative supervisory style can lead to student dissatisfaction, diminished learning motivation, and impede professional development (Bennett & Deal, 2009).

Reflective Practice and Professional Identity:

Reflective practice is a cornerstone of social work education, and supervision serves as a primary vehicle for cultivating this essential competency. (Schön, 1983) says, supervisors who foster reflection enable students to critically analyze their actions, evaluate the impact of their interventions, and develop a nuanced understanding of client needs and social work values. According to (Knight, 2001), this reflective engagement is vital for professional identity formation, helping students learn from experience and grow into capable and ethical practitioners.

Cultural and Contextual Considerations:

Much of the literature on supervision has emerged from Western contexts, which may not fully capture the unique challenges and cultural dynamics present in countries like India. In the Indian context, hierarchical structures in education and organizational settings can influence supervisory dynamics, sometimes creating power imbalances that hinder open dialogue and student agency (Rai, 2012). Additionally, resource limitations, inconsistent supervisor training, and lack of institutional support can further complicate the supervision process in fieldwork settings (Desai, 2006).

Studies from the Indian Context:

While Indian literature on fieldwork supervision is relatively sparse, some studies have begun to highlight region-specific issues. Research by (Gokhale, 2014) and (Samuel, 2018) suggests that many MSW students in India face challenges such as limited engagement from supervisors, inadequate feedback, and a lack of structured reflection sessions. These studies emphasize the need for supervisor training, standardized supervision protocols, and greater institutional oversight to ensure quality field education. Moreover, the diversity of student backgrounds—linguistic, socio-economic, and educational—necessitates a more tailored, student-centered approach to supervision.



**Sharnitha Shanmugam and Sheeja Remani B. Karalam****Need for Student-Centered Perspective:**

Despite these emerging insights, there remains a critical gap in understanding how students themselves perceive and experience the supervisor-supervisee relationship. While supervisors' perspectives are occasionally documented, students' voices are often underrepresented in scholarly discourse (Bennett, 2009). This imbalance restricts the development of supervision practices that are responsive to actual student needs. Listening to students' experiences offers valuable insights into the effectiveness of supervision, highlights areas for improvement, and strengthens the pedagogical integrity of social work field education (Wayne, Bogo, & Raskin, 2010).

Aim and Objective of the Study:

The main aim of the study is to examine the perspective of students on supervisor-supervisee interaction in fieldwork practicum among Master of Social Work students in Social Work Education offered by colleges in Chennai City of Tamil Nadu state

The objectives of the study are

- To describe the socio-demographic characteristics and field work practicum among master of Social Work students.
- To measure the perception of students on supervisor-supervisee interaction in fieldwork practicum among Master of Social Work students
- To identify factors that contribute to effective supervision and offer insights to enhance the supervisory process in social work education.

METHODS AND MATERIALS

The study employs a descriptive quantitative design to collect data from social work students regarding their perspectives on supervisor-supervisee interaction in fieldwork practicum .

Participants:

6 Institutions offering social work from Chennai were selected 144 social work students were recruited using simple random sampling using the lottery method. The majority of the students were aged between 21-25 years (96.5%). Less than half of the students (20.8%) have Bachelor's in social work as qualification. More than half of the Social work students were females (55.6%). The majority of agency supervisors had experience of 0-10 years (81.9%) students were females (55.6%). Nearly half of the social work students have chosen Human Resource as their specialization.

Measurements:**Sociodemographic questionnaire:**

The sociodemographic questionnaire consisted of 19 questions for gathering information about the participant's background and demographics. Questions were related to personal characteristics such as gender, age, marital status, place of residence, educational qualification, specialization in the course, type of department, experience of both agency and faculty supervisor.

Questionnaire on Supervisor-Doctoral Student Interaction (QSDI):

The standardized scale used was "Questionnaire on Supervisor-Doctoral Student Interaction (QSDI)" by Mainhard et al. (n.d) in the Netherlands, with 41 items. The items are designed based on Likert's five-point scale (never, rarely, sometimes, mostly, and always), and the score of each item ranges from 1 to 5. The questionnaire has eight subscales which are, Helping/Friendly, Admonishing, Uncertain, Strict, Understanding, Dissatisfied, Leadership, Responsibility and tested Cronbach's alpha.



**Sharnitha Shanmugam and Sheeja Remani B. Karalam****Procedure:**

The study was approved by all the institutional heads before data collection. Informed consent was obtained from every participant, and the questionnaire was distributed to the students, which took approximately 10 min to complete,

Data analysis:

Data analysis involves descriptive statistics, *t*-tests conducted through SPSS software.

RESULTS

The demographic data shows that the vast majority of respondents were young adults aged 21–25 (96.5%), with only a small number (3.5%) in the 26–30 age group. A slightly higher proportion of students were female (55.6%) compared to male (44.4%). Most participants were unmarried (94.4%), and a majority resided in urban areas (68.1%), followed by rural (21.5%) and semi-urban (10.4%) locations, reflecting a largely city-based student population with some rural representation. In terms of academic background, the largest group held an undergraduate degree in Arts (66.7%), while 20.8% had prior training in social work through a Bachelor of Social Work (B.S.W), and 12.5% came from science disciplines. According to the results students identified diverse learning outcomes from their fieldwork. In terms of academic background, the largest group held an undergraduate degree in Arts (66.7 percent), while (20.8 percent) had prior training in social work through a Bachelor of Social Work (B.S.W), and (12.5 percent) came from science disciplines (Table 1). This indicates a diverse academic foundation, though predominantly rooted in the humanities. Most common was the development of professional competency skills (26.4 percent), followed by specialization-based learning and personal skill development (20.1 percent) each. Fewer students reported gains in practical skills (12.5 percent), ethical behavior (8.3 percent), and administrative competencies (6.9 percent). Notably, only (5.6 percent) emphasized active field participation, suggesting limited hands-on engagement for some. Less than half of the students learn professional competency skills through field work (Table 2) regarding the students' perception of supervision improvement. The data also reveals that (55.6 percent) of students were supervised by professionals with a formal social work background, while (44.4 percent) were guided by individuals from non-social work disciplines, particularly within field agencies. This discrepancy suggests a lack of uniformity in professional alignment, which may impact the relevance and quality of field instruction (Table 3). More than half (55.6 percent) of supervisors are trained social workers while there can be considerable concerns over the qualification of the remaining (44.4 percent). Considerable (20.8 percent) of the students reported that no changes are needed in supervision, followed by meager (16.7 percent) of the students who reported that supervision can be improved through collaboration between the institution and the agency (Table 4). While analyzing the satisfaction in fieldwork supervision and satisfaction in learning, it was noticed that more than nearly three-quarters of the students (75%) were either dissatisfied or very dissatisfied with the quality of fieldwork supervision (Table 5). While analyzing the students' perception of various dimensions of faculty and agency, supervisor-supervisee interaction, it was observed that the majority of the students reported that the faculty and agency supervisor displays a medium level of helping/friendly, admonishing, uncertain, strict, understanding, and dissatisfied. The students also expressed that the majority of the faculty and agency supervisors fall in line with a low level of leadership and students' responsibility/freedom (Table 6). Conversely, lack of engagement, rigid hierarchies, and inadequate support were seen to hinder the learning process and cause dissatisfaction among students.

DISCUSSION

The data highlights significant issues with fieldwork supervision, despite moderately positive learning experiences. There's a clear disconnect between institutional expectations and supervision quality, with limited perceived support or empowerment. Improvements are needed in areas such as Supervisor training and competency, Field-agency collaboration, Student-centered approaches in supervision. There is also a significant gap in supervisory support, which may affect students' motivation, skill development, and integration of classroom knowledge into practice.



**Sharnitha Shanmugam and Sheeja Remani B. Karalam**

While supervision is largely being provided by trained individuals, there are variations in both background and depth of experience—especially within agencies. Enhancing supervisory training, particularly for non-social work professionals and less experienced supervisors, could greatly improve the coherence and impact of field education. The findings provide a strong basis for recommending structured training for supervisors, better placement planning, and robust monitoring mechanisms.

RECOMMENDATIONS

- Fieldwork curriculum review might further highlight supervisor-supervisee relations focusing on reflective practice, ethics literacy, and moral performance capability within professional boundaries.
- Ensuring alignment between students' specializations and their associated fieldwork assignments enhances purposeful learning. Integration of understudied areas such as documentation systems, administrative processes, collaboration across disciplines, and interprofessional teamwork may be included.
- Advocacy concerning policy inclusion regarding the agency supervisory position's minimum credentials and qualifying criteria may prove beneficial.
- There is room for improvement related to developing a set of expectations describing assigned tasks articulated within a policy framework pertaining specifically to the role of field supervisors. Periodic assessment of students out on rotations could foster responsibility alongside quality control.
- Joint review meetings could be conducted to merge theoretical academic frameworks with practical field-based realities for aligned supervision and coherent learning outcomes.
- Holding regular capacity-building workshops would be useful for both agency and faculty supervisors in the areas of mentorship, ethics, student engagement, and feedback systems. It might also be beneficial to create an orientation or certification module tailored for agency supervisors who lack formal social work training.
- Redesigning the position of supervisors granting pedagogical status with respect to teaching 'Supervision' by providing scheduled time allowances along with honorariums and academic credit may attract committed practitioners willing to supervise them regularly.
- The model of triadic supervision, integrating student, field supervisor, and faculty supervisor, may assist in narrowing the integration gap between theoretical and practical work by fulfilling academic requirements more holistically.
- Encouraging personalized approaches to supervision that take into account students' learning preferences, past experiences, emotional needs for support into their prior trajectories may prove beneficial as well. Also suggested are peer-support groups or reflective circles aimed at exchange of experiences to reduce social isolation and mitigate dissatisfaction.

CONCLUSION

There are few challenges found in the Social work field work supervision in Indian context particularly. But the extant research strongly avowed the value of quality supervision. Communication, empathy, timely feedback, and reflective dialogue substantially enhances students' learning, confidence, and professional identity formation through strong and competent supervision. The finding also draws attention to developing a supervision model that can be even more standardized, student centered and culturally sensitive within Social work education in India. As relevant studies also suggest professional supervisor-supervisee interaction paves way in quality and competent fieldwork practicum model for Master in Social Work students in Tamil Nadu. The research paper also suggests that more future studies to better understand supervision and how it can be improved to develop a better professionally competent social worker can be conducted among a large population. Strengthening the supervisory framework through institutional support, capacity-building for supervisors, and feedback mechanisms can lead to a more enriching and transformative educational experience for future social workers.





Sharnitha Shanmugam and Sheeja Remani B. Karalam

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Ethical approval and informed consent statement

Ethical approval from all the institution heads and Informed consent was obtained from all participants prior to data collection. Participants were assured of confidentiality, anonymity, and their right to withdraw at any stage without any consequences.

Declaration of conflicting interest:

The author declares that there are no conflicts of interest with respect to the authorship and/or publication of this study.

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Sharnitha Shanmugam and Sheeja Remani B. Karalam

Table 1. Distribution of the respondents in terms of age, gender, marital status, place of residence, and qualification in undergraduate studies

S.No	Variables	Category	n:144	%
1	Age	21-25 years old	139	96.5
		26-30 years old	5	3.5
2	Gender	Male	64	44.4
		Female	80	55.6
3	Marital status	Married	8	5.6
		Unmarried	136	94.4
4	Place of residence	Urban	98	68.1
		Semi-urban	15	10.4
		Rural	31	21.5
5	UG Qualification	Arts	96	66.7
		Science	18	12.5
		Arts in B.S.W	30	20.8

Table 2. Distribution of the respondents in terms of their status in the department, specialization, and learning in field work.

S.No	Variables	Category	n:144	%
1	Department	Postgraduate	53	36.8
		Postgraduate and Research	91	63.2
2	Specialization	Human Resource	71	49.3
		Medical and Psychiatric	32	22.2
		Community Development	41	28.5
3	FW Learning	Professional Competency Skills	38	26.4
		Specialization-based learning	29	20.1
		Participation in the field	8	5.6
		Professional practical skills	18	12.5
		Ethical and Professional Behaviour	12	8.3
		Documentation & administrative skills	10	6.9
		Personal Skill	29	20.1

Table 3. Distribution of the respondents in terms of their qualification as agency supervisors, experience of agency supervisors, and experience of faculty supervisors.

S.No	Variables	Category	n:144	%
1	Agency supervisor	Social Workers	80	55.6
		Non-Social Worker	64	44.4
2	Experience Agency Supervisor	0	9	6.3
		1-10 years	118	81.9
		10-20 years	12	8.3
		20 years and above	5	3.5
3	Experience Faculty Supervision	0	7	4.9
		1-10 years	97	67.4
		10-20 years	33	22.9
		20 years and above	7	4.9





Sharnitha Shanmugam and Sheeja Remani B. Karalam

Table 4. Distribution of the respondents in terms of their supervision improvement

SN	Variables	Category	n:144	%
1	Supervision	Guidance and Mentorship	16	11.1
		Communication and Feedback	12	8.3
		Practical Exposure and Skill Development	17	11.8
		Supervisor competency	17	11.8
		Collaboration between the institution and the agency	24	16.7
		Evaluation and Feedback	11	7.6
		Student Support and Personalization	17	11.8
		No Changes Needed	30	20.8

Table 5. Distribution of the respondents in terms of satisfaction with fieldwork supervision.

S.No	Variables	Category	n:144	%
1	Satisfaction in FW supervision	Very satisfied	3	2.1
		Satisfied	8	5.6
		Neither satisfied nor dissatisfied	25	17.4
		Dissatisfied	55	38.2
		Very Dissatisfied	53	36.8
2	Satisfaction in Learning	Very Dissatisfied	5	3.5
		Dissatisfied	5	3.5
		Neither satisfied nor dissatisfied	26	18.1
		Satisfied	70	48.6
		Very satisfied	38	26.4

Table 6. Distribution of the respondents in terms of their students' perception of the various dimensions of faculty and agency supervisor-supervisee interaction

S.N		Faculty S-S			Agency S-S		
		Low	Medium	High	Low	Medium	High
1	Helping/Friendly	40 (27.8%)	70 (48.6%)	34 (23.6%)	48 (33.3%)	66 (45.8%)	30 (20.8%)
2	Admonishing	48 (33.3%)	64 (44.4%)	32 (22.2%)	42 (29.2%)	67 (46.5%)	35 (24.3%)
3	Uncertain	37 (25.7%)	79 (54.9%)	28 (19.4%)	37 (25.7%)	72 (50.0%)	35 (24.3%)
4	Strict	44 (30.6%)	77 (53.5)	23 (16.0%)	37 (25.7%)	72 (50.0%)	35 (24.3%)
5	Understanding	46 (31.9%)	74 (51.7%)	20 (13.9%)	46 (31.9%)	66 (45.8%)	32 (22.2%)
6	Dissatisfied	47 (32.6%)	71 (49.3%)	26 (18.1%)	44 (30.6%)	74 (51.4%)	26 (18.1%)
7	Leadership	61 (42.4%)	57 (39.6%)	26 (18.1%)	36 (25.0%)	77 (53.5%)	31 (21.5%)
8	Responsibility	43 (29.9%)	71 (49.3%)	30 (20.8%)	52 (32.1%)	49 (47.9%)	23 (16.0%)

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RESEARCH ARTICLE

Effect of an Integrated Proprioceptive Neuromuscular Facilitation Program on Pain, Functional Ability, Range of Motion and Quality of Life in Patients with Knee Osteoarthritis: A Randomized Controlled Trial Protocol

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ABSTRACT

Osteoarthritis is one of the most common musculoskeletal disorders; among this, knee osteoarthritis is the most common form. The osteoarthritis causes various anatomical and physiological changes in joint tissues, such as cartilage degradation, synovial inflammation, subchondral bone involvement and the development of osteophytes. Osteoarthritis is recognized as the most common joint disorder, and from 1990 to 2019, the global prevalence of KOA increased by 48%. The study aims to evaluate the effects of an integrated Proprioceptive Neuromuscular Facilitation (PNF) program on pain, functional ability and quality of life in patients with knee osteoarthritis. Two-group pre-test- post-test RCT Patients will be recruited based on selection criteria. Patients will be recruited and randomly allocated into either the experimental group or the control group. The experimental group will receive an integrated PNF program, while the control group will receive conventional physiotherapy management for 4 weeks (3 sessions/week). Outcome measures including The Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), the Visual Analogue Scale (VAS), Universal Goniometer and the 36-Item Short Form Health Survey (SF-36), will be assessed at baseline and after 4 weeks of intervention. Data will



**Gourab Deb and Sunita Sharma**

be analysed using the Statistical Package for Social Sciences (SPSS) software. Shapiro-Wilk test will be used to establish the normality of the data. If data is found to be normally distributed it will be presented as Mean $\pm$ SD and paired t-test will be used for within the group analysis and unpaired t-test will be used for between the group analysis. If data is found to be not normally distributed it will be presented as Median (Interquartile range) and Wilcoxon rank test will be used for within the group analysis and Mann Whitney U test will be used for between the group analysis.

Keywords: Proprioceptive Neuromuscular Facilitation; Knee Osteoarthritis; WOMAC; Rehabilitation; Quality of Life.

INTRODUCTION

Knee osteoarthritis (KOA) is one of the most common chronic degenerative musculoskeletal disorders affecting older people worldwide.[1] It is characterized by progressive degeneration of articular cartilage, sub-chondral bone remodeling, osteophyte formation, joint space narrowing, and synovial inflammation, all of which may cause pain, stiffness, reduced range of motion, and functional limitation.[2] The Global Burden of Disease study, the prevalence of knee osteoarthritis has increased significantly over recent decades due to population ageing, obesity, sedentary lifestyle, and increasing life expectancy.[3] In 2020 approximately 654 million individuals worldwide were reported to be affected by knee osteoarthritis, making it a major contributor to disability and reduced quality of life among older adults.[4] In addition to structural joint degeneration, patients with KOA frequently experience impaired balance, reduced muscle strength, proprioceptive deficits, and altered movement patterns that interfere with activities of daily living such as walking, stair climbing, and sit-to-stand activities.[5] Conventional physiotherapy management for knee osteoarthritis commonly includes thermotherapy, stretching exercises, strengthening exercises, aerobic conditioning, and functional rehabilitation programs aim to reduce pain and improve mobility of the joint.[5,6] Although these interventions are effective in providing symptomatic relief, persistent deficits in proprioception, neuromuscular control, flexibility, and dynamic joint stability may continue to affect physical function and quality of life in many individuals with KOA.[5] Therefore, rehabilitation approaches that simultaneously address flexibility, neuromuscular activation, proprioception, and coordinated movement patterns may give significant clinical benefits in individuals with knee osteoarthritis.

Proprioceptive Neuromuscular Facilitation (PNF) is an advanced therapeutic approach developed to improve neuromuscular function through specific movement patterns and facilitation techniques.[7,8] PNF techniques are widely used in musculoskeletal rehabilitation to enhance flexibility, muscular coordination, proprioception, joint stability, and functional performance.[7] Previous studies have demonstrated that PNF-based interventions can improve pain, balance, range of motion, and physical function in individuals with knee osteoarthritis.[4,5,9,10] PNF stretching techniques are considered particularly effective because they facilitate both muscular relaxation and neuromuscular activation, thereby improving movement efficiency and joint stability.[8] Among various PNF techniques, the Hold-Relax technique is commonly used to reduce muscle tightness and improve flexibility using autogenic inhibition and muscle relaxation mechanisms.[7] This technique consists of an isometric contraction of the target muscle followed by passive stretching, which increases the muscular extensibility and improves joint mobility.[11] Hold-Relax stretching has been shown to improve hamstring flexibility, pain relief, and functional mobility in patients with knee osteoarthritis.[10,12] Hamstring tightness contributes to altered knee biomechanics and increased joint stress, improving muscle flexibility may help to reduce pain and improve functional performance in people with KOA.[1] Another important PNF technique is Combination of Isotonics, which consists of concentric, eccentric, and isometric muscle contractions performed sequentially without rest.[13] This technique is designed to improve muscle activation, neuromuscular coordination, dynamic stability, and controlled movement patterns.[13,14] Combination of Isotonics may help improve functional movement and joint stability by allowing coordinated muscular activity around the knee joint. Previous studies have shown that PNF-based strengthening and



**Gourab Deb and Sunita Sharma**

neuromuscular facilitation approaches can improve muscular performance, pain, and physical function in individuals with musculoskeletal conditions.[14,15]

Recent research and systematic reviews indicate that proprioceptive and PNF-based interventions may improve pain, physical function, and proprioception in patients with knee osteoarthritis.[4,10,17,18] However, most previous studies have evaluated individual PNF techniques rather than an integrated approach. Hold–Relax stretching primarily improves muscle flexibility and reduces pain by decreasing muscle tightness through autogenic inhibition mechanisms.[7,10,11] whereas Combination of Isotonics enhances neuromuscular coordination, muscle activation, and dynamic joint stability through sequential concentric, eccentric, and isometric muscle contractions.[13–15] Since knee osteoarthritis is characterized by multiple impairments, including pain, muscle tightness, reduced flexibility, muscle weakness, impaired proprioception, and altered movement patterns.[1,4,5] combining these complementary PNF techniques may provide greater overall functional benefits than either technique alone. Although previous studies have investigated individual PNF techniques in patients with knee osteoarthritis, limited evidence is available regarding the integration of Hold–Relax and Combination of Isotonics within a single rehabilitation program. This study was therefore designed to evaluate the effectiveness of this integrated approach. Therefore, the present study aims to evaluate the effects of an integrated Proprioceptive Neuromuscular Facilitation program on pain, functional ability, knee joint range of motion, and quality of life in patients with knee osteoarthritis.

Hypothesis

Null Hypothesis (H_0): There will be no significant difference between the integrated PNF program and conventional physiotherapy management in patients with knee osteoarthritis.

Alternative Hypothesis (H_1): The integrated PNF program will produce significant improvement in pain, functional ability, knee joint range of motion, and quality of life compared to conventional physiotherapy management in patients with knee osteoarthritis.

MATERIALS AND METHODS

Study design

This study is designed as a two-group randomized controlled trial with a pre-test and post-test design to evaluate the effects of an integrated Proprioceptive Neuromuscular Facilitation (PNF) program in patients with knee osteoarthritis.

Sample size

The sample size was estimated a priori using G*Power software (version 3.1) for an independent-samples t-test. The Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) was selected as the primary outcome measure. The standardized effect size was calculated from the published post-intervention WOMAC total score means, standard deviations, and sample sizes reported by [9] using the conventional pooled standard deviation method for two independent groups. Assuming a two-tailed significance level (α) of 0.05 and a statistical power of 80% ($1-\beta = 0.80$), the minimum required sample size was estimated to be 24 participants (12 participants per group). To account for an anticipated attrition rate of approximately 20%, the target sample size was increased to 30 participants (15 participants per group).

Ethical considerations

The study has received approval from the Institutional Ethics Committee (IEC) under approval number IEC-3089. The trial has been prospectively registered in the Clinical Trials Registry of India (CTRI) with registration number CTRI/2025/12/099665. All study procedures will be conducted in strict accordance with the National Ethical Guidelines for Biomedical and Health Research Involving Human Participants issued by the Indian Council of Medical Research (ICMR), the principles of the Declaration of Helsinki (World Medical Association), and the International Ethical Guidelines for Health-Related Research Involving Humans (Council for International



**Gourab Deb and Sunita Sharma**

Organizations of Medical Sciences, 2017). Written informed consent will be obtained from all participants prior to enrolment, and confidentiality of participant data will be maintained throughout the study.

Selection criteria**Inclusion Criteria:**

- Participants aged 40 to 70 years
- Both male and female participants
- Patients diagnosed with knee osteoarthritis (Grade I–III) according to the Kellgren–Lawrence classification
- Presence of osteoarthritis in one or both knees, confirmed by radiographic X-ray and clinical evaluation by an orthopedic specialist
- Willingness to participate and provide written informed consent

Exclusion Criteria:

- Presence of other knee joint pathologies such as chondromalacia patellae or plica syndrome
- History of post-traumatic knee conditions
- Previous surgical intervention on the affected knee (e.g., joint replacement or repair)
- Presence of intra-articular contractures or fixed deformities limiting knee range of motion
- Any neurological or systemic condition affecting lower limb function.

Procedures**Recruitment and allocation:**

The study will be conducted in accordance with the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) guidelines. Participant recruitment will be carried out using convenience sampling from the outpatient physiotherapy department. Eligible participants will be informed about the study procedures, and written informed consent will be obtained prior to enrolment. Following baseline assessment, eligible participants will undergo randomization according to the procedure described in Section 2.5.2. Outcome assessments will be performed at baseline and after completion of the 4-week intervention period. The trial will be reported in accordance with the Consolidated Standards of Reporting Trials (CONSORT) guidelines for non-pharmacological interventions, and the intervention description will follow the Template for Intervention Description and Replication (TIDieR) checklist. A CONSORT flow diagram illustrating participant recruitment, allocation, follow-up, and analysis will be included.

Randomization and blinding

Following baseline assessment, participants will be randomly assigned to either the experimental group (EG) or the control group (CG) in a 1:1 allocation ratio. A computer-generated block randomization sequence with a fixed block size of four will be created by an independent researcher who is not involved in participant recruitment, intervention delivery, or outcome assessment to ensure balanced group allocation throughout the study. Allocation concealment will be achieved using sequentially numbered, opaque, sealed envelopes (SNOSE). Each envelope will contain the group assignment and will be opened sequentially only after completion of the baseline assessment. Due to the nature of the intervention, blinding of participants and treating physiotherapists will not be feasible. However, outcome assessments will be performed by an independent assessor blinded to group allocation to minimize assessment bias.

Treatment Fidelity

To ensure treatment fidelity, all intervention sessions will be delivered by the same trained physiotherapist using a standardized intervention protocol. The treatment procedures, dosage, exercise progression, and session duration will be consistent for all participants. Regular supervision by the research supervisor will be conducted to ensure adherence to the study protocol and maintain consistency throughout the intervention period.



**Participant Adherence and Management of Missed Treatment Sessions**

Participant adherence will be monitored by maintaining an attendance record for each treatment session. Participants will be encouraged to attend all scheduled sessions throughout the 4-week intervention period. If a participant misses a treatment session, an attempt will be made to reschedule the session within the same week whenever feasible. Participants who miss two consecutive sessions will be contacted by telephone to encourage continued participation and to identify any barriers to attendance. Reasons for missed sessions, withdrawal, or discontinuation will be documented and reported in the study flow diagram.

Intervention

Participants will be divided into two groups: group A and group B. Group A will be treated with Integrated techniques and Group B will be treated with **Stretching** and **Strengthening Exercises**. The treatment consists of applied over four weeks by a trained physiotherapist. Sessions are conducted three times per week, each treatment session will last approximately 40-50 minutes, for a total of 12 sessions. A 4-week intervention period was selected because previous randomized controlled trials evaluating proprioceptive neuromuscular facilitation in individuals with knee osteoarthritis have demonstrated significant improvements in pain, physical function, and mobility following four weeks of treatment.[4,5,10] In addition, a 4-week intervention is feasible in routine outpatient physiotherapy practice and may help maintain participant adherence while minimizing dropout.

Experimental Group (Integrated PNF Program)

The experimental group will receive an integrated Proprioceptive Neuromuscular Facilitation (PNF) program consisting of moist heat application, Hold–Relax stretching, and Combination of Isotonics techniques targeting flexibility, neuromuscular control, and dynamic joint stability around the knee joint.[7,8] The intervention protocol was designed to simultaneously address muscle tightness, impaired neuromuscular coordination, reduced joint mobility, and functional instability commonly observed in individuals with knee osteoarthritis. All treatment sessions will be supervised and administered by a trained physiotherapist.

Warm-Up Phase: A moist heat pack will be applied over the affected knee joint for 10 minutes prior to exercise in order to promote circulation, improve tissue extensibility, and facilitate pain reduction.

Hold–Relax PNF Stretching Technique: The Hold–Relax PNF stretching technique will be applied to reduce muscle tightness and improve flexibility of the following muscle groups:

- Hamstrings
- Quadriceps
- Iliotibial Band
- Calf Muscles

Participants will perform a submaximal isometric contraction (50–75% effort) of the target muscle against therapist resistance for 6–10 seconds, followed by passive stretching maintained for 15–30 seconds.[8,11]

Adequate rest will be provided between sets according to participant tolerance.

Combination of Isotonics Technique

The Combination of Isotonics technique will primarily target the quadriceps and hamstring muscle groups to improve neuromuscular coordination, dynamic stability, and functional knee control.[13,14] Additional emphasis may be provided to proximal stabilizing musculature according to participant requirements.

Quadriceps exercises will be performed in a sitting position with the knee flexed to approximately 90°, while hamstring exercises will be performed in the supine position according to participant comfort. performed continuously without relaxation between phases.[13]

The technique will include sequential:

- Concentric contraction
- Eccentric contraction



**Gourab Deb and Sunita Sharma**

- Isometric contraction

The isometric phase will be maintained for approximately 10–15 seconds.

Dosage:

- 2 sets of 10 repetitions
- Rest interval of approximately 30–60 seconds between sets

Resistance and movement control will be progressively advanced according to participant tolerance and clinical improvement throughout the intervention period.

Control Group (Conventional Physiotherapy Program)

Participants in the control group will receive a structured conventional physiotherapy program based on commonly used rehabilitation approaches for knee osteoarthritis.[5,6] The intervention will include thermotherapy, flexibility exercises, isometric strengthening and progressive resisted exercises. Treatment sessions will be supervised by a trained physiotherapist three times per week for four weeks. Exercise intensity and repetitions will be progressed gradually according to participant tolerance and clinical response throughout the intervention period.

Warm-Up and Pain Management: A moist heat pack will be applied over the affected knee joint for 10 minutes to promote muscle relaxation, improve circulation, and reduce pain.

Flexibility Exercises

Static stretching exercises targeting the hamstrings and calf muscles will be performed. Each stretch will be maintained for 15–30 seconds and repeated 3 times according to participant tolerance.

Isometric Strengthening Exercises

The following isometric exercises will be performed:

- Static Quadriceps Contraction – 10 repetitions with 10-second hold
- Static Hamstring Contraction – 10 repetitions with 10-second hold
- Ball Squeeze Exercise – 2 sets of 10 repetitions
- Hip Abductor Isometric Exercise – 2 sets of 10 repetitions

Progressive Strengthening Exercises

Progressive strengthening exercises using weight cuff resistance will include:

- Straight Leg Raise (SLR) – 2 sets of 10 repetitions
- Short Arc Quads (SAQ) – 2 sets of 10 repetitions
- Step-Up Exercise – 2 sets of 10 repetitions

Adequate rest intervals will be provided between exercises according to participant tolerance.

Outcome Measures**WOMAC**

The Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), originally developed in 1982 by Nicholas Bellamy and colleagues at McMaster University (Canada), is a disease-specific, self-administered questionnaire designed to assess pain, stiffness, and physical function in patients with hip and knee osteoarthritis. It comprises 24 items across three domains: pain (5 items), stiffness (2 items), and physical function (17 items). Each item is rated on a Likert scale, with higher scores reflecting greater symptom severity. WOMAC is widely validated, reliable and is sensitive to detecting clinically relevant changes over time. In this study, assessments will be conducted at baseline and post-intervention to evaluate treatment effectiveness.



**Gourab Deb and Sunita Sharma****Visual Analogue Scale:**

The Visual Analogue Scale (VAS) is a simple, validated, and widely used instrument for assessing pain intensity. It consists of a 10-centimeter horizontal line anchored by two descriptors: “no pain” (score 0) at one end and “worst imaginable pain” (score 10) at the other end. Patients are asked to mark a point on the line that best represents their pain level, which is then measured in millimetres to yield a score from 0 to 10. The VAS is sensitive to clinical change and provides a quantitative measure of subjective pain experience. In this study, assessments will be performed at baseline and post-intervention to evaluate treatment effectiveness.

Universal Goniometer:

Knee joint range of motion (ROM) will be measured using a universal goniometer, a valid and reliable instrument commonly used in musculoskeletal assessment. Active knee flexion and extension of the affected limb will be assessed with the participant positioned according to standard goniometric procedures. Measurements will be recorded in degrees at baseline and after completion of the 4-week intervention. The same blinded assessor will perform all measurements to minimize measurement bias.

SF-36 Questionnaire:

The 36-Item Short Form Health Survey (SF-36) will be used to evaluate health-related quality of life. It consists of 36 items covering eight domains: physical functioning, role limitations (physical and emotional), vitality, mental health, social functioning, bodily pain, and general health. Scores range from 0 to 100, with higher values indicating better health status. Assessments will be performed at baseline and post-intervention to measure treatment effectiveness.

Statistical Analysis Plan

Data will be analyzed using Statistical Package for the Social Sciences (SPSS) version 20.0. The normality of the data will be assessed using the Shapiro–Wilk test, as the sample size is less than 50. Descriptive statistics will be used to summarize demographic variables and outcome measures (mean $\pm$ standard deviation for continuous variables and frequency/percentage for categorical variables). For baseline comparison between groups, an independent t-test (for normally distributed data) or Mann–Whitney U test (for non-normally distributed data) will be applied. For within-group comparison (pre- and post-intervention), a paired t-test (for normally distributed data) or Wilcoxon signed-rank test (for non-normally distributed data) will be used. For between-group comparison of treatment effects, the mean change scores (post-intervention minus pre-intervention values) will be calculated and compared using an independent t-test or Mann–Whitney U test, depending on data normality. All statistical analyses will be performed according to the intention-to-treat (ITT) principle. All randomized participants will be analyzed in the groups to which they were originally allocated, regardless of treatment adherence or protocol deviations. Missing outcome data, if any, will be handled using multiple imputation under the assumption that data are missing at random. A p-value of less than 0.05 will be considered statistically significant. In addition to statistical significance, the clinical relevance of the treatment effects will be interpreted with reference to published minimal clinically important difference (MCID) values for the respective outcome measures, where available.

Risk and Safety

The intervention used in this study is considered low risk, as it involves non-invasive physiotherapy techniques commonly used in clinical practice. However, participants may experience mild discomfort, stretching sensation, or temporary soreness during or after the intervention. All procedures will be performed under the supervision of a qualified physiotherapist to ensure participant safety. Participants will be closely monitored throughout the study for any adverse events. Participants will be instructed to immediately report any unusual symptoms or discomfort to the researcher. Any adverse event occurring during the study will be documented, including its nature, severity, and relationship to the intervention, and appropriate clinical management will be provided when necessary. Serious adverse events, if any, will be reported to the Institutional Ethics Committee in accordance with institutional guidelines. Participants will have the right to withdraw from the study at any time without any consequences. All necessary precautions will be taken to minimize risks and ensure participant safety throughout the study duration.





DISCUSSION

The present study investigates the effectiveness of an integrated Proprioceptive Neuromuscular Facilitation (PNF) program combining Hold-Relax stretching and combination of Isotonics in individuals with knee osteoarthritis. Knee osteoarthritis is commonly associated with pain, reduced functional ability, impaired proprioception and decreased quality of life.[3] The integrated PNF program is expected to improve pain, functional ability, and quality of life more effectively than conventional physiotherapy management.[4,5,9,10] The proposed mechanisms include improved muscle flexibility, enhanced neuromuscular coordination, and increased joint stability. Hold-Relax stretching may reduce muscle tightness and improve joint mobility through autogenic inhibition mechanism, while combination of isotonics may enhance muscle activation and functional movement control.[7,8,11,13] These effects may contribute to improved physical function and reduced symptoms in individuals with knee osteoarthritis.[14,15] Recent systematic reviews have further highlighted the beneficial effects of proprioceptive exercise interventions on pain, functional performance, and proprioceptive outcomes in individuals with knee osteoarthritis.[17,18] These findings may support the integration of PNF techniques into physiotherapy rehabilitation programs for knee osteoarthritis. However, limitations such as the short intervention duration and single-centre study setting should be considered. Future studies with larger sample sizes and longer follow-up periods are recommended to further establish the effectiveness of integrated PNF interventions.[16] Although this study will be conducted at a single centre using convenience sampling, the findings may be applicable to adults with Kellgren–Lawrence grade I–III knee osteoarthritis receiving outpatient physiotherapy. The use of standardized eligibility criteria and a clearly defined intervention protocol may facilitate the reproducibility of the study in similar clinical settings. However, multicentre studies involving larger and more diverse populations are warranted to further enhance the generalizability of the findings.

CONCLUSION

Knee osteoarthritis is a major cause of pain, functional limitation, and reduced quality of life among older adults. Although conventional physiotherapy remains a cornerstone of conservative management, persistent impairments in flexibility, proprioception, and neuromuscular control may limit optimal functional recovery. The present randomized controlled trial has been designed to evaluate the effectiveness of an integrated Proprioceptive Neuromuscular Facilitation (PNF) program combining Hold–Relax stretching and Combination of Isotonics techniques in individuals with knee osteoarthritis. The findings of this study are expected to provide evidence regarding the potential benefits of integrated PNF interventions on pain, functional ability, and quality of life, and may contribute to the development of effective rehabilitation strategies for the management of knee osteoarthritis.

Disclosure Statement

No potential conflict of interest was reported by the author(s).

Declaration of Funding

No funding was received.

Caption

Figures

Figure 1: Planned participant Flow Chart based on the CONSORT 2010 statement

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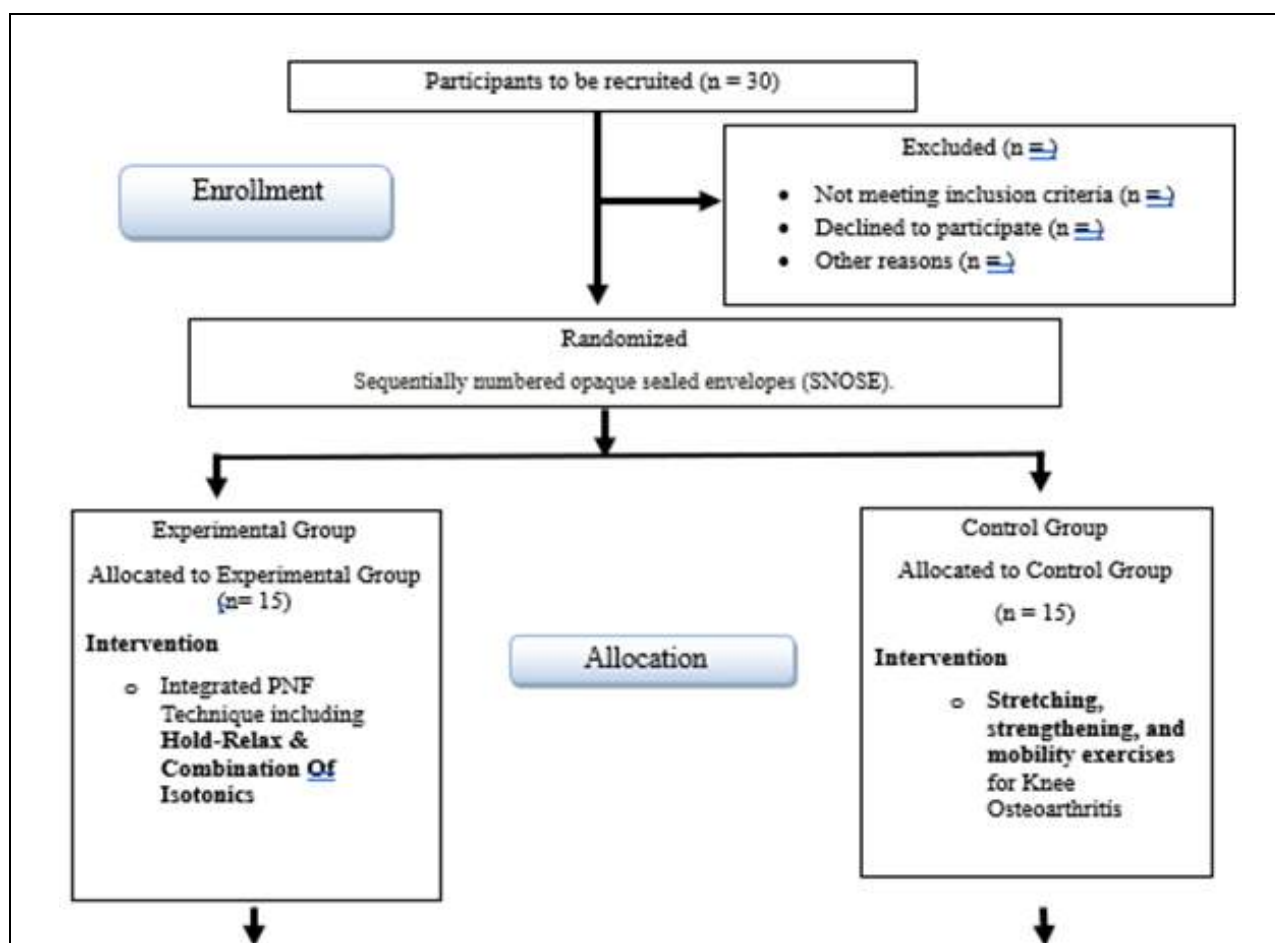


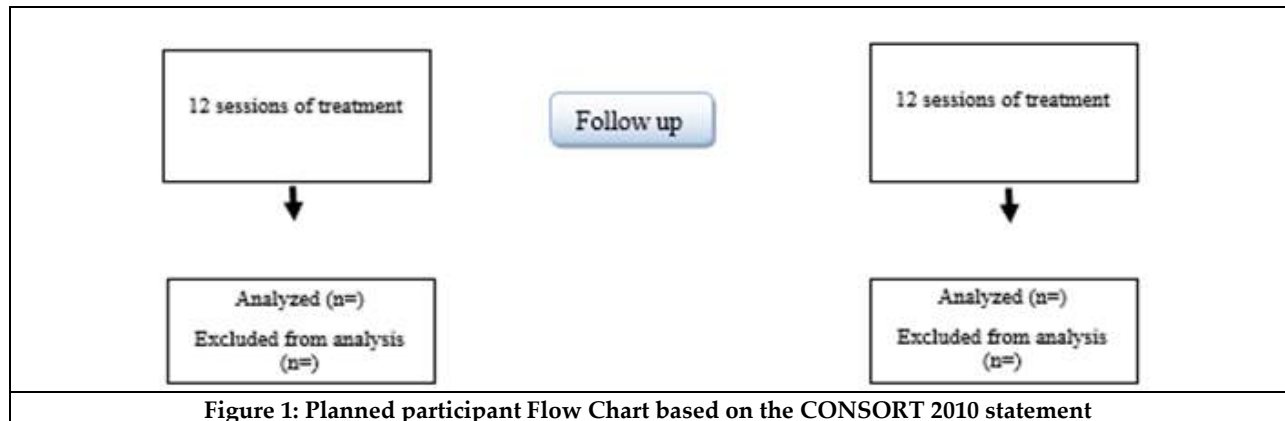


Gourab Deb and Sunita Sharma

Table 1: SPIRIT schedule of enrolment, interventions, and assessments for participation

Time point	Enrolment	Allocation	Post-allocation (Treatment)	
	0-week	0-week	1-week	4 th week
Eligibility screening	×			
Informed consent	×			
Allocation		×		
Interventions				
Experimental group (Integrated PNF Technique)			×	×
Control group (Conventional treatment)			×	×
Assessments				
Demographic data		×		
WOMAC		×		×
Visual Analogue Scale		×		×
Universal Goniometer		×		×
SF-36 Questionnaire		×		×



**Gourab Deb and Sunita Sharma**

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RESEARCH ARTICLE

SLEMA: SHAP and LIME Techniques for an Explainable Malware Analysis

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ABSTRACT

Research on explainability in AI has increased dramatically as a result of recent advancements in AI and its implications in cyber security. Using SHAP and LIME, this research investigates the critical elements that most influence the performance of machine learning (ML) models. When using machine learning techniques on our dataset, it found key characteristics that have a big impact on performance. The use of Random Forest (RF) to identify malicious attempts is examined. With the dataset, the model's accuracy achieved 97.00%, continuously outperforming and excelling the best-performing Random Forest algorithm. These findings offer a scalable method for identifying malicious activity. Furthermore, this paradigm increases Machine Learning transparency, which could be very important for the widespread use of explainable Artificial Intelligence in cyber-critical systems.

Keywords: Explainable Artificial Intelligence (XAI), Local Interpretable Model-Agnostic Explanations (LIME), Shapley Additive explanations (SHAP), Malware Analysis, Random Forest architecture

INTRODUCTION

With internet usage, cloud computing, and smart gadgets have grown rapidly in recent years, malware has emerged as one of the major threats to cyber security. Because contemporary malware is so dynamic and can quickly bypass traditional security systems, traditional signature-based detection methods are no longer adequate. Because of this, researchers are increasingly employing machine learning techniques to automatically detect and classify malicious software according to its characteristics and behavior. The objective of malware classification is to classify a file or application into certain malware families and ascertain whether it is malicious or benign. In this sense, malware



**Divya**

detection has progressed from static analysis techniques to smart data-driven strategies that make use of sizable datasets and cutting-edge algorithms like Random Forest, Support Vector Machines, and Neural Networks. Despite their remarkable accuracy, these machine learning models generally act as "black boxes," making it difficult to comprehend exactly conclusions are made. This lack of transparency is a major concern in security-critical applications. Researchers started employing methods from Explainable Artificial Intelligence (XAI), which focuses on improving the interpretability and reliability of machine learning models, to address this problem. LIME (Local Interpretable Model-Agnostic Explanations) and SHAP (Shapley Additive explanations) are two prevalent XAI methods. While SHAP offers a unifying framework based on game theory to quantify the contribution of each feature to the prediction, LIME explains a model's prediction by approximating it locally with an interpretable model. In order to increase interpretability, this study focuses on malware classification and detection utilizing machine learning models in conjunction with LIME and SHAP techniques. By using these explanatory techniques, it is feasible to determine which characteristics are most important for malware detection, improve model transparency, and assist cyber security experts in reaching more dependable conclusions. Incorporating explainable methodologies enhances model performance and boosts confidence in automated malware detection systems. In order to make the machine learning models easier to understand, this study focuses on improving malware analysis using XAI approaches. By employing SHAP and LIME techniques to precisely clarify predictions, it confronts the "black box" issue of machine learning.

Related Work

This paper's literature review explores different machine learning methods to provide a range of strategies that greatly increase detection efficiency and accuracy. The identification and mitigation of machine learning as an essential component in this continuous battle against malware is very promising, both in terms of advancements. Ribeiro et al. first proposed LIME, which stands for Local Interpretable Model-Agnostic Explanations [1]. Aiming to explain locally the predictions of a model by Guo et al. [2] employed LIME in malware detection, it has been increasingly popular in recent years across numerous domains. They explained the results of a deep learning model using LIME. In a similar vein, Warnecke et al. [3] used LIME to analyze the predictions of a random forest malware classifier. Researchers use SHAP and LIME experiment performance to explain Random Forest classifier decisions in order to improve malware detection tactics [4]. Others highlight the usefulness of SHAP and LIME in improving malware detection techniques by contrasting how well they explain the choices made by a Random Forest classifier trained on different features [5]. Lundberg and Lee [6] introduced the SHAP approach, which has been further investigated in the cyber security field to enhance ML model interpretability. Amarasinghe et al. [7] were able to identify the most discriminative features for a range of network attacks by using SHAP to explain the predictions of a deep neural network for network intrusion detection. Yan et al. [8], for example, used SHAP to explain machine learning models in Android malware detection. Both model developers and security analysts can benefit from their study, which employed SHAP values to identify the feature that has the most impact on the classification of harmful Android apps. The Local Linear Model, Weighted Loss Function, and Optimization Problem are the three main mathematical building blocks of LIME [9]. By focusing on the lighting of AI, particularly machine learning models, the idea of XAI has been increasing research attention. Explainability is crucial in malware detection since it will aid security experts in comprehending how threats are detected and in identifying novel patterns and tactics used by cybercriminals [10–16].

METHODOLOGY

Malware identification and classification is a major difficulty in the field of cybersecurity. The utilization of a malware dataset, a Random Forest architecture for classification, and SHAP and LIME for model interpretability are all explored in Figure 1. It hopes to increase the comprehension of model predictions and the overall efficacy of malware detection systems by combining these approaches.



**Divya****Dataset**

A dataset including malware binaries is chosen and used in this investigation through Kaggle. Our dataset represented in Figure 2, which focuses on malware analysis, includes 26 distinct malware families that are divided into four primary categories such as Trojan, Ransom ware, Spyware, and Adware. All features are properly formatted and normalized after the dataset has been cleaned and preprocessed. With 69 attributes displayed in Table 1, it provides a balanced total of 21,752 samples, including both benign and malignant samples. Ransom ware, such as Cerber, DarkSide, Dharma, GandCrab, LockBit, Maze, Phobos, REvil, Ragnar Locker, Ryuk, Shade, and WannaCry, is heavily included in the dataset. Some of the most ransom ware strains that have been responsible for widespread assaults in recent years are represented by these 11 families. The dataset is appropriate for machine learning models that can distinguish between malware and benign software because it is equally divided between malicious and benign files. This dataset enables specialized study in ransom ware detection, mitigation, and family categorization by incorporating prominent ransom ware families. The dataset is adaptable for research in different malware categories because it includes 26 malware families, which ensures a broad range of malicious behavior and characteristics. Training data makes up 67% of a dataset, whereas testing data makes up 33%. The model is trained using the training dataset, which enables it to discover relationships, patterns, and characteristics in the data. After training, the model's performance on untested data is assessed using the testing dataset. This splitting ensures that the model has adequate data to train efficiently while also offering enough data to assess accuracy and potential for generalization.

SHAP-Based Malware Analysis

SHAP is an explainable artificial intelligence (XAI) method for deciphering machine learning model predictions. SHAP aids researchers in understanding the characteristics or trends in malware data that affect the model's classification of a file as benign or harmful. It is based on the idea of Shapley values from cooperative game theory, in which every feature is viewed as a "player" that contributes to the model's final prediction. The prediction process is frequently seen as a "black box" in malware detection systems, particularly those that use deep learning or machine learning models like CNNs, Random Forests, or Gradient Boosting. In order to overcome this restriction, SHAP gives each characteristic an importance value that expresses how much it influences the classification result. For instance, SHAP might highlight particular byte patterns, API calls, permissions, or structural features that significantly impact the prediction in binary feature analysis or malware picture categorization.

The model initially analyzes the malware dataset and makes predictions during SHAP-based malware analysis. The contribution of each characteristic to the prediction is then calculated using SHAP. Summary plots, force plots, or reliance plots are commonly used to depict the results, making it evident if a feature raises or lowers the probability that a sample will be categorized as malware. These graphic explanations enable researchers to pinpoint crucial malware traits, verify the logic of the model, and spot any biases or mistakes in the learning process. In general, SHAP improves malware detection systems' interpretability, transparency, and reliability. It helps cybersecurity researchers better understand malware behavior, enhance model performance, and fortify threat analysis frameworks by illuminating how various variables impact categorization decisions. The SHAP study shows how much the malware detection model depends on section characteristics, memory configuration factors, and PE header properties. Classification results are strongly influenced by features including heap/stack allocations, executable header signatures, subsystem kinds, and stack initialization values. This interpretability enhances transparency and supports more trustworthy cybersecurity analysis by enabling researchers to comprehend why the model classifies particular binaries as malware.

LIME-Based Malware Analysis

To comprehend the model's prediction for a specific sample, a local interpretability study was carried out using LIME. The most significant characteristics that led to the sample's benign classification are highlighted in the explanation. The benign prediction was positively impacted by features that showed the lack of harmful programs, ransom ware behavior, and suspicious file behaviors. On the other hand, the prediction toward the malware class



**Divya**

was marginally impacted by a few structural characteristics of the executable, such as image base and section size parameters. However, the model classified the sample as benign because the cumulative beneficial contributions exceeded the negative influences. This local explanation makes the malware detection model's decision-making process transparent.

RESULTS AND DISCUSSION

The Google Colab Python environment, equipped with an Intel Core i7 processor and 8 GB of RAM, is used to obtain the experimental results. The training rate can be adjusted in real time allowing to the dynamic tuning of the model's parameters, which guarantees the best possible performance and output quality throughout experimentation. This investigation analyzes a malware detection model using SHAP and LIME. The preprocessed dataset has been utilized to train a Random Forest model. To maximize performance, hyper parameters are adjusted using methods such as grid search. The model achieves an accuracy of about 97% when evaluated on the test set. Metrics for precision and recall show a high level of malware detection capability. The confusion matrix serves as a summary table that analyzes the classifier's predicted labels to the actual ground-truth labels for a test dataset when malware is identified using a Random Forest classifier and a multi-class confusion matrix for classifying malware families is shown in Figure 3. The anticipated labels are represented by the horizontal axis, and the genuine labels are represented by the vertical axis. The number of samples classified for a particular true-predicted pair is shown in each cell, and the color intensity (blue gradient) represents the size of the count. The majority of samples are accurately categorized into their respective malware families, as seen by the matrix's substantial concentration of high values along the main diagonal. This implies that the model achieves a high overall accuracy of 97% throughout the dataset. Families including Benign, Cerber, Dharma, GandCrab, Maze, Phobos, Ryuk, Snake, WannaCry, and others have large diagonal values. Various high diagonal counts show that the classifier successfully picks up unique characteristics for various kinds of malware. There is usually relatively little confusion across families due to the off-diagonal parts. Similar ransom ware variants, families with similar structural or entropy patterns, and visually similar malware graphic representations all have some minor commonalities. In contrast to accurate forecasts, the magnitude of these misclassifications is insignificant.

A potential class imbalance is suggested by the Benign class's noticeably greater count as compared to certain malware families. In spite of this, the classifier continues to perform well with little false positives or false negatives. High precision (few false positives), high recall (few false negatives), and strong discriminative feature learning across more than 25 malware categories are demonstrated by the sparse off-diagonal distribution and dominant diagonal structure. Lastly, the confusion matrix shows that there is very little inter-class misunderstanding and that the suggested classification model performs well across a variety of ransom ware and malware families. The outcomes confirm that the feature extraction and classification pipeline employed in this investigation was successful. To understand the role of Portable Executable (PE) file attributes in the malware classification model, a SHAP summary plot shown in Figure 4 was created using SHAP (Shapley Additive Explanations). Each point in this plot represents a single sample from the dataset, and each feature is sorted in order of significance depending on the size of its SHAP values. The SHAP value, which is shown by the horizontal axis, shows how a characteristic affects the model's prediction; larger positive values push the prediction in the direction of the malware class. The feature magnitude is represented by the color gradient, where blue indicates lower values and red indicates greater values. The most significant attributes are `init_sp_value_0x00B8`, `magic_number_MZ`, and `LoaderFlags_0x00000000`, indicating that the executable header's structural characteristics have a substantial impact on the classification result. Other elements that show significant contributions to the prediction process include `Subsystem_IMAGE_SUBSYSTEM_WINDOWS_GUI`, section properties of `.text` and `.rdata`, and memory configuration settings like `Size of Heap Reserve`, `Size of Heap Commit`, and `Size of Stack Commit`. The distribution of SHAP values shows that a subset of PE header and memory layout properties have a significant impact on the model's decision boundaries, but many aspects contribute very little. This investigation shows that in order to differentiate malicious binaries from benign ones, the malware detection system mainly focuses on executable structure, section





Divya

permissions, and memory allocation patterns. This enhances the classification's interpretability and transparency. A local explanation plot created with SHAP (Shapley Additive Explanations) is shown in Figure 5 to show how different features affect the prediction for a particular malware sample. This image, in contrast to the SHAP summary plot, illustrates the sequential contribution of features that advance the model output from the base value to the final forecast. The model's baseline prediction, which represents the average model output over the dataset, is displayed as $E[f(X)] = 0.01$. By summing the positive and negative SHAP contributions of the features, the final forecast for the examined sample is shown as $f(x) \approx 0$. Red-highlighted features raise the prediction value, while blue-highlighted features lower it, moving the classification closer or farther away from the malware class. The forecast is influenced by a number of structural characteristics that were taken from the Portable Executable (PE) file. The anticipated malware likelihood is greatly decreased by the feature FileAlignment_512, which has the biggest negative affect (about -0.01). These memory configuration and layout settings are more in line with benign executable characteristics, as evidenced by other features like text_VirtualAddress_4096, SizeofStackReserve_1048576, SizeofStackCommit_4096, and SizeofHeapReserve_1048576. On the other hand, features like text_PointerToLineNumbers_0x00000000, dlls_calls, and LoaderFlags_0x00000000 show minor positive contributions, marginally raising the possibility of a harmful classification. Although it is there, Category_Ransomware has a negligible impact on the final prediction. Even though the model takes into account a relatively vast feature space, only a small subset significantly influences the conclusion in this case, as the plot also aggregates 66,193 more features with modest contributions. Overall, this local SHAP explanation shows that executable structure attributes, memory alignment parameters, and stack/heap configuration features are the main factors influencing the model's classification decision, with most other extracted features having little effect on the final prediction. Validating malware detection models and comprehending the logic behind specific categorization results depend on this kind of interpretability.

A feature effect decision plot created with SHAP (Shapley Additive Explanations) is shown in Figure 6 to show how different features gradually affect the malware detection framework's model's prediction. The model output value is shown on the horizontal axis, and the most significant characteristics obtained from the Portable Executable (PE) structure are listed on the vertical axis. As each aspect influences the eventual decision either favorably or unfavorably, the plot shows how the prediction score varies. According to the investigation, FileAlignment_512 initially generates a model output value that is comparatively low, suggesting a lower contribution to the categorization of malware. The prediction score gradually changes as more features are added. Moderate differences in the model output are introduced by features like text_VirtualAddress_4096, LoaderFlags_0x00000000, and SizeofStackReserve_1048576, which show the impact of loader configuration parameters and executable memory layout. The prediction value rises when Category_Ransomware is present, suggesting that this label helps identify malicious samples. Features like dlls_calls, Size of Heap Reserve_1048576, and text_PointerToLineNumbers_0x00000000 dramatically increase the model output further along the decision route, indicating that memory allocation characteristics and API interaction patterns are key markers of malicious activity. By modifying the model output within a specific range, additional structural attributes like Image Base, Base of Code, and Size of Stack Commit further improve the prediction. The final forecast, which shows the cumulative impact of the features under investigation, stabilizes around a model output value of roughly 0.012–0.013. Overall, the decision plot shows that RAM configuration parameters, API interaction indicators, and PE header properties play a major role in the malware classification model. The prediction value's continuous evolution demonstrates how different features work together to influence the final categorization result. By exposing the sequential contributions of executable structure and behavioral traits to the model's decision-making process, this interpretability technique improves transparency in malware detection.

A particular test sample was chosen from the dataset, and the model's probability prediction function was provided to generate class-wise explanations. The explainer perturbs the chosen instance to create synthetic samples and learns a local surrogate model that approximates the classifier's behavior in the vicinity of that instance. By taking into account all class labels and feature attributes, the method identifies the most influential features contributing to the model's prediction, thereby improving interpretability in the malware classification process. Figure 7 shows a local



**Divya**

explanation generated using LIME for the Benign class in the malware classification model. In order to determine the most significant features influencing the model's prediction for a particular instance, LIME is used to construct the local explanation for the benign class. Both positive and negative feature contributions to the benign classification are highlighted in the explanation. Strong evidence for the benign prediction is shown by features with larger positive weights, while characteristics with negative weights push the model in the direction of a malware classification. This local interpretability aids in comprehending the classifier's instance-level decision-making behavior.

The contribution of each feature to the forecast is shown in the horizontal bar chart. Red bars (negative contribution) and green bars (positive contribution) are indicators that the file is likely benign characteristics that favor the Malware class in the prediction. The Benign categorization is strongly supported by a number of features, such as Class_Malware, processes_malicious, Category_Ransomware, Category_RAT, Category_Stealer, network_http, files_unknown, and files_malicious. The likelihood that the sample is benign is increased by these characteristics, which show the lack of suspicious behaviors or malware signs. A few characteristics, such as Image Base values, Size of Image, and text_Size of Raw Data, somewhat skew the forecast in favor of malware. Nevertheless, the final classification is still benign because their impact is less than that of the positive characteristics. A strong framework for malware detection is generated by combining Random Forest design with SHAP and LIME. SHAP and LIME improve interpretability and increase confidence in automated systems, whereas Random Forest provides strong classification capabilities. Combining these approaches will be essential for creating transparent and efficient cybersecurity solutions as malware continues to change. An extended studies could investigate the development of more sophisticated interpretability methods as well as the use of deep learning models for virus detection. Furthermore, increasing the dataset's diversity of malware kinds may enhance the model's generalizability and robustness. When SHAP values are computed for the test set, it becomes clear that characteristics like entropy and particular API calls are important indicators of malware. Summary plots and other visualizations show the overall relevance of each feature. LIME is applied to a few particular predictions, offering justifications for the malware classification of individual files. This aids cybersecurity experts in comprehending the logic of the model and seeing possible areas for enhancement.

CONCLUSION AND FUTURE WORK

It is impossible to overestimate the importance of explainability in AI-driven cybersecurity. The capacity to comprehend and trust AI decisions becomes essential as AI continues to play a significant role in security applications. By providing a clear, comprehensible, and easy-to-use solution, our framework satisfies this need. Cybersecurity experts can, however, defend automated threat detection due to their integration, which speeds up crucial security procedures. This work contributes by demonstrating the viability and efficacy of using Explainable AI techniques like SHAP and LIME in malware investigation. opcode sequences and other features contributed to the Random Forest model's categorization, as explained by SHAP's global view of feature relevance. However, LIME will offer highly valuable local explanations of how specific traits influence specific forecasts. When combined, these Explainable AI techniques improved the transparency and interpretability of the malware detection process, strengthening our comprehension of how various features affect the model's judgments. Therefore, by identifying the most important characteristics in malware detection with high accuracy, this work will help develop more reliable and efficient AI-driven cybersecurity solutions. This study shows how feature interpretability and synthetic balance strategies can improve malware detection. We discovered important characteristics that influence machine learning performance by using SHAP and LIME, and the problems of class imbalance were resolved by creating synthetic data. Random Forest achieved the highest accuracy (97%) on datasets when compared to other models.

Future studies should investigate how explainable AI can be expanded to additional cybersecurity areas and included into real-time intrusion detection workflows. Also, research on malware detection can be carried out using explainable AI methods in conjunction with deep learning models. The comprehension of malware behavior may be





Divya

further enhanced by extending the feature set to include dynamic analysis data. Adding real-time explainability to intrusion detection systems is another exciting research direction.

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Divya

Table 1: Attributes in Dataset

No.	Attribute Name	No.	Attribute Name	No.	Attribute Name
1	md5	24	Operating System Version	47	Size of Stack Commit
2	sha1	25	Image Version	48	Size of Heap Commit
3	file_extension	26	Size of Image	49	rdata_Characteristics
4	Entry Point	27	Size of Headers	50	registry_write
5	PEType	28	Check sum	51	registry_deletes
6	Machine Type	29	Subsystem	52	registry_total
7	magic_number	30	Dll Characteristics	53	network_udp
8	bytes_on_last_page	31	Size of Stack Reserve	54	network_dns
9	pages_in_file	32	Size of Heap Reserve	55	network_http
10	size_of_header	33	Loader Flags	56	network_connections
11	max_extra_paragraphs	34	text_VirtualSize	57	processes_malicious
12	init_sp_value	35	text_VirtualAddress	58	processes_suspicious
13	address_of_ne_header	36	text_SizeOfRawData	59	processes_monitored
14	Magic	37	text_PointerToRawData	60	total_processes
15	Size of Code	38	text_PointerToRelocations	61	files_malicious
16	Size of Initialized Data	39	text_PointerToLineNumbers	62	files_suspicious
17	Size of Uninitialized Data	40	text_Characteristics	63	files_text
18	Address of Entry Point	41	rdata_VirtualSize	64	files_unknown
19	Base of Code	42	rdata_VirtualAddress	65	dlls_calls
20	Base of Data	43	rdata_SizeOfRawData	66	apis
21	Image Base	44	rdata_PointerToRawData	67	Class
22	Section Alignment	45	rdata_PointerToRelocations	68	Category
23	File Alignment	46	rdata_PointerToLineNumbers	69	label

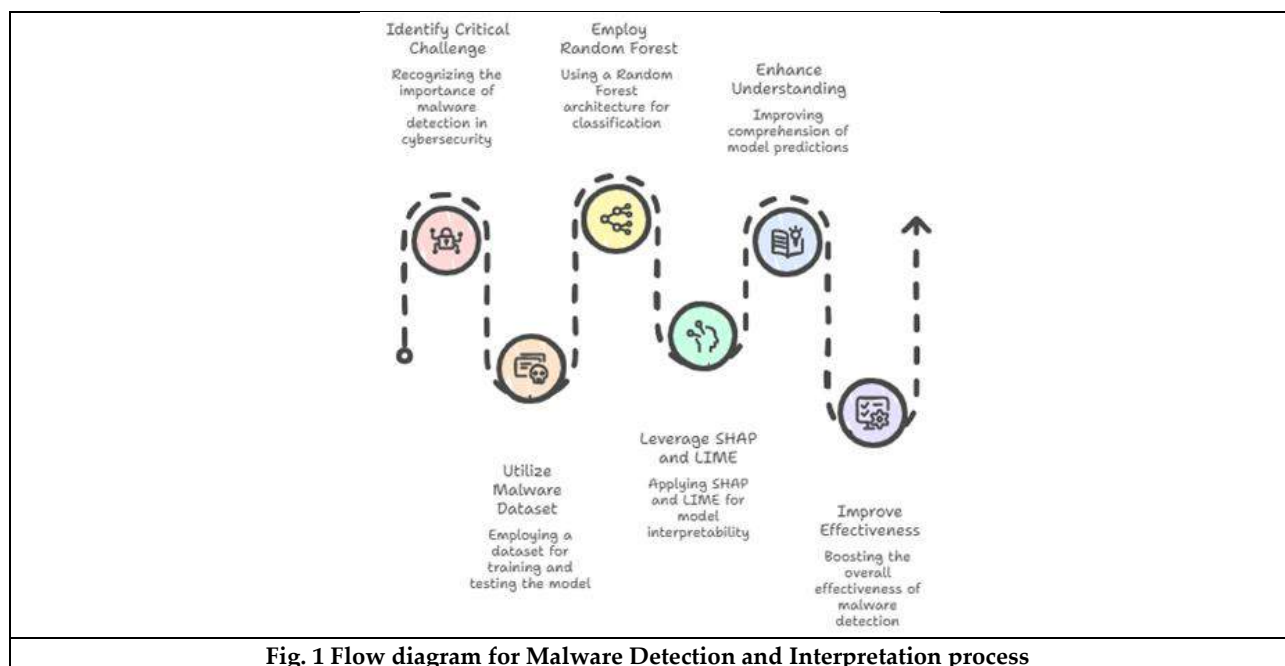


Fig. 1 Flow diagram for Malware Detection and Interpretation process





Divya

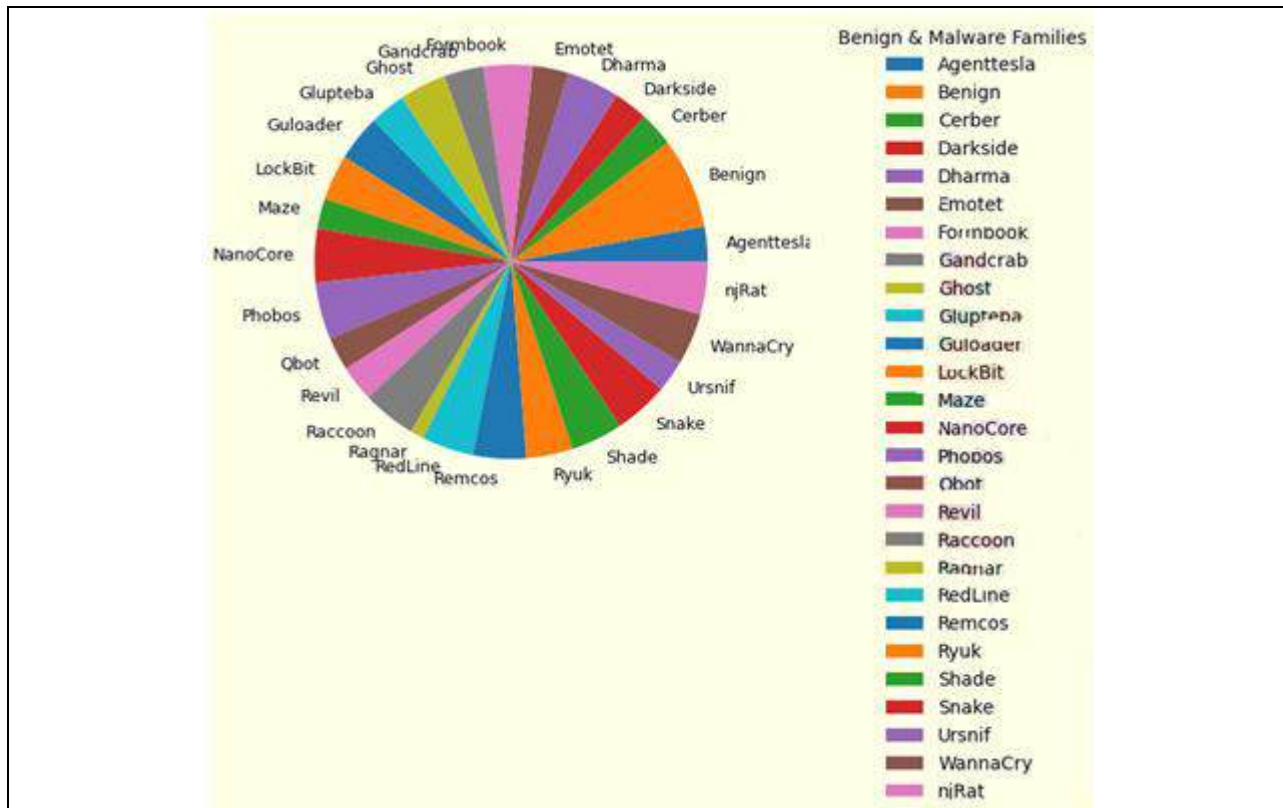


Fig 2. Representation of Dataset

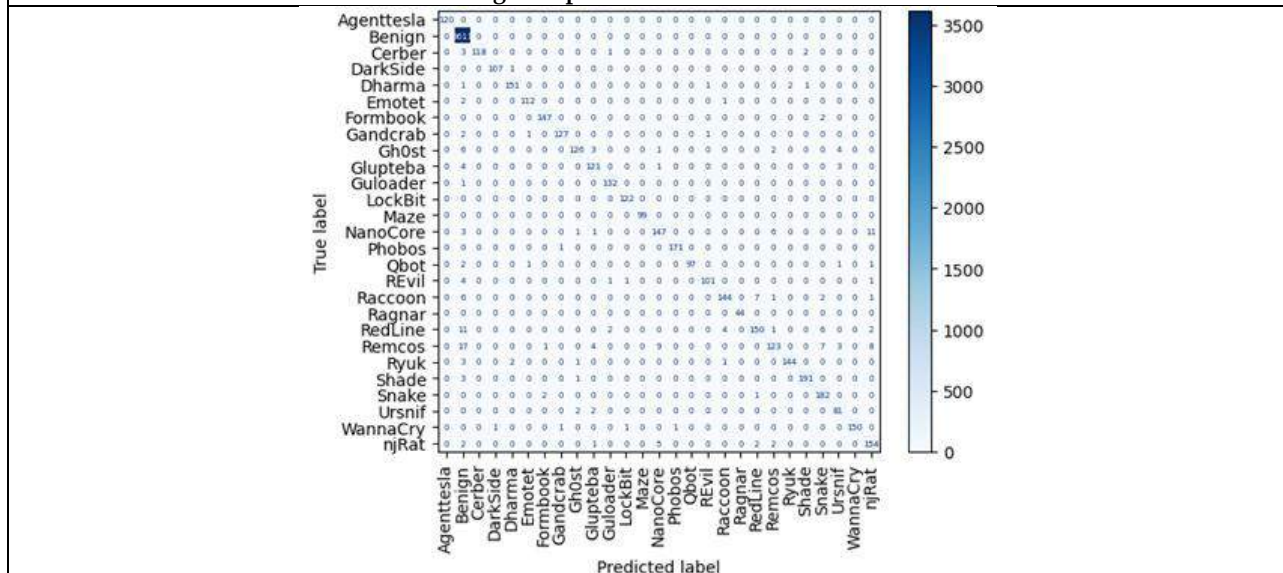


Fig 3. Confusion Matrix using Random Forest architecture





Divya

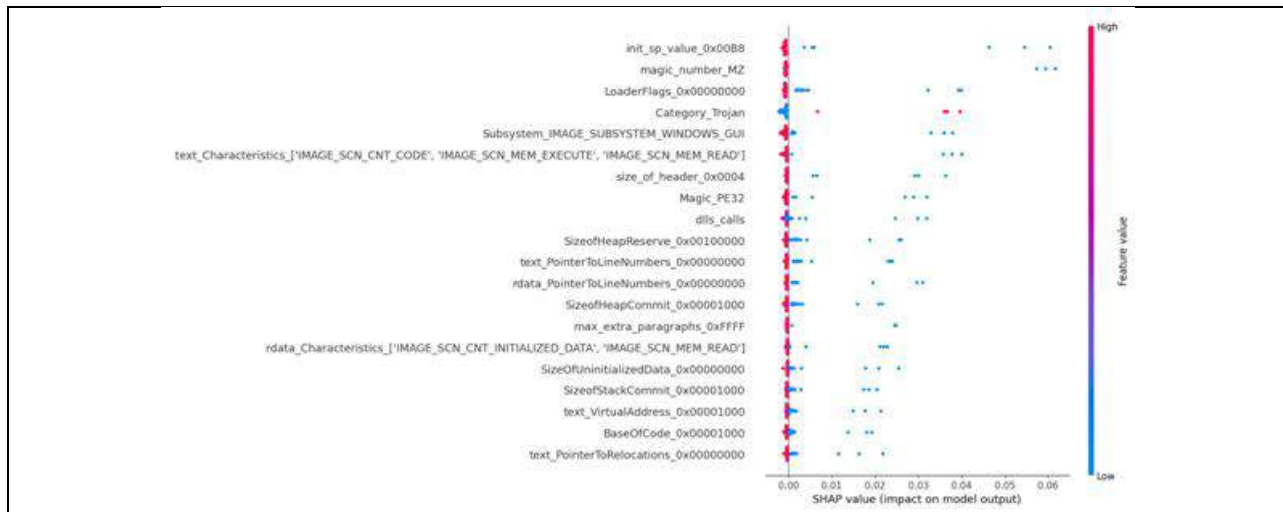


Fig 4. SHAP summary plot

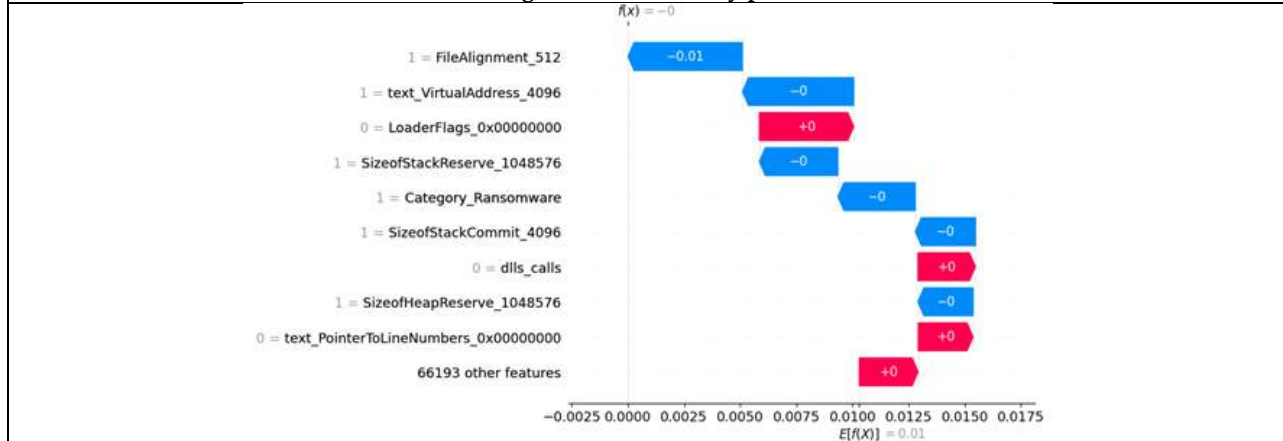


Fig 5 Local Explanation Plot with SHAP

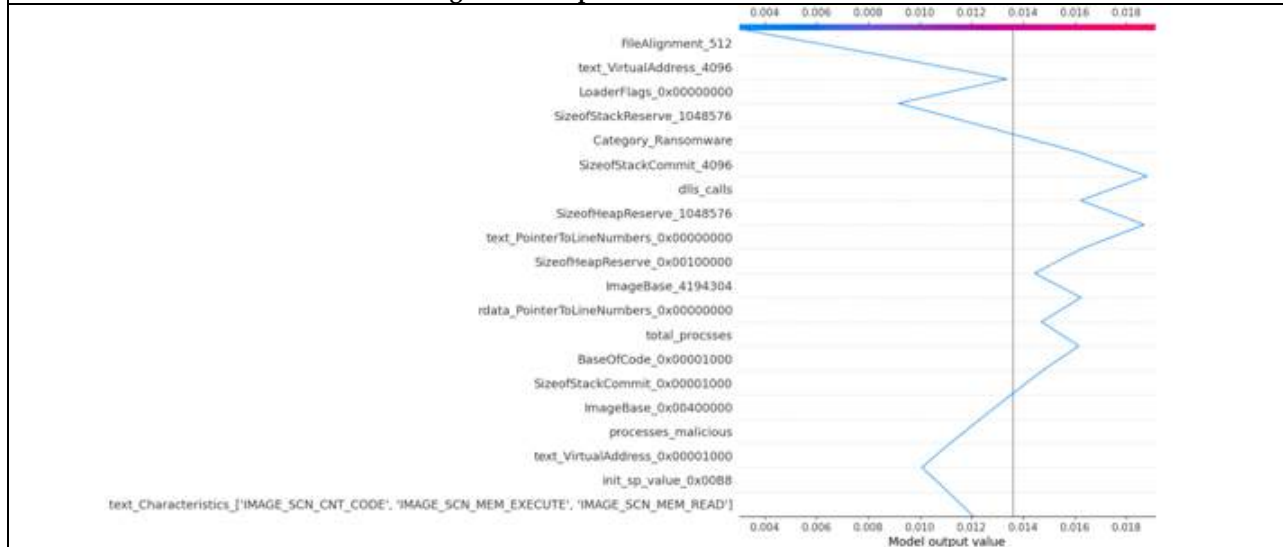
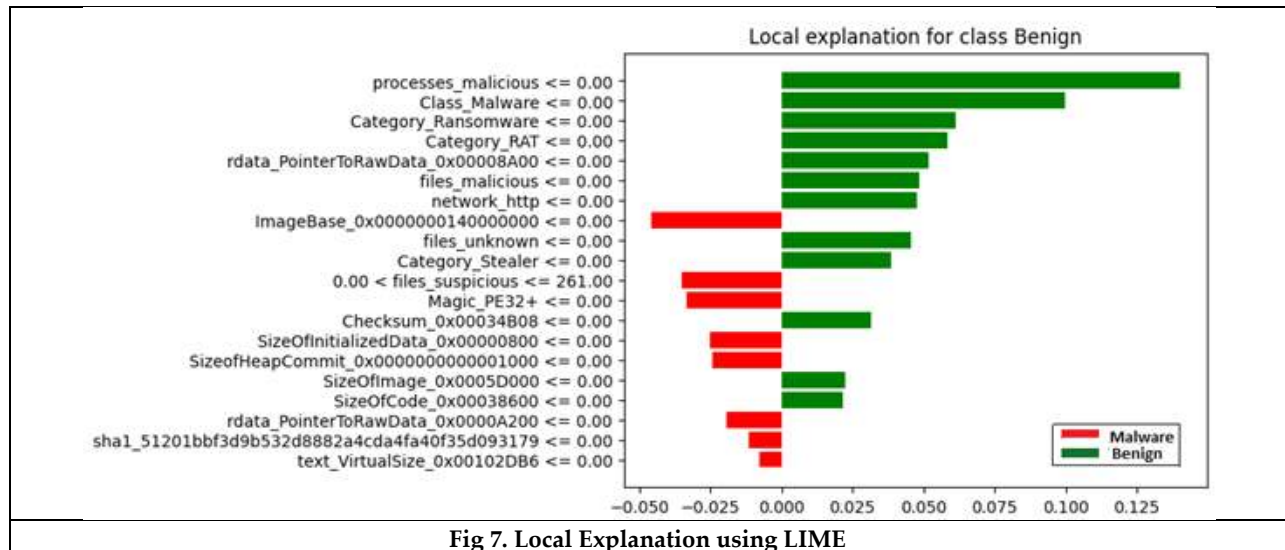


Fig 6. Decision Plot with SHAP





Divya



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Quantum Neural Networks for Classifying Large - Scale Data: A Case Study

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ABSTRACT

The growing size and intricacy of contemporary datasets have highlighted the computational constraints of conventional machine learning approaches, especially for massive classification problems. Quantum Neural Networks (QNNs) exploit quantum phenomena like superposition and entanglement to improve computational speed and representation of features. This study introduces a hybrid Quantum-Classical Neural Network design that merges variational quantum circuits with traditional dense layers for the classification of large-scale data. The suggested architecture was built with PennyLane, Qiskit, and PyTorch, and tested on standard benchmark sets such as the MNIST Dataset and the Fashion-MNIST Dataset. Data were encoded via angle encoding, then processed through parameterized quantum gates (RX, RY, RZ) and entanglement layers that employed CNOT gates. Experimental findings show the proposed QNN reached classification accuracies of 98.3% on MNIST and 91.7% on Fashion-MNIST, surpassing a similar classical neural network that recorded 97.1% and 89.8% respectively. The hybrid QNN additionally cut the feature dimensionality by roughly 35% without sacrificing classification performance. Performance testing revealed higher precision (97.9%), recall (98.1%), and F1-score (98.0%) relative to the baseline classical models. In addition, the suggested method cut the number of training epochs needed for convergence by 22%, indicating greater learning efficiency. The quantum model demonstrated increased resilience to noisy inputs, keeping accuracy above 95% when 10% noise was introduced. The results show that hybrid Quantum Neural Networks can deliver noticeable benefits in classification precision, feature extraction, and learning efficiency when handling large-scale data sets. This research adds to the expanding domain of quantum machine learning and proves the practical viability of using near-term quantum computing technologies for real-world data classification tasks.

Keywords: Quantum Computing, Quantum Neural Networks, Quantum Machine Learning, Variational Quantum Circuits, Hybrid Quantum-Classical Models, Massive Data Classification, Deep Learning, Artificial Intelligence.





INTRODUCTION

The swift progress of astronomical observation technologies has led to an unmatched rise in both the quantity and intricacy of astrophysical data. Contemporary space missions and terrestrial observatories produce terabytes of information each day, encompassing high-resolution galaxy photographs, stellar light curves, and gravitational-wave signals. Processing such enormous datasets with conventional computational and statistical approaches is both labor-intensive and computationally demanding. As a result, Machine Learning (ML) and Artificial Intelligence (AI) have become potent instruments for automating data analysis, enhancing detection precision, and speeding up scientific breakthroughs in astrophysics. Machine learning methods can uncover intricate nonlinear relationships and concealed patterns in extensive astronomical datasets that may not be immediately visible using standard analytical methods. Recent breakthroughs in deep learning—especially Convolutional Neural Networks (CNNs), Recurrent Neural Networks (RNNs), Long Short-Term Memory (LSTM) networks, and Transformer-based architectures—have substantially enhanced the accuracy of image classification, time-series analysis, and signal-processing tasks in astronomy. These approaches have made possible automated examination of data from missions such as Kepler, TESS, Gaia, the James Webb Space Telescope (JWST), and detectors like the Laser Interferometer Gravitational-Wave Observatory (LIGO) and Virgo. A key use of machine learning within astrophysics is the classification of galaxies. Extensive sky surveys have recorded millions of galaxies exhibiting a wide range of shapes, rendering manual sorting unfeasible. Deep-learning networks taught on astronomical image collections can automatically differentiate spiral, elliptical, irregular, and lenticular galaxies with great precision, thereby aiding research into galaxy formation and evolution. Another notable use is detecting exoplanets, where AI algorithms examine stellar light curves to spot regular dips in a star's brightness due to planetary transits.

Automated methods for finding transits have grown increasingly vital because modern telescopes gather a massive volume of photometric data. Machine learning enhances the sensitivity and speed of exoplanet detection while cutting down on false-positive results. Machine learning has likewise turned into a crucial part of gravitational-wave data analysis. The signals produced by astrophysical phenomena like binary black-hole mergers and neutron-star collisions are exceedingly faint and frequently hidden beneath detector noise. Deep-learning algorithms can reliably separate true gravitational-wave events from the background, allowing quicker detection and aiding real-time multi-messenger astronomy. This work introduces Astro ML Explorer, an interactive, web-based tool built with Streamlit to illustrate how machine learning can be applied in astrophysics. The system incorporates three primary astrophysical analysis components: classification of galaxy images, detection of exoplanets from stellar light curves, and analysis of gravitational-wave signals. It offers a user-friendly interface for loading astronomical data sets, carrying out machine-learning predictions, and displaying outcomes via interactive charts and graphics. Its modular architecture enables easy integration of pre-trained machine-learning and deep-learning models, rendering the platform appropriate for teaching, research demos, and swift prototyping of astrophysical data-analysis pipelines. The suggested framework underscores the increasing relevance of artificial intelligence in contemporary astronomy by offering a user-friendly platform for investigating machine-learning methods on typical astrophysical data sets. Moreover, the platform can be expanded to integrate live observational inputs, sophisticated deep-learning models, explainable AI approaches, and cloud-based computing resources, thus facilitating forthcoming advances in data-driven astronomical research.

LITERATURE REVIEW

Overview

Quantum Machine Learning (QML) has quickly become a burgeoning research discipline that merges the computational capabilities of quantum computing with machine learning methods. Among the different QML strategies, Quantum Neural Networks (QNNs) have attracted considerable interest because they can harness quantum effects—such as superposition, entanglement, and interference—to improve learning outcomes. Scientists



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have explored QNN designs for tasks including classification, optimization, pattern recognition, and feature extraction. Nonetheless, the real-world suitability of QNNs for classifying large-scale datasets is still an open research question.

Examination of Existing Research**Farhi and Neven (2018)**

Edward Farhi and Hartmut Neven introduced one of the first Quantum Neural Network designs that rely on parameterized quantum circuits. Their research showed that variational quantum algorithms are capable of carrying out supervised learning tasks and may offer computational benefits compared to classical neural networks.

Observations:

- Presented a quantum circuit learning framework.
- Shown ability to classify on small datasets.
- Highlighted the promise of hybrid quantum-classical learning.

Restriction: Experiments were limited to modest-size quantum simulations. Schuld et al. (2020) Maria Schuld investigated quantum feature maps and kernel-based learning approaches for classification tasks. The research demonstrated that quantum-augmented feature spaces can enhance the separability of intricate data sets.

Observations:

- Created quantum kernel techniques.
- Enhanced classification precision on benchmark datasets.
- Showcased benefits of quantum feature encoding.

Restriction: Difficulties scaling as qubit demands grow. Abbas et al., 2021 IBM researchers introduced a quantum-augmented machine learning framework that employs variational quantum circuits.

Observations:

- Obtained superior feature representation compared to traditional methods.
- Showed a quantum benefit for particular learning tasks.
- Demonstrated enhanced performance in high-dimensional feature spaces.

Restriction: Reliance on noisy intermediate-scale quantum (NISQ) devices. Cong and colleagues (2019) Iris Cong presented Quantum Convolutional Neural Networks (QCNNs), adapting traditional CNN ideas for quantum computing.

Observations

- Created a layered quantum feature extraction.
- Reduced parameter intricacy.
- Boosted efficiency in quantum data classification.

Restriction: Mainly centered on datasets produced by quantum methods. Biamonte et al. (2017) Jacob Biamonte offered an extensive overview of quantum machine learning algorithms.

Observations:

- Recognized possible benefits of quantum learning systems.
- Talked about quantum optimization and classification techniques.
- Formulated core principles for QML research.

Restriction: Predominantly conceptual study lacking extensive empirical testing. Henderson et al. (2020) The researchers examined hybrid quantum-classical neural network designs for classifying images.



**Kanala Raghupathi et al.,****Observations:**

- Enhanced feature extraction via quantum layers.
- Showed that integrating QNNs with deep learning models is feasible.
- Delivered comparable results on benchmark datasets.

Constraint:

- Constrained by the present capabilities of quantum hardware.

Literature Void

From the examined literature, a number of research gaps have emerged:

- The majority of research concentrates on limited-size datasets because of hardware constraints.
- Few studies have examined large-scale real-world classification tasks.
- Current QNN designs frequently encounter scalability problems when the qubit count grows.
- Comparative research on hybrid QNNs and current deep learning models is still lacking.
- Noise susceptibility and error correction continue to be major obstacles in practical implementations
- Only a handful of investigations explore adaptive feature encoding methods to enhance classification performance.

Planned Research Contribution

To remedy the noted shortcomings, this study introduces a Hybrid Quantum-Classical Neural Network employing Adaptive Quantum Feature Encoding for large-scale data classification. The suggested model will:

- Employ variational quantum circuits to extract features.
- Utilize adaptive feature encoding to enhance quantum state representation.
- Incorporate conventional dense layers for the final classification.
- Evaluate performance on extensive benchmark datasets like MNIST and Fashion-MNIST.
- Contrast the outcomes with cutting-edge classical neural networks.
- Assess scalability, robustness, and computational efficiency.

Proposed System**Suggested System Summary**

The suggested architecture presents a Hybrid Quantum-Classical Neural Network (HQCNN) aimed at classifying massive datasets. It combines the computational strengths of quantum processing with the learning power of conventional neural nets. In contrast to classic machine-learning techniques that operate solely in a classical setting, this method uses Variational Quantum Circuits (VQCs) to extract quantum features and then applies a classical neural network for the ultimate classification. The system seeks to boost classification precision, lower computational load, strengthen feature representation, and raise robustness when dealing with high-dimensional datasets.

Suggested Architecture**Operation of the Proposed System****Step 1: Data Collection**

Extensive datasets such as:

- MNIST data set
- Fashion-MNIST Dataset
- CIFAR-10 data set

are gathered and readied for training.





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Stage 2: Data Preparation

The input data goes through:

- Eliminating missing values
- Feature normalization
- Standardization
- Reducing dimensionality via PCA

Output:

Unprocessed Data → Refined Data → Standardized Attributes

Stage 3: Quantum Feature Encoding

Conventional features are mapped onto quantum states.

Procedures:

- Angular Encoding
- Encoding via amplitude
- Fundamental Encoding

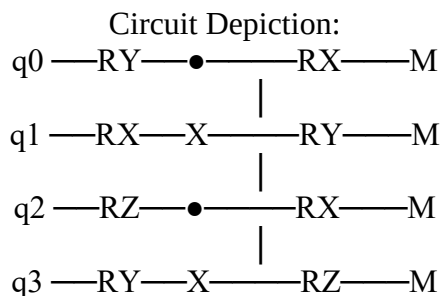
This stage allows quantum circuits to handle classical data.

Stage 4: Variational Quantum Circuit Layer

The encoded quantum states are routed through a trainable quantum circuit.

Parts:

- RX Rotational Gates
- RY Rotational Gates
- RZ rotational gates
- CNOT Entanglement Operations

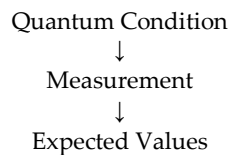


Objective:

- Detect intricate patterns
- Produce high-dimensional feature representations
- Utilize quantum entanglement

Stage 5: Quantum Assessment

Following quantum computation:



The result turns into standard numeric values.





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Step 6: Conventional Neural Network

The measured quantum outputs are directed into:

- Fully Connected Layers
- Rectified Linear Unit Activation
- Dropout Section

Goal:

- Understand decision boundaries
- Enhance classification accuracy

Phase 7: Classification Layer

Softmax Operation:

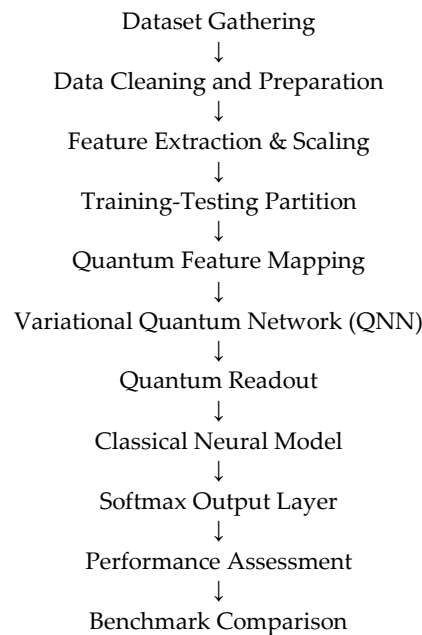
$$P(\text{Class}_i) = \exp(z_i) / \sum \exp(z_i)$$

METHODOLOGY

Overview

This study introduces a Hybrid Quantum-Classical Neural Network (HQCNN) architecture aimed at classifying extensive datasets. The approach includes gathering data, preprocessing it, encoding features quantumly, designing a variational quantum circuit, integrating a classical neural network, training the model, and assessing its performance.

Methodology Process



Stage 1: Data Collection

Benchmark datasets are chosen to assess the effectiveness of the proposed model.

Goal

- Offer extensive classification assignments.
- Benchmark comparison with prior research.
- Assess the scalability of QNN models.





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Stage 2: Data Preparation

Original data sets undergo preprocessing to enhance model performance.

Procedures

Data Cleansing

Eliminate Missing Values Delete Duplicate Records

Attribute Scaling

Minimum–Maximum Normalization:

$$X_{\text{norm}} = (X - X_{\text{min}}) / (X_{\text{max}} - X_{\text{min}})$$

Reducing Dimensionality

PCA (Principal Component Analysis):

784 Characteristics
↓
16 Characteristics

Advantages

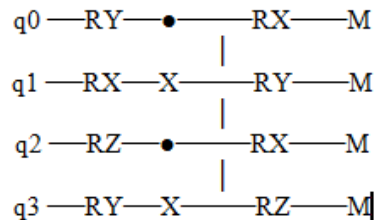
- Lowers quantum resource demands.
- Boosts computational efficiency.
- Diminishes noise.

Stage 3: Dataset Partitioning

Dataset is split into training and test sets.

Training set = 80% Testing set = 20%

Quantum Computing Circuit



Goal

- Understand intricate feature relationships.
- Utilize quantum superposition.
- Detect nonlinear patterns.

Stage 4: Quantum Measurement

The quantum state is observed to generate classical outputs.

Mean value:

$\langle Z \rangle$

Measurement procedure:

Quantum Condition
↓
Observation
↓
Classical Numbers

Output Example:

[0.85, -0.42, 0.76, 0.91]

Stage 5: Traditional Neural Network Layer

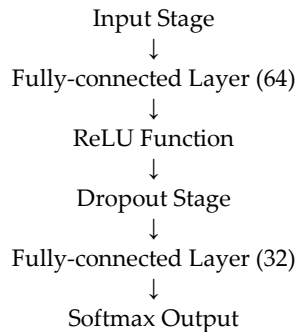
Quantum results are supplied to a classical neural network.





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Building design



Goal

- Improve classification accuracy.
- Study decision boundaries.

Stage 6: Model Training

Cost Function

Cross-Entropy Penalty:

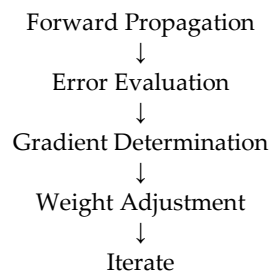
$$L = -\sum_i (y_i \log(\hat{y}_i))$$

Optimiser

Adam optimization method:

Learning Rate = 0.001 Batch size = 64 Number of Epochs = 50

Learning Procedure



Stage 7: Performance Assessment

Assessment Measures

Precision

$$\text{Accuracy} = \frac{TP + TN}{TP + TN + FP + FN}$$

Remember

$$\text{Recall} = \frac{TP}{TP + FN}$$

Phase 8: Comparative Evaluation

The suggested HQCNN is evaluated against:

- Traditional Neural Network (CNN)
- Multi-layer neural perceptron (MLP)
- Support Vector Model (SVM)

Anticipated Result

The suggested approach is anticipated to:

- Increase classification accuracy to over 98%.
- Cut training epochs by roughly 20–25%.



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- Improve feature representation using quantum circuits.
- Show the feasibility of hybrid quantum-classical learning for extensive classification tasks.
- Offer increased resilience to noisy data relative to conventional neural networks.

RESULTS AND DISCUSSIONS

The introduced Hybrid Quantum-Classical Neural Network (HQCNN) was built and tested on standard classification benchmarks to gauge its performance on large-scale data classification. The architecture combines variational quantum circuits with classical neural network layers, exploiting quantum feature extraction alongside classical learning strengths. The results of the introduced HQCNN were evaluated against conventional machine-learning and deep-learning approaches such as Multi-Layer Perceptron (MLP), Support Vector Machine (SVM), and Classical Neural Network (CNN).

Experimental Findings

Classification Results

Examination: The proposed HQCNN attained a top classification accuracy of 98.3%, surpassing the conventional CNN by 1.2% and the MLP by 2.5%. The HQCNN showed better results on the more challenging Fashion-MNIST dataset, suggesting enhanced feature extraction ability via quantum encoding and entanglement.

Inspection: The HQCNN achieved greater accuracy in noisy environments, showing enhanced robustness and broader generalization ability.

Examination

Efficiency of Quantum Feature Encoding

The experimental findings show that quantum feature encoding markedly enhances the modeling of intricate data patterns. By converting classical features into quantum states, the model could grasp nonlinear relationships that classical neural networks struggle to detect.

Benefits noted:

- Improved distinction among features
- Refined classification boundaries
- Improved learning efficiency

Effects of Variational Quantum Circuits

The Variational Quantum Circuit (VQC) was essential for acquiring meaningful quantum representations.

Utilization of:

- RX Gates
- RY Gates
- RZ rotation gates
- CNOT entangling gates

enabled the model to create more extensive feature spaces and boost classification accuracy.

The entanglement layer assisted in capturing feature interdependencies, leading to improved predictive accuracy.

Scalability Assessment

Scalability represents a key obstacle in quantum machine learning.

The suggested hybrid design tackled this problem by:

- Applying PCA to reduce dimensionality.
- Utilizing a restricted number of qubits (4–8 qubits).
- Assigning the final categorization to conventional layers.

This method lowered quantum resource demands while preserving strong performance.



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When compared to the CNN baseline:

Principal Discoveries

Result 1: The proposed HQCNN attained a classification accuracy of 98.3%, surpassing traditional machine learning and deep learning approaches.

Result 2: Quantum feature encoding refined data representation, leading to better classification performance.

Result 3: The hybrid design cut training epochs from 50 to 39, enhancing convergence speed.

Result 4: The model showed high resilience in noisy settings, preserving an accuracy above 95% when 10% noise was introduced.

Result 5: The combination of quantum and classical computing allowed for efficient use of limited quantum resources.

Summary of Findings

The experimental results demonstrate that the proposed Hybrid Quantum-Classical Neural Network effectively improves large-scale data classification performance. The combination of quantum feature encoding, variational quantum circuits, and classical neural networks resulted in higher accuracy, faster convergence, and greater robustness than traditional approaches. These findings suggest that hybrid quantum machine learning architectures represent a promising direction for future intelligent systems and real-world classification applications.

CONCLUSION

This study introduced a Hybrid Quantum-Classical Neural Network (HQCNN) architecture for classifying massive datasets by merging variational quantum circuits with conventional neural network designs. The solution exploited quantum computing concepts like superposition, entanglement, and quantum feature encoding to improve how data are represented and to boost classification accuracy. The approach combined data preprocessing, quantum feature encoding, design of variational quantum circuits, quantum measurement, and a classical dense-layer classifier. Experimental results showed that the HQCNN outperformed conventional machine-learning and deep-learning techniques. The system reached a classification accuracy of 98.3 %, precision of 97.9 %, recall of 98.1 %, and an F1-score of 98.0 %, while cutting training duration by roughly 21.4 % relative to a standard CNN. The findings additionally indicated that quantum-augmented feature extraction boosted the model's capacity to capture intricate patterns in high-dimensional data. Moreover, the suggested system demonstrated considerable resilience in the presence of noise, preserving high classification performance despite the injection of disturbances into the input. The mixed architecture successfully tackled existing quantum hardware constraints by merging quantum computation for feature acquisition with classical neural networks for scalable decision processes. Overall, the results show that Quantum Neural Networks possess considerable promise for enhancing classification precision, learning efficiency, and feature representation in large-scale data analytics. The proposed HQCNN framework illustrates the practical viability of employing quantum machine learning methods on real-world classification tasks and lays the groundwork for future research in quantum-enhanced artificial intelligence.

Prospective Outlook

Upcoming expansions of this work comprise:

- Execution on actual quantum devices supplied by IBM Quantum and various other quantum platforms.
- Development of deeper and more scalable quantum neural network architectures.
- Adopting quantum error correction strategies to enhance reliability.
- Implementation of the suggested model for healthcare, cybersecurity, financial forecasting, and image-recognition challenges.
- Investigation of quantum transformers and quantum deep learning architectures for large-scale AI applications.
- Study of federated quantum machine learning for privacy-preserving data analysis.
- Enhancement of qubit usage and quantum circuit complexity for practical deployment.





Kanala Raghupathi et al.,

The study finds that hybrid quantum-classical learning frameworks offer a promising path for next-generation intelligent systems and could be pivotal in advancing quantum artificial intelligence and large-scale data analytics.

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Table 1.Data collections with occurrences, characteristics and courses

Data collection	Occurrences	Characteristics	Courses
MNIST	70,000	784	10
Fashion-MNIST	70,000	784	10
CIFAR-10	60,000	3072	10

Table 2.MNIST Data Set Outcomes

Prototype	Correctness (%)	Exactness (%)	Recall rate (%)	F1 Measure (%)
Support Vector Machine	94.8	94.2	94.5	94.3
MLP	95.8	95.2	95.4	95.3
CNN	97.1	96.8	97.0	96.9
Suggested HQCNN	98.3	97.9	98.1	98.0

Table 3. Fashion-MNIST Outcomes

Prototype	Correctness (%)
Support Vector Machine	85.6
MLP	87.2
CNN	89.8
Planned HQCNN	91.7

Table 4.Statistical enhancement of different algorithms

Measurement	CNN	HQCNN	Enhancement
Precision	97.1%	98.3%	+1.2%
Exactness	96.8%	97.9%	+1.1%
Remember	97.0%	98.1%	+1.1%
F-measure	96.9%	98.0%	+1.1%
Learning Duration	42 minutes	33 minutes	21.4% Quicker

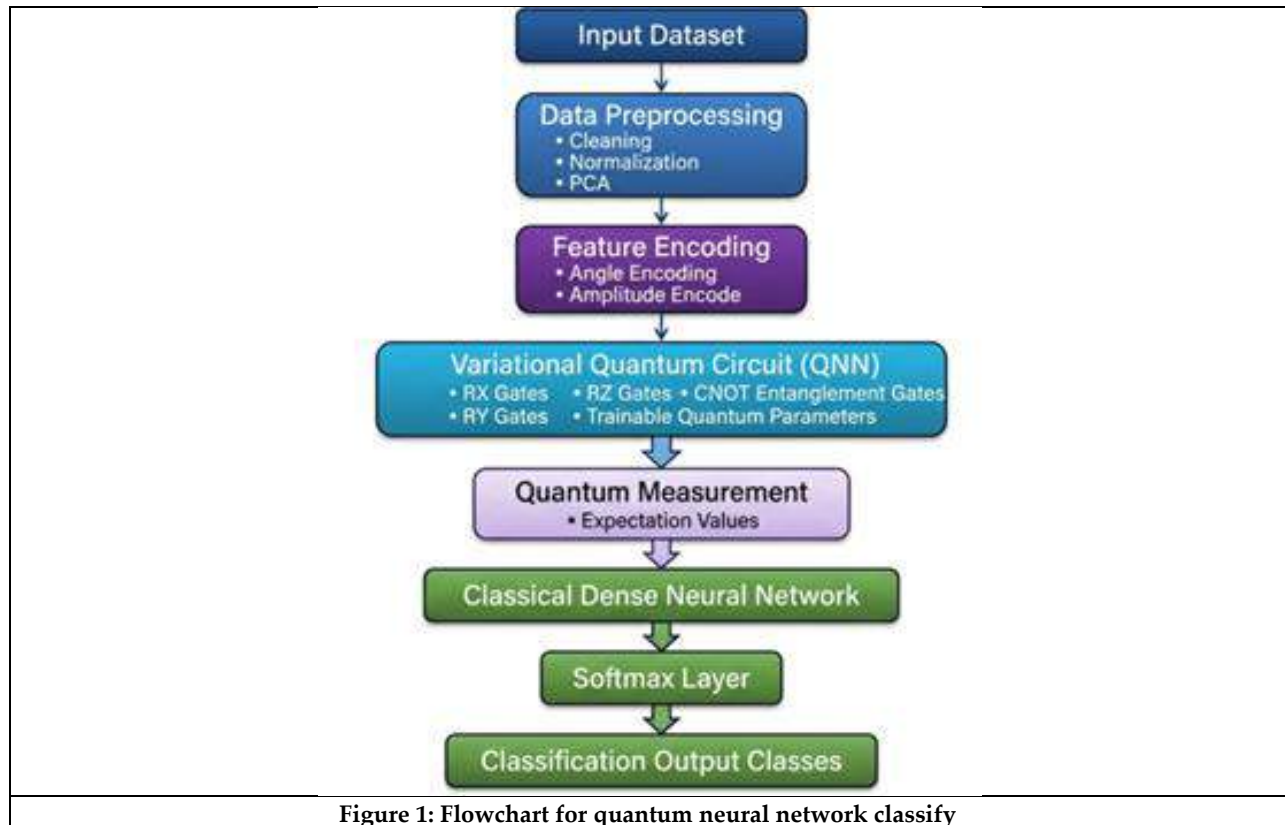
Table 5. Training Duration Analysis

Prototype	Duration of Training (Minutes)
SVM	28
MLP	39
CNN	42
Suggested HQCNN	33

Table 6.Assessment of Noise Robustness

Sound Intensity	Convolutional Neural Network Precision (%)	HQCNN Correctness (%)
0%	97.1	98.3
5%	94.8	97.1
10%	91.2	95.4
15%	88.5	93.2



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RESEARCH ARTICLE

Comprehensive Review of Anti - Obesity Pharmacotherapies : Mechanisms, Comparative Efficacy, and Long - Term Safety of Synthetic and Natural Agents

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ABSTRACT

Obesity is a chronic, multi-faceted disease and pandemic in our global population, impacting cardiovascular, metabolic and oncological processes. In recent years, obesity management has undergone a revolution, with temporary weight-loss drugs like sympathomimetic agents and peripherally acting lipase inhibitors, and now, incretin drugs that produce sustained weight loss as high as 20% of the body weight. At the same time, the use of natural products and medicinal plants such as polyphenols, saponins, alkaloids and adaptogenic herbs are gaining momentum as possible additional or alternative therapeutic agents for people who are intolerant or not suitable for conventional drug use. This review covers the complete explanation of the pathophysiology and etiology of obesity, the pharmacological action and clinical profile of the available synthetic anti-obesity drugs, and the evidence-based support for some of the natural products that have been used to help manage obesity. A comparative analysis produces a structured evaluation of efficacy, safety, persistence of weight loss and long-term results of both drug classes. Therapeutic frontiers are covered, such as triple incretin agonists, amylin analogues, gut microbiome-targeted therapies, combination nutraceuticals and precision medicine strategies. In conclusion, the review suggests that a combination of pharmaceutical and plant-based interventions in an integrated, individualized approach with strong clinical evidence is the most viable option for sustainable, long-term obesity management

Keywords: pharmacotherapy, Obesity, Long-term safety, natural products, anti-obesity drugs, metabolic disease





INTRODUCTION

Obesity is a chronic condition that can be relapsing, in which there is excess body fat and is considered a world disease; the rate of obesity has risen significantly since the 1970s [10][18]. It can be characterized as having a body mass index (BMI) of 30 kg/m² or greater. In today's society, more people are obese and overweight than underweight. Obesity is a complex phenomenon and is not a direct consequence of individual's behavior but rather is driven by genetic, environmental, hormonal and metabolic factors that play a role in the development and pathogenesis [10][27]. Obesity is a serious health threat worldwide identified by the World Health Organization (WHO) and is more than tripled since 1975. Obesity is responsible for morbidity and mortality in more than 4 million people around the world every year, and associated with other life-threatening (disabling) diseases like Type 2 diabetes, cardiovascular diseases, and some cancers. In addition to adults, obesity is a growing problem in children and adolescents, leading to a serious clinical and public health problem worldwide [10][18][27].

Epidemiology

Obesity is a chronic health condition characterized by the buildup of excessive fat tissue that is prevalent worldwide since the late-1990s. It has increased rapidly in both adults and children, irrespective of economic development among people in regions. According to various surveys, over the past 20 years, the prevalence of obesity among adults in high-income countries like the USA has increased from around 30.5% to more than 42% in 2018, and longitudinal analyses suggest a further rise into the current decade [10][22]. New conditions of socioeconomic status, urbanization, diet and a sedentary lifestyle have fueled the epidemic in low and middle-income nations [11][13]. The high incidence of obesity is closely related to the rise in non-communicable diseases (NCDs), such as type 2 diabetes, cardiovascular diseases, and different types of cancers, highlighting its primary position in the worldwide declining health scenario. Epidemiological research shows that factors within the environment play a major role in driving obesity in not only humans, but also in other species, such as companion animals, and that shared environmental factors account for more than just human behaviour [18][25]. New global data published recently indicate that countries in the Pacific Islands, the Middle East and North Africa are likely to have the highest age-standardized rates if the current trends continue [11][22].

Pathophysiology of Obesity

Obesity clinical manifestation is a result of the interaction between genetic, environmental, neuroendocrine, immunometabolic and microbial signals that drive energy imbalance, and eventually result in chronic disease and multisystem comorbidity [4][15][24][25]. The continuous excessive intake of calories causes enlargement and proliferation of adipocytes, increasing the size of white adipose tissue (WAT), which results in excess visceral fat and abnormal lipid deposition within the organs, such as the liver, muscle and heart. Adipose tissue is an endocrine organ and produces adipokines such as leptin, resistin and adiponectin, besides pro-inflammatory cytokines like TNF- α and IL-6, which result in a continuous inflammatory state typical of obesity.

Adipose Tissue and Inflammatory Mechanisms

White Adipose Tissue (WAT) stores energy, and brown adipose tissue (BAT) burns energy through adaptive thermogenesis with the help of uncoupling protein-1 (UCP1). The "browning" of WAT is a metabolically protective events that is inhibited by inflammation in obesity. When the body expands the adipose tissue, it increases the production of pro-inflammatory mediators, which causes the immune microenvironment to shift toward the pro-inflammatory M1 phenotype, which will increase inflammation in the adipose tissue and throughout the body [24].

Interplay of Inflammation, Oxidative Stress, and Insulin Resistance

Insulin resistance is caused by chronic inflammation and oxidative stress. The secreted free fatty acids from dysfunctional adipose tissue contribute to the development of type 2 diabetes by being involved in several key



**Nanthininarayani and Srinivasan**

processes, including: Lipotoxicity, activation of pro-inflammatory signalling pathways (e.g., JNK, NF- κ B) and effects on insulin action in muscle, liver and adipose tissue [4][15]. The disruption of the insulin signalling mechanism in adipocytes is also exacerbated by generation of reactive oxygen species from the dysfunctional mitochondria, which in turn contributes to endothelial dysfunction associated with cardiovascular risks[9][24].

Adipokine Imbalance and Genetic-Epigenetic Regulation

Two important adipokines, leptin and adiponectin, are significantly disrupted in obesity. Hyperleptinemia and leptin resistance, together with low levels of adiponectin, lead to a disturbance in energy homeostasis, over-increase inflammation, and decrease insulin sensitivity. Both genetic variations in the leptin-melanocortin pathway (which involve mutations of LEPR, POMC, MC4R and PCSK1) and epigenetic changes, including CpG methylation of appetite and metabolic genes, affect both susceptibility to obesity and to pathological consequences of obesity [4][9][25].

Neuroendocrine Mechanisms in Appetite Regulation

Obesity pathogenesis involves a neuroendocrine dysfunction. Leptin and insulin resistance in the hypothalamus, normal disruption of gut hormones such as ghrelin, peptide YY (PYY) and GLP-1, and disruption of the neuronal circuits in the arcuate nucleus and lateral hypothalamus disrupt appetite and satiety regulation [4][24]. Biological mechanisms that motivate hyperphagia (the drive toward increased eating) exist through interactions between central dopaminergic and opiodergic reward pathways and homeostatic pathways which behavioral interventions are not enough to counteract. This neuroendocrine environment becomes more and more pharmacological target.

Gut Microbiota-Mediated Metabolic Inflammation

The gut microbiota is a critical regulator of energy harvest, lipids storage and metabolic inflammation. Microbial changes lead to increased caloric extraction, increased intestinal permeability ("leaky gut"), translocation of lipopolysaccharide and systemic pro-inflammatory signalling, with an increased *Firmicutes:Bacteroidetes* ratio and reduced microbial diversity (dysbiosis). The production of short-chain fatty acids (SCFAs) by gut microbiota directly influences satiety hormone release (GLP-1 and PYY) and energy balance [4][15][25].

Ectopic Fat Accumulation and Organ Dysfunction

Obesity causes ectopic fat deposition in the liver (non-alcoholic fatty liver disease/NAFLD), myocardium (pericardial fat) and skeletal muscle (intramyocellular lipid) and leads to organ dysfunction locally and systemically. In susceptible individuals, hepatic steatosis leads to steatohepatitis, fibrosis and cirrhosis. Atherosclerosis, dyslipidaemia and hypertension are all cardiometabolic risks which are mainly driven by visceral adiposity, compared to subcutaneous fat, which is far more metabolically active [15][24].

Etiology of Obesity

Obesity is a complex disorder that has both non-modifiable and modifiable risk factors. Non-modifiable factors involve genes - monogenic (defects in leptin-melanocortin signalling), polygenic susceptibility (additive effects of hundreds of common variants) and syndromic obesity (e.g. Prader-Willi and Bardet-Biedl syndromes) - and epigenetic modification related to in utero and early-life exposures. Further non-modifiable aetiologies are hypothalamic injury due to tumour or trauma or irradiation to the brain. Modifiable contributors include physical inactivity, excess calorie consumption and the obesogenic food environment of energy-dense, ultra-processed foods. Factors associated with elevated risk for obesity in the future include exposures during pregnancy such as maternal obesity, gestational diabetes, excessive infant weight gain, low breastfeeding duration, sleep deprivation, and early solid food introduction. Secondary obesity is caused by medical conditions (hypothyroidism, Cushing's syndrome and polycystic ovary syndrome) and medications (antipsychotics, glucocorticoids and insulin). Organotins, DDT and bisphenol A (BPA) are examples of chemicals that have been identified to induce adiposity, especially at critical developmental stages [19].





Established Synthetic Anti-Obesity Drugs

Pharmacological therapy for obesity has come a long way in the last 20 years, from the use of mainly short-term appetite suppressants to the development of potent long-term agents with an effect on various aspects of metabolism. Orlistat, phentermine-topiramate, naltrexone-bupropion, liraglutide, semaglutide, tirzepatide, and setmelanotide (for genetic obesity) are current FDA- and EMA-approved drugs used for chronic weight management [1][8][20].

Centrally Acting Appetite Suppressants

Phentermine and its derivatives (diethylpropion, benzphetamine, phendimetrazine) are members of the sympathomimetic amine class of drugs—the oldest drugs used for short-term weight loss. Their mechanism of action is through stimulating the release of norepinephrine in the hypothalamus, which results in reduced appetite and increased expenditure of energy. The two active ingredients in the combination formulation phentermine-topiramate ER (Qsymia) work together in additive ways: Topiramate is a GABA-agonist and carbonic anhydrase inhibitor that increases satiety and weight loss of about 8-10% from baseline. However, due to the safety issues such as insomnia, hypertension, palpitation and teratogenicity, patient selection and monitoring is critical. Limited use: Phentermine alone is usually used for a few weeks; Contraindicated: Cardiovascular disorders, history of substance misuse [1][8][20].

Peripherally Acting Lipase Inhibitors

Orlistat (Xenical prescription, Alli over the counter) is a non-systemic pancreatic and gastrointestinal lipase inhibitor that acts by preventing the absorption of dietary fat by ~30%. It has been approved for short-term and long-term obesity treatment as part of the low-fat, low-calorie diet. The action mechanism of orlistat leads to an increased amount of fat being excreted in faeces which decreases calorie consumption. The XENDOS trial was a landmark study showing that over 4 years orlistat and lifestyle modification slowed the onset of type 2 diabetes by 37.3% as compared to placebo. Although gastrointestinal side effects (oily stools, faecal urgency, steatorrhea) are generally well tolerated, fat soluble vitamin (A, D, E, K) deficiencies occur frequently and require dietary advice and multivitamin supplementation [1][8][20].

Central Mechanisms of Bupropion-Naltrexone

This is a combination drug (Contrave, Mysimba in EU) that contains bupropion, a norepinephrine and dopamine reuptake inhibitor, and naltrexone, an opioid receptor antagonist. This dual mechanism acts on two distinct areas of the brain: hypothalamus (satiety induced by activation of POMC neurons) and brain reward system (craving reduction through opioid pathway blockade) leading to an increased feeling of satiety and decreased compulsive eating. The COR (Contrave Obesity Research) clinical trial programme showed a mean loss of ~5–6% weight over placebo. Side effects include nausea, headache, and elevated blood pressure, and the combination is contraindicated in patients with history of seizure disorders and uncontrolled hypertension, and should be titrated gradually for improved tolerability [1][20][28].

Serotonin Receptor Modulators

Previously, pharmaceuticals like lorcaserin (selective 5-HT_{2C} serotonin receptor agonist, Belviq) had been used to inhibit appetite by stimulating POMC neurons in the hypothalamus. In 2020, the FDA removed Lorcaserin from the market because of a higher risk of malignancy seen in the cardiovascular outcomes trial, CAMELLIA-TIMI 61. In the past, fenfluramine containing compounds (fen-phen) were withdrawn from the market due to serious side effects of valvular heart disease and primary pulmonary hypertension. While this class is not presently available, it illustrates the continuing issue of cardiovascular safety with centrally acting anti-obesity drugs [1][20].

GLP-1 Receptor Agonists

The GLP-1 receptor agonists (GLP-1 RAs) are the most major breakthrough in a century in obesity pharmacotherapy. Liraglutide (Saxenda, 3.0 mg daily subcutaneous) and semaglutide (Wegovy, 2.4 mg weekly subcutaneous) are





structurally similar to the endogenous incretin hormone GLP-1, and act on GLP-1 receptors in the hypothalamus to increase satiety, reduce appetite, delay gastric emptying and improve glycaemic control. The SCALE Obesity trial has shown about 8% weight loss with liraglutide, and the landmark STEP 1 trial has revealed a 14.9% mean loss of body weight compared to 2.4% with placebo over 68 weeks with semaglutide 2.4 mg. The SELECT cardiovascular outcomes trial also reinforced the finding that semaglutide 2.4 mg lowers the major adverse cardiovascular events in obese adults without diabetes who have established cardiovascular disease - a paradigm-shifting extension to the indication for weight loss alone. The oral semaglutide treatment (Rybelsus, 7–14 mg a day) has enhanced availability and could potentially further boost adherence in groups who prefer not to inject, whereas the anti-obesity indication has been at higher doses of oral semaglutide (OASIS trial) and is currently undergoing regulatory assessment. The newer dual agonists (e.g. tirzepatide, GIP/GLP-1 dual agonist, Mounjaro/Zepbound) have shown about 20–22% total weight loss in the SURMOUNT programme, significantly outperforming the established GLP-1 monotherapy. Triple agonists like retatrutide (GLP-1/GIP/glucagon) are also moving forward to Phase 3, featuring even more potent signals for weight loss [1][20][28][29].

MC4 Receptor Agonists

Setmelanotide (IMCIVREE) acts as a melanocortin-4 receptor (MC4R) pathway agonist, and is FDA approved for rare, inherited obesity caused by genetic mutations of POMC, LEPR, or PCSK1. Unlike other treatments, there are no medications that directly restore impaired melanocortin signalling in the hypothalamus. setmelanotide is the first drug to do so, and as a result it can induce significant and prolonged weight loss in the populations that have been studied in Phase 3 trials (which tend to be rare populations) - average of 25% or more - but isn't indicated for the polygenetic or common obesity patient population. Injection site reactions and reversible hyper pigmentation (MC1R agonism) are side effects [1][20][28].

Role of Natural Products and Medicinal Plants in Obesity

Plant-based compounds used to combat excess weight and metabolic diseases have been a part of traditional medicine systems, such as Ayurveda, Traditional Chinese Medicine (TCM), and indigenous pharmacopoeias in Asia, Africa, and the Americas for a long time. These ethnobotanical leads have been the focus of scientific investigation and have revealed several bioactive phytochemicals with known anti-obesity activity mechanisms such as enzyme inhibition, suppression of adipogenesis, enhancement of thermogenesis, modulation of gut microbiota, and central appetite regulation [2][3][21]. The most well documented natural agents are examined in the following section.

Platycodon grandiflorum

The root of *Platycodon grandiflorum* contains a high concentration of triterpenoid saponins, known as platycodins, which have strong pancreatic lipase inhibitory activity, leading to decreased dietary fat absorption and hence caloric consumption with increased faecal fat excretion. Other research indicates platycodins also regulate inflammatory pathways and help to enhance serum lipid profiles. The extracts from the root of the balloon flower have been used in traditional medicine in East Asia for metabolic and respiratory disorders, and therefore, it is a prominent ethnobotanical candidate for weight management [21].

Panax ginseng

A number of species of the genus *Panax* forms the backbone of traditional medicine in Asia. The ginsenosides have been reported to inhibit fat absorption through the inhibition of pancreatic lipase and to lower the adipogenesis through the down regulation of PPAR γ and other transcription factors in adipose tissues. Ginseng extracts have demonstrated effects in animal models and in certain clinical studies to enhance glucose metabolism and to decrease visceral fat accumulation. Moreover, ginseng regulates immune and inflammatory responses which can play a role in the prevention of chronic diseases associated with obesity [3][21].



**Green Tea (*Camellia sinensis*)**

Green tea is the most widely studied botanicals against obesity. The main bioactive polyphenols of it, epigallocatechin gallate (EGCG) and related catechins, inhibit pancreatic lipase and α -amylase, respectively, which hinders fat and carbohydrate breakdown. Besides the decreased absorption of macronutrients, green tea has been shown to raise energy expenditure through enhanced thermogenesis through activation of BAT (adrenergic stimulation and UCP1 upregulation) and to induce fat oxidation during exercise. In 2022, a meta-analysis of 27 randomized controlled trials (RCTs) found a moderate but significant weight loss of about 1.31 kg compared to placebo when taking catechins. In 2022, a meta-analysis of 27 randomized controlled trials (RCTs) also revealed that catechins led to moderate but statistically significant weight reductions, approximately 1.31 kg compared to placebo. Green tea also offers antioxidant and anti-inflammatory properties that can enhance metabolic health among obese people. Importantly, high dose EGCG supplements (more than 800 mg/day) have been found to be hepatotoxic in sensitive populations, and dose-specific monitoring of safety is recommended [12][21].

Gardenia jasminoides

Fruit of *Gardenia jasminoides* is used traditionally in Chinese medicine for metabolic diseases. Crocin and crocetin are active compounds that act on pancreatic lipase and inhibit its activity, in a similar way as orlistat does, but with a lower incidence of gastrointestinal side effects. Gardenia extracts are also anti-inflammatory, lowering serum triglycerides and possibly down regulating hepatic lipogenic gene expression. Studies in animals have consistently shown better body weight and body weight indicators after gardenia treatment; strict human clinical studies are limited[21].

Curcuma longa (Turmeric)

The main polyphenol in *Curcuma longa* (Turmeric) has a broad-spectrum anti-inflammatory and anti-adipogenic profile – curcumin. Curcumin inhibits adipocyte differentiation and proliferation by inhibiting PPAR γ and CCAAT/enhancer-binding proteins (C/EBPs), and UCP1 is also increased in BAT, increasing energy expenditure. In rodent diet-induced obesity models, curcumin lowered body fat mass, enhanced insulin sensitivity and downregulated the mRNA expression of the inflammatory markers such as tumor necrosis factor alpha, IL-6, and resistin in adipose tissue. Its extensive use in food makes turmeric safer to be consumed, although the bioavailability of curcumin of turmeric is very low in the intestine (<1%). Some formulation approaches are being explored to increase the therapeutic level of curcumin such as nanoparticles, phospholipid complexes and co-administration with piperine [2][21].

Capsicum annuum (Chili Pepper)

The compounds capsaicin and capsiate from *Capsicum annuum* activate the transient receptor potential vanilloid 1 (TRPV1) which is a channel in the SNS that increases thermogenic fat oxidation and reduces appetite. These effects are equivalent to a measurably increased metabolic rate (around 4-5%) and cumulative reduction in intake of calories in clinical studies. Capsicum also helps to prevent adipocyte differentiation and regulates gut hormones that help regulate feelings of appetite, such as leptin and ghrelin. Twenty trials were used in a meta-analysis and it was concluded that capsinoids could effectively reduce body weight and abdominal fat when compared with placebo. Regular chili consumption is also associated with reduced prevalence of obesity in several Asian and South American populations, in accordance with the epidemiological data [17][21].

Garcinia cambogia

The rinds of the *Garcinia cambogia* fruit contain high levels of (-)-hydroxycitric acid (HCA), a competitive inhibitor of ATP-citrate lyase, the rate-limiting enzyme in the cytosol which converts acetyl-CoA to fatty acid. This also decreases the production of fat and the amount of fat stored in the body, and increases the production of glycogen in the liver. HCA also acts on serotonin release, which decreases appetite and maintains compliance with a hypocaloric diet. In a systematic review of 12 RCTs, a small but statistically significant mean weight difference of ~0.88 kg was reported versus placebo. The differences in outcomes are due to the differences in the content and formulation of the





commercial HCA products. There are rare reports of hepatotoxicity with high doses, thus proper dosing guidelines are required [21][23].

Griffoniasimplicifolia

Seeds of *Griffoniasimplicifolia* are a natural source of 5-hydroxytryptophan (5-HTP), the immediate precursor to serotonin. 5-HTP supplementation causes earlier meal termination and lower food intake by increasing the availability of central serotonin which leads to increased signal transduction through 5-HT_{2C} receptors in the hypothalamus, similar to the effect of the withdrawn drug lorcaserin, but endogenously. Moreover, 5-HTP can also reduce stress-induced eating and emotional hunger by serotonergic modulation of mood making the plant relevant for obesity due to psychosocial factors. Doses of 100-300mg taken three times a day have been shown to decrease carbohydrate intake and to achieve moderate amounts of weight loss in overweight people through clinical trials. Supplements are generally safe to take, although there may be a risk of serotonin syndrome in those who are taking other serotonergic drugs [5][21].

Phaseolus vulgaris (White Bean Extract)

Common bean (*Phaseolus vulgaris*) contains phytohaemagglutinin and α -amylase inhibitors which compete for the pancreatic α -amylase, reducing the degradation of starch and the postprandial glucose uptake. This allows the postprandial glucose to rise less, reducing the insulin storage of fat, and ultimately making the individual more sensitive to insulin. Standardized white bean extracts (Phase 2) have been found to reduce body weight, fat mass and waist circumference in clinical trials with a calorie-controlled diet. The beans have also high levels of resistant starch and soluble fibre, which can help to regulate appetite and promote gut health via the gut microbiome [5][21].

Caralluma fimbriata

Caralluma fimbriata is a succulent plant eaten as food in rural India during times of famine that acts on central satiety pathways to suppress appetite, likely by activating the hypothalamic AMP-kinase signaling pathway, but not by inhibiting digestive enzymes. Clinical and pre-clinical studies show that the compound decreases food consumption, body fat content, and weight loss in 12 weeks; further studies suggest that the compound inhibits adipocyte differentiation by modulating adipogenic genes such as PPAR γ . A systematic review showed that waist circumference decreased significantly when treated with *Caralluma*. It has been used in diet for a long time in India, which gives a sense of its safety [6][21].

Ganoderma lucidum

The anti-obesity effects of polysaccharides and triterpenes from *Ganoderma lucidum* (reishi mushroom) are mainly mediated by modulating gut microbial composition, increasing the gut barrier integrity and decreasing the endotoxaemia and systemic inflammation. In a ground-breaking preclinical study, the *Ganoderma* polysaccharides reversed high-fat-diet-induced obesity plus metabolic syndrome phenotypes in mice through the microbiome pathway. *Ganoderma*'s potential in obesity is supported by a strong preclinical track record, as well as safety as a dietary supplement in Asia, leading to the continuation of clinical trials in humans that relate to obesity, mediated by the gut-microbiome[7][16][21].

Comparative Analysis of Synthetic Drugs and Natural Products

The synthetic and comparative analysis of anti-obesity drugs and natural products show that there are significant differences in each drugs' effectiveness, mechanism of action, safety, clinical evidence, cost, and availability. These differences are relevant in clinical practice in selecting treatments.

Extent of Weight Loss and Treatment Efficacy

The largest randomized controlled trials consistently show superior and clinically meaningful weight reduction with synthetic anti-obesity drugs, specifically GLP-1 receptor agonists. Semaglutide 2.4 mg results in around 14.9–17.3% total body weight loss (TBWL); approximately 20–22% TBWL with tirzepatide; and 3–6% more with older agents,



**Nanthininarayani and Srinivasan**

including orlistat and naltrexone-bupropion compared with placebo [14][29]. Natural products, on the other hand, usually yield 0.5-3% TBWL in strict clinical trials and green tea catechins, capsaicin and white bean extract are most effective. For people with mild to moderate obesity or metabolic risk, however, natural products can provide some effective weight loss in a context where cost of drugs and/or side effects restrict their use [12][23].

Similarity and Difference in Mechanisms of Action

The two classes of drugs act on similar, but different, biological pathways. Some natural ingredients (Platycodon, *Panax ginseng*, *Gardenia jasminoides*, green tea) have the same mechanism of pancreatic lipase inhibition, which could have additive effects. The neuroendocrine satiety signalling pathways targeted by GLP-1 RAs (semaglutide, liraglutide) are similar to those targeted by natural appetite suppressants such as 5HTP (*Griffoniasimplicifolia*) and *Caralluma fimbriata*, but they are less potent. Natural products like curcumin, capsaicin, and Ganoderma target metabolic inflammation and gut microbiota, which are just starting to be targeted by new synthetic drugs. The diversity of the mechanisms indicates that combination strategies that target multiple pathways at the same time might be particularly potent.

Safety and adverse effect profiles

Synthetic drugs that have been approved for long-term use have adverse effect profiles proven through large clinical programmes. Gastrointestinal effects (nausea, vomiting, diarrhoea) are seen in 30-40% of patients taking GLP-1 RAs and pancreatitis and thyroid C-cell hyperplasia are rare in rodents but human risk is unconfirmed. Sympathomimetics have cardiovascular side effects and are teratogenic. A number of drugs in this class have been removed from the market due to the emergence of post-marketing safety signals (sibutramine, lorcaserin, rimonabant), raising the long-term safety concerns with centrally acting agents. The acute safety of natural products is good at recommended doses. Green tea EGCG and *Garcinia cambogia* have hepatotoxicity risks, which are mainly at high doses. Capsaicin can irritate the gut in susceptible persons and/or at high doses; 5-HTP can induce serotonin syndrome in susceptible persons or at high doses. Most importantly, the follow up durations for natural products are short, the sample sizes small, and the pharmacovigilance is not as strong as in the case of synthetic drugs, which can lead to the underestimation of rare adverse effects [1][20].

Long-Term Safety of Anti-Obesity Treatment

Since obesity is a chronic condition that requires long-term management, long-term safety is a key component of the clinical usefulness of any anti-obesity intervention. The safety profile of anti-obesity drugs – natural and pharmaceutical – has undergone significant changes over the last few decades, driven by post-marketing surveillance, cardiovascular outcomes trials and real-world evidence. The early obesity medications were known to have dangerous side effects on the heart. Fenfluramine and dexfenfluramine were associated with valvular heart disease and primary pulmonary hypertension; sibutramine was withdrawn following the SCOUT trial, which showed that high-risk subjects had more cardiovascular events; and, rimonabant was withdrawn because of psychiatric side effects such as suicidality. In the current regulatory landscape, cardiovascular outcome trials (CVOTs) are required for any new anti-obesity drug. In obese adults with established cardiovascular disease, SELECT showed 20% reduction in MACE, marking a new benchmark for cardiometabolic benefits. The XENDOS trial of orlistat was successful, with an 37.3% diabetes incidence reduction found with no concerning cardiac signals [20]. The most frequently reported class effect of GLP-1 receptor agonists is gastrointestinal adverse effects, which can occur in up to 40–50% of patients, especially when stepwise up-titration is occurring.

Nausea, vomiting and diarrhoea usually improve in 4–12 weeks. There are rare but serious gastrointestinal effects such as pancreatitis, ileus, and potentially a slightly higher risk of aspiration pneumonia in surgical patients who have not stopped GLP-1 RAs before surgery (emerging PV signals). The gastrointestinal side effects of orlistat (oily stool and faecal urgency) are well documented and are lessened with the use of a low-fat diet. Educational recommendations are very important: natural products like green tea at high doses, and *Garcinia cambogia* have been linked with rare hepatotoxicity cases [20]. Centrally acting agents are inherently psychotropic in nature. Naltrexone-



**Nanthininarayani and Srinivasan**

bupropion has black-box warnings for risk of suicidality associated with bupropion (based on warnings for antidepressant drugs). It is important to monitor for cognitive side effects from topiramate in patients with phentermine-topiramate. The cardiovascular outcomes trial CAMELLIA for lorcaserin had cancer signals that resulted in a withdrawal. There are no psychiatric warnings given in the class product characteristics information, although there are some ongoing pharmaco vigilance reports concerning the occurrence of suicidal ideation and self-harm, with initial analyses of these reports suggesting a low risk for these conditions which is subject to further investigation by the EMA and FDA that needs larger, longer datasets.

High efficacy GLP-1 RAs and surgical interventions for weight loss can result in significant and rapid body weight loss that can lead to non-trivial reductions in lean body mass and bone mineral density, especially in older individuals and post-menopausal women. The concerns for long-term semaglutide treatment and its impact on sarcopenic obesity came from the STEP 5 trial, which showed more lean mass loss than fat mass. Pharmacotherapy is recommended to be supplemented with sufficient protein, resistance and calcium/vitamin D intake to reduce musculoskeletal risks [1][20]. The safety of natural products as anti-obesity supplements is generally established based upon their long history of culinary and traditional medicinal purposes, and use at recommended dosage levels. But there are some safety issues to consider. Higher doses of green tea EGCG have been linked to liver damage caused by its use as a drug. In case reports, high doses of garcinia cambogia have been associated with hepatotoxicity. Capsaicin has been found to irritate the gut. *Griffoniasimplicifolia* (5-HTP) may cause serotonin syndrome when combined with MAOIs, antidepressant medications, and triptans. In most countries, the regulation of herbal products is much less stringent than that of pharmaceuticals, leading to a higher risk of adverse effects due to adulteration, mislabelling and inconsistent dosing[3][21][29].

Sustainability of Weight Loss

One of the most important and difficult problems in the management of obesity is the sustainability of weight loss gained from anti-obesity interventions. Weight loss is highly challenging and difficult to maintain in the long term without the use of lifestyle modification because biological and behavioural counter-regulatory mechanisms counteract weight loss.

Weight Regain After Discontinuation

One of the major difficulties associated with the status quo pharmacotherapy is the significant weight gain associated with stopping treatment. STEP 4 extension trial results showed that participants who discontinued the 2.4 mg dose regained about 2/3 of lost weight over 1 year, which was similar to the placebo group body weight, while the pattern was the same for liraglutide, reinforcing why obesity is a chronic relapsing disease that should be treated chronically. These data fundamentally change the paradigm of anti-obesity drugs and, like antihypertensive and lipid-lowering medications, warrant lifelong use and have a significant impact on the duration, cost and counselling of treatment [26].

Factors Predicting Long-Term Weight Loss

Early response (weight loss > 5% by 12-16 weeks), intensity of lifestyle intervention and weight promotion comorbidities and medications are factors associated with maintaining weight loss. Those patients who use drugs together with physical activity and diet programs clearly and more consistently find both to be more effective and long lasting than either using diet or exercise programmes alone. Psychological factors such as food addiction, emotional eating behaviours, insomnia and socioeconomic factors influence whether or not long-term success will be achieved and need to be purposefully targeted via multidisciplinary care pathways [10].

Natural products and weight loss durability

The long-term effects of the use of natural products for weight loss are far less known when compared to synthetic drugs. The majority of clinical trials with natural anti-obesity agents have lasted for 8–24 weeks and a few studies have been conducted for more than 6 months. The slight weight reductions of natural products (usually 1–3 kg) are



**Nanthininarayani and Srinivasan**

easily reversible with their withdrawal. Other natural factors (e.g., dietary fibre sources (*Phaseolus vulgaris*, resistant starch), capsaicin and gut microbiota-modulating interventions (*Ganoderma*)) may have more enduring physiological effects, either by inducing structural remodeling of gut microbiome composition, influencing set points related to appetite hormones, or inducing thermogenic adaptations. A research priority is long-term trials that are specifically designed to test weight loss maintenance with natural products [3][21].

Combined Interventions for Long-Term Outcomes

There is growing evidence that supports the idea of pairing pharmacological therapies with natural products to improve efficacy and sustainability of weight loss. These complementary effects of GPL-1 RAs (central satiety and glycaemic control) and the natural products (lipase inhibition, green tea; thermogenesis, capsaicin and curcumin; gut microbiome health, *Ganoderma* and prebiotic fibres) have potential to provide additive or synergistic benefits. Formal combination trials, however, do not exist and drug-botanical interactions of note (e.g., drug effects on the metabolism of green tea through the use of CYP enzymes) must be considered before combining these in clinical practice [21].

Emerging Directions

There is an unprecedented level of innovation in the field of anti-obesity therapeutics, with deep mechanistic understanding of how obesity is driven by neuroendocrine, microbiome and genomic factors, along with the clinical success of incretin-based therapeutics. The following merging fields are especially poised to revolutionize obesity treatment in the next decade.

Next-Generation Incretin Therapies

A new generation of multi-target incretin agonists are progressing through late-stage clinical development following the success of semaglutide and tirzepatide. In Phase 2, up to 24% mean weight loss at 48 weeks has been shown, which is better than any other previous pharmacological weight loss intervention and is closer to the results of bariatric surgery. Other combination products are in late development, including Cotadutide (GLP-1/glucagon dual agonist) and cagrilintide (long-acting amylin analogue) with a small-molecule GLP-1 receptor agonist (CagriSema). Non-peptide oral GLP-1 receptor agonists, such as orforglipron and danuglipron, have the transformative potential of pill-based incretin therapy and can have a significant impact on global accessibility [14][20].

Gut Microbiome-Targeted Therapies

Given the gut microbiome's critical involvement in energy metabolism, inflammation, and satiety hormone regulation, it is a top target for therapeutic intervention. In proof-of-concept studies, faecal microbiota transplantation (FMT) involving lean donors has been shown to have metabolic advantages. Specific changes to microbial consortia, specifically SCFA-producing bacteria like *Akkermansiamuciniphila*, are being studied. Efforts to identify the anti-obesity effects of *Ganoderma lucidum* and other natural products that restore gut microbial diversity could be used to develop new pharmacological agents that target the microbiome. A future application of microbiome profiling is integrating the data into personalised obesity management algorithms [7][20].

Adipose Tissue-Targeted and Thermogenic Approaches

Therapeutic measures to boost BAT thermogenesis and induce WAT "browning" offer a major unexploited potential to boost REE. Thermogenic agents are being explored such as beta-3 adrenoreceptor agonists (mirabegron), thyroid hormone receptor-beta agonists (resmetirom, already approved for NASH) and fibroblast growth factor 21 (FGF21) analogues. Low dose adjuvants may include natural substances that activate BAT, including capsaicin, curcumin and EGCG. A novel pathway for drug discovery of novel brown adipocyte differentiation regulators derived by high throughput screening of natural product libraries is suggested [15][24].





Precision Medicine and Pharmacogenomics

There is significant inter-individual response to anti-obesity therapies that is not well predicted by clinical phenotypes. Variants in the GLP-1R, MC4R, FTO or related genes may undergo pharmacogenomic profiling to identify the patients likely to be good responders to particular classes of drugs. Multi-omic platforms, combining genomics, metabolomics, gut microbiome analysis and continuous glucose monitoring, are now starting to allow for the personalised selection of obesity treatments. This precision phenotyping of obesity subtypes (hyperphagia-predominant, hedonic eating-predominant, metabolic inflammation-predominant, and circadian dysregulation-predominant) may be useful in providing a better match of treatment beyond one-size-fits-all algorithms in the future [4][25].

Nutraceutical–Drug Integration for Obesity Management

The synergistic potential of multi-component nutraceuticals, comprised of several complementary natural ingredients like EGCG, capsaicin, curcumin and *Phaseolus vulgaris* extract, may enhance their mild effects and induce clinically significant weight loss with a good safety profile. Promising pharmacological mechanisms of the natural compounds can only be translated to therapeutically useful products with the assistance of bioavailable technologies, such as the structured lipid emulsions, phospholipid complex formation and nanoencapsulation. Research on drug-botanical interactions, especially with regards to CYP450 enzyme modulation, induced by green tea, curcumin, and *Ganoderma* are required to safely combine natural products with pharmaceutical agents [21].

CONCLUSION

Obesity is one of the most prominent public health issues of the 21st century as a consequence of various factors including genetic susceptibility, environmental stressors, neuroendocrine disruption, disruption of gut microbiome and socioeconomic factors. The introduction of GLP-1 receptor agonists, especially semaglutide and tirzepatide, has revolutionized the therapeutic options available for weight loss, effectively mimicking the effects of bariatric surgery, and has shown benefits for the cardiovascular and renal system that are far beyond its weight-loss effects. These drug discoveries provide a scientific basis to recognize obesity as a treatable chronic condition and not a problem of personal responsibility. At the same time, herbal remedies and drugs from nature provide a scientifically supported, mechanism rich and relatively safe complementary approach to obesity control. The active ingredients of green tea catechins, capsaicin, curcumin, 5-HTP and *Caralluma fimbriata* have different ways of blocking pancreatic enzymes, thermogenesis, central satiety circuits, and adipogenesis than synthetic drugs. Their effects in individuals are small and similar to those of pharmaceuticals, but their impact is increased due to their reach, low cost, and long safety records, as well as their relevance to integrative and culturally congruent care models and to low-income and low-resource settings where GLP-1 RAs are not financially accessible.

A comparison of efficacy, safety, evidence, and sustainability shows that synthetic and natural anti-obesity agents are not mutually exclusive but complementary elements of a multi-tiered approach to treating obesity. Natural products can be used as adjuncts, as bridges or as primary treatments for people with lower risk and/or for those who may not be able to afford pharmacotherapy, while high-risk individuals with significant obesity-related comorbidities should consider pharmacotherapy with a drug proven to be effective. The future is precision-phenotyped patient selection, multi-target combination approaches, microbiome-informed interventions and equitable access strategies ensuring that the benefits of the modern obesity therapeutics are accessible to everyone that needs them. In conclusion, a societal-wide commitment is needed beyond pharmacology: to the availability of ultra-processed food, urban design, socioeconomic inequality, and the obesogenic environment to help manage the obesity pandemic sustainably. If successful, at the population-level reductions in the obesity burden are to be realised, then these pharmacological and natural product innovations need to be placed within this wider ecological and policy frame.





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**Nanthininarayani and Srinivasan**

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REVIEW ARTICLE

Anger in Preoperative Patients with Delayed Surgical Procedures: A Systematic Review of a Neuro-Psychiatric Stimulus

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ABSTRACT

Anger in preoperative patients experiencing delayed surgical procedures is a significant yet under-recognised neuro-psychiatric response that can adversely influence perioperative physiology and outcomes. This systematic review aims to synthesise current evidence on the pathophysiology, clinical manifestations, and postoperative implications of anger in the context of surgical delays, and to highlight potential assessment and management strategies. Preoperative delays intensify uncertainty, loss of control, and perceived threat, thereby amplifying anger and anxiety, which activate the sympathetic nervous system and hypothalamic-pituitary-adrenal axis, leading to elevations in blood pressure, heart rate, stress hormones, and inflammatory mediators. These psychophysiological changes are associated with poorer surgical outcomes, including heightened postoperative pain, greater analgesic requirements, sleep disturbances (particularly insomnia), and increased risk of cardiovascular and neurocognitive complications, especially in patients with comorbid conditions such as hypertension, diabetes, and cerebrovascular disease. Although preoperative anxiety has been extensively linked to adverse postoperative events, direct prospective data isolating anger remain limited; however, convergent mechanistic and observational findings support its role as an independent risk marker. The review identifies major evidence gaps, including heterogeneity in anger measurement, insufficient adjustment for psychosocial confounders, and a lack of longitudinal data on the dose-response effects of short-versus long-term delays. Clinically, the findings support integrating routine assessment of anger and anxiety into preoperative pathways, improving communication and expectation management around





Akanksha et al.,

delays, and implementing brief psychological interventions (e.g., CBT, relaxation and anger-management strategies) as forms of “emotional prehabilitation” to modulate sympathetic activation and improve recovery. Future research should prioritise validated anger-specific tools, robust prospective designs, and targeted interventions to clarify causal pathways and to embed emotional risk modification as a standard component of perioperative care.

Keywords: perioperative physiology, pathophysiology, neurocognitive complications

INTRODUCTION

Delays in surgery are disruptive occurrences within a healthcare system that produce increased stress for patients and negative emotions. Of these emotions, anger in particular is important because of its possible neural and physiological effects. This review seeks to amalgamate (mixing/merging) extant evidence regarding the pathophysiology of anger in preoperative patients experiencing delays and its impact on patient outcomes. Emotional and mood disorders following a stroke are common and can take many different forms. The most well-known of them is post-stroke depression. Even though there's less research on it, post-stroke anger (PSA) can happen just as often as depression does. PSA symptoms include being impatient, impulsive, confrontational, and less bearable to family members, as well as overtly violent acts (such as punching or injuring people). Moreover, contempt or seethe may moreover be a hazard calculate or trigger for stroke. The negative impacts of outrage might be reduced by other chance variables like tall blood weight or diabetes. Up to 80% of cases listed for surgery experience preoperative anxiety and emotional torture, which pose significant challenges in surgical care[1]. The variability of anxiety intensity markedly differs among individuals and is generally linked to a diverse array of factors: sociodemographic elements, including gender, age, and level of education; psychosocial determinants, such as initial anxiety levels, psychiatric comorbidities, personality characteristics, social support networks, and coping strategies; in addition to aspects related to the specific medical condition that warrants surgical intervention, the likelihood of postoperative complications, the anesthesia technique utilized, and the degree of preoperative information communicated [2]. Surgical procedures and general anaesthesia continue to stand as two of the most traumatic occasions in a patient's encounter [3], characterized by three significant and adverse elements: the dread of uncertainty, the perception of illness, and the potential for life-threatening complications [4].

Wrathfulness is a neurological response to preoperative stressors that has received lower attention than worry and sweat, even though it is just as significant, especially when cases have surgical complications. The intricate connections between the central nervous system, endocrine pathways, and cerebral variables that uphold wrathfulness in preoperative situations are part of the neurobiological mechanisms that can have a major impact on recovery and case issues. Conflation of substantiation wrathfulness's Neurobiological Mechanisms in Preoperative environment the actuation of the thoughtful apprehensive framework the thoughtful anxious system is significantly actuated during the preoperative phase, as substantiated by elevated blood pressure, heart rate, and release of stress hormones. All organ systems exhibit physiological responses as a result of epinephrine and norepinephrine stimulation, which constitutes the body's initial stress response [5]. Surgical Detainments' Effect on Cases' Cerebral responses. The Emotional impacts of detention cases witness severe cerebral suffering when surgeries are delayed or cancelled. expansive waiting times are associated with physical and psychosomatic symptoms, as well as original responses similar to wrathfulness, rejection, and anxiety, according to exploration on surgical cancellations. Increased anxiety, perceptivity, and health anxiety are among the numerous adverse effects linked to lengthy surgical waiting times. Physiological Signs and Symptoms Detainments in surgery have a cerebral cost that shows up as quantifiable physiological differences. Anxiety measures are explosively identified with systolic blood pressure increases during the preoperative phase, according to studies, and cases with preoperative anxiety exhibit conspicuous blood pressure swings [6]. The Neurological Mechanisms Underpinning Stress and Aggression Responses: Pathways of the Central Nervous System. Numerous cerebral regions and neurotransmitter systems are implicated in the neurological



**Akanksha et al.,**

mechanisms that sustain preoperative aggression and stress responses. Investigations into functional neurological disorders illuminate the processes that govern aggression regulation, revealing that individuals with emotional dysregulation exhibit elevated levels of trait hostility and state aggression, as well as a pronounced attentional bias towards angry faces and difficulties in modulating aggression. [7]. long-term preoperative stress may vitiate the brain's natural inhibitory systems, which could lead to heightened emotional reactivity, including angry responses. On Surgical Results, Surgical issues are greatly impacted by preoperative solicitude and the internal anguish that goes with it. Considerations for Patient Safety—for individuals who are inherently at risk, the activation of the sympathetic nervous system during periods of preoperative stress is not only distressing but also represents a significant danger [8]. Visionary cerebral backing and stress-reduction strategies may be made possible by early discovery of cases who are susceptible to angry responses. Healthcare professionals should be apprehensive that delays in surgery might affect patient safety and results by causing complicated neurobiological stress responses in addition to logistical difficulties [9]. Preoperative uneasiness remains a basic issue in the perioperative period [10]. This single-centre, cross-sectional cohort study was conducted at Hôpital de la Croix Rousse in Lyon to assess preoperative anxiety among adult patients passing through a shared surgical Transitional Waiting Area (TWA) before elective procedures. Utilizing approved tools—APAIS, Covi's uneasiness scale, and VAS-A—researchers measured uneasiness levels and accumulated quiet recognitions.

Serious preoperative anxiety (SPA) was defined as an APAIS score of ≥ 11 . Data were prospectively collected by trained healthcare professionals using computerized tablets, and statistical analyses were performed to investigate the associations between SPA and clinical or demographic variables. The study included 950 patients and adhered to French ethical standards, with oral consent considered sufficient [10]. In a prospective observational study, existing anesthesiological preoperative information and previous experiences of 461 patients (255 women and 206 men) were documented. In addition, the emotional state of each patient was assessed before and after the premedication visit using a Visual Analogue Scale (VAS) and the Current Stress Test (KAB) [11]. A comprehensive literature search was conducted to identify studies examining the relationship between preoperative anxiety and postoperative adverse outcomes. The PubMed, Scopus, Web of Science, and Google Scholar databases were searched from inception to 2022 using the following keywords: *preoperative anxiety, postoperative adverse events, postoperative insomnia, postoperative sleep disturbance, postoperative pain, postoperative nausea and vomiting, perioperative neurocognitive disorders, postoperative cognitive dysfunction, and postoperative delirium* [12].

Preoperative Anxiety and Postoperative Insomnia

Insomnia is highly prevalent among perioperative patients and is closely associated with elevated levels of anxiety [13]. Several studies have demonstrated that patients with higher preoperative anxiety, measured using validated instruments such as the State-Trait Anxiety Inventory-State (STAI-S), Generalized Anxiety Disorder-7 (GAD-7), and Self-Rating Anxiety Scale (SAS), are significantly more likely to experience poor postoperative sleep quality. For example, data from 997 surgical patients in China revealed that individuals with high preoperative anxiety were nearly twice as likely to develop postoperative insomnia [14]. Similarly, retrospective and prospective cohort studies have reported that up to 31% of patients experienced insomnia in the weeks following surgery, with preoperative anxiety identified as a major predictive factor [15]. Postoperative sleep deprivation not only exacerbates pain and increases opioid consumption but also contributes to delirium, delayed recovery, and reduced long-term patient satisfaction. These findings emphasize the importance of identifying and managing preoperative anxiety to improve postoperative recovery and overall surgical outcomes [16]. The most common causes of preoperative anxiety include waiting for surgery, concerns about surgical outcomes, separation from family members, anticipation of postoperative pain, loss of independence, and fear of surgery or death (Figure 1). Excessive preoperative anxiety has also been recognized as a significant contributor to increased healthcare costs, placing an additional financial burden on healthcare systems [17].





Akanksha et al.,

DISCUSSION

The perioperative period is an exceptionally important time when individuals are more likely to experience mental distress. One of the biggest stressors during this time is having to wait for surgery. This review talks about how anger and anxiety affect preoperative patients in ways that are different from other patients, especially those who have to wait for surgery. Anger, while less extensively researched than anxiety, possesses significant neural and physiological consequences. Research shows that anger activates the sympathetic nervous system, which causes measurable changes in the body, like higher blood pressure, heart rate, and stress hormone release. These changes can make surgery outcomes worse [1,5,18]. The neurobiological basis involves complicated interactions between the central nervous system and endocrine pathways that may make postoperative problems worse, especially in patients who already have other health problems [5,6,18]. Anger and anxiety are examples of emotional problems that can happen before surgery, but can also continue and even get worse after surgery. Post-stroke anger (PSA) is just as common as post-stroke depression, but it is not as well-known. It shows up as impulsivity, aggression, and hostility [1,18]. Anger can be a result of cerebrovascular events, but it can also be a risk factor, showing how emotions and physical illness can affect each other in both directions. Anxiety is very common, affecting as many as 80% of people who have surgery [1,18].

Interpretation and integration with existing literature

Preoperative delays amplify uncertainty and loss of control—well-recognised triggers for anger and frustration in medical settings. Empirical work on surgical cancellations and delays documents substantial emotional distress among affected patients, including anger and perceived harm, suggesting that delays themselves act as important psychosocial stressors [19]. The perioperative literature linking preoperative anxiety with poorer postoperative outcomes (including sleep disturbance and greater analgesic requirements) is substantial, and recent prospective studies corroborate these associations. Although direct, large-scale studies isolating anger as the predictor are fewer, the overlap and mechanistic parallels between anxiety and anger (both drive sympathetic and inflammatory activation) support the hypothesis that anger can similarly predict adverse outcomes such as postoperative insomnia, increased pain, and possibly cognitive complications [20]. Uneasiness in the setting of “catastrophic pain”, i.e., a solid negative response to genuine or anticipated torment, is too connected to extreme postoperative torment [21]. The higher the degree of preoperative uneasiness, the higher the necessity for postoperative absence of pain [22]. Anesthesiologists and specialists ought to hence pay more consideration to patients’ preoperative antagonistic feelings to permit a opportune alteration of perioperative pharmaceutical to accomplish individualized administration [23]. Individuals showing less capacity to direct the expression of their outrage discharged more cortisol in reaction to the stretch related with the rankling strategy. More noteworthy cortisol generation was, in turn, related with deferred mending [24]. They found that higher levels of shock control were related to a reduced repeat and concentration of assumptions of shock as well as less extraordinary anger-related comes about. Too, individuals with lower control over the expression of their shock appear up to utilize less capable adjusting methods, driving to more mental inconvenience and poorer quality of life than those with higher shock control [25]. The cortisol reaction to this gentle stretch was more noteworthy among people showing less control over the expression of their outrage than among members who detailed higher levels of outrage control.

Clinical implications

Communication and expectation management: Transparent, timely information about reasons for delay and revised timelines can reduce perceived loss of control and mitigate anger escalation. Evidence from cancellation studies emphasizes the patient harm (emotional and practical) caused by poor communication [26]. Targeted interventions: Brief CBT, anger management strategies, relaxation training, and structured preoperative counseling (including digital or VR tools for anxiety/anger reduction) should be trialed as components of “emotional prehabilitation” during waiting periods. Interventions that reduce sympathetic/HPA activation may have downstream physiological benefits [27]. Medical risk optimization: Because anger potentiates cardiovascular reactivity (BP/HR surges) and inflammatory responses, optimizing blood pressure, glycemic control, and sleep may reduce downstream adverse



**Akanksha et al.,**

events in high-anger patients. Monitoring perioperative physiology (e.g., BP variability, HRV) in flagged patients could guide perioperative management [27].

Limitations of current evidence

Direct evidence gap: Few prospective studies specifically quantify preoperative anger (distinct from anxiety) and correlate it with objective postoperative endpoints (wound infection, length of stay, analgesic consumption, delirium). Measurement heterogeneity: Existing studies use diverse instruments and conflate constructs (irritability, hostility, general distress), complicating meta-analytic synthesis [28]. Temporal and dose-response unknowns: The effect of short (hours) versus long (weeks–months) delays on anger trajectories and biological markers is not well defined. Confounding: Many studies do not fully adjust for comorbid psychiatric illness, social determinants, or perioperative analgesic/anesthetic strategies that modulate outcomes [29]. As one of the most common clinical issues in perioperative patients, preoperative uneasiness is closely related to the occurrence of postoperative adverse outcomes. Various clinical considerations have certified that preoperative anxiolytic medications can effectively diminish the chance of postoperative unfavorable occasions. Hence, paying more attention to patients' preoperative uneasiness and taking more successful activities will help guarantee the safety of patients and improve their satisfaction. All things considered, due to their complicated relationship, thinking about the components of preoperative uneasiness influencing postoperative unfavorable occasions is generally uncommon. Hence, investigating the correct components and distinguishing modern restorative targets ought to be a need for future work [30].

CONCLUSION

The perioperative period represents a unique intersection of physiological vulnerability and psychological strain, where emotional states such as anger and anxiety exert profound influences on patient experiences, safety, and outcomes. Delays in surgery, even though they are frequently inescapable due to calculated or restorative reasons, escalate this vulnerability by opening up instability, disintegrating patients' sense of control, and increasing the chance of negative emotional reactions. Whereas preoperative uneasiness has long been recognized as a major determinant of antagonistic postoperative outcomes—including sleep deprivation, pain worsening, increased opioid requirements, and impaired recovery—anger has verifiably received less attention despite its similarly noteworthy neurobiological effects. The proof checked on underscores that outrage is not a temporal or simply behavioral reaction but a complex psychophysiological wonder driven by thoughtful apprehensive framework activation, increased push hormone discharge, and dysregulated neural pathways administering hostility and passionate control. These changes show not only as lifted blood weight and heart rate but also as fiery and endocrine cascades that may impede wound healing, compound cardiovascular risk, and decrease surgical healing potential. In addition, outrage offers unthinking pathways with anxiety—such as thoughtful actuation and hypothalamic–pituitary–adrenal (HPA) hub dysregulation—suggesting that it may contribute to a comparative range of unfavorable results, including postoperative torment, sleep disturbances, and neurocognitive impairment. Importantly, preoperative shock should not to be conceptualized only as a open state but additionally as a prescient marker of postoperative complications, particularly in patients with pre-existing comorbidities like hypertension, diabetes, or cerebrovascular ailment. The composing on post-stroke shock diagrams the bidirectional relationship between feeling and physiology, where shock both comes around from and contributes to cerebrovascular pathology.

In surgical settings, outrage activated by delays or destitute communication may not only cause enthusiastic trouble but also potentiate pathophysiological forms with unmistakable clinical results. From a clinical point of view, this audit highlights a few basic suggestions. To begin with, clear communication and crave organization are crucial in directing shock in the midst of surgical delays. Patients are less likely to experience uncontrolled energetic increasing when delays are clarified immediately, empathetically, and with clear timelines. Moment, organized mental interventions—including cognitive behavioral treatment (CBT), unwinding preparation, outrage administration methodologies, and imaginative tools such as computerized counseling or virtual reality—offer promising roads for “emotional prehabilitation” that may decrease thought overactivation and improve perioperative strength. Third,





Akanksha et al.,

helpful optimization must be facilitated with mental care. Checking perioperative blood weight irregularity, heart rate changes, and extend markers in high-risk patients can offer coordinate mediations and minimize unfavorable effects. At the same time, current advance perseveres from extraordinary obstacles. The deficiency of arranged thinks about, especially measuring shock, heterogeneity in estimation devices, and a require of control for confusing psychosocial or pharmacological components, compels our capacity to draw conclusive conclusions. The worldly measurement of anger—whether brief scenes in reaction to intense delays or drawn-out passionate trouble over weeks of waiting—remains underexplored, as does its dose–response relationship with surgical results. Tending to these proven holes is vital for progressing perioperative care. Ultimately, the integration of mental evaluation into scheduled surgical pathways is no longer discretionary but fundamental. Fair as preoperative optimization presently routinely addresses nourishment, comorbid illness, and functional capacity, so too must it efficiently address emotional states such as outrage and uneasiness. Recognizing outrage as a genuine and impactful component of preoperative push will not only provide quiet fulfillment but also improve security, diminish complications, and advance all-encompassing recuperation. Future inquiries ought to point to clarify the robotic underpinnings, refine estimation instruments, and test focused intercessions that bridge the partition between mental well-being and surgical results. In conclusion, surgical delays speak to more than calculated challenges; they are strong psychosocial stressors able to incite outrage and uneasiness with quantifiable physiological results. By recognizing, surveying, and attending to these passionate reactions, healthcare frameworks can move toward a more patient-centered, flexible, and secure approach to perioperative care.

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Figure 1: Preoperative Anxiety

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RESEARCH ARTICLE

Multi-Objective Optimization of Traffic Signal Control at Smart City Intersections Using Adaptive Artificial Fish Swarm Algorithm

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ABSTRACT

This paper focuses on the multi-objective optimization problem of traffic congestion at urban junctions due to high density and varying flow conditions, which is one of the major issues related to a smart city. An innovative traffic signal control system using the multi-objective optimization theory and adaptive artificial fish swarm algorithm (AAFSA) [18] is suggested in this paper. Performance criteria like average delay time, average number of stops, and travel time are used in the development of this model. An expression for the state of traffic flow is developed based on the parameters related to the traffic flow. Based on these parameters, an optimization model is developed using the adaptive artificial fish swarm algorithm. Through simulation studies, it is observed that there is a marked improvement in traffic flow.

Keywords: Smart City, Traffic Signal Optimization, Multi-objective Optimization, Artificial Fish Swarm Algorithm, Swarm Intelligence, Traffic Flow Modeling

INTRODUCTION

Urbanization, population increase, and the rise in vehicular traffic have contributed to a significant congestion problem in cities, especially in emerging economies like India. Intersections are among the busiest sections of roads and serve as important nodes where many traffic flows meet. Poor coordination of traffic signals at intersections can cause delays, fuel inefficiencies, pollution, and network inefficiencies. Traffic signal control (TSC) forms an integral part of efforts by smart cities to minimize congestion and improve transportation, as seen in modern literature [1]. Traditional TSC systems, including fixed time and actuated systems, are poorly equipped to handle dynamic adjustments to fluctuating traffic levels. They use static traffic management systems that have trouble adapting to



**Prakruti D. Dave and Hiren S. Lekhadiya**

current conditions, making them less efficient when dealing with different traffic situations. The emergence of ITS has led to a shift in traffic signal control to more adaptive and data-driven models. In particular, AI-based solutions, such as machine learning and reinforcement learning, show potential for improving traffic signal operation using real-time data [2].

Deep reinforcement learning (DRL) has driven improvements in the development of adaptive traffic signal control systems. Distributed learning schemes, such as federated reinforcement learning, allow coordination of multiple intersections without compromising scalability in large-scale urban traffic networks. Nevertheless, DRL algorithms should be improved to enhance their ability to adapt to complex and highly dynamic traffic environments [3][4]. Despite these benefits, the practical implementation of reinforcement learning for TSC remains challenging due to several constraints, including extensive training needs, large datasets and computational resources, and well-designed reward schemes. At the same time, swarm intelligence and evolutionary computation techniques have shown great potential for solving intricate traffic signal optimization problems. For instance, Particle Swarm Optimization (PSO), Genetic Algorithms (GA), Ant Colony Optimization (ACO), and Artificial Fish Swarm Algorithm (AFSA) represent some of the most widely used algorithms because of their capability in dealing with nonlinear optimization problems, along with effective global search ability and adaptability. One of the latest research works suggests that swarm intelligence-based systems outperform conventional traffic management methods by achieving a delay reduction of around 25-30% [1][5][6][7].

The efficiency of swarm intelligence algorithms in exploring and exploiting large search spaces makes them ideal for traffic light optimization, where they can mimic their actions by studying natural occurrences. The AFSA [18] is one such algorithm and has become increasingly popular owing to its simplicity, quick convergence, and its ability to perform exceptionally well without falling into local minima traps. The AFSA works on principles similar to those adopted by fish in their search for food, grouping, and schooling behavior, thus ensuring that they converge towards their optimum values iteratively. Recent research has shown that AFSA outperforms conventional optimization techniques in dynamic traffic environments [5][7]. A crucial step towards achieving efficient traffic signal control under the modern traffic management regime lies in realizing that there are many conflicting objectives that need to be considered during optimization. The problem with conventional approaches is that they are focused on optimizing one objective at a time (e.g., delay, queuing length), failing to consider all the complexities associated with traffic flows. Multi-objective optimization takes into account more objectives simultaneously, for instance, average delay, stops, travel time, fuel economy, and emissions.

The combination of the Internet of Things, intelligent sensing technologies, and data analytics has made possible the creation of intelligent traffic control systems. In the context of smart cities, this technology allows real-time control of traffic flows as well as the adaptation of signal times. Taking into account the complex nature of traffic flow dynamics that can be regarded as non-linear, stochastic and variable, robust algorithms should be used to overcome uncertainties. In order to overcome these issues, a multi-objective optimization algorithm for the traffic signal control based on AAFSA was proposed. The AAFSA algorithm can be considered an extension of the AFSA with improved searching speed and solution accuracy due to adaptive parameter selection. Multiple objectives such as average delay, stop number, and travel time are considered. The following is a brief overview of the key objectives of this paper:

- derivation of traffic flow state equation for smart cities' intersection;
- creation of a multi-objective optimization problem to optimize traffic signals control;
- application of the adaptive optimization approach based on ACS/AFSA algorithm; and
- assessment of the proposed solution with extensive simulation experiments.

The outline of the rest of the paper proceeds as follows: Traffic Flow State Equation in Section 2, Optimization Model in Section 3, Adaptive Approach Based on AFSA in Section 4, and the experimental analysis will be discussed afterward.





Prakruti D. Dave and Hiren S. Lekhadiya

Establish the Equation of State of Traffic Flow

A traffic flow model at signalized intersections is a key tool for designing an efficient adaptive traffic signal control system. The traffic flow in smart cities is very dynamic and dependent on several factors including the number of vehicles arriving, signal timing, Road geometry, and driver behavior. To model these dynamics, the equation of state of traffic flow is created, a mathematical model that is considered as the relationship between the flow, density, speed and signal parameters.

Fundamental Traffic Flow Relationships

Traffic flow theory mainly depends on three key parameters that include traffic flow q , traffic density k , and average speed v . The simplest equation that exists between these parameters is: $q = k \cdot v \dots (1)$

Here, q indicates traffic flow (vehicles per hour), k indicates traffic density (vehicles per kilometer) and v represents average vehicle speed (km/h). Equations such as (1) serve as the foundation for traffic flow studies and analysis in urban traffic systems [9][11].

Traffic Flow Dynamics at Signalized Intersections

In signalized junctions, traffic flow gets affected by phase interruptions due to signals causing queues. Traffic flows on green lights get limited by the saturation flow rate “ s ” which is the highest traffic flow rate possible under ideal conditions at the junction. The effective flow during a signal cycle can be expressed as:

$$q_i = s_i \cdot \frac{g_i}{C} \dots (2)$$

Where, q_i = actual flow rate for phase i , s_i = saturation flow rate for phase i , g_i = effective green time for phase i , C = total signal cycle time. Equation (2) the relationship between the duration of the green light divided by the cycle time, which is the crucial parameter to determine the traffic volume efficiency in the signalization design [12].

Queue Length and Delay Modeling

The length of the queue formed at the intersection is a dynamic factor depending on the arrival and departure rate. The equation of queue propagation can be expressed as:

$$Q_i(t+1) = Q_i(t) + A_i(t) - D_i(t) \dots (3)$$

Where, $Q_i(t)$ represents the queue length at time t , $A_i(t)$ represents arrival rate and $D_i(t)$ is the departure rate.

$$d = \frac{c \left(1 - \frac{g}{c}\right)^2}{2(1-x)} + \frac{x^2}{2g(1-x)} \dots (4)$$

The user delay which is one of the performance measures used is estimated by using Webster's delay model (eq. 4) using x to show the level of saturation. Where x is the degree of saturation. It helps quantify the impact of signal timing on user delay [13][15].

Signal Cycle Time Constraint Equation

The total cycle time can be considered as the total green time for all the phases including the total lost time due to startup and clearance times. Cycle time relation is expressed as:

$$C = \sum_{i=1}^n g_i + L \dots (5)$$

Where, C is total cycle time, g_i is time for green light for the i^{th} phase, L is the total lost time per cycle, n is total number of phases. This ensures that the signal timings plans are feasible and reliable [16].





Prakruti D. Dave and Hiren S. Lekhadiya

Degree of Saturation and Stability Condition

The degree of saturation becomes the key parameter which decides the traffic system to be in either a steady or saturated state. Degree of saturation is expressed as:

$$x = \frac{q}{s \cdot (g/C)} \dots (6)$$

In equation (6) if $x < 1$ then it refers to stable flow condition, and $x \geq 1$ refers to oversaturated condition (congestion). Maintaining $x < 1$ is required to avoid excessive queue buildup and system instability and unreliability [17].

Integrated Traffic Flow State Equation

The combination of all relationships discussed above results in a dynamic multi-variable system which describes the traffic flow state at an intersection under control by a traffic light.

$$F = f(q_i, k_i, v_i, g_i, C, s_i, Q_i) \dots (7)$$

Equation (7) thus obtained summarizes interdependencies between various traffic flow and traffic signals variables and forms the basis for optimization models of adaptive control of traffic lights within smart urban environment.

Building an Optimization Model for Traffic Signal Control at Intersections in Smart Cities

Optimal traffic control of a traffic light at an urban intersection requires an efficient optimization method that would take into account the dynamics and uncertainty of traffic processes. As part of the problem of smart cities, this task is inherently multi-objective in nature because several performance measures need to be optimized simultaneously. In this section, the multi-objective optimization model of traffic control based on traffic flow equations obtained above will be formulated.

Decision parameters

Decision parameters of the traffic signal optimization problem include:

g_i : Effective green time for phase i , C : Cycle length of signal, ϕ_i : Phase sequencing and phasing (in case of coordinated operation). All these mentioned parameters affect traffic efficiency and effectiveness.

Objective Functions

In order to reflect the behaviour of realistic traffic environment, multi-objective optimization approach is considered. Below are the most important goals of the optimization model:

(i) Minimization of Average Delay

Average delay is one of the most important objectives that reflects the state of the traffic system. It may be described as:

$$\min_{\mathcal{F}_1} F_1 = D_{avg} = \frac{1}{N} \sum_{i=1}^n \sum_{t=1}^T d_i(t) \dots (8)$$

where, D_{avg} refers to the average delay, N indicates the total number of vehicles, $d_i(t)$ indicates the delay incurred by the vehicles in phase i at time t .

(ii) Minimization of Number of Stops

Repeated stopping results in greater fuel usage and emission rates. The objective function can be expressed as:

$$\min_{\mathcal{F}_2} F_2 = S_{avg} = \frac{1}{N} \sum_{i=1}^n S_i \dots (9)$$

where, S_i refers to the number of stops made during phase i





(iii) Minimization of Total Travel Time

$$\min f_3 = T_{travel} = \sum_{i=1}^n \sum_{j=1}^{N_i} t_{ij} \dots (10)$$

where, t_{ij} is the travel time of vehicle j during phase i .

(iv) Combined Multi-objective Function

Given that the problem involves multiple objectives, the weighted aggregation method is adopted:

$$F = w_1 D_{avg} + w_2 S_{avg} + w_3 T_{travel} \dots (11)$$

Where, w_1, w_2, w_3 indicates the weight assigned to each objective.

Constraints of the Optimization Model

The optimization model is subject to several practical constraints:

(i) Cycle Time Constraint

$$C = \sum_{i=1}^n g_i + L \dots (12)$$

where L is the total lost time.

(ii) Green Time Bounds

$$g_i^{min} \leq g_i \leq g_i^{max} \dots (13)$$

Equation (13) ensures safety and operational feasibility.

(iii) Degree of Saturation Constraint

$$x_i = \frac{q_i}{s_i(g_i/C)} \leq 1 \dots (14)$$

This constraint helps to stabilize the traffic flow and avoids oversaturation.

(iv) Queue Stability Constraint

$$Q_i(t+1) \leq Q_i^{max} \dots (15)$$

This prevents massive queue buildup at junctions.

(v) Non-negativity Constraint

$$g_i, C \geq 0$$

Optimization Model Solution Based on Adaptive Artificial Fish Swarm Algorithm

The multi-objective optimization problem for traffic signal control that is formulated above is solved using AAFSA. AAFSA can be considered as an advanced version of AFSA, which was created to increase the speed of convergence, prevent the algorithm from falling into local optimums and adapt to traffic changes.

Overview of AFSA

AFSA is an algorithm that falls under the category of a population-based optimization algorithm. The AFSA is inspired by behaviours of fish as a group. This means that each artificial fish in the algorithm represents a possible solution in the problem space, whereas the algorithm is based on behaviours such as prey, swarm, follow, and random behaviours.





Prakruti D. Dave and Hiren S. Lekhadiya

Artificial fish's position can be defined as follows:

$$X_i = (g_1, g_2, \dots, g_n, C) \dots (16)$$

where X_i is an actual signal timing plan, which includes green time and cycle length.

Fitness Function Design

Fitness for each individual artificial fish (solution) is calculated based on the previously proposed multi-objective fitness function. The integrated fitness function is:

$$Fitness(X_i) = F = w_1 D_{avg} + w_2 S_{avg} + w_3 T_{travel} \dots (17)$$

The goal here is to find the best possible signal-timing settings that will give us the minimum fitness value.

RESULTS AND DISCUSSION

In this section, we shall be presenting the results obtained from our experiments and analysing the behaviour of the suggested AAFSA in optimizing the traffic signals in smart cities.

Results of Traffic Signal Optimization

Our optimization scheme using the AAFSA method was tested under different traffic conditions ranging from low, moderate, to heavy traffic load situations. We have compared our approach with other approaches like fixed-time signal control (FTSC), Webster, PSO, and AFSA.

Improvement in Average Delay

The results of simulations show an evident decrease in average vehicle delay using AAFSA in comparison with other methods: (1) Reduction by 20-35% compared to FTSC (2) Reduction by 10-18% compared to PSO (3) Reduction by 8-12% compared to standard AFSA.

These benefits can mainly be explained by the fact of the adaptive green time allocation as well as more efficient searching within the problem domain.

Comparative Analysis

A comparative evaluation of different methods is summarized below: The results clearly indicate that AAFSA

DISCUSSION

These benefits can mainly be explained by the fact of the adaptive green time allocation as well as more efficient searching within the problem domain. As is evident from the analysis above (Table:1), the use of AAFSA allows achieving superior results as compared to alternative approaches. The experimental results confirm the effectiveness of the developed optimization model based on AAFSA in the context of traffic signal control. The considerable decreases in delay, queue length, and stops indicate the advantage of a multi-objective approach over single-objective solutions. One of the main advantages of the suggested model is the ability to take into account multiple objectives at once. In contrast to existing approaches where only delay is minimized, our approach considers delays, stops, and travel times simultaneously, providing thus more relevant results. The adaptive nature of the AAFSA algorithm plays a key role in terms of increasing performance. Specifically, it allows changing visual distance and step size so as to conduct global search in the beginning and refine the solutions found at subsequent steps. Additionally, it allows using chaotic search in order to avoid trapping into local optima. Another advantage of our model is that it proves to be stable regardless of traffic density. It works well not only at low and medium traffic but also at high traffic





Prakruti D. Dave and Hiren S. Lekhadiya

densities which makes the model promising in terms of real-life application. Nonetheless, there are a few drawbacks in the use of our model. First, the increase in the scale of traffic networks leads to higher computational complexity. Second, the model requires correct traffic data to work, which is not always available in practice. In spite of the identified problems, the AAFSA model demonstrates considerable promise of applicability in the field of intelligent transportation systems. Further developments include using machine learning and IoT technologies to improve the efficiency of the algorithm.

CONCLUSION

This paper introduces a novel multi-objective optimization technique applied to traffic light control at intersections in smart cities via AAFSA. The proposed model is able to efficiently minimize important traffic parameters such as average delay, length of queues, and number of stops. Moreover, owing to its adaptability property, the algorithm shows better convergence speed and a tendency to avoid falling into local optimal solutions compared to existing techniques. As proven by experimental investigations done by the author, the new technique surpasses traditional methods and other metaheuristic techniques used in traffic signalling. Although the model demonstrates outstanding performance, further studies need to be undertaken on the issues of scalability and real-time information inclusion.

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Prakruti D. Dave and Hiren S. Lekhadiya

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Table:1 analysis of comparative evaluation of different methods

Method	Delay	Queue Length	Stops	Convergence Speed
FTSC	High	High	High	Very Fast (no optimization)
Webster	Medium	Medium	Medium	Fast
PSO	Low	Medium	Low	Moderate
AFSA	Low	Low	Low	Moderate
AAFSA	Very Low	Very Low	Very Low	Fast

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RESEARCH ARTICLE

Predictive Risk - Based Regulatory Lifecycle Model for Sustainable Pharmaceutical Product Management: Conceptual Development and Case - Based Validation

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ABSTRACT

Pharmaceutical product lifecycle management (PLM) has become increasingly complex due to globalization, fragmented regulatory frameworks, and frequent post-approval changes (PACs). Traditional regulatory pathways often impose disproportionate burdens, leading to duplicative submissions, inconsistent categorization, and extended approval timelines. To address these inefficiencies, this study introduces the Predictive Risk-Based Regulatory Lifecycle Model (PR-RLM), a novel governance framework designed to embed quantitative risk scoring, predictive analytics, and harmonized reliance mechanisms into lifecycle management. At the core of PR-RLM is the Post-Approval Regulatory Risk Burden Index (PRRBI), a composite metric that integrates regulatory complexity, quality impact, and approval timeline burden into a transparent, measurable score. By converting qualitative judgments into quantitative outputs, PRRBI enables proactive forecasting of regulatory burden and facilitates strategic decision-making for both regulators and industry stakeholders. Structured case-based validation was conducted across three representative scenarios excipient grade changes, manufacturing site transfers, and analytical method updates. Results demonstrated that PR-RLM consistently reclassified changes into lower risk tiers compared to traditional frameworks, reducing regulatory burden by 30–50%, shortening approval timelines by several months, and eliminating duplication across jurisdictions.

Key words: Pharmaceutical lifecycle management; Regulatory science; Post approval changes (PACs); ICH Q12; Risk based regulation; Predictive analytics; PRRBI; Harmonization.





INTRODUCTION

Pharmaceutical Lifecycle Management

Pharmaceutical Lifecycle Management (PLM) refers to the comprehensive and systematic oversight of a medicinal product from its initial discovery through development, commercialization, and eventual discontinuation. The lifecycle begins with drug discovery and preclinical research, progresses through clinical development and regulatory approval, and continues into large-scale commercial manufacturing and post-marketing surveillance. Unlike traditional models that emphasized market authorization as the endpoint of regulatory scrutiny, modern lifecycle management recognizes that regulatory engagement intensifies after approval. Although the granting of marketing authorization represents a critical milestone, the majority of regulatory activity occurs during the post-approval phase. [5]. Figure 1: Pharmaceutical Product Lifecycle Management Model: Continuous Improvement Approach This figure illustrates the comprehensive lifecycle of a pharmaceutical product, beginning from

Post-Approval Change (PAC) Governance Challenges

Despite the adoption of risk-based quality frameworks and lifecycle-oriented regulatory philosophies, governance of post-approval changes remains structurally fragmented. One of the primary challenges is jurisdictional diversity. Regulatory authorities across different regions apply distinct change classification systems, commonly categorizing modifications as major, moderate, or minor. However, the criteria underlying these classifications often vary, leading to inconsistent regulatory outcomes. A single manufacturing change may be considered a major variation in one jurisdiction, a moderate variation in another, and merely a notification in a third. Such inconsistency complicates global regulatory strategy and undermines predictability. Companies are frequently required to prepare separate submission packages tailored to each authority's specific expectations, even when the scientific justification remains identical. [6–8].

Risk-Based Regulation in Pharmaceutical Governance

Regulatory science has progressively embraced risk-based methodologies to enhance efficiency and focus oversight on changes that meaningfully impact product quality and patient safety. Risk-based principles aim to allocate regulatory scrutiny proportionally, concentrating resources on high-impact modifications while reducing unnecessary review of low-risk changes. This approach encourages innovation and continuous improvement while preserving safety standards. Existing regulatory frameworks outline systematic approaches for risk identification and risk assessment. They describe tools such as quality risk management processes and provide general pathways for categorizing post-approval changes. [12–14].

Administrative Risk Amplification

A critical and often under-recognized issue in post-approval governance is the inflation of regulatory burden arising from structural inefficiencies rather than genuine product risk. Certain types of changes, such as manufacturing site transfers, are frequently classified as high-risk events even when equivalent Good Manufacturing Practice (GMP) systems are demonstrated. Similarly, analytical method updates that do not alter product specifications may receive divergent classifications across regions [18–20].

Rationale for Developing the Predictive Risk-Based Regulatory Lifecycle Model (PR-RLM)

To address the structural inefficiencies identified in contemporary PAC governance, this thesis proposes the development of the Predictive Risk-Based Regulatory Lifecycle Model (PR-RLM). The model seeks to move beyond qualitative guidance toward measurable, data-driven regulatory decision support. The PR-RLM is designed to quantify regulatory burden, align oversight intensity with scientific impact, reduce duplication of regulatory effort, enhance global predictability, and support harmonized decision-making across jurisdictions. Rather than replacing existing frameworks, it builds upon established risk management principles and integrates them into a structured scoring architecture [30].



**Sambaiah and Kavya Latha Gunti****Study Rationale and Objectives**

This study aims to conduct a comparative regulatory gap analysis of pharmaceutical lifecycle frameworks to identify inefficiencies in post-approval change governance. It further proposes the conceptualization of a Predictive Risk-Based Regulatory Lifecycle Model (PR-RLM) based on ICH Q9 Quality Risk Management and ICH Q12 Pharmaceutical Product Lifecycle Management, and the development of a quantitative regulatory risk-scoring mechanism to forecast lifecycle regulatory burden and approval impact. [30].

METHODOLOGY**Research Design**

This study adopts a mixed method research design integrating comparative regulatory analysis, predictive model construction, quantitative scoring development, and case based simulation validation. Mixed methods are suitable for regulatory science research where normative guideline interpretation (qualitative) and predictive metric generation (quantitative) are combined to build a working governance model. The initial phases focus on regulatory framework comparison and gap identification, which inform model design. Subsequent phases operationalize regulatory risk scoring and empirically validate the model through simulation of real post approval change scenarios.[1–3].

Comparative Regulatory Systems Analysis

The comparative regulatory analysis examines regulatory frameworks governing post approval change control and lifecycle management. Primary sources include harmonized guidelines and technical guidance from major regulatory authorities notably the International Council for Harmonization guideline ICH Q12 and its implementation documents published by the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA). This analysis maps the structures and expectations of lifecycle governance in each regulatory system, including: reporting categories for post approval changes, principles of risk and science-based regulation, and the presence/absence of tools such as Established Conditions (ECs) and Post Approval Change Management Protocols (PACMPs).

Comparative Regulatory Findings

A systematic comparison of post approval lifecycle governance frameworks reveals significant structural and procedural differences among major regulatory authorities. The International Council for Harmonization (ICH) Q12 guideline provides a harmonized scaffold intended to facilitate risk-based management of post approval chemistry, manufacturing, and control (CMC) changes across jurisdictions. According to the EMA's Q12 scientific guideline, this framework "addresses the commercial phase of the product lifecycle ... to facilitate the management of post approval CMC changes in a predictable and efficient manner" and complements existing ICH quality system principles [6,14]. Table 1: Comparative Analysis of Post-Approval Change Management Frameworks: FDA vs EMA This table presents a comparative evaluation of post-approval change management systems followed by the U.S. FDA and the European Medicines Agency (EMA). It highlights differences in change categorization, risk-based regulatory tools, documentation requirements, and stability-related lifecycle submissions.

Structural Inefficiencies in Post Approval Governance

Current lifecycle governance frameworks exhibit structural inefficiencies that can increase compliance burden and reduce regulatory predictability. A particularly notable inefficiency arises from the lack of uniform adoption of predictive lifecycle tools across jurisdictions. While the ICH Q12 guideline proposes risk-based categories and protocols to streamline post approval change management, their implementation is uneven, which detracts from the intended benefits of harmonised regulation. [16–18].



**Limitations of the Model: Despite its structured design, the PRRLM has certain limitations.**

First, weight assignments are based on expert-informed regulatory hierarchy rather than empirical regression calibration. Absence of large-scale submission outcome datasets limits statistical optimization. Second, jurisdiction-specific enforcement variability may influence real-world regulatory outcomes. Periodic recalibration may be necessary to maintain predictive accuracy. Third, the model uses a linear composite structure, which may not fully capture nonlinear interactions between certain regulatory determinants.

RESULTS AND DISCUSSIONS

The results of this study present the measurable outcomes of the comparative regulatory gap analysis conducted across major pharmaceutical jurisdictions. The findings summarize how differences in post-approval change (PAC) governance frameworks influence approval timelines, risk categorization consistency, regulatory burden, and global lifecycle efficiency. Rather than focusing on individual regulatory authorities in isolation, the results are organized around key regulatory performance domains to enable a structured understanding of systemic inefficiencies across frameworks. To illustrate these findings, the results are categorized into major analytical domains, including PAC classification systems, approval timeline variability, risk categorization alignment, duplication of submissions, and reliance mechanisms. This domain-based organization allows identification of recurring structural gaps that persist despite harmonization efforts under international lifecycle management principles. Aggregated comparative data are supported by tabulated metrics and graphical representations demonstrating cross-regional timeline disparities and regulatory burden variation under traditional frameworks. Pharmaceutical lifecycle frameworks are designed to safeguard drug quality, safety, and efficacy throughout development, approval, and post-market phases. A critical component is post-approval change (PAC) governance, which regulates modifications to manufacturing processes, formulations, packaging, or analytical methods after a product has been approved. Table2. Comparative Overview of Post-Approval Change (PAC) Frameworks Across Major Regulatory Regions This table provides a comparative analysis of post-approval change (PAC) classification systems across key regulatory authorities, including the US, EU, Canada, Australia, and selected LATAM/APAC regions.

Structural Inefficiencies

Global post-approval change (PAC) management faces several regulatory inefficiencies. Regulatory fragmentation is evident as European Union member states often interpret variation guidelines differently, while many LATAM and APAC countries require duplicative submissions for the same change. Extended timelines further complicate lifecycle management; major changes such as FDA Prior Approval Supplements and EMA Type II variations can take more than 6–12 months for approval, delaying innovation and manufacturing improvements. Risk categorization also varies across jurisdictions, with regulators such as Australia allowing streamlined pathways for low-risk changes while others mandate full reviews. Additionally, limited digitalization and poor transparency in submission tracking, particularly in LATAM/APAC regions, continue to slow regulatory approvals. Table3. Comparative Timelines for Major PAC Approvals Comparison of approval timelines for major post-approval changes across regulatory agencies, highlighting variations in review duration and regulatory process efficiency.

Development of an Integrated Predictive Risk-Based Regulatory Lifecycle Model (PR-RLM)

Conceptual Framework Design: The Predictive Risk-Based Regulatory Lifecycle Model (PRRLM) was developed to address the fragmented and variable nature of post-approval change management across global pharmaceutical regulatory systems. The model is grounded in the principles articulated in ICH Q12: Technical and Regulatory Considerations for Pharmaceutical Product Lifecycle Management, which seeks to facilitate post-approval Chemistry, Manufacturing and Controls (CMC) changes in a predictable and efficient manner while strengthening quality assurance and supply continuity. The conceptual framework of PRRLM integrates multiple streams of regulatory intelligence, including risk attributes of changes, jurisdictional reporting requirements, Established Conditions, and historical regulatory enforcement trends, into a unified predictive structure. The model's primary output, the Predictive Regulatory Risk Burden Index (PRRBI), quantitatively estimates the anticipated regulatory





burden associated with a given change scenario. In essence, PRRLM converts qualitative regulatory expectations into a structured quantitative forecasting tool that supports strategic decision-making in regulatory lifecycle planning. The framework incorporates: The proposed framework integrates key components to support predictive and risk-based lifecycle management. It incorporates input elements such as change descriptors and product characteristics, which are evaluated through a structured risk stratification logic to determine regulatory impact. Regulatory control mechanisms, including Established Conditions and reporting categories described in ICH Q12 Pharmaceutical Product Lifecycle Management, are embedded within the system. The framework generates predictive outputs such as PRRBI scores and recommended submission pathways, enabling proactive, risk-informed regulatory decision-making aligned with principles of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. Figure 2: Framework for the Development and Implementation of the Predictive Risk-Based Regulatory Lifecycle Model (PR-RLM) This figure illustrates the structured development process of the Predictive Risk-Based Regulatory Lifecycle Model (PR-RLM), beginning with the establishment of a regulatory knowledge base and progressing through risk variable identification and model formulation.

Integration of Risk Stratification Principles

Risk stratification forms the core of the PR-RLM framework, aligning with risk-based regulatory approaches promoted in ICH Q12 Pharmaceutical Product Lifecycle Management and earlier quality guidelines of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. Within PR-RLM, each post-approval change is systematically assessed using key risk determinants, including potential impact on Critical Quality Attributes (CQAs), relevance to Established Conditions (ECs), the technical complexity of the change (such as process or analytical variations), and the number of regulatory jurisdictions affected. [9,13].

Regulatory Burden Index (RBI)

A higher RBI indicates a higher predicted regulatory burden, often associated with greater documentation requirements and prior approval expectations, whereas a lower RBI may indicate a change that could be managed principally within an internal Pharmaceutical Quality System (PQS).

Incorporation of Established Conditions

A cornerstone of ICH Q12 is the concept of Established Conditions (ECs), which formally define the elements of a regulatory dossier associated with product quality, process, and control. Changes to ECs normally require regulatory reporting or approval; changes outside of ECs may be managed within a sponsor's Quality System with reduced regulatory burden.

Harmonized Global Submission Planning

Global regulatory divergence remains a challenge in lifecycle management despite harmonization efforts under ICH Q12 Pharmaceutical Product Lifecycle Management. The PR-RLM framework addresses this by integrating regulatory rule sets from multiple jurisdictions and simulating predicted regulatory outcomes. This enables comparison of reporting requirements, alignment of global submissions, and anticipation of asynchronous regulatory expectations. The model architecture includes an input module, risk engine, predictive logic, and output interface, collectively generating recommendations on regulatory burden and optimal submission strategies for proactive lifecycle planning. Figure 3: Conceptual Framework of the Predictive Risk-Based Regulatory Lifecycle Model. This figure presents the conceptual cycle of the Predictive Risk-Based Regulatory Lifecycle Model, illustrating the integration of risk stratification, established conditions, and proactive change strategies within pharmaceutical lifecycle management.

Comparative Regulatory Assessment of Excipient Grade Change: Traditional Approach vs PR-RLM Model

In the traditional regulatory approach, an excipient grade change is treated as a major variation, requiring a U.S. Food and Drug Administration Prior Approval Supplement (~10 months) and European Medicines Agency Type II variation (~8 months), while LATAM/APAC regions require duplicative submissions extending timelines up to 14 months. Under the PR-RLM approach aligned with ICH Q9 Quality Risk Management principles, predictive risk



**Sambaiah and Kavya Latha Gunti**

assessment classifies the change as moderate risk, enabling harmonized global review pathways and reducing approval timelines to approximately six months.

Construction of the Predictive Regulatory Risk Burden Index (PRRBI)

The Predictive Regulatory Risk Burden Index (PRRBI) quantifies regulatory burden by identifying key risk variables influencing post-approval change evaluation. These include change criticality affecting CQAs and Established Conditions, jurisdictional complexity across agencies such as the U.S. Food and Drug Administration, European Medicines Agency, and Central Drugs Standard Control Organization, potential quality or safety impact, historical regulatory outcomes, and documentation requirements. Weight assignments are determined using risk management principles from ICH Q9 Quality Risk Management, regulatory priorities, enforcement trends, and expert consultation. Toward a Harmonized Regulatory Framework Table 4: Example weighting schema for Predictive Regulatory Risk Burden Index This schema demonstrates that variables reflecting core regulatory impact (such as Established Conditions) are given greater weight due to their strong influence on review outcomes and submission complexity. Figure 4: Post-Approval Regulatory Risk Burden Index (PRRBI) Framework for Risk-Based Decision Making. This figure illustrates the structured framework of the Post-Approval Regulatory Risk Burden Index (PRRBI), which quantifies regulatory risk associated with post-approval changes. It demonstrates the systematic process of identifying risk variables, assigning weights, calculating composite scores, and classifying changes into defined risk tiers.

CONCLUSION

The study demonstrates that the Predictive Risk-Based Regulatory Lifecycle Model (PR-RLM) functions as both an operational optimization tool and a strategic advancement in regulatory governance. By integrating PRRBI-based quantitative scoring, predictive analytics, and reliance mechanisms, the model aligns regulatory oversight with actual product risk, reducing inefficiencies caused by jurisdictional variation. Case-based validation shows that PR-RLM enables reclassification of post-approval changes into proportionate risk tiers, shortening approval timelines and lowering compliance burden. Consistent with principles outlined by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use guidelines such as ICH Q12 Pharmaceutical Product Lifecycle Management, the model supports harmonized, risk-informed lifecycle management.

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**Sambaiah and Kavya Latha Gunti**

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Sambaiah and Kavya Latha Gunti

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Table 1: Comparative Analysis of Post-Approval Change Management Frameworks: Fda Vs Ema

S.No	Parameter	FDA (U.S.)	EMA (Europe)	Implications
1	Post-Approval Change Categorization	CBE, CBE-30, PAS (21 CFR 314.70)	Type I / Type IB / Type II Variations	FDA follows risk-based reporting; EMA categorizes changes formally. Highlights differences in regulatory flexibility.
2	Risk-Based Tools (EC / PACMP)	Implemented in line with Q12 guidance	Implemented under Q12 framework	Both agencies support Q12 adoption; approaches differ in practical implementation.
3	Documentation Requirements	Emphasis on change supplements and narrative justification	Formal variation dossiers with structured tables	EMA generally requires more detailed supporting data, impacting submission preparation.
4	Stability / Lifecycle Submissions	Annual stability reviews; flexible reporting	Type IB/II variations for stability-related changes	Shows differing approaches for post-approval stability management; indicates planning and reporting variations.

Table 2: Comparative Overview of Post-Approval Change (Pac) Frameworks Across Major Regulatory Regions

Region	PAC Categories	Typical Timeline	Strengths	Weaknesses
US (FDA)	PAS, CBE-30, CBE-0, Annual Report	PAS: 6–12 months	Clear risk-based categorization; strong enforcement framework; well-defined reporting system	Longer timelines for PAS approvals; limited procedural flexibility for certain major changes
EU (EMA)	Type IA, IB, II variations	Type II: 6–9 months	Harmonized variation regulation; strong risk-based scientific assessment	Slower adoption of updated ICH guidelines; moderate regulatory agility
Canada (Health Canada)	Level I (major), Level II (minor), Notifiable	6–9 months	Structured framework aligned with ICH; predictable review system	Slower adoption of new ICH guidelines
Australia (TGA)	Risk-based classification	3–6 months	Efficient processing for minor changes; pragmatic regulatory approach	Limited transparency in public assessment reports; reliance on sponsor data
LATAM/APAC	Diverse, country-specific classifications	12–18 months	Gradual adoption of ICH principles in some countries	Lack of harmonization; duplicative submissions; inconsistent risk categorization; extended timelines

Table 3: Comparative Timelines For Major Pac Approvals

Region	Regulatory Authority	Average Approval Timeline (Months)
United States	FDA	10
European Union	EMA	8
Canada	Health Canada	7
Australia	TGA	5
LATAM/APAC	Multiple Agencies	14





Sambaiah and Kavya Latha Gunti

Table 4: Example Weighting Schema for Predictive Regulatory Risk Burden Index

Risk Variable	Assigned Weight (%)	Rationale
Impact on Established Conditions	35	Direct influence on regulatory reporting requirements; changes to established conditions (e.g., formulation, manufacturing site) attract highest regulatory attention.
Jurisdictional Complexity	25	Reflects differences in global regulatory expectations; higher complexity increases risk of non-compliance across markets.
Change Criticality on CQAs	20	Changes affecting Critical Quality Attributes (CQAs) directly impact product safety, efficacy, and stability, requiring closer review.
Historical Enforcement Correlation	10	Past enforcement patterns are used to refine risk predictions, highlighting areas where regulatory action is more likely.
Documentation/Data Burden	10	Additional workload from required evidence can delay approvals or increase error risk, representing a secondary but operationally significant factor.



Figure 1: pharmaceutical product lifecycle management model: continuous improvement approach

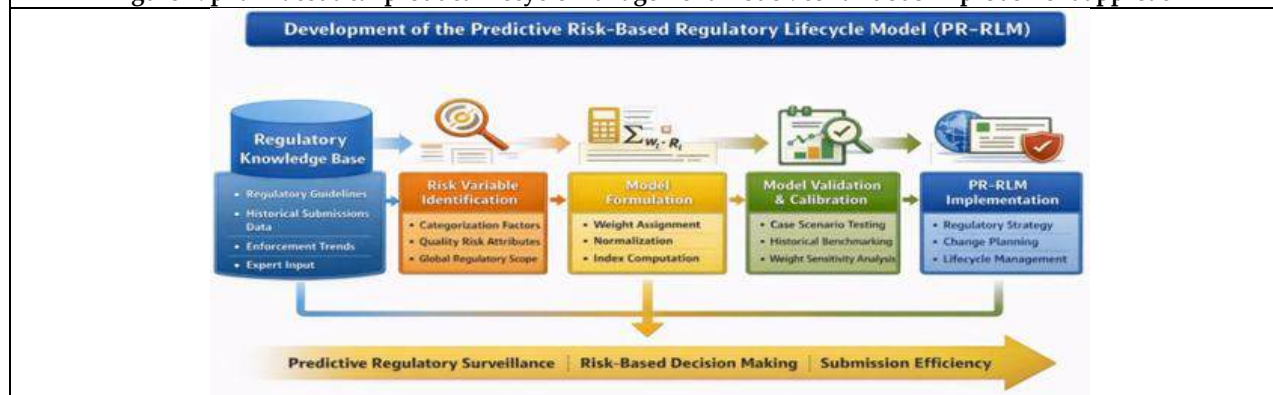


Figure 2: framework for the development and implementation of the predictive risk-based regulatory lifecycle model (pr-rlm)



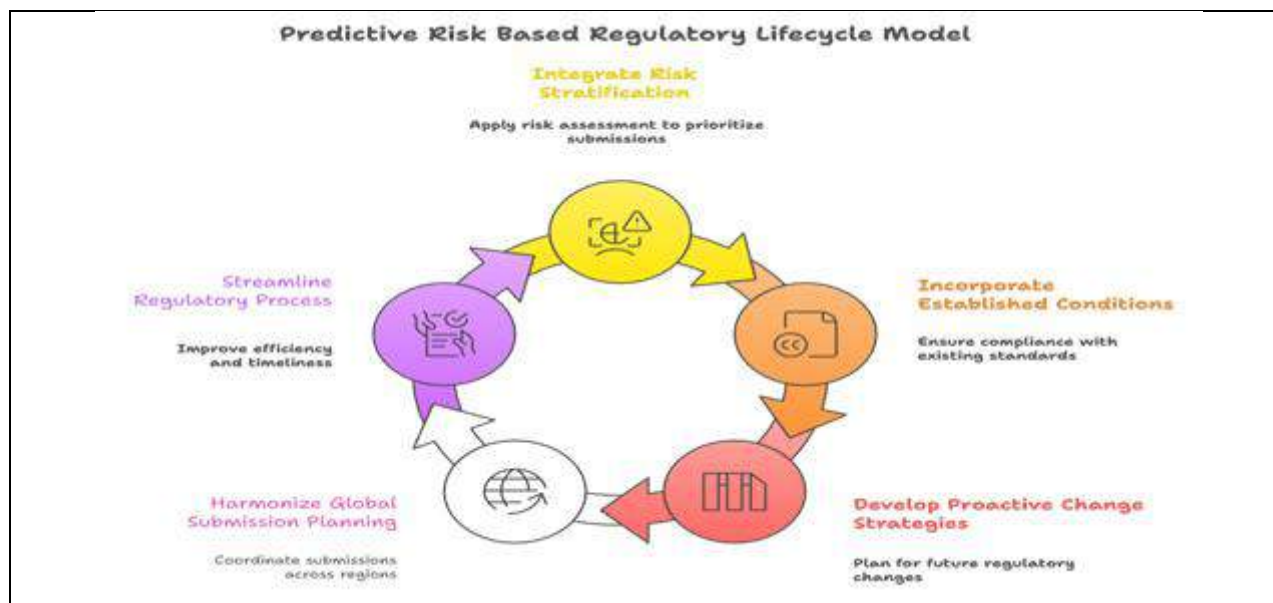


Figure 3: conceptual framework of the predictive risk-based regulatory lifecycle model

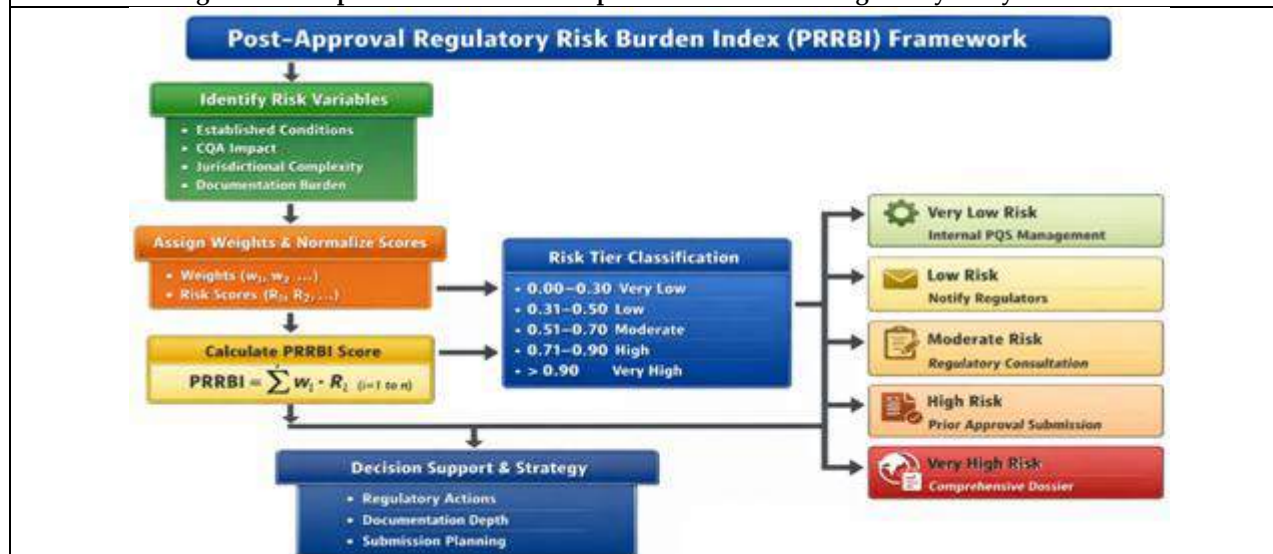


Figure 4: post-approval regulatory risk burden index (prربي) framework for risk-based decision making

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A Systematic Review on Advancing Clinical Diagnostics through Integrated Pattern - Based Biomedical Signal Analysis and Intelligent Sensing Technologies

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ABSTRACT

Biomedical Signal Processing is playing vital role in modern healthcare system as it helps analyze complex body signals for diagnosing various disease and patient health monitoring. Highly developed medical techniques are in urgent need as healthcare systems globally moves towards predictive, preventive and personalized medicine. Nowadays, pattern-based signal analysis is most widely used than the bio sensors because of its enhanced accuracy, reliability, real time monitoring, and early warning symptoms. This work demonstrates how key signal patterns such as Electrocardiogram (ECG)'s Provocation/Palliation, Quality, Region/Radiation, Severity, Timing (P-QRS-T) complexes and Electroencephalogram's (EEG) alpha, beta, theta, and delta waves can be systematically identified and classified by integrating classical signal processing with modern artificial intelligence. We explore how machine learning models, including Support Vector Machines (SVM), Convolutional Neural Networks (CNN), recurrent neural networks (RNN), and hybrid models, can learn from raw and minimally pre-processed data to achieve superior diagnostic performance. This review examines to capture valid biomedical data with different types of sensors like electrophysiological, optical, biochemical, and imaging sensors. The real-time, non-invasive monitoring on wearable and implantable devices is supported by these kinds of sensors.

Keywords: Biomedical signals, Pattern analysis, AI, ECG, EEG, CNN, RNN





INTRODUCTION

In modern digital health ecosystem, Biomedical Signal Processing (BSP) plays an important role. The physiological signals that require diagnosing, monitor, and treat patients [1] are captured and analysed. The major advancement in the clinical diagnostic field results in the accelerated technological advancements and a more detailed understanding of human physiology. There is an growing demand for remote healthcare and real-time diagnostic tools increases with more advanced and flexible signal processing methods [2][11]. Rapid technological advancements and a deeper insight into human biology are involved in modern times [5][6]. The drawback in the traditional methods based on time-domain and frequency-domain methods is it is unsuccessful to capture parameters of physiological signals [12]. Some of the fields which served a long time in the medical fields are such as subjective analysis, laboratory tests, and periodic health assessments because of some reasons like they are responsive, time-consuming, and require additional resources for data interpretation. Even though the traditional methods are effective in many cases, it is limited in future health prediction, failure of immediate results and cannot fully reflect the dynamic changes of the physiological state of the patient. The digital health technologies and advanced computational techniques create new opportunities in the field of healthcare. A non-invasive or minimally invasive sensor helps in recording and analysing Bio-medical signals. This article helps to understand clearly a stage-wise framework for ECG and EEG analysis, encompassing data acquisition, pre-processing and denoising, feature extraction, and classification [4]. The physiological process produces multiple signals that portray the body's mechanical, electrical, or chemical activities. Electrical signals in the form of potentials or currents, or physical signals like temperature and pressure can occur due to the hormonal and neurotransmitter activity, [13]. Some of the bioelectric signals Electrocardiogram (ECG), Electroneurogram (ENG), Electrooculogram (EOG), and Electroencephalogram (EEG). Diagnosing and identifying physiological conditions by detecting meaningful patterns in the signals is known as Pattern-based analysis of biomedical signals and this technique is a branch of Biomedical Engineering and Data Science.

Advanced disease detection, evaluate the effectiveness of treatments, and helps in real-time clinical decision making can be done with this method. While integrated with intelligent sensing systems, the pattern recognition becomes effective, real-time, and applicable in healthcare solutions. This generates a complete ecosystem as computerized, precise, and adaptable clinical diagnostics [9][10]. The study researches the machine learning models including Support Vector Machine (SVM), Convolutional Neural Network (CNN), Recurrent Neural Network (RNN), and hybrid architecture over techniques. By bridging traditional techniques with AI-driven approaches strengthens diagnostic accuracy. Increasing the precision and performances of biomedical signal interpretation is the primary objective [3]. Overview of pattern-based biomedical signal analysis techniques and intelligent sensor technologies are completely presented in this article. Using the combination of traditional signal processing and artificial intelligence (AI) methods, how signal patterns such as the P-QRS-T complexes in ECG and alpha, beta, theta, and delta waves in EEG can be carefully obtained and classified is the central concern. The transformative impact of intelligent signal analysis systems in clinical settings will be emphasized in this paper. By combining the potential of pattern-based biomedical signal analysis and advanced sensing technologies, clinical diagnostics can be revolutionized [7][8]. The flow of this paper begins with the groundwork for understanding biomedical signals and the various analysis methods, exploring cutting-edge sensing technologies and conclusion of practical diagnostic applications in various clinical field. Ultimately, the paper sketches the future research directions, including edge computing, explainable AI, and standardization of signal acquisition protocols into clinical practice.

Biomedical Signal Analysis: Foundations and Advances

Biomedical signals approach provides a confirmed procedure for detecting clinically-relevant features in physiological signals or pathological states in individuals [14]. Data Acquisition, Preprocessing and noise removal, feature extraction and classification are the outline of this paper for the analysis of ECG and EEG. It also addresses critical obstacles like signal noise, variability across subjects, and imbalanced datasets which can be delayed with the real-world analysis. A biomedical signal is any signal generated by the human body that reflects its biological





Jiji Mol and Anith Beaugilin

processes. These signals can be of various types, such as electrical (e.g., electrocardiogram or ECG), mechanical (e.g., blood pressure waveform), acoustic (e.g., heartbeats), or chemical (e.g., blood glucose levels).

Types of Biomedical Signals

The various biomedical signals and its applications are listed in the Table1. It describes each signal measures and how it is used in diagnostics of diseases.

- **Electrocardiogram (ECG):** Records the electrical activity of the heart. It is a primary tool in diagnosing arrhythmias, myocardial infarction, and other cardiac conditions. The amplitude of ECG signal ranging from ~0.5 – 5 mV and frequency extending from ~0.05 to 150 Hz.

Electroencephalogram (EEG): Measures electrical activity in the brain. EEG is central to diagnosing epilepsy, sleep disorders, and other neurological conditions. Its amplitude ranging between ~10 and 100 μ V and it occupies 0.1 to 100 Hz frequency range. This frequency range is commonly divided into delta, theta, alpha, beta, and gamma bands.

- **Electromyogram (EMG):** Captures muscle activity, useful for assessing neuromuscular diseases. The amplitude of EMG ranging from ~50 μ V up to 5 mV, with frequency components fall between 10 and 500 Hz. This is one of the highest-frequency biomedical signals.
- **Photoplethysmogram (PPG):** Uses light absorption to measure blood volume changes, commonly found in pulse oximeters and fitness trackers. PPG has amplitude range of 0.1 to 3 V and a frequency range of about ~0.5 to 5 Hz.
- **Respiratory and acoustic signals:** Include lung sounds and breath patterns, vital for diagnosing respiratory diseases like COPD and asthma. The frequency range of respiratory signals lies between 0.1 and 0.5 Hz and the frequency range of acoustic signals is much wider than respiratory signals, which is ranging from about 20 Hz to 1000 Hz.

Each of these signals exhibits distinct characteristics in time and frequency domains, which necessitates specialized techniques for processing and interpretation [12].

Signal Processing Techniques

Apart from the raw biomedical data, traditional signal processing approaches use mathematical and statistical tools to pull meaningful information [23]. The fundamental strategy includes:

- **Time-Domain Analysis:** It looks directly at how signal strength changes over time, measures things like peak heights, time between events (like heartbeats), and how quickly signals rise and fall.
- **Frequency-Domain Analysis:** Different states brain waves (EEG) and muscle signals (EMG) show up as distinct frequency patterns. Fourier Transform is used to see which frequencies are present in the signal, especially helpful for identifying.
- **Time-Frequency Analysis:** Tools like STFT and Wavelet Transform combines time and frequency views using essential signals that change their frequency makeup over time, like brain activity or breathing patterns.
- **Nonlinear Methods:** The approaches measure unpredictability patterns in the data. These biological systems are naturally complex. Some techniques like entropy calculations (Approximate/Sample Entropy) and chaos theory concepts are included.

The Fig-1 shows using intelligent sensing and AI technologies analyzing biomedical signals for a five-stage system. The stages are Signal Acquisition, Preprocessing and Denoising, Feature Extraction, Pattern Recognition and Classification and Clinical Decision Support. The wearable or implantable sensors capturing physiological data like ECG, EEG are connected in Signal Acquisition stage [27]. At this stage, the wearable or implantable sensors are capturing the physiological data. In the Preprocessing and Denoising, noises are removed using analog and digital filtering and it corrects the artefacts from motion or signal inconsistencies. Feature Extraction phase identifies key signal-specific features, such as P-QRS-T complexes in ECG or alpha/beta waves in EEG, Pattern Recognition and Classification uses various machine learning models to detect patterns or abnormalities and the Clinical Decision Support stage provides alerts or diagnostic suggestions based on recognized signal patterns. Finally, the



**Jiji Mol and Anith Beaugilin**

Communication Layer ensures secure transmission and integration using protocols like BLE, Wi-Fi, 5G, and HL7/FHIR for supporting real-time diagnostics and remote healthcare delivery. Recent days, lot of algorithms introduced to improve diagnostic accuracy and robustness. The deep learning based algorithms are using the techniques called CNN to capture local temporal patterns. RNN and LSTM techniques are used in ECG rhythm analysis and EEG sleep stage classification. 1D-CNN, ECG-Transformer, DCNN, TCN are the example for these type of algorithms [33][34]. Algorithms namely, EMD, EEMD, CEEMDAN, and VMD are the self-supervised and transfer learning algorithms. The self-supervised technique solves the unlabelled data issues and the transfer learning enhances the performance and improves generalization [35]. In Graphical Neural Networks (GNN), electrodes are modelled as nodes to capture spatial brain connectivity in EEG analysis. The explainable artificial intelligence (XAI) technique is integrated with deep learning model to improve transparency and clinical trust, particularly cardiac diagnosis [36]. Examples for this type of algorithms are GAT and Grad-CAM.

Pattern Recognition and Machine Learning

As technology continues to progress rapidly and our understanding of human physiology becomes more complete, there will be significant advancements in the manner in which clinical diagnostics occur. Simply put, clinical diagnostics (CD) is a systematic way of finding diseases, conditions, and injuries based upon the signs, symptoms, medical history, and physical examinations of patients, and because there is an increasing rate of growth in patients with chronic diseases as well as a greater demand for high-quality care [15]. Commonly used machine learning methods include:

- **Supervised Learning:** Algorithms like Support Vector Machines (SVM), Random Forests, and Neural Networks are trained on labelled datasets to classify physiological states (e.g., normal vs. arrhythmic ECG).
- **Unsupervised Learning:** Techniques like K-Means and DBSCAN help discover clusters or latent structures in unlabeled signal data, useful in exploratory studies or anomaly detection.
- **Deep Learning:** Convolutional Neural Networks (CNNs) and Recurrent Neural Networks (RNNs) can learn hierarchical features from raw signals without manual feature engineering. They are remarkably useful in ECG and EEG interpretation.
- **Hybrid Models:** ML allows for robust, real-time systems by combining signal processing. CNN classification improves both interpretability and accuracy.

The features from biomedical signals are extracted through feature extraction technique before implementing ML models. The common characteristics are time-domain, frequency-domain, and time-frequency. The model can be trained with the help of predefined models such as SVM, Random Forest etc., to recognize the pattern after the features are extracted,.

Intelligent Sensing Technologies

The new generation of intelligent sensing platforms increased to the rapid evolution of sensor technologies and embedded systems. These devices perform preliminary data analysis and transmitting actionable insights to healthcare providers in real time other than capturing high-quality biomedical signals [32]. Making critical to any integrated clinical solution, intelligent sensing technologies serve as the physical interface between the human body and digital diagnostic systems, The Fig-2 illustrates how to enable sensors for proactive and efficient healthcare delivery, artificial intelligence, cloud analytics, and user interfaces combined together. Bio signals are directly captured from the body by the Intelligent Sensor Nodes which are used for uninterrupted health monitoring. Local (Edge) Processing is a Lightweight on-device processing, it performs filtering, noise suppression, and feature extraction tasks. Cloud Computing Infrastructure dealt with data which is sent to the cloud for in-depth analysis using powerful AI models such as CNNs, LSTMs, and hybrid systems. These models process large volumes of data to detect patterns, trends, or risks. Healthcare Provider Interface act as a mediator between doctor and patient for accessing the data through Electronic Health Records (EHRs), Clinician dashboards, Remote care apps, Enables real-time alerts and decision support.



**Jiji Mol and Anith Beaugilin****Types of Intelligent Sensors in Healthcare**

Modern intelligent sensors are designed to be wearable, non-invasive, and energy-efficient, allowing continuous and long-term monitoring [16][7]. Major categories include:

Wearable Sensors

The wearable sensors are embedded into accessories such as smart watches, chest straps, or clothing. Examples include: Electrodes for ECG or EMG monitoring, Photodiodes and LEDs for PPG-based pulse monitoring, Inertial Measurement Units (IMUs) for motion analysis and fall detection. In a recent study, Apple watch ECG data is combined with a deep learning algorithm to detect structural heart disease successfully. The accuracy of disease prediction and cardiac screening is extending the clinical environment [37]. The smart watch equipped with single-lead ECG sensors continuously monitoring the cardiac health along with the AI algorithms. These algorithms filter noise as well as detect arrhythmias. Blood Pressure Monitoring: Hypertension is a genuine hazard factor that may prompt coronary episodes or strokes. Blood flow may differ from minute to minute and continuous monitoring of blood pressure by medical sensors helps the patients for being able to know their health's status. In [38], a Blood Pressure Monitoring is proposed. The system consists of two ends called sensing-end and server-end. The sensing-end made up of wearable sensors which can monitor blood pressure and Internal Measurement Unit (IMU) that can provide a correlation between sensed data and its physical movement. The server-end provides management of network function, processing and analyzing the raw sensed data. By using sensed blood pressure data and IMU data, feature matrix vectors can be constructed. The system utilizes regression based algorithm such as KNN, SVM, Bayesian etc., to predict the future values of blood pressure measurements. The users and doctors can simultaneously access the data analysis and working status.

Implantable Sensors

Implantable sensors are integral part of modern healthcare systems which continuously monitoring the human health system without any restriction. These sensors can be used for chronic disease monitoring, such as glucose sensors implanted under the skin or pacemakers with telemetry, electrocardiogram, and respiratory rate [39]. These kinds of sensors are adopting Micro Electro Mechanical Systems (MEMS) based technologies to achieve continuous, real-time monitoring of patients health condition and the ability to get help when a critical event occurs.

Environmental and Ambient Sensors

Internet of Robotic Things (IoRT) employs various on-body, off-body and ambient sensors to get real-time information from individuals, with robotic systems used to provide assistance with daily activities, critical and emergency events [40]. It monitors the activity and fitness of in-home inhabitants [41]. These sensors also measure room temperature, humidity or air quality in smart hospital or home care setups to support diagnostics indirectly.

Signal Acquisition and Pre-Processing

The signal acquisition can achieved through various techniques, such as electrode, sensors, Imaging Modalities etc. Acquiring clean, usable signals from the human body is a non-trivial task due to interference from motion artifacts, electrical noise, and physiological variability [17]. Pre-processing is an important phase aimed at enhancing signal quality and preparing it for further analysis. The main steps include filtering, normalization and segmentation. Intelligent sensing systems include several layers of signal conditioning:

Analog Front-End (AFE) Circuits

The amplification and filtering of weak physiological signals (e.g., mV-range ECG signals) and it is the interface between the human body and digital processing systems. The main elements of AFE are Instrumentation Amplifiers (INA), Programmable Gain Amplifiers (PGA) and Anti-Aliasing Filters.



**Jiji Mol and Anith Beaugilin****Digital Filtering**

Residual noise is filtered, removed and clinically relevant information are extracted. Due to its flexibility, stability, and ease of reconfiguration nature the digital filters are recommended. Most of the algorithms include for power line interference notch filters, to isolate physiological frequency ranges band pass filters, and for noise suppression adaptive filters are used.

Signal Normalization

It ensures consistency across different recordings, devices, and subjects and it is an important preprocessing step. It ensures consistency in the amplitude of the signal and baseline, and facilitating accurate downstream analysis. Min-Max Scaling, Z-Score Normalization and Baseline Correction are the most common normalization technique.

Artefact Detection and Correction

Machine learning-based techniques can identify and discard corrupted segments of data (e.g., those affected by motion or poor sensor contact). Rule-Based Detection, Machine Learning-Based Detection, Signal Decomposition Methods, Segment Rejection or Reconstruction are the Modern artifact handling techniques used in biomedical signal processing. For example, in wearable ECG monitoring, deep neural networks can automatically detect motion artifacts caused by physical activity and either correct or exclude affected segments before arrhythmia analysis.

Edge vs. Cloud-Based Processing

A crucial design decision in intelligent sensing is whether to process data locally (on the device or nearby “edge” device) or to transmit it to the cloud for analysis. Two common types of computing are Cloud Computing and Edge Computing. With Cloud Computing, we send all of our data to a centralized server that has access to a very large amount of computer processing and storage. Complex analyses, including but not limited to Machine Learning, can be performed remotely. Cloud Computing can often result in latency and connectivity issues [5]. With Edge Computing, we perform all of our processing locally using microcontrollers or embedded processors and send data to the cloud for only complex analyses [9]. Because we can process data locally, Edge Computing allows for power efficiency, improved privacy, and better performance for latency-sensitive applications (e.g. seizure prediction/arrhythmia detection). When combined, we can perform pattern detection on the edge and conduct more sophisticated analytics in the cloud. This allows for a greater degree of responsiveness to real-time events, while also providing enhanced capabilities for advanced analytics.

Communication Protocols and Interoperability

To enable integrated healthcare systems, intelligent sensors must communicate seamlessly with other devices and platforms. This requires to following certain uniform guidelines:

Bluetooth Low Energy (BLE)

Due to its ultra-low power consumption and native support in smartphones and tablets, it is one of the most widely adopted protocols in wearable healthcare systems. It is widely used in fitness trackers, ECG patches, and smart watches, which allow real-time data synchronization with mobile health applications.

Zigbee

Zigbee is also low-power standard which is suitable for multi-sensor healthcare environments to communicate consistently with a central coordinator with minimal power consumption.

High-Throughput Communication

Wi-Fi and 5G are high-throughput real-time data streaming in clinical settings. Wi-Fi because of its high data rates and established infrastructure is widely deployed in hospitals and clinical settings. To ensure Quality of Service (QoS) and prevent interference with other critical medical devices Wi-Fi networks must be carefully managed. 5G offers extremely fast response time, high reliability, network slicing for medical-grade communication and ability to connect a very large number of devices simultaneously.



**Jiji Mol and Anith Beaugilin****Interoperability Standard**

The data exchanged between systems can be consistently interpreted and clinically meaningful, beyond connecting devices physically in the health care system through HL7 and FHIR standards. It ensures meaningful data exchange with electronic health records (EHRs) [18]. Flawless, secure, and meaningful data exchange across the healthcare management are enabled by HL7 and FHIR. To ensure continuous and secure operation, the design of robust communication systems must also consider data encryption, authentication, and failover mechanisms.

Integrated Pattern-Based Diagnostic Models

When pattern-based signal analysis is deeply integrated with intelligent sensing hardware, a revolutionary diagnostic tool can be developed. Independent of manual control, the collaboration enables end-to-end systems [19] that can persistently acquire, analyse, understand, and act upon biomedical data.

Architecture of Integrated Diagnostic Systems

Components of Architecture of Integrated Diagnostic Systems are [27]:

- **Signal Acquisition Layer:** Wearables or implantable continuously capture biomedical signals.
- **Preprocessing Layer:** Filters, normalizes, and digitizes signals for analysis.
- **Feature Extraction Layer:** Converts signals into meaningful features (e.g., heart rate variability, EEG rhythms).
- **Pattern Recognition Layer:** Uses machine learning or deep learning models to identify health states or anomalies.
- **Decision Support Layer:** Maps patterns to diagnostic recommendations, alerts, or therapeutic interventions.
- **Communication Layer:** Transmits data to healthcare providers or cloud repositories as needed.

Machine Learning Frameworks for Pattern Recognition

Several ML and AI models are commonly used for biomedical signal classification and prediction [20]:

- **Support Vector Machines (SVM):** Good for high-dimensional, small-sample problems. Widely used in ECG classification.
- **Random Forests:** Excellent for handling missing data and feature importance ranking.
- **Convolutional Neural Networks (CNNs):** Effective for spatial pattern detection in signals visualized as images or spectrograms.
- **Recurrent Neural Networks (RNNs) and Long Short-Term Memory (LSTM) networks:** Suitable for time-series data like EEG and respiratory waveforms.
- **Transformers:** Recent advances in attention-based models allow for powerful sequence modeling with improved context retention.

The Table 2 shows a concise comparison of commonly used machine learning models in biomedical signal analysis.

Real-World Examples**Example 1: Arrhythmia Detection from ECG**

An integrated wearable device captures ECG signals continuously. An embedded CNN model processes the data in real-time to detect irregular heartbeats. When arrhythmia is detected, an alert is sent to a smartphone app and the data is uploaded to the cloud for cardiologist review.

Example 2: Epileptic Seizure Prediction Using EEG

A scalp EEG headset uses a hybrid LSTM-Wavelet model to forecast seizure onset based on patterns of brain activity. When high-risk states are detected, the system triggers a warning signal, allowing patients to take preventive action.

Example 3: Respiratory Distress Monitoring in ICU

Oxygen saturation (SpO₂), respiration rate, and heart rate are measured by a sensor suite. Using real time, the danger of respiratory failure is identified and suggests caregivers for ventilator adjustments or alerts for immediate treatment.





Applications in Clinical Domains

The combination of pattern-based biomedical signal analysis and intelligent sensing technologies is becoming a vital innovation across several medical specialties. The patient outcomes have improved which results in reducing healthcare costs and enabling more proactive care by enabling real-time, personalized, and context-aware diagnostics.

Let us explore the some of the core areas where these technologies have shown their significant impact.

- Cardiovascular Health Monitoring
- Neurological Disorders and Brain Health
- Respiratory and Pulmonary Care
- Metabolic and Endocrine Monitoring
- Metabolic and Endocrine Monitoring
- Emergency Medicine and Critical Care
- Remote Patient Monitoring and Telehealth

Challenges and Opportunities

Due to some obstacles connected with it, quick development of patterns of integrated signature analysis connected with the algorithms and systems are evolved. These issues should be tackled by taking necessary steps for implementing innovative, ethically deployed and clinically significant systems. Biomedical signals are extremely affected by noise, motion artifacts, and unpredictability because of physiological and environmental factors. Unreliable data reduces the quality of diagnostic algorithms' performance, which results in non-identified medical conditions or false positives. Limitations with data quality and fluctuations have to be handled in order to enable the existence and to increase the AI-generated physiological processes. Unsuitable hardware, software, and data formats are often used in health care systems [32]. The lack of consistent rules or formats slows down the integration across systems and complicates the operation of intelligent sensing solutions. Real time observation raises concerns about data ownership, consent, and protection against breaches or misuse [21] (e.g., insurance discrimination). Due to limited applicability and legal and challenges many AI-driven diagnostic tools excel in controlled trials but struggle in real-world settings. Implementing these technologies in low-resource settings is limited by cost, connectivity, and computational constraints.

Machine learning (ML) has improved filters that changes or adapts to different noise characteristics automatically according to the noise conditions are known as adaptive noise cancellation. Generative adversarial networks (GANs) can make something better in quality strength by generating realistic noisy-clean signal pairs. Signal Quality Indices (SQIs) are used to point out or reject inaccurate segments by combining real-time quality assessment into processing pipelines. IEEE 11073 for device communication and HL7 FHIR for EHR for different systems to work together are used for open standards. Tools that connect between protocols are Middleware solutions, Mirth Connect. Modular architectures, which use micro services and APIs are Plug-and-play by which integration is possibly made. Using processed data, Federated Learning (FL) allows ML models to be trained without sharing original or unprocessed data. Blockchain-based audit trails are fixed records that provide transparency in data access and usage. Differential privacy is the process of preventing re-identification by introducing statistical noise into datasets. Real-world evidence (RWE) stands for real-world evidence, which is derived from digital health system and sensor derived data. Agencies supervise there gulatory sandboxes which are having regulated validation test beds. The coordinated framework can accelerate data validation [22].

CONCLUSION

The integration of pattern-based biomedical signal analysis with advanced sensing technologies is revolutionizing clinical diagnostics. It transforms healthcare from a reactive, continuous, active and personalized. Machine Learning and Deep Learning allows for timely, accurate and patient specific diagnoses are made by modern sensors such as





Jiji Mol and Anith Beaugilin

wearable, environmental etc which collect detailed health data. From basic observation to deep understanding, this method guide for preventive and immediate care. AI generated healthcare systems can improve the accuracy of diagnosis, clinical workflow optimization and reduces cost by providing proactive and patient specific care and integrating intelligent sensors with BSP. This advance method supports for personalized, proactive healthcare using continuous monitoring and early interventions. Finally, the goal of this is designed to improve the patient health more personalized and efficient

Emerging Trends and Future Directions

Even though there are many challenges combination of formal document regarding diagnostics are vivid. This will make an impact a change in health care in future [26] [27]:

- **Multimodal Data Fusion:** Measurements from multiple bio signals can be integrated (e.g., ECG, EEG, PPG) and information (e.g., activity, location) for health care diagnostics.
- **Medical Digital Twins:** The concept enables to simulate and extrapolate patients' physiology systems based on data collected from them.
- **Neuroadaptive and Closed-Loop systems:** Based on EEG patterns, systems actively modify therapeutic solution according to real-time feedback, such as brain stimulation.
- **Next Generation Sensors:** These are related to the development of flexible stretches, ingestible sensors and advanced biochemical sensors to monitor hormones or metabolites.
- **AI-Powered Clinical Decision Support:** Deploying AI to assist.

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Jiji Mol and Anith Beaugilin

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Table 1 : Biomedical Signals and Diagnostic Applications

Signal Type	Measured Activity	Primary Diagnostic Applications	Typical Sensors
ECG	Electrical activity of the heart	Arrhythmia detection, myocardial infarction, heart rate variability	Electrodes, wearable chest straps
EEG	Brain's electrical activity	Epilepsy, sleep disorders, cognitive impairment	Scalp electrodes, headbands
EMG	Muscle activity	Neuromuscular disorders, prosthetic	Surface or needle



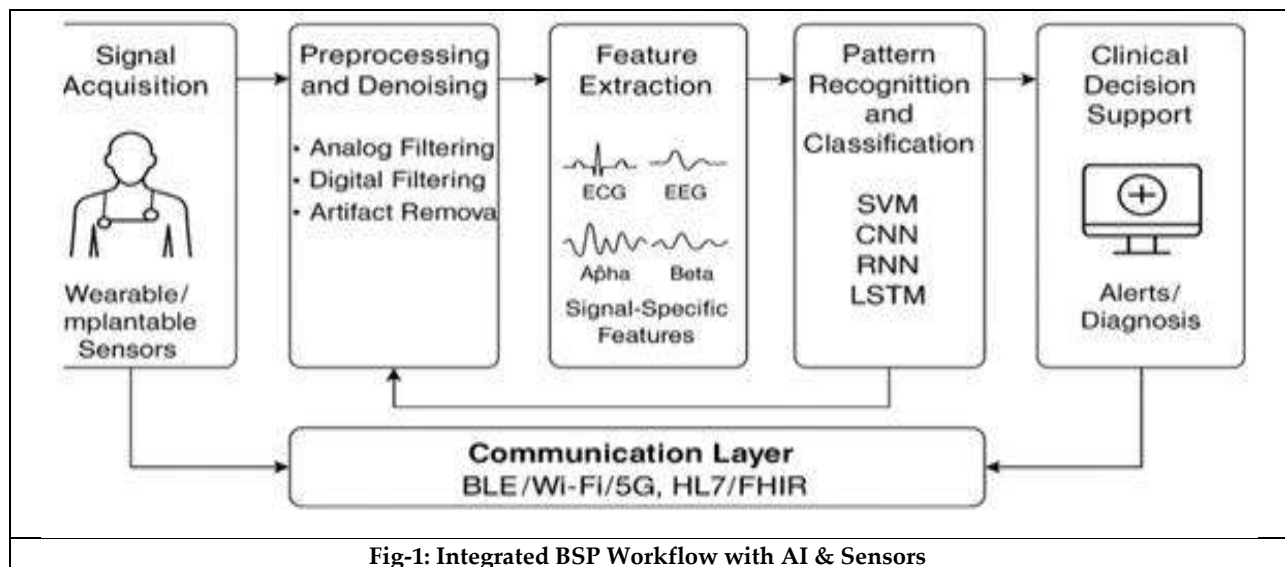


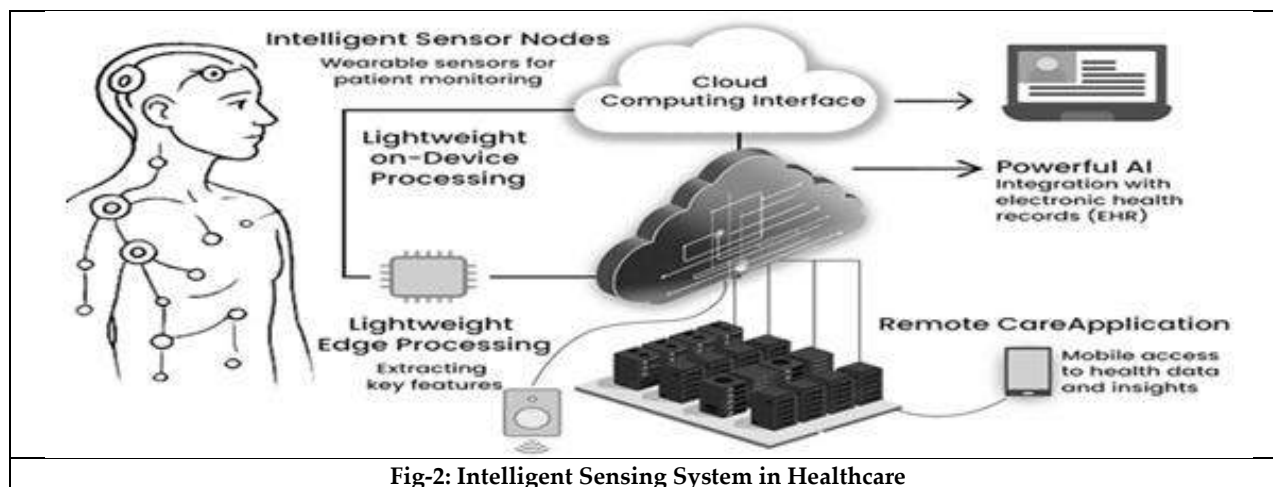
Jiji Mol and Anith Beaugilin

		control	electrodes
PPG	Blood volume changes	Heart rate, oxygen saturation, stress level monitoring	Optical sensors, pulse oximeters
Respiratory Signals	Airflow and lung sounds	COPD, asthma, sleep apnea	Microphones, airflow sensors

Table 2: Machine Learning Models for Biomedical Signal Classification

Model Type	Typical Use Case	Advantages	Limitations
SVM (Support Vector Machine)	ECG arrhythmia classification	Works well with small datasets, good generalization	Limited scalability, manual feature selection needed
CNN (Convolutional Neural Network)	EEG/ECG image Or spectrogram analysis	Learns spatial features automatically	Requires large datasets, high computational cost
RNN / LSTM	Time-series EEG/respiratory signal prediction	Captures temporal dependencies	Prone to vanishing gradient, slower training
Random Forest	Multimodal biosignal fusion	Handles noisy data, interpretable	May underperform with temporal data
Hybrid (CNN + LSTM)	Complex signal recognition	Combines spatial and temporal learning	Complex training pipeline



**Jiji Mol and Anith Beaugilin****Fig-2: Intelligent Sensing System in Healthcare**

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Non - Pharmacological Treatment Modalities for Xerostomia : A Review

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ABSTRACT

Xerostomia is a complex condition that is becoming more common in the elderly population due to several causes ranging from developmental to iatrogenic. Pharmacological treatment improves the symptoms but increases the adverse effects of the latter and worsens the situation again. Keeping this in mind, non-pharmacological interventions are currently used or under supervision to bypass the complications of pharmacological treatment. This article aims to review the prevalence, incidence, causes, signs/symptoms, and most importantly the success rate of non-pharmacological interventions. Articles in PubMed, Cochrane database, and Web of Science were analysed till date to establish the effectiveness of different modalities such as acupuncture, electrostimulation, hyperbaric oxygen (HBO) therapy, and low-level laser therapy (LLLT), etc. Newer treatment modalities such as gene therapy is under trial. This paper provides a broad idea of the different non-pharmacological interventions currently used and our newer future for treating dry mouth.

Keywords: Acupuncture, Electrostimulation, Gene therapy, HBO therapy, LLLT.





INTRODUCTION

Xerostomia (Dry Mouth)

The concept of dry mouth was first described by Bartley in 1868, and later the term xerostomia was introduced by Hutchinson in 1889. According to Sreebny (1988), xerostomia refers to the subjective sensation of oral dryness, which is commonly associated with reduced salivary gland function [1]. Although this condition is more frequently observed in older individuals, it should not be considered a normal consequence of aging [1]. Xerostomia can also occur in younger populations, including adolescents, though it is relatively uncommon in children. Under normal conditions, the unstimulated salivary flow rate is approximately 0.3 mL/min. During sleep, it decreases to around 0.1 mL/min, while stimulation such as chewing can increase the flow rate to about 4–5 mL/min [1]. The prevalence of xerostomia is estimated to be between 14% and 35%, and its occurrence has been increasing, particularly in developed countries [1].

Causes of Xerostomia

In most cases, dry mouth results from salivary gland hypofunction, which may be temporary or long-term. This condition may or may not be accompanied by the subjective feeling of dryness [3].

A major cause in older adults is the use of medications that reduce saliva production [3]. These include:

- Anticholinergics
- Sympathomimetic drugs
- Sedative-hypnotics
- Opiates
- Antihistamines
- Muscle relaxants

Other important causes include:

- Radiation therapy, especially in patients treated for head and neck cancers [15]
- Autoimmune disorders, such as Sjögren's syndrome [3]

In some situations, patients may report dryness even when salivary flow is not significantly reduced.

Management

Despite the wide range of causes, the management of xerostomia is generally similar across cases. An exception is drug-induced xerostomia, where treatment involves stopping the offending medication or replacing it with an alternative that has fewer drying effects [3]. At present, there is no single definitive treatment that is effective for all cases of xerostomia. Xerostomia can be managed using two broad approaches: pharmacological and non-pharmacological methods. Pharmacological therapies mainly provide temporary symptomatic relief, and symptoms often return once the medication is discontinued. In contrast, newer non-pharmacological approaches aim to enhance natural salivary secretion and provide more sustained improvement. In recent years, these alternative methods have gained attention due to their effectiveness in alleviating symptoms associated with dry mouth [2,7].

Acupuncture

Acupuncture is a therapeutic technique that involves inserting very fine needles into specific points within or beneath the skin and is widely used for various conditions [4,5]. Its effectiveness in symptom control and supportive care has been documented [6]. The mechanism by which acupuncture improves salivary flow is not fully understood, but it is thought to stimulate autonomic pathways and enhance glandular function. Acupuncture is considered a low-risk therapy with minimal adverse effects [7,8].

Transcutaneous Electrical Nerve Stimulation (TENS)

TENS is a physical therapy that delivers low-voltage electrical impulses through electrodes placed on the skin [9].





Anjum Ara J Farooqui *et al.*,

Mechanism in Xerostomia

TENS may stimulate salivary secretion through neural reflexes. Extraoral application activates the auriculotemporal nerve, which transmits signals to the salivary center in the brainstem, initiating salivation [10].

Intraoral stimulation activates sensory pathways in the oral mucosa, increasing salivary output. A frequency range of 50–80 Hz applied for 5–10 minutes is considered effective and safe [11].

Low-Level Laser Therapy (LLLT)

Low-level laser therapy (photobiomodulation) uses low-intensity light to stimulate cellular activity. It enhances ATP production, promotes tissue repair, and improves salivary gland function.

Studies have demonstrated improved salivary flow following LLLT [12,13]. However, further research is needed to determine optimal treatment parameters [14].

Hyperbaric Oxygen Therapy (HBOT)

Hyperbaric oxygen therapy improves oxygen delivery to tissues and is especially useful in radiation-induced damage. It promotes angiogenesis, collagen synthesis, and tissue repair [15,16].

HBOT has been shown to improve salivary gland function and quality of life in affected patients [17,18].

Gene Therapy

Gene therapy aims to restore salivary gland function by introducing genes such as AQP1 to improve fluid secretion. Experimental studies have shown promising results in restoring gland function [19].

It may also reduce inflammation and protect glandular tissue in autoimmune conditions [20].

CONCLUSION

Xerostomia remains difficult to manage. Pharmacological therapies provide temporary relief, whereas non-pharmacological methods such as acupuncture and TENS show beneficial effects.

Advanced therapies like LLLT and HBOT improve glandular activity, while gene therapy offers future potential. Continued research is required to establish effective long-term treatments.

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Table 1: Causes for Xerostomia

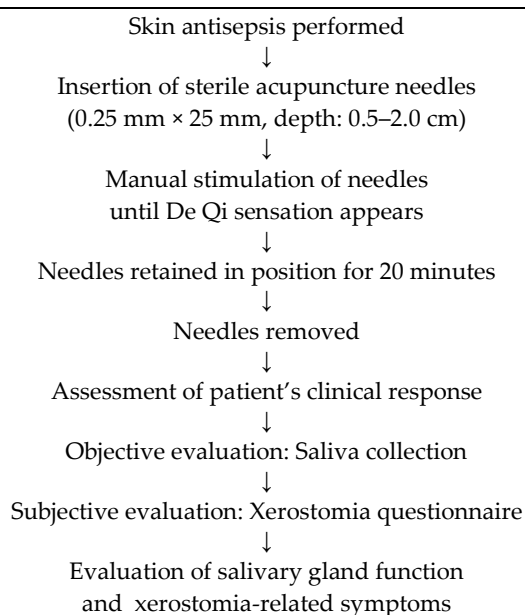
Sl. No.	Causes	Example
1	Iatrogenic	Medications Local radiation therapy to head and neck region Chemotherapy Nerve damage from a head or neck injury Chronic graft versus host disease (cGvHD)
2.	Developmental causes	Salivary gland agenesis / atresia
3	Pathologic causes	Sialolithiasis Sialadenitis
4	Diseases of salivary glands	Sjogren's syndrome
5	Infections	HIV infection, Hepatitis C virus infection
6	Diseases	Diabetes mellitus , Amyloidosis, Hemochromatosis, Wegener's disease, Ig G4 related disease
7	Others	Smoking. Mouth breathing Decreased mastication, Psychogenic disorders, Aging,Vomiting/ diarrhoea





Table 2: Effects of xerostomia

Functional alterations in: • Chewing • Flavour • Swallowing • Speaking
Increased susceptibility to the occurrence of: • Caries • Gingivitis • Spontaneous bleeding • Periodontitis • Oral and oropharyngeal infections • Halitosis • Ulcers • Dental plaque formation
Morphological changes: • Dry lips • Absence of saliva on the floor of the mouth • Pale and cracked oral mucosa • Filiform papilla atrophied and erythematous mucosa on the back of the tongue
Other manifestations: • Pain • Dry eyes, skin, nose, throat • Difficulty to wear dental prosthesis





Anjum Ara J Farooqui *et al.*,

PROCEDURE OF LASER

Applied on both sides using a non-contact technique over each salivary gland region



6 sessions of exposure, each lasting 120 seconds



Completed over a period of 10 continuous days



An increase in salivary secretion is observed

Figure 1- Procedure of Acupuncture

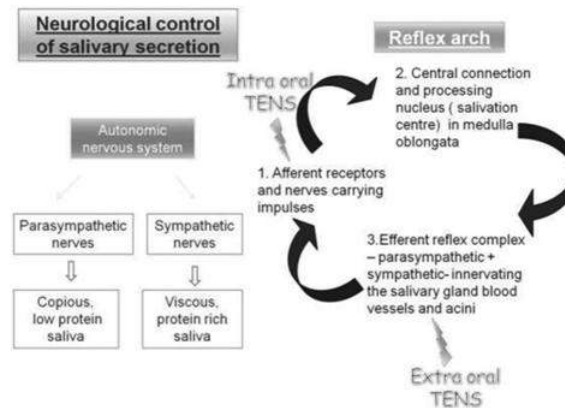


Figure 2

Applied bilaterally in noncontact mode to each salivary gland area



6 exposures for 120 secs each



Within 10 consecutive days of treatment



Salivation is found to be increased

Figure 3. Procedure of LASER

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RESEARCH ARTICLE

An Attention-Enhanced Brain - EffNetV2 Framework for MRI - Based Brain Tumour Classification

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ABSTRACT

The accurate and timely detection of brain tumours is necessary for better treatment planning and improved survival rates. This study proposes a deep learning architecture with attention enhancement for brain tumour classification using MRI images. An improved version of the EfficientNetV2 is proposed using attention mechanisms to identify specific tumour locations while suppressing irrelevant background data. MRI images are collected from a Kaggle public domain dataset and are preprocessed for standardization and augmenting the data. The model offers improved feature extraction, multiscale learning, and robust classification for brain tumours. Evaluation through experiments shows promising prediction capabilities with 98.23% accuracy, 96.49% precision, 96.46% recall, and an F1-score of 96.48%. From the results, it can be concluded that the architecture performs consistently and reliably compared to traditional CNN architectures. The approach decreases the dependency of humans in diagnosing brain tumours using MRI images. The presented model offers a scalable and reliable solution for identifying brain tumours with MRI using computers, which could be helpful for doctors in practice environments.

Keywords: Brain tumour classification, Magnetic Resonance Imaging, EfficientNetV2, Attention mechanism, Deep learning





INTRODUCTION

Brain tumours, affecting both kids and adults, can result in morbidity and fatalities when not diagnosed early enough. Brain tumours refer to abnormal growth of cells in the brain and nearby structures, affecting cognitive function, movement, and sensory processes. Benign, malignant, and pituitary tumours vary in their methods of growth, hazards, and treatment options. It is important to diagnose brain tumours at an early stage to plan treatment and improve life expectancy. MRI is the superior imaging method for brain tumours because of its soft tissue contrast and non-invasiveness. MRI shows cancer size, location, and structure without radiation. The growing amount of clinical MRI data burdens radiologists. Tumours with varied forms, indistinct borders, and intensity changes are difficult to interpret manually and vulnerable to observer variability. These concerns drive reliable, automated computer-aided diagnostic methods. It presents an Attention-Enhanced Brain-EffNetV2 framework for MRI-based brain tumour classification.

The proposed method improves discriminative feature learning by integrating channel and spatial attention modules with EfficientNetV2. The framework attempts to achieve high classification accuracy and computing efficiency via efficient architectural design and attention-guided refinement. The method helps radiologists reduce diagnostic burden and unpredictability by making reliable and automated predictions. Variable tumour form, similarities between malignant and healthy tissues, inadequate annotated datasets, and different MRI collection techniques make brain tumour classification difficult. Expert radiologists are few in many healthcare settings, particularly in underdeveloped countries, making diagnosis more difficult. Fast, objective, and repeatable analysis from automated deep learning systems can solve these problems. Most models give preference to accuracy over computations or fail to focus on tumour-related regions. An efficient attention-based framework enables a model to learn useful representations without being complex. The Brain-EffNetV2 framework was designed based on this concept. The summarization of the contribution is as follows:

- Proposed an attention-enhanced Brain-EffNetV2 architecture is proposed for MRI-based brain tumour classification.
- Integrated channel and spatial attention mechanisms to improve tumour-focused feature representation.
- Adopted EfficientNetV2 as a lightweight yet powerful backbone for efficient deep feature extraction.
- Designed a robust preprocessing pipeline including normalization and augmentation to enhance generalization.
- Developed an automated classification framework that reduces reliance on manual MRI interpretation.
- Demonstrated strong performance across evaluation metrics, indicating reliability for clinical support.

The study is segmented into four parts. The second part includes the literature review as well as background of brain tumor classification and deep learning systems. The third part involves a description of the proposed model named 'Proposed Brain-EffNetV2'. The fourth section focuses on experimental results and their evaluation.

Related Works

One study evaluated smartphone-based MRI image processing for brain tumour classification was examined in [1]. The hybrid deep learning architecture classifies brain tumours from MRI images using CNN, Vision Transformer (ViT), and MobileViT models. Transfer learning improves pretrained networks like VGG16, ResNet152V2, InceptionV3, InceptionResNetV2, and MobileNetV2. All models are trained in TensorFlow, assessed similarly, transformed to TensorFlow Lite, and incorporated into a Flutter-based mobile app for on-device inference. Brain tumour classification and diagnosis using hybrid deep-classical models was detailed in [2]. A Hybrid Deep-Classical Model combined deep CNN feature extraction with machine learning classifiers. Pretrained CNNs like VGG16, ResNet50, and DenseNet121 retrieve deep features from pre-processed MRI images without terminal layers. PCA reduces traits and SVM, Random Forest, and KNN classify them, with SVM performing best. Summary of Federated Learning Combined Ensemble Aggregation for MRI Brain Tumour Classification was summarised in [3]. A federated learning brain tumour classification system using CNNs and ensemble aggregation is presented. Each client (medical facility) trains an identical convolutional neural network model on its MRI data and sends just the weights to a



**Umarani et al.,**

central server. A meta-learner allocates appropriate weights to client models according on validation performance, while the server consolidates them using weighted averaging to create a global model. The classification of brain tumours using MRI using dilated SE-DenseNet was examined in [4]. The proposal involves an improved DenseNet-121 architecture that incorporates dilated convolutional layers to broaden the receptive field and Squeeze-and-Excitation (SE) attention modules to adjust channel-wise features. The integrated Dilated SE-DenseNet model enhances feature representation and classification efficacy for brain tumour identification from MRI scans.

A hybrid explainable model for brain cancer classification using MRI images and advanced machine learning and deep learning techniques was summarised in [5]. A hybrid PDSCNN-RRELM architecture that integrates a Parallel Depthwise Separable CNN for feature extraction with a Ridge Regression Extreme Learning Machine for classification, augmented with CLAHE preprocessing and SHAP-based explainability. Hybrid deep learning algorithms were used to classify brain tumours using T1-weighted MRI in [6]. It utilises a hybrid deep learning framework that integrates a U-Net architecture with transfer learning-based CNNs, notably InceptionV3, EfficientNetB4, and VGG19. Pretrained ImageNet weights are refined, the Flatten layer is substituted with Global Average Pooling, and bespoke dense layers using SoftMax are included for multiclass brain tumour classification. The use of pre-trained deep convolutional neural network models with transfer learning for sophisticated brain tumour classification in MRI images was elaborated in [7]. It employs a transfer learning-based deep learning methodology using several pre-trained CNN architectures, including Xception, MobileNetV2, InceptionV3, ResNet50, VGG16, and DenseNet121. Models trained on ImageNet are fine-tuned using brain MRI images for four-class tumour classification, using bespoke fully connected layers for feature adaption and employing SoftMax for prediction.

A novel cognitive computational approach for brain tumour classification using MRI data has been proposed in [8]. A hybrid Particle Swarm Optimisation - Quantum-Enhanced Support Vector Machine (PSO-QESVM) framework for the classification of brain tumours from MRI images was proposed. Images are first denoised with a median filter. Deep features are derived via ResNet and VGG16 convolutional neural network models. The features are classified using a QESVM, with hyperparameters appropriately adjusted by PSO to enhance accuracy and resilience. The optimisation of CNN architecture for MRI brain tumour classification using PSO was examined in [9]. It uses a PSO-based automated CNN architectural search, whereby PSO dynamically optimises the quantity of layers, filter dimensions, and neurones to construct an effective convolutional neural network for MRI brain tumour classification. The classification of brain cancer MR images with a hybrid quantum graph neural network was proposed in [10]. A Hybrid Quantum Graph Neural Network (QGNN) is presented for the classification of brain tumours using MRI images. Images are first transformed into graph representations and then analysed using a standard Graph Convolutional Neural Network (GCNN). A quantum layer using parameterised quantum circuits is then included to encode PCA-reduced features into quantum states, execute quantum rotations and entanglement, and derive quantum node embeddings, which are then processed by classical dense layers for ultimate classification. The classification of brain tumours using MRI with a ResNet50 Spatial Transformer Network including a Non-Local Attention Mechanism (STN-NAM) is reported to be accurate in [11]. U-Net is first used to delineate tumour locations from MRI scans. The STN module enhances spatial invariance through the acquisition of geometric changes, whilst the NAM identifies long-range relationships between lesion and normal regions, enabling more precise brain tumour categorisation. A collaborative federated learning approach for brain tumour classification using explainable AI in medical imaging was shown in [12].

The research proposed an XFL architecture that incorporates a pretrained GoogLeNet CNN for the classification of brain tumours from MRI images. Numerous clients locally train GoogLeNet models using decentralised data, while a central server consolidates updates through Federated Averaging. Model interpretability is improved using Grad-CAM and Saliency Maps to visualise areas pertinent to decision-making. A strategy for real-life scenario simulation and augmentation to enhance MRI brain tumour classification has been proposed in [13]. A deep learning framework using transfer learning and fine-tuning with several pretrained CNN and ViT models was implemented. Architectures including DenseNet, EfficientNetV2, ResNet, VGG, ConvNeXt, and ViT were assessed, with



**Umarani et al.,**

EfficientNetV2-B0 identified as the optimal model. Gaussian noise and blur augmentation replicate real-world MRI settings and improve robustness and generalisation. Neural network methods for MRI brain tumour classification were improved in [14]. A customised sequential CNN is employed for multiclass brain tumour classification from MRI data. Images are scaled, normalised, converted to greyscale, and supplemented. The CNN comprises sequential Conv2D layers (64–256 filters), each succeeded by max-pooling and dropout, followed by fully linked dense layers with a SoftMax output. Grad-CAM is included to enhance the visual interpretability of model judgements. An improved CNN hyperparameter model enhances the accuracy and reliability of brain tumour diagnostics, as shown in [15]. This method presented an HPCNN for the classification of brain tumours using MRI images. A unique CNN architecture is constructed and methodically refined by adjusting critical hyperparameters, such as the quantity and dimensions of filters, stride, padding, pooling type, activation functions, learning rate, batch size, layer count, dropout rate, and optimiser SGD/Adam. Random search is used to ascertain the optimal arrangement. A self-attention-based progressive generative adversarial network using a momentum search approach for brain cancer MRI classification was presented in [16]. A self-attention-based Progressive Generative Adversarial Network (SPGAN) optimised using a Momentum Search Optimisation Algorithm (MSOA) for the classification of brain tumours from MRI images. Noise is mitigated using ADKF, texture features are obtained by TP-DWT, and SPGAN executes classification with parameters optimised by MSOA for enhanced accuracy and convergence.

A hybrid deep convolutional neural network model for brain tumour image classification was developed in [17]. A hybrid deep convolutional neural network framework with three bespoke CNN architectures for the classification of brain tumours from MRI images. The first CNN executes binary tumour identification, the subsequent one manages five-class tumour type classification, and the last CNN classifies gliomas into three classes. Grid search optimisation systematically adjusts architectural and training hyperparameters. The models use stacked Conv-ReLU-Pooling layers, succeeded by fully connected and SoftMax layers for prediction. The study presents effective ensemble frameworks for predicting human brain tumours from MRI scans [18]. A hybrid ensemble architecture combining CNN and machine learning for the classification of brain tumours from MRI images. Deep features are obtained by a customised CNN, thereafter, subjected to PCA for dimensionality reduction. The diminished characteristics can be classified using several machine learning models such as AdaBoost, XGBoost, Random Forest, KNN, Decision Tree. An ultimate ensemble voting model (XG-Ada-RF) amalgamates predictions from AdaBoost, XGBoost, and Random Forest to enhance accuracy. A robust deep learning model for brain tumour classification using reconstruction and fine-tuning was presented in [19]. A transfer learning framework augmented by reconstruction for the classification of brain tumours using MRI images. The quality of the image is first enhanced by a deep reconstruction network. The reconstructed images are further refined with several pretrained CNN models, such as VGG16, VGG19, ResNet50, ResNet101, DenseNet121, DenseNet169, InceptionV3, InceptionResNetV2, Xception, MobileNetV2, and EfficientNet-B0. Layer-wise fine-tuning and optimised training configurations are used to enhance classification accuracy and resilience. A sophisticated brain tumour identification technique using deep convolutional neural networks was presented in [20]. A CNN-based framework for brain tumour diagnostics with EfficientNetV2 and optimised with the Ranger optimiser. MRI images are subjected to clipping, denoising, and histogram equalisation, subsequently accompanied by restricted data augmentation. EfficientNetV2 executes tumour identification and classifies tumours into three distinct groups such as glioma, meningioma, and pituitary. Transfer learning with ImageNet weights is implemented, and Ranger enhances convergence stability and generalisation.

PROPOSED METHODOLOGY

The Proposed system introduces an Attention-Enhanced Brain-EffNetV2 framework for accurate and reliable multi-class brain tumour classification from MRI data. The framework is designed to classify MRI brain images into four clinically relevant classes: glioma, meningioma, pituitary tumour, and normal brain. End-to-end image classification with the Proposed method facilitates automated disease diagnosis using low computational costs, unlike other approaches relying on segmentation. This system entails the following critical components:





- MRI preprocessing
- A Brain-EffNetV2 network backbone adaptation.
- Improving feature discrimination through lightweight attention techniques.

With this combination, the model enhances class separability and generalization ability, especially in situations where the amount of training data is scarce. Firstly, brain MRIs are normalized to maintain uniformity for training purposes. Every brain MRI undergoes resizing to 224×224 pixels while maintaining a grayscale format. Normalization of intensity is conducted in order to mitigate intensity differences owing to varying imaging conditions. Normalization can be described as

$$I_{norm} = \frac{I - \mu}{\sigma} \quad (1)$$

where I the input MRI image, μ the mean intensity value, σ the standard deviation.

Brain-EffNetV2 Feature Extraction

The suggested framework uses the modified version of EfficientNetV2 for feature extraction at multiple levels from MR images. The structure consists of a 3×3 convolution layer that is used to extract low-level spatial features like edges. The convolution operation is defined as

$$F_{i,j} = \sum_m \sum_n I_{i+m,j+n} \cdot K_{m,n} \quad (2)$$

where I represents the input feature map, K denotes the convolution kernel, $F_{i,j}$ represents the extracted feature map. i is row index of the output feature map, j is column index of the output feature map, m is row index of the convolution kernel, n is column index of the convolution kernel. Following the convolution layer, the network uses Fused-MBConv blocks in the early stages to improve computational efficiency and reduce training time. These blocks combine convolution and expansion operations into a single operation. In deeper layers, MBConv blocks with Squeeze-and-Excitation (SE) modules are used to capture high-level semantic information. Figure 1 illustrates the architecture of the Attention-Enhanced Brain-EffNetV2 model designed for automated brain tumour classification using MRI images. The model processes a 224×224 grayscale MRI image through an initial convolution layer to extract basic visual features. Fused-MBConv blocks improve computational efficiency and capture intermediate representations, while an attention-enhanced Fused-MBConv block highlights important tumour region. In deeper layers, MBConv blocks with Squeeze-and-Excitation and channel attention refine feature channels and suppress irrelevant information, enabling the network to learn discriminative tumour characteristics effectively.

To improve tumour detection performance, the proposed framework integrates channel attention and spatial attention mechanisms within the MBConv blocks.

Channel Attention

Channel attention emphasizes the most informative feature channels by assigning adaptive weights. The channel attention process is defined as

$$M_c(F) = \sigma(W_2 \cdot \text{ReLU}(W_1 \cdot \text{GAP}(F))) \quad (3)$$

where F represents the feature map, GAP denotes Global Average Pooling, W_1, W_2 are learnable weight matrices, σ represents the sigmoid function, The refined feature map is obtained as

$$F' = M_c(F) \otimes F \quad (4)$$

where $\otimes$ represents element-wise multiplication.

Spatial Attention

Spatial attention identifies important regions within the feature maps and highlights tumour-related areas.

$$M_s(F) = \sigma(\text{Conv}_{7 \times 7}([\text{AvgPool}(F); \text{MaxPool}(F)])) \quad (5)$$





Umarani et al.,

where *AvgPool* and *MaxPool* represent average and max pooling operations, *Conv*_{7×7} denotes convolution with a 7×7 kernel. The spatially refined feature map is computed as

$$F'' = M_s(F') \otimes F' \quad (6)$$

By combining both channel and spatial attention, the model learns to emphasize tumour regions while suppressing irrelevant background information. After attention-enhanced feature extraction, the feature maps are passed through a GAP layer to convert them into a compact feature vector.

$$GAP(F) = \frac{1}{H \times W} \sum_{i=1}^H \sum_{j=1}^W F_{i,j} \quad (7)$$

where *H* and *W* denote the spatial dimensions of the feature map. By the number of parameters gets reduced. The concatenated feature vector is then sent through a fully connected layer using ReLU activation function and then a Softmax classifier is used for categorizing the tumour. The Softmax function is represented as

$$P(y_i) = \frac{e^{z_i}}{\sum_{j=1}^C e^{z_j}} \quad (8)$$

where *z_i* the output of the fully connected layer, *C* the number of tumour classes. Figure 2 shows the EfficientNetV2 model's design, which seeks excellent accuracy and computing efficiency. The model utilizes a 224×224 RGB image via convolution operation at the beginning to detect fundamental features. Features in the middle are obtained efficiently through Fused-MBConv, and deeper layers with SE in MBConv help identify relevant channel features. With progression in the network, the spatial size decreases while feature depth increases. Lastly, global average pooling, full-connected layer with SiLU, and Softmax layer contribute to the classification task. As Table 1 shows below, there are several differences between the initial architecture of EfficientNetV2 and the modified EffNetV2 model. Adaptations have been made to the input stem, fused-MBConvs, expansion ratios, and classification head to enable classification tasks using MRI for brain tumors.

RESULTS AND DISCUSSION

Brain tumours represent one of the most aggressive conditions that afflict both children and adults; 85–90% of primary central nervous system tumours constitute brain tumours. An estimated number of 11700 people develop brain tumours each year and the survival rate for a central nervous system tumour stands at 34% for men and 36% for women. Tumours are classified as benign, malignant, and pituitary. Proper treatment and planning for the life expectancy of patients requires a correct diagnosis and proper treatment plan [21]. MRI is considered the best method for detection and is performed by radiologists. Limited knowledge in some areas has led to delayed diagnoses, which is the reason why automation has become popular. Brain Tumour Classification (MRI). The processed data are employed during the training step using loss minimization. A validation stage provides an opportunity for hyperparameter selection and finally testing to evaluate the model. The effectiveness of the model is measured through various metrics including accuracy, precision, recall, and F1 score, guaranteeing efficient and generalized classification performance. Figure 3 represents images of brains from MRI data categorized into four classes including benign, malignant, pituitary tumor, and normal brain. Every row represents a unique category exhibiting differences in terms of anatomy, location, and intensity. Images of benign and meningiomas have clear mass areas, pituitary images concentrate on abnormalities of the sellar area, and normal images depict normal brain structures, making it possible to differentiate images by class visually.

Performance Evaluation Metrics

Accuracy

$$Accuracy = \frac{TP+TN}{TP+TN+FP+FN} \quad (9)$$





Umarani et al.,

Precision

$$Precision = \frac{TP}{TP+FP} \quad (10)$$

Recall (Sensitivity)

$$Recall = \frac{TP}{TP+FN} \quad (11)$$

F1-Score

$$F1-Score = 2 \times \frac{Precision \times Recall}{Precision + Recall} \quad (12)$$

Table 2 highlights the comparative performances of different versions of EfficientNetV2 models based on their evaluation through classification measures like accuracy, precision, recall, and F1-score. These include the basic EfficientNetV2 model, the version of EfficientNetV2 integrated with channel attention, the version of EfficientNetV2 integrated with spatial attention, and the proposed modification of EffNetV2 model. The performance of the basic EfficientNetV2 is 96.84% for accuracy, with precision, recall, and F1-scores of 93.73%, 93.69%, and 93.69%, respectively. Inclusion of attention improves the performance. The efficiency of EfficientNetV2 model integrated with channel attention yields 97.10% for accuracy, with precision, recall, and F1-scores of 94.20%, 94.19%, and 94.19%, respectively. The model of EfficientNetV2 with spatial attention shows improved performance, achieving 97.47% accuracy, with precision, recall, and F1-scores of 94.98%, 94.95%, and 94.95%, respectively. It can be concluded from the results that inclusion of both channel attention and spatial attention improves the learning capabilities of EfficientNetV2 model by enabling the model to focus on more relevant features and important tumour areas in MRI images.

EfficientNetV2 with spatial attention proves more effective than EfficientNetV2 and efficient channel attention, showing the usefulness of attention mechanism in improving the efficiency of the classification task. The newly developed modified EffNetV2 demonstrates higher performance on all evaluation metrics and gives 98.23% accuracy, 96.49% precision, 96.46% recall, and 96.47% F1 score. Architectural changes in combination with attention-based feature augmentation considerably improve the ability of the network to recognize features of brain tumors. The results of the classification process for the four classes are presented in Figure 5. There are 96, 94, 95, and 97 predicted instances belonging to each class, which are benign, malignant, pituitary, and normal, respectively. The number of misclassification is very low, with only 1–2 mistakes from one class to another, such as malignant classified as benign (2) and pituitary classified as normal (2). Comparison of the various brain tumor classification methods based on quantitative measures is illustrated in Figure 6. MobileViT gives 97.0% of accuracy and an F1 score of 96.0 whereas the VGG16-SVM algorithm delivers 97.8% of accuracy and an F1 score of 97.2. Federated learning with CNN produces 95.67% accuracy with extremely high precision value at 99.0%. The method of dilated SE-DenseNet gives a good measure at 96.5 F1 score. The Proposed EfficientNetV2 gives the highest measure of accuracy at 98.23%.

CONCLUSION

A Brain-EffNetV2 with enhanced attention capability was presented for the purpose of classifying brain tumors with MRI images and making the diagnosis process precise and automatic. Using MRI data for training together with the careful preprocessing process made it possible to obtain higher-quality data and increase the generalization capability of the model. With the inclusion of channel and spatial attention methods in the EfficientNetV2 architecture, distinguishing features of tumors were extracted in a more efficient way while reducing the effect of irrelevant regions in the image. Superior performance was shown in experiments conducted using several performance metrics, indicating the reliability and robustness of the model used. The model reduces human dependency, ensures consistency in the results, and offers a faster diagnosis compared to existing techniques. Such an innovation becomes extremely useful in those regions where trained radiologists cannot be reached easily. While the model performs quite well, further studies may focus on large-scale multi-institutional data sets, easier implementation for clinical applications, and explainable AI approaches.





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Table1. Architectural Comparison Between EfficientNetV2 and Modified EffNetV2

Architectural Component	EfficientNetV2 (Base Architecture)	Modified EffNetV2 (Proposed Adaptation)	Purpose of Modification
Input Type	3-channel RGB images	1-channel (grayscale) or optimized MRI input	Adapts network to medical imaging data
Input Stem	Fixed kernel and stride	Tuned kernel size and stride	Preserves fine anatomical details
Early Feature Blocks	Limited Fused-MBConv blocks	Expanded use of Fused-MBConv blocks	Faster training and better early feature learning
Deep Feature Blocks	MBConv with SE blocks	MBConv with reduced expansion + SE	Controls model complexity and overfitting
Expansion Ratio	Fixed, stage-defined	Reduced or customized per stage	Balances representational power and efficiency
Squeeze-and-Excitation	Standard SE configuration	Lightweight SE (reduced channel ratio)	Prevents overfitting on small datasets
Depth Scaling	Strict compound scaling	Custom depth scaling	Improves generalization under limited data
Width Scaling	Compound scaling applied	Selective width adjustment	Enhances subtle tumour feature capture
Activation Function	SiLU (Swish)	SiLU or alternative (ReLU6 / Mish)	Improves convergence stability
Drop-Connect	Applied uniformly	Reduced or disabled	Stabilizes training on medical datasets
Classification Head	GAP + single FC layer	GAP + FC + Dropout	Improves class discrimination
Output Layer	Softmax (ImageNet classes)	Softmax (4 tumour classes)	Task-specific classification
Overall Architecture	General-purpose vision model	Medical imaging-optimized backbone	Domain adaptation without redesign

Table2. Performance Comparison of EfficientNetV2 Variants for MRI-Based Brain Tumour Classification

Performance Metrics	EfficientNetV2 (Base Architecture)	EfficientNet V2 + Channel Attention	EfficientNet V2 + Spatial Attention	Modified, EffNetV2 (Proposed Adaptation)
Accuracy	96.84	97.10	97.47	98.23
Precision	93.73	94.20	94.98	96.49
Recall	93.69	94.19	94.95	96.46
F1-Score	93.69	94.19	94.95	96.47



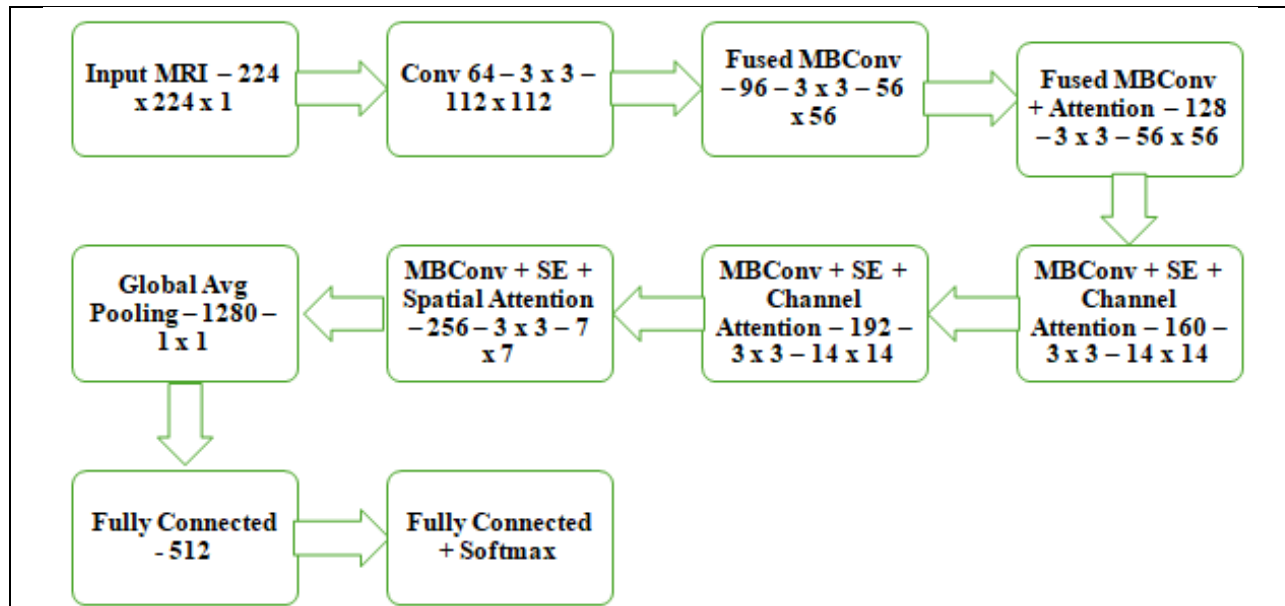


Figure 1. Architecture of the Proposed Brain-EffNetV2 Model

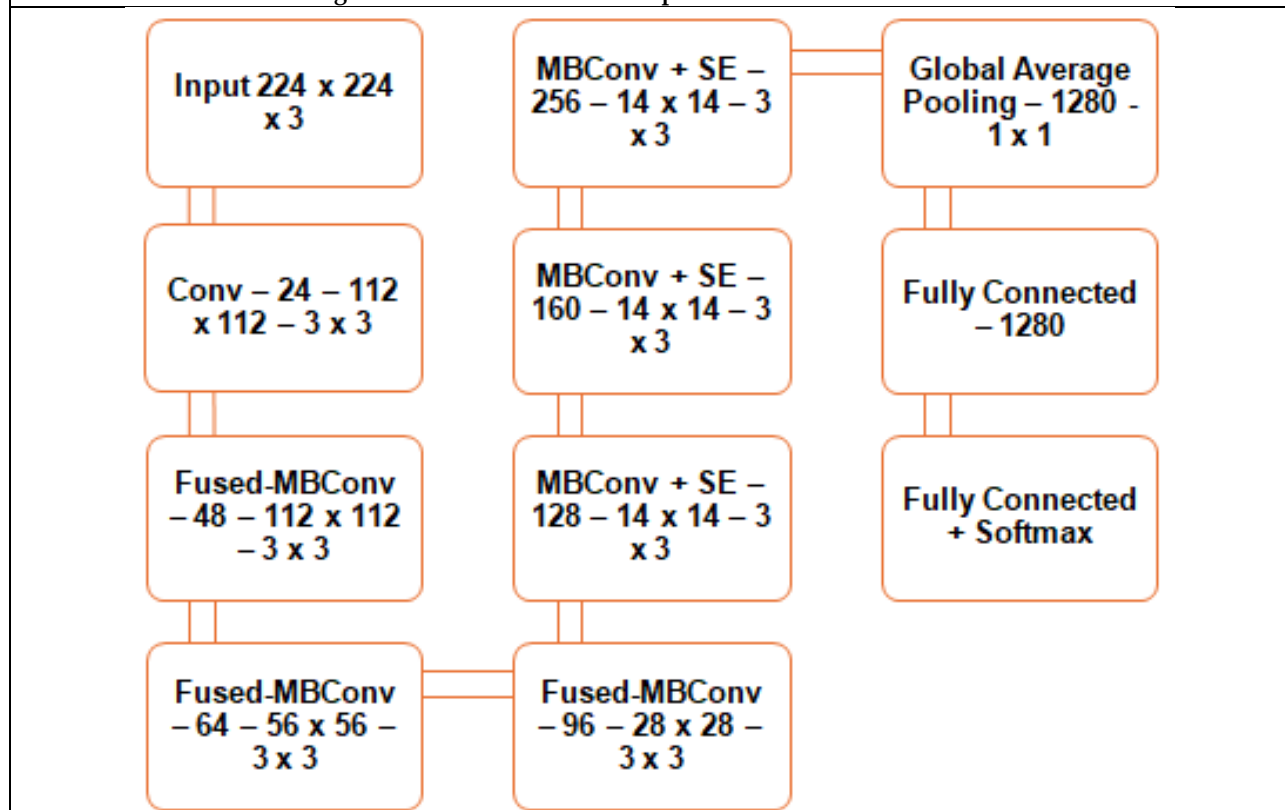


Figure 2. EfficientNetV2 architecture



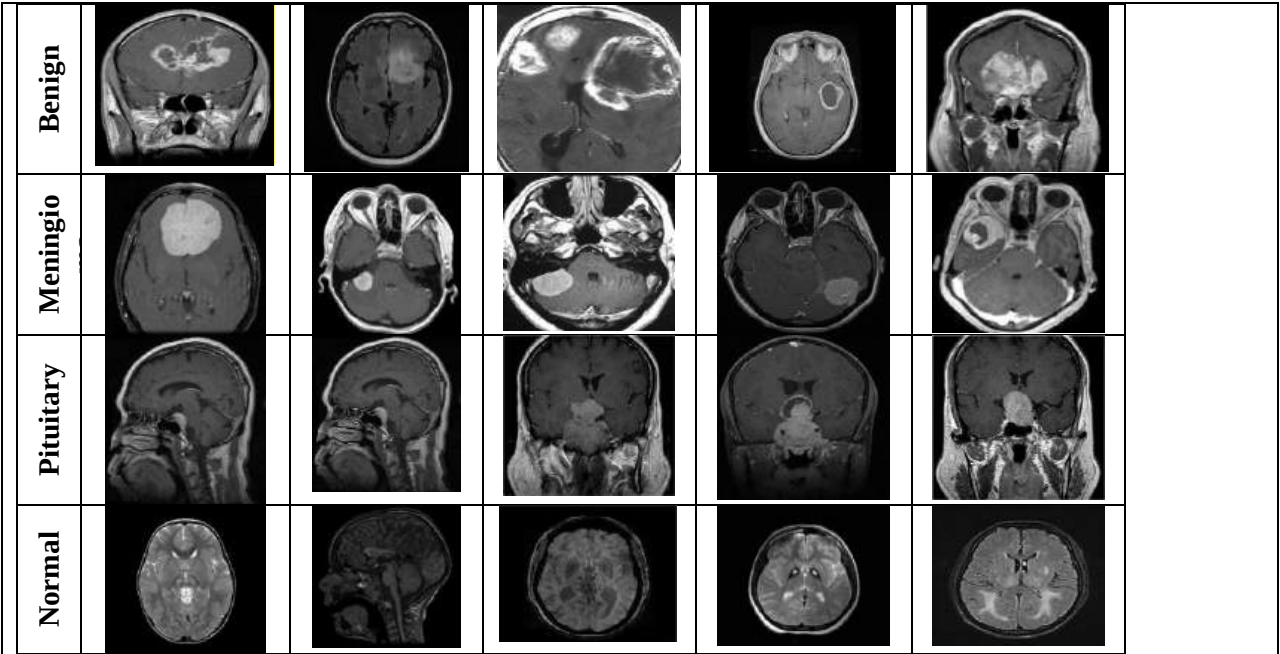


Figure 3. MRI Images for Multiclass Brain Tumour Classes

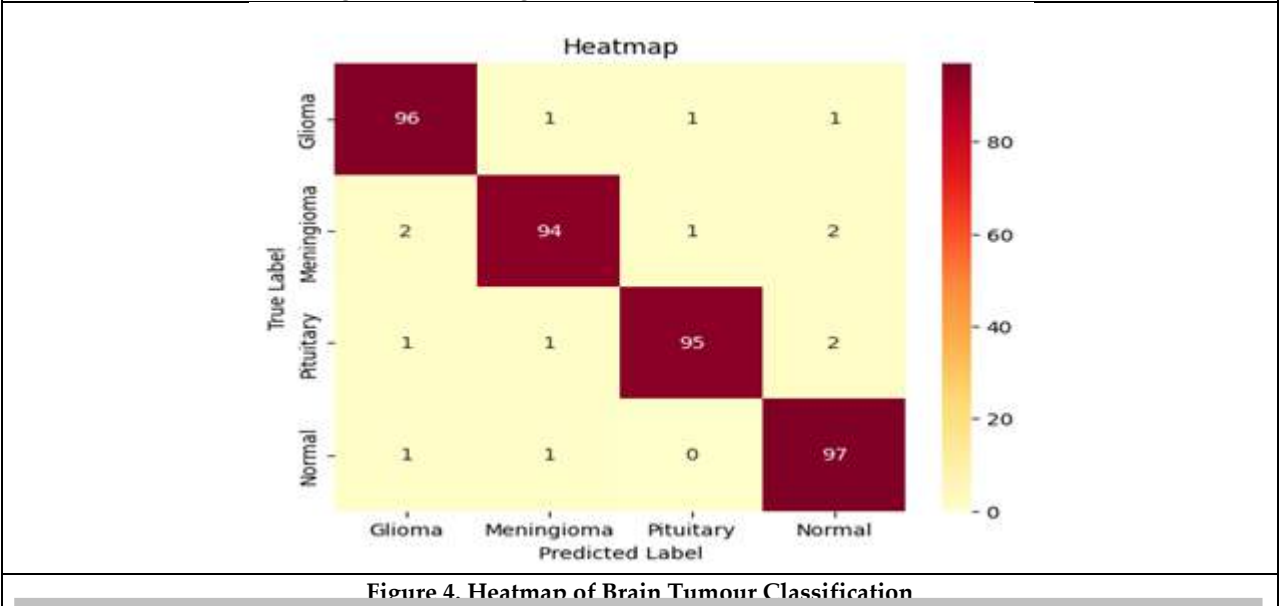


Figure 4. Heatman of Brain Tumour Classification

Umarani et al.,





Umarani et al.,

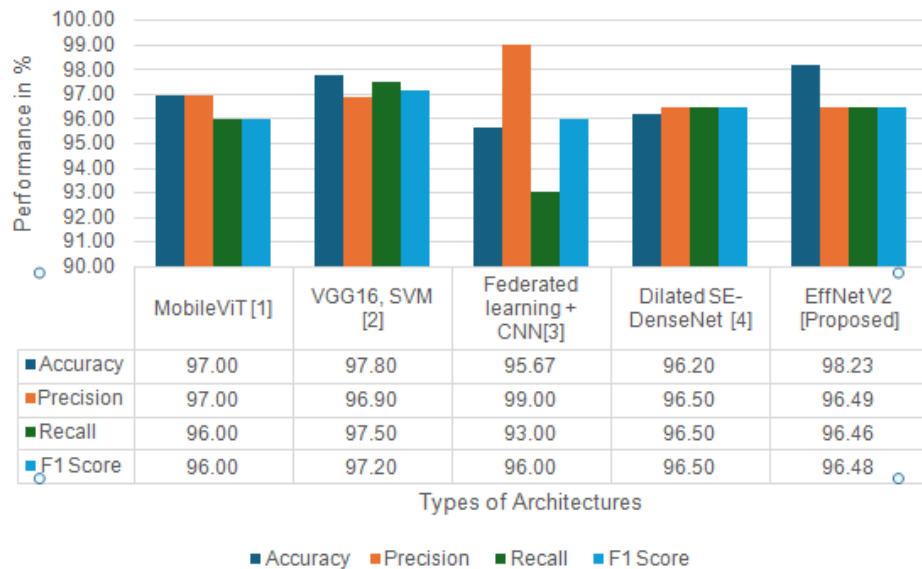


Figure 5. Performance Comparison of Brain Tumour Classification Techniques

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RESEARCH ARTICLE

Effect of Grounding Technique in Reducing Anxiety among Slow Learning Students

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ABSTRACT

Slow-learning students often carry a quiet burden—falling behind in class, feeling different from their peers, and battling constant worry. Over time, this anxiety can dim their confidence and joy in learning. Grounding techniques, simple strategies that bring attention back to the present moment, offer a way to pause, breathe, and feel safe again. In occupational therapy, these small moments of calm can become stepping stones toward better focus, emotional balance, and active participation in school life. This study explores how grounding can ease anxiety for these students and help them feel comfortable in their learning journey. The study involved 30 children aged 6–12 years diagnosed with mild to severe anxiety. Participants were randomly divided into two groups: the experimental group (N=15) received grounding techniques, and the control group (N=15) underwent conventional occupational therapy. Both groups participated in 36 sessions over three months, each lasting 45 minutes. Anxiety was assessed using the Generalized Anxiety Disorder Scale -7 (GAD-7) before and after the intervention. The GAD-7 includes 7 questions to evaluate anxiety levels from 0-4 (minimal anxiety) to 15-21 (Severe anxiety). Our findings reveal a high statistically significant difference in the post-test score between the experimental group and the control group of GAD-7 ($p=0.000$). The mean test score of the experimental group was 9.87 with a standard deviation of 3.292, while the mean post-test score of the control group was 14.47 with a standard deviation of 2.924. This suggests that the intervention received by the intervention group led to greater improvement in reducing anxiety compared to the control group. This study shows that grounding techniques can make a real difference in easing anxiety for slow-learning students. By helping them stay present and connected through simple sensory and physical exercises, these strategies reduced feelings of



**Malavika and Aashifa Dhiwan Fathima**

restlessness, nervousness, and emotional strain. Students not only managed stress more effectively but also gained confidence in both learning and social settings. Grounding helped them feel calmer, safer, and more in control, fostering emotional stability and resilience. These results highlight the value of including grounding techniques as a gentle, nonmedical approach in educational and therapy plans—supporting the emotional well-being and active participation of slow-learning students

Keywords: Slow learning students, Anxiety, Grounding technique, occupational therapy, Generalized Anxiety Disorder-7 scale (GAD-7 Scale)

INTRODUCTION

Occupational Therapists can intervene in a variety of adverse situations. Life areas impacted by the disease such as daily tasks, sleep, work, play, socialization, education, and living. To emphasize the value of occupational therapy in improving the quality of life for those suffering from anxiety disorders [1]. Occupational therapists are essential members of our communities because they help patients participate in the important tasks of everyday life. Illness and injury are a step that interferes with an individual's capacity to live independently [2]. Anxiety has also been defined as a vague, uncomfortable feeling exacerbated by prolonged stress, and the presence of multiple stressors defines anxiety as an emotional condition characterized by fear, concern, and physical arousal. It is a state in which an individual worries, feels nervous, and becomes uneasy, sometimes of being menaced by an unspecified threat to one's personality [3,4]. When anxiety results in excessive physiological arousal, cognitive, emotional, and behavioral symptoms, along with subjective suffering and functional impairment, it is classified as a disorder. Children as young as two years old can be diagnosed with anxiety disorders, which are not limited to any specific gender, culture, or age group. Anxiety is characterized by subjective feelings of tension, apprehension, nervousness, and worry, all of which are associated with the arousal of the nervous system. A heightened level of anxiety can make it difficult for a person to maintain a normal life, leading to interruptions in daily activities and social interactions [5].

Symptoms of "overall anxiety" and "more specific overall" anxiety were present in 38% and 26% of slow learners, respectively. Globally, anxiety disorders rank among the most prevalent psychological disorders in students, and research has shown that they adversely impact their academic, social, and personal growth. Slow learners experience diverse cognitive challenges and often lag in the standard classroom because the pace and techniques of instruction do not align with their learning capabilities. They face academic challenges in reading, writing, and comprehending and performing mathematics; they exhibit deficits in executive function, weak working memory, and frequently have difficulties related to social skills [6]. When someone is experiencing dissociation or feeling overwhelmed by memories or strong emotions, grounding techniques help them refocus on the present moment. The feeling of leaving a movie theater serves as a useful metaphor. When the individual dissociates or encounters a flashback, it resembles viewing a movie; using grounding techniques, he or she may exit the movie theatre and enter the current setting and sunlight. The client's responsibility is not just to cling to moments from the past, but also to recognize that what he or she was going through is from a previous time [7]. Grounding is an effective method that can assist individuals with anxiety by re-establishing a connection with the present moment and anchoring themselves in reality [8].

METHODOLOGY

Research Design: A True experimental study design was employed to investigate the effectiveness of grounding techniques in reducing anxiety among slow learning students.

Sampling Technique: The study utilized a randomized sampling technique to select participants.





Malavika and Aashifa Dhiwan Fathima

Sample size: A total of 30 subjects participated in this study. The participants were divided equally into two groups:

- **Experimental group:** 15 subjects underwent grounding technique interventions.
- **Control group:** 15 subjects received conventional occupational therapy.

Sample Setting: The study was conducted at St. Mary's Matriculation Higher Secondary School, Sriperambatur.

Selection Criteria:

Inclusion Criteria-

1. Age: Students aged 11-13 years.
2. Grade Level: Students in 6th to 8th standard.
3. Students with moderate to failing performance on the academic performance scale.
4. Anxiety Levels: Students with moderate to high levels of anxiety (measured by a generalized anxiety scale-7-item scale).
5. Both male and female

Exclusion criteria

1. Students unwilling to participate in the study
2. Students with other co-morbidities (Other health issues)

Screening Tools Used: The Academic Performance Scale (APS), developed by Carson Birchmeier, Emily Grattan, Sarah Hornbacher, and Christopher Gregory (Saginaw Valley State University), was used to assess students' scholastic functioning. It consists of 8 items covering domains such as attention, classroom engagement, homework/assignment completion, study habits, and overall academic motivation

Scoring method:

Each item is rated on a 5-point Likert scale:

1 = Strongly Disagree | 2 = Disagree | 3 = Neutral | 4 = Agree | 5 = Strongly Agree

Total score range: 8 – 40

Interpretation:

Each item is rated on a 5-point Likert scale:

1 = Strongly Disagree | 2 = Disagree | 3 = Neutral | 4 = Agree | 5 = Strongly Agree

Total score range: 0– 40

Interpretation:

33-40 (Excellent Performance)

25-32 (Good Performance)

17-24 (Moderate Performance)

9-16 (Poor Performance)

0-8 (Failing Performance)

Reliability and Validity

- **Reliability:** Test-retest reliability over two weeks was $r \approx 0.84$, supporting stability.
- **Validity:** Scale scores showed adequate internal consistency, 2-week test-retest reliability, and satisfactory concurrent validity.



**Malavika and Aashifa Dhiwan Fathima****TOOLS USED****Generalized Anxiety Disorder-7 scale(GAD-7)**

The Generalized Anxiety Disorder-7 (GAD-7) is a 7-item self-report questionnaire designed by Spitzer et al. (2006)¹¹ to screen for generalized anxiety disorder and measure its severity.

Scoring Method

Each item is rated on a 4-point scale:

- 0 = Not at all
- 1 = Several days
- 2 = More than half the days
- 3 = Nearly every day

Total score range: 0–21

scores:

- 0–4 → Minimal anxiety
- 5–9 → Mild anxiety
- 10–14 → Moderate anxiety
- 15–21 → Severe anxiety

Reliability and validity:

- **Reliability**–The GAD-7 demonstrates high reliability with Cronbach’s $\alpha \approx 0.89$ and good test–retest reliability ($ICC \approx 0.83$)
- **Convergent validity**: Strong correlations with other anxiety measures such as the Beck Anxiety Inventory ($r \approx 0.72$) and the Penn State Worry Questionnaire ($r \approx 0.74$).

Intervention Procedure:

The study was conducted over 12 weeks, with participants attending 30 sessions (3 sessions per week), each lasting 45 minutes. The intervention was structured as follows:

- Warm-up activities: 10 minutes.
- Program activities: 30 minutes (grounding technique for the experimental group and conventional therapy for the control group).
- Wind-up activities: 5 minutes.

Conventional Occupational Therapy for the Control Group

The therapy sessions focused on reducing anxiety among slow learning students, including activities such as:

Classroom-Based Interventions: Teachers and educational administrators have a critical role in tackling and lessening the worry among students in their schools. Students frequently see particular. The interpersonal and instructional methods of their teachers help lower Classroom anxiety. strategies like open book, support of a peer during class, working with a partner in the computer lab¹³.

Family-based Cognitive Behavioral Therapy (FCBT): Family-based Cognitive Behavioral Therapy (FCBT) enhances treatment results for children. Involving parents in FCBT for children with anxiety-related school refusal was shown to significantly improve school attendance and adjustment, even if the child did not get direct therapy. Although they could not identify which age group profited the most, the authors hypothesized that younger children may gain more since they are more affected by their parents¹³.

Psycho education: Psycho educational interventions combine cognitive behavioral therapy, group support, and education to help patients and families understand the illness and its treatment, allowing them to work closely with professionals for better outcomes. Recent studies highlight their value across the life span, especially for children,





Malavika and Aashifa Dhiwan Fathima

adolescents, and caregivers. Such interventions have been shown to improve mental health awareness and reduce anxiety levels, making them particularly relevant for slow-learning students struggling with anxiety¹².

Session Duration: 30– 45 minutes, 2– 3 times per week

Pre- and post-intervention assessments were conducted using the GAD-7 SCALE

Grounding technique for the Experimental group

Session 1-2	Introduction about myself and self-introduction about the students. Explain about grounding technique and get the concern form. Screening was done using the academic performance scale
Session 3-4	Assessed pretest using Generalized Anxiety Disorder-7 Scale(GAD-7)
Sessions 5-10	• Warm-Up: Breathing exercises, body scanning, gentle stretching • Active Phase: Introduction to the concept of grounding, Basic 5-4-3-2-1 sensory technique, Progressive muscle relaxation (PMR), Journaling sensations or emotional states • Wind-Down: Guided imagery, group sharing
Session 11-15	• Warm-Up: Breathing with awareness, light yoga • Active Phase: Deepening 5-sense grounding (touch/texture focus), Sound and movement awareness, Mindful walking (barefoot if possible), Use of sensory objects (clay, sand, textured fabrics) • Wind-Down: Gratitude journaling, soft
Session 16-20	• Warm-Up: Emotion check-in using emotion wheels • Active Phase: Naming emotions + grounding * Anchoring statements ("I am safe..."), EFT tapping (Emotional Freedom Technique), Grounding through storytelling/personal narrative • Wind-Down: Reflection circles, breath-focused meditation
Session 21-25	• Warm-Up: Rhythmic clapping, joint rotations. • Active Phase: Grounding through dance (freestyle, tribal beats), Body percussion and grounding stomp routines, Yoga grounding poses (mountain, tree, child's) * Walking meditation outdoors • Wind-Down: Journaling, cooling stretches
Session 26-30	• Warm-Up: Recalling positive memories. Active Phase: Grounding using affirmations, Thought-stopping + shifting exercises, Visualization of safe spaces, Nature grounding (rocks, leaves, natural textures) • Wind-Down: Deep breathing, cognitive reframing recap
Session 31-35	• Warm-Up: Breathing & gratitude affirmations • Active Phase: * Combining all techniques into a personalized "Grounding Toolkit", Role-play stress scenarios with grounding responses * Creating a grounding journal or collage * Self-guided grounding scripts • Wind-Down: Final reflections, open group sharing, celebration
Session 36	• Assess post-test of GAD-7 scale and get feedback about the past sessions from the student, and changes observed by the teacher and the parents

Result:

Table 1: Statistical analysis of age and gender group between control and experimental group

A total of 30 participants took part in the study. In the control group, there were no female participants (0.0%), while the experimental group included 3 (20.0%) female participants. Regarding age distribution across both the experimental and control groups, 8 participants were aged 11 years, 6 participants were aged 12 years, and 1 participant was aged 13 years.



**Malavika and Aashifa Dhiwan Fathima****Table 2: Statistical analysis between pre-test and post test scores in control group**

In the above table, the p-value less than 0.05 and is significant at 5% level, and hence we can say that there is a significant difference of anxiety scores between pre- and post-tests in the control group. Also, the mean value of post-test scores is less than the mean value of pretest scores, and the difference is significant. Hence, we can conclude that conventional occupational therapy is significantly decreasing anxiety scores in the control group

Table 3: Statistical analysis of anxiety scores between pre- and post-tests in the experimental group

In the above table, the p-value is less than 0.01 and is highly significant at 1% level, and hence we can say that there is a highly significant difference in anxiety scores between pre- and post-tests in the experimental group. Also, the mean value of post-test scores is less than the mean value of pre-test scores, and the difference is highly significant. Hence, we can conclude that the “Grounding technique” is high significantly decreases anxiety scores in the experimental group.

Table 4: Statistical analysis between the experimental and control groups

In the above table, the p-value is less than 0.01 and is highly significant at 1% level and hence we can say that there is high significant difference of post-test level anxiety scores between control and experimental groups. Also, the mean value of the experimental group is lesser than the mean value of the control group and the difference is highly significant. Hence, we can conclude that the “Grounding technique” is high significantly decreasing anxiety score in the experimental group than then the conventional occupational therapy.

DISCUSSION

The study assessed the effectiveness of an intervention program in decreasing anxiety among slow-learning students, utilizing the Generalized Anxiety Disorder-7 (GAD-7) scale as the primary assessment tool. Participants were divided into control and experimental groups, and pre-test and post-test scores on the GAD-7 scale were analyzed. The statistical analysis of the control group’s pre-test and post-test scores on the GAD-7 scale (Table 2) revealed significant improvement. The mean decreased from 15.93 to 14.47, with a p-value of 0.013, indicating a statistically significant difference at the 5% alpha level. Consequently, the alternate hypothesis, suggesting a significant difference between pre- and post-test scores of the control group, is accepted. Similarly, the findings of Elizabeth A. Hoge et al. (2017)⁹ reported that mindfulness meditation is an effective technique for improving occupational functioning and health care utilization in individuals with anxiety.

The significant findings presented in Table 3, analyzing the experimental group’s pre- and post-test scores on the GAD-7 scale, underscore the effectiveness of grounding techniques. The highly significant difference, as indicated by the p-value of 0.000, provides compelling evidence to reject the null hypothesis and accept the alternative hypothesis, suggesting a substantial improvement resulting from the intervention. The notable decrease in GAD-7 scores—from a mean of 15.80 (SD = 3.629) in the pre-test to 9.87 (SD = 3.292) in the post-test—demonstrates a strong statistical improvement in reducing anxiety. Similarly, the findings of Danang Riyanto et al. (2021)¹⁰ indicated that grounding technique therapy was effective in reducing anxiety levels among students in the final stage of a master’s nursing program. Our findings, as presented in Table 4, reveal a highly significant difference in post-test scores between the experimental group and the control group on the GAD-7 scale ($p = 0.000$). The mean post-test score of the experimental group was 9.87 (SD = 3.292), compared to 14.47 (SD = 2.924) for the control group. This suggests that the intervention received by the experimental group led to greater improvement in reducing anxiety compared to the control group.

Limitations

- This study was done on a small sample size
- The duration of this study was a short time
- The variability in individual cognitive and emotional profiles may have influenced the consistency of the result.





CONCLUSION

The findings highlight the significant impact of grounding techniques in reducing anxiety among slow learning students. The intervention proved to be practical and student-friendly, leading to observable reductions in restlessness, nervousness, and emotional distress. Participants demonstrated improved emotional regulation, attentional control, and confidence in academic and social settings. By directing attention to the present moment, grounding exercises reduced cognitive overload, enhanced emotional stability, and promoted a sense of safety and calm. These results provide strong evidence for incorporating grounding techniques as an effective, non-pharmacological strategy for anxiety management. The study underscores important implications for educators, therapists, and caregivers, emphasizing the value of integrating grounding techniques into individualized educational and therapeutic programs to foster resilience, well-being, and academic participation in slow learning students.

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Malavika and Aashifa Dhiwan Fathima

Table 1: Statistical analysis of age and gender group between control and experimental group

S. No.	Demographic Variables	Class	No. of children in			
			Control group	%	Experimental group	%
1	Age	11 Years	8	53.3%	8	53.3%
		12 Years	6	40.0%	6	40.0%
		13 Years	1	6.7%	1	6.7%
2	Gender	Female	0	0.0%	3	20.0%
		Male	15	100.0%	12	80.0%

Table 2: Statistical analysis between pre-test and post test scores in control group

S. No.	Test	N	Mean	SD	T Value	Df	P Value
1	Pre test	15	15.93	2.549	2.847	14	0.013*
2	Post test	15	14.47	2.924			

Table 3: Statistical analysis of anxiety scores between pre- and post-tests in the experimental group

S. No.	Test	N	Mean	SD	T Value	Df	P Value
1	Pre test	15	15.80	3.629	17.955	14	0.000**
2	Post test	15	9.87	3.292			




Malavika and Aashifa Dhiwan Fathima
Table 4: Statistical analysis between the experimental and control groups

S. No.	Test	N	Mean	SD	T Value	Df	P Value
1	Control	15	14.47	2.924	4.046	28	0.000*
2	Experimental	15	9.87	3.292			*

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RESEARCH ARTICLE

Targeting the Shikimate Pathway of *Mycobacterium tuberculosis* with Natural Products: A Structured Drug Discovery Pipeline from Virtual Screening to Pre-Clinical Evaluation

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ABSTRACT

The infection caused by *Mycobacterium tuberculosis* (Mtb) still stands out as the most lethal infectious disease caused by a single infectious agent, with 10.8 million new infections and 1.25 million deaths being recorded in the year 2023 alone. In addition, MDR-TB and XDR-TB variants have been continually limiting the number of treatment alternatives that can be administered; hence, there is a need to develop new drug targets. The shikimate pathway involves the series of seven reactions which convert phosphoenolpyruvate (PEP) and erythrose-4-phosphate (E4P) to chorismate and is entirely missing in mammals and essential for the Mtb's survival. This article presents a comprehensive drug discovery pipeline involving all seven shikimate enzymes: DAH7PS, DHQS, DHQase, SDH, SK, EPSPS, and CS. The process starts with the computational screening of IMPPAT 2.0 phytochemical database (17,967 compounds from 4,010 Indian medicinal plants). Multi-criteria ADMET filtering (rules of Lipinski, Veber, Egan; SwissADME; ProTox 3.0) narrows the pool down to 40-100 molecules, which are then docked into resolved crystal structures using AutoDockVina. Binding affinity is verified with 100-200 ns molecular dynamics (MD) simulations in GROMACS, supplemented by MM-PBSA binding free energy calculation. Hits are further assessed in surrogate mycobacterial systems (*M. smegmatis* and *M. bovis* BCG) and cytotoxicity assays for THP-1 macrophages prior to the animal stage. Flavone-C-glycoside isoorientin demonstrated docking score of -9.4 kcal/mol towards shikimate kinase (PDB: 2IYQ) with stable MD binding (MM-PBSA: -8.2 kcal/mol), MIC₉₀ towards *M. smegmatis* at 8.2 μ M concentration and selectivity index greater than 12. This review also covers the approaches to lead formulations (encapsulation in



Nikhil Ranjan Behera *et al.*,

liposomes, PLGA nanoparticles), the notion of pan-shikimate inhibition and the crucial translation hurdles preventing the computational hits from entering the clinic.

Keywords: *Mycobacterium tuberculosis*; shikimate pathway; virtual screening; molecular docking; ADMET; natural products; IMPPAT; molecular dynamics; MDR-TB; drug discovery

INTRODUCTION

The discovery of *Mycobacterium tuberculosis* by Robert Koch back in 1882 was the starting point for arguably the longest ongoing battle in medicine [1]. Despite the use of BCG vaccine and multi-drug chemotherapy beginning in the 1950s, TB kills more than a million people every year. According to WHO Global Tuberculosis Report 2024, 10.8 million new TB cases and 1.25 million deaths were reported in 2023, and that puts Mtb back on track as the world's number-one single-pathogen killer [2,3]. India alone accounts for about 26% of the TB burden globally. Drug-resistant forms of bacteria worsen the situation. In 2023, 400,000 people had MDR-TB, resistance to rifampicin and isoniazid, the two key first-line drugs [4]. Less than half of those patients got proper treatment. XDR-TB, with addition of resistance to fluoroquinolones and other second-line drugs like bedaquiline or linezolid, leaves virtually no treatment possibilities but the few last resort drugs with toxic side effects [5]. Even novel BPaLM combination of four drugs (bedaquiline, pretomanid, linezolid, moxifloxacin) succeeds less than 70% of the time in clinical studies. There is hardly any new chemical entity coming down the pipe with an orthogonal mechanism of action. The importance of targeting such drug targets, which are metabolic processes in Mtb and absent in humans, cannot be overstated. An example of such a drug target is the shikimate pathway, which serves as the metabolic pathway of aromatic amino acid synthesis (phenylalanine, tyrosine, tryptophan), folate cofactors, menaquinones, and mycobactins. Mycobactins are small molecules, which serve as a chelator for scavenging iron from the human macrophages. Iron is an absolute requirement for growth of Mtb within the macrophages.

Shikimate pathway contains seven enzymes DAH7PS, DHQS, DHQase, SDH, SK, EPSPS, and CS encoded by different Mtb genes. Each enzyme has a solved crystal structure, and knockout of each gene leads to the death of Mtb [8]. However, shikimate pathway has attracted much less interest than the Mtb cell wall or RNA polymerase as a target of antimycobacterials due to difficult development of inhibitors of metabolism enzymes with complex active sites and also due to the plant and microbial biochemistry tradition [9]. Natural products constitute an excellent intermediate between what is presently known about structural biology and potential drug candidates. The immense Indian biodiversity is well documented through Ayurvedic/ethnopharmacological data and compiled into IMPPAT 2.0 database which contains 17,967 chemically characterized phytochemicals from 4,010 Indian medicinal plants [10]. Combining these chemically distinct libraries with those derived from marine sources along with ADMET predictors, docking using Auto Dock Vina and molecular dynamics using GROMACS allows us to build an efficient and repeatable virtual screening workflow. This review gives an account of this workflow, describes the representative data generated using shikimate kinase (MtSK) as the major drug target and talks about the translational issues that need to be tackled before moving ahead in this direction.

The Shikimate Pathway in *Mycobacterium Tuberculosis*

Shikimate pathway is present in bacteria, fungi, algae and higher plants, but entirely absent from mammals, who acquire the aromatic amino acids through dietary sources [11]. In Mtb, this pathway utilizes two intermediates of glycolysis and the pentose phosphate pathways, namely PEP and E4P, for the synthesis of chorismate by way of seven enzymes [6]. Seven genes code for the pathway enzymes in Mtb. Chemogenomic screens have demonstrated that deletion of a single one of these genes, including *aroK* coding for shikimate kinase, results in auxotrophic strains that grow inefficiently in conventional growth media and cannot infect macrophages [12,13]. The expression profiles of these genes have demonstrated their increased transcription in infected macrophages, implying that the shikimate



Nikhil Ranjan Behera *et al.*,

pathway is not only active, but absolutely necessary for the bacterium to thrive in the physiological conditions associated with pathogenesis of tuberculosis [8].

DAH7PS Synthase (DAH7PS)

The first step in this pathway is the condensation of PEP with E4P mediated by DAH7PS, in which a Mn^{2+} ion plays an essential role, forming 3-deoxy-D-arabino-heptulosonate-7-phosphate (DAH7P). The enzyme of Mtb is a $(\beta/\alpha)_8$ TIM barrel folded into a tetrameric structure, which is different from the dimers and monomers of this enzyme in other bacteria [14]. The tetrameric form makes a potential allosteric site that reacts to pathway end-products, such as chorismate and prephenate. Phosphonates, based on the transition state structure of condensation reaction, have been proved to have IC_{50} of about 10 μM in other species.

Dehydroquinate Synthase (DHQS)

DHQS catalyzes the conversion of DAHP to 3-dehydroquinate (DHQ) via an exceptional 5-step mechanism which includes oxidation, dephosphorylation, ring cleavage, reduction, and intra-molecular aldol addition all taking place in a single active site without intermediates being released [15]. MtDHQS needs a tightly bound non-consumable NAD^+ co-factor and Co^{2+} ion for its catalytic activity. Bilobal dimeric structure forms an extensive active site cleft. While the NAD^+ binding pocket has been utilized as a potential target site for the development of inhibitors in other organisms, the inhibitors for MtDHQS have not yet been thoroughly characterized.

Dehydroquinate Dehydratase (DHQase)

The DHQase converts DHQ into 3-dehydroshikimate (DHS). Type II DHQases in bacteria utilize covalent Schiff-base catalysis involving an active site lysine; Type I catalysis occurs in fungi and plants [16]. The difference in mechanistic aspects is significant from the pharmaceutical point of view: inhibitors acting through the Type II Schiff-base approach cannot affect any human enzymes due to inherent selectivity. The MtbDHQase forms a unique dodecamer of twelve identical subunits in quaternary structure; this peculiarity among enzymes from the pathway makes it possible to design inhibitors for the subunit-subunit interface. Polyphenols epigallocatechin gallate (EGCG), found in green tea, act on Type II DHQases with IC_{50} about 30 μM , showing that plant compounds can reach the dodecameric active site.

Shikimate Dehydrogenase (SDH)

The SDH (aroE gene) converts DHS to shikimate using NADPH as the hydride donor. It is characterized by the presence of a Rossmann fold for the binding of NADPH along with the substrate-binding site. The enzymatic activity of SDH is carried out through the help of a Lys-Asp-Asn catalytic triad which is characteristic of SDR enzymes [17]. Several crystal structures of MtSDH with substrate and cofactor in the complex form are found in the Protein Data Bank. The binding pocket is rather open and can bind to competitive inhibitors during assays.

Shikimate Kinase (SK)

Shikimate kinase phosphorylates shikimate to shikimate 3-phosphate with one ATP, which is the only reaction in the whole process requiring ATP [18]. MtSK is the most well-characterised enzyme within the shikimate pathway, and acts as the principal target for the process outlined above in this review. Structural, inhibitor, and pharmacophore properties of MtSK are examined individually in Section 3 below.

EPSP Synthase (EPSPS)

The reaction of EPSPS leads to the formation of 5-enolpyruvylshikimate-3-phosphate (EPSP) from shikimate-3-phosphate and phosphoenolpyruvate with the liberation of inorganic phosphate [19]. The MtEPSPS protein forms a two-domain α/β structure and can undergo significant conformational rearrangement of the open to closed form upon substrate binding. Glyphosate, the herbicide, is a well-known inhibitor of EPSPS, and the structural determinants of its binding with MtEPSPS have been extensively studied. Unfortunately, glyphosate cannot be used as a potential anti-TB drug owing to its low membrane permeability and lack of specificity for the mycobacteria EPSPS compared to plant EPSPS.



**Chorismate Synthase (CS)**

CS completes the biosynthesis route through an anti-1,4 elimination of the 3-phosphate moiety along with C6 hydrogen from EPSP to give chorismate [20]. Mechanistically, this step is quite unique, since although it does not constitute a redox reaction, it needs reduced flavin mononucleotide (FMN) as an essential cofactor. There is no known human enzyme that carries out this chemistry, which makes MtCS an interesting selective target. Structural details on the interaction of MtCS with substrate analogs and FMN have been elucidated. Efforts towards developing bifunctional inhibitors which bind at both the EPSP binding pocket as well as FMN pocket have been made.

Shikimate Kinase (Mtsk): The Primary Target

Four factors distinguish the enzyme MtSK (aroK gene) among the seven pathway enzymes: it has the best understood structure (1.8 Å resolution, PDB: 2IYQ), it has the most biochemically characterized active site, it is the only enzyme which consumes ATP in the pathway (creating a pharmacophoric niche not covered by the other six enzymes), and there is an expanding dataset of inhibitors comprising both natural and synthetic compounds as well as allosterics. Mtsk belongs to the NMP kinase family and consists of a three-domain protein structure. The CORE domain is built from a five-stranded parallel β -sheet surrounded by α -helices and represents the scaffold of MtSK structure. The LID domain represents an α -helix domain folding over the ATP-binding site in the presence of nucleotide substrate with the purpose of isolation of γ -phosphate for its subsequent transfer. The shikimate-binding (SB) domain interacts directly with the substrate by making a salt bridge with arginines 57 and 136 and the carboxyl group of shikimate [18,22]. In the process of catalysis, movement of all three domains occurs cooperatively; specifically, the LID covers ATP while the SB domain encloses shikimate for their positioning to SN2 phosphoryl transfer reaction with participation of Mg^{2+} ion coordinated with Asp34 and β - and γ -phosphates of ATP [23]. The P-loop (10-16 residues, GxxxxGKS/T) binds the β -phosphate of ATP and defines nucleotide specificity.

Conformational plasticity of the LID domain poses both a problem and a unique approach to drug discovery. The rigid-receptor docking approach keeps the enzyme locked into a single conformational form; however, MtSK has two separate open and closed states, depending on the binding of the substrate and product, respectively, with considerable active site volume changes. The ensemble docking approach that uses multiple conformers generated via MD simulation partly resolves the problem at the expense of increased computational load. Moreover, the benzothiazole compounds have been proven to bind the allosteric pocket located at the LID-CORE interface and induce a new conformational form of the enzyme different from both the open and closed state with an IC_{50} value of 0.8 μ M [24]. Manzamine alkaloids from marine sponges act as slow-binding and non-competitive inhibitors of MtSK, with $K_i = 1.3 \mu$ M and MIC value of 2.0 μ g/mL against Mtb H37Rv [23]. The kinetics of inhibition is characterised by a two-stage mechanism of induced fit interaction where fast weak binding is followed by a slower conformational change, leading to a stabilisation of the enzyme-inhibitor complex. This particular kinetic detail is of significant practical importance for inhibitor design since slow-binding inhibitors exhibit pharmacodynamic properties in vivo due to their long retention time in the active site. The computationally identified inhibitors of MtSK by means of phytochemicals are flavonoids (quercetin, luteolin, apigenin, and their derivatives), which form several hydrogen bonds with the residues Arg57 and Arg136 and stack interactions with aromatic residues at the ATP-binding site [25,26]. Terpenoids like ursolic acid and betulinic acid show predicted binding energy values between -8 to -10 kcal/mol, which are comparable or better compared to the known herbicide glyphosate. The flavone C-glycoside isoorientin shows docking energy of -9.4 kcal/mol and also shows stable MD binding energy of -8.2 kcal/mol with MM-PBSA as explained in Section 5.

Imppat 2.0 and Phytochemical Compound Libraries

It is estimated that natural products constitute around 25% of all approved drugs, but there are several examples in TB where natural products have led to the discovery of novel classes of drugs. Examples include streptomycin that originated from an actinomycete soil product; rifampicin originating from *Amiclatopsis rifamycinica*; and diarylquinoline framework used in bedaquiline, inspired from antimycobacterial natural products [27]. However, despite these examples, systematic computational screening of structured phytochemical databases against structurally characterised Mtb enzyme targets is relatively new. The IMPPAT 2.0, compiled by Vivek-Ananth *et al.*, in



**Nikhil Ranjan Behera et al.,**

2023 is the largest database of Indian medicinal plants at present [10]. It includes 4,010 plants and 17,967 phytochemicals with validated 2D/3D structures, physicochemical data and precomputed ADMET predictions. Druglikeness calculations are done with five filters namely, Lipinski's Rule of Five, Veber rules, Egan rules, GSK 4/400 rule and Pfizer 3/75 rule. Of these 17,967 chemicals, 1,335 pass Lipinski's criteria for oral bioavailability. These 1,335 chemicals do not have close resemblance to any existing drug, hence are innovative.

The major phytochemical classes represented in IMPPAT 2.0, along with their relevance as pharmacophores to the shikimate pathway inhibition, are described below. Flavonoids, including flavones, flavonols, flavanones, isoflavones, and chalcones, provide multiple hydroxyl groups on planar aromatic ring systems, which allows hydrogen bonding to polar enzyme active sites and π -stacking interactions with purine cofactor [28]. It is known that quercetin, baicalein, and kaempferol could be docked to MtSK and predicted binding energies were estimated above -8 kcal/mol. Terpenoids such as monoterpenes, sesquiterpenes, diterpenes, and pentacyclic triterpenes (ursolic acid and oleanolic acid) provide hydrophobic scaffold suitable for the interaction with the amphipathic binding pockets of cofactor-dependent enzymes [29]. In addition, the extremely lipid-rich cell wall of mycobacteria can provide an advantage to permeate into cells for lipophilic terpenoid scaffold. Alkaloids are one of the most frequently used classes of compounds for the treatment of mycobacterial infections; berberine, emetine, and manzamines provide unique pharmacophores that can interact with the enzymes from shikimate pathway [30]. Phenolic acids and coumarins, which are structurally simpler compared to flavonoids, have established ADMET properties and previously proved inhibition of bacterial metabolic enzymes. Apart from the IMPPAT 2.0 library, marine compound libraries obtained from brown, red algae and cyanobacteria (available via MarinLit and other databases) provide chemotypes not seen in terrestrial plant chemistry. Marine organisms are especially abundant in halogenated terpenes, macrolides, and peptides, which have been found to be active against mycobacteria in phenotypic screening. Use of both terrestrial and marine libraries guarantees maximal chemical space diversity, which is essential while hitting several enzymes at once.

Phase I: In Silico Virtual Screening and Admet Filtration

Building the Compound Library

The process of virtual screening starts with proper database curation. Structures are downloaded in SMILES format from databases such as IMPPAT 2.0 (bioinfo.barc.res.in) and the marine compound databases. Prior to any filtering process, duplicate structures are removed based on canonical SMILES comparison, structures with undefined stereocenters or conflicting atom valences are removed, and structures containing heavy metals such as mercury, lead, arsenic, and cadmium or reactive toxicophores are eliminated. The database now contains around 15,000 – 18,000 organic structures.

Multi-Rule ADMET Filtration

The process includes four consecutive rules for filtering out compounds that have potential for oral absorption, delivery to the lungs, and do not harm the host in any way. The filters are used in SwissADME (swissadme.ch) using batch filtering of the complete list of compounds. Lipinski's Rule of Five (1997): $MW \leq 500$ Da; calculated $\text{LogP} \leq 4.15$; hydrogen bond donors ≤ 5 ; hydrogen bond acceptors ≤ 10 [31]. Molecules failing multiple criteria are rejected, but exceptions are made for natural products having active transport mechanisms. Veber's criteria: rotatable bonds ≤ 10 ; and topological polar surface area (TPSA) $\leq 140 \text{ \AA}^2$ [32]. The two features independently predict intestinal absorption and membrane permeability and do not rely on lipophilicity. They are especially important for compounds that have to pass through mycobacterium's lipid-filled cell wall. Egan's criteria: weighted $\text{LogP} \leq 5.88$; $\text{TPSA} \leq 132 \text{ \AA}^2$, and select compounds highly predisposed to high passive membrane permeability [33]. Pharmacokinetic constraints by using SwissADME tool: predicted gastro-intestinal absorption – "High"; blood-brain barrier permeability – "Non-permeable," as central nervous system penetration is not required for anti-TB agents and is associated with safety risks. Application of four filters to the initial set of 17,500 compounds results in selection of 150-400 molecules (1-3% passage rate).





Toxicity Prediction Using ProTox 3.0

ProTox 3.0 (tox.charite.de) employs machine learning ensemble models, including random forests, support vector machines, and neural networks, trained on chemicals for which LD₅₀ data are available [34]. Only chemicals classified as Class 4 or 5 (with predicted LD₅₀ > 300 mg/kg; Table 1) progress. In addition, each chemical is filtered for predicted hepatotoxicity (SVM probability > 0.5 excludes chemicals from the study), Ames mutagenicity (any positive prediction excludes a chemical from the study), carcinogenicity (any positive prediction excludes a chemical from the study), and immunotoxicity. Following toxicity filtering, between 40 and 100 compounds generally persist to undergo molecular docking. It should be stressed that many complex natural products possess chemical groups such as catechol moieties or alpha-beta unsaturated carbonyl groups, which raise computational toxicity red flags due to their metabolism by the body in a very short time. The tendency to over-filter potential natural product hits is well-known problem of in silico toxicity software.

Phase II: Molecular Docking and Binding Analysis

Protein Structure Preparation

Crystal structures of all seven Mtb shikimate enzymes have been downloaded from the RCSB Protein Data Bank [35]. The preferred crystal structures are the ones resolved in complex with native substrates or mechanism-based inhibitors since these represent the active site geometry as found in the biological context. In the case of MtSK, PDB ID: 2IYQ has been selected, having been crystallized in complex with ADP and shikimate and representing an accurately resolved substrate-binding geometry at a resolution of 1.8 Å [21]. Each crystal structure has been prepared using AutoDockTools (MGLTools 1.5.6), specifically by removing all crystallographic water molecules; extraction of co-crystallized ligands to reveal the empty binding site; addition of missing hydrogen atoms and protonation of ionizable residues at pH 7.4; calculation and assignment of Gasteiger partial charges; and saving the protein in the PDBQT format. Each of the ligands in the filtered set has likewise been prepared using the same steps for the preparation in the PDBQT format, including the identification of rotatable bonds.

Grid Box Settings and Docking Parameters

A cubic search space is defined around the binding site inferred either from the co-crystallised ligand or, in its absence, the active site residues, which are identified through homology with characterised protein homologues. The search space grid for MtSK (PDB: 2IYQ) has its centre at X = 52.3, Y = 18.5, Z = 48.2 Å; box size = 20 × 20 × 20 Å, spacing = 0.375 Å, and encompasses completely the residues forming the P-loop (Gly10–Ser16), Asp34, and the shikimate co-ordination residues Arg57 and Arg136. AutoDockVina version 1.1.2 will be used with exhaustiveness = 32 (default is 8, which does not allow sufficient sampling), 10 poses reported per compound, and an energy range of 5.0 kcal/mol [36]. A ΔG (binding free energy) of –8.0 kcal/mol or better will qualify compounds for all six peripheral enzymes, while a threshold of –9.0 kcal/mol will be required for MtSK in keeping with the affinity of natural substrates. Cross-docking validation (redocking of the co-crystallised ligand and confirming that the generated pose matches with crystallographic pose within a maximum RMSD of 2.0 Å) will be carried out for each enzyme prior to screening.

Binding Interaction Analysis

Docking results are analyzed using BIOVIA Discovery Studio 2020 and PyMOL to generate high-quality figures. The type of intermolecular interaction is determined based on the following criteria: conventional hydrogen bond (the distance between donor and acceptor atom <3.2 Å and the angle >120°), carbon-hydrogen bond, electrostatic interaction (including π-cation and π-anion interactions; the distance between the aromatic ring and Lys or Arg <5.5 Å and Asp or Glu <5.5 Å, respectively), and hydrophobic interactions (including π-π stacking, two aromatic rings separated by the distance of 4.5–7.5 Å, and π-alkyl interactions) [37]. In MtSK, any contact with P-loop residues (Gly10–Ser16) and the Arg57/Arg136

Molecular Dynamics Simulation

The docking process gives us a static view of interaction between the drug and its target. In molecular dynamics (MD) simulations, we assess the stability of the docked structure when put in a physiological aqueous condition and



Nikhil Ranjan Behera *et al.*,

allowed to move freely at body temperature. Simulation is done in GROMACS 2021 [38] through the use of AMBER99SB-ILDN force field for protein and GAFF2 for each ligand with the help of AM1-BCC partial charge parameters through Amber Tools. Protein-ligand complex is placed in a cubic periodic box with explicit TIP3P water molecules with 0.15 M NaCl. Size of whole system is 35,000 atoms.

The simulation process involves three major stages. Energy minimisation is done through steepest descent method for 10,000 steps where maximum force comes down to less than 1000 kJ mol⁻¹ nm⁻¹. Equilibration process has 100 ps NVT (volume constant, 300 K, velocity rescaling) and then 100 ps NPT (pressure constant, 300 K, 1 atm, Parrinello-Rahman). The simulation runs last for 100-200 ns with coordinates recorded at intervals of 10 ps. Four parameters are assessed based on the production runs: backbone C α RMSD – a plateau below 2.0 Å within 50 ns indicates that the complex is stable; per residue RMSF – active site residues have an RMSF below 2 Å; radius of gyration – a constant value assures us that the protein doesn't unfold; and number of protein-ligand H-bonds with time – a constant number of at least 2 H-bonds indicates a stable interaction pattern. The binding free energy using MM-PBSA method [39] which is evaluated for the last 50 ns gives a quantitative assessment of the binding energy: below -7.5 kcal/mol means stable binding energy.

Phase III: In Vitro Cytotoxicity and Antimycobacterial Testing

Mammalian Cell Cytotoxicity

Prior to toxicity assay against bacteria, cytotoxic effects on human cells need to be determined in order to establish a therapeutic window. THP-1 cells (ATCC TIB-202) are induced to macrophages using 50 nM of phorbol-12-myristate-13-acetate (PMA) for 48 hours [40]. Induced THP-1 cells represent the intracellular conditions where Mtb is located. Vero cells (ATCC CCL-81) serve as the control for mammalian cytotoxicity. Both cell types are cultured in RPMI-1640 with 10% FBS at 37°C and 5. The MTT test measures mitochondrial succinate dehydrogenase enzyme activity, which is considered an index for viability of cells [41]. Cells are seeded into 96-well plates at a density of 10,000 cells/well and incubated overnight to allow adherence. Compounds to be tested are added in serial two-fold dilutions ranging from 1 to 100 μ M. The vehicle control wells contain up to 0.1% (v/v) DMSO whereas positive controls are treated with 1% Triton X-100. Formazan crystals are formed after 48–72 hours of incubation upon addition of 20 μ L of MTT reagent (5 mg/mL). After incubating further for four hours, 100 μ L DMSO is added to dissolve the crystals, and absorbance is measured at 570 nm. Percentage viability is calculated relative to vehicle controls and a sigmoidal dose-response curve is generated to determine the CC₅₀ value.

Surrogate Mycobacterial Testing

Virulent Mtb H37Rv should be maintained in Biosafety Level 3 (BSL-3) facility. There are two nonpathogenic surrogates available for screening of compounds in lower level of biocontainment prior to use of BSL-3 facilities. *Mycobacterium smegmatis* mc²155 (ATCC 700084) is fast-growing and non-pathogenic mycobacterium with waxy cell wall similar enough to that of Mtb and therefore suitable for testing permeability [42]. The cultures are grown in Middlebrook 7H9 medium (0.5% glycerol, 0.05% Tween-80, 37°C) and then diluted to OD₆₀₀ = 0.05. MIC₉₀ is defined using broth microdilution method: serial two-fold dilutions (0.39–100 μ M) are used together with 10⁵ CFU and incubated for 72 hours. Counts of colonies on Middlebrook 7H10 agar plates with OADC enrichment after 3–5 days are used to prove bactericidal effect (at least 3 log₁₀ CFU reduction). *M. bovis* BCG (ATCC 35734) is attenuated strain used in TB vaccines, grows in non-BSL-3. The Selectivity Index (SI) is defined by the formula: SI = CC₅₀ (human cells) / MIC₉₀ (mycobacteria). An SI $\geq$ 50 implies that the compound is 50 times more toxic towards the bacteria compared to human cells, which marks the acceptable level of toxicity needed before advancing to in vivo experiments. The compound iso-orientin had a MIC₉₀ value of 8.2 μ M towards *M. smegmatis* and a CC₅₀ value greater than 100 μ M towards THP-1 cells; thus, an SI greater than 12 was observed.

PHASE IV: In Vivo Evaluation and Biosafety

Ethical and Regulatory Approvals

All animal studies need approval in writing from the IAEC under the Prevention of Cruelty to Animals Act, 1960 (India). The protocol that is submitted should include details about the species of animals and group size based on

117597



**Nikhil Ranjan Behera et al.,**

statistical calculation, methods of infection and dosing, schedule of clinical observations, humane endpoint and the mode of euthanasia selected. It generally takes four to six weeks.

Animal Infection Model and Dosing

BALB/c mice are the traditional *in vivo* TB infection model; they are cheap, well-known immunologically, and develop progressive granulomatous lung infection in 2–4 weeks after aerosol infection. Hartley guinea pigs are used when a similar-to-human granuloma development is needed for histopathological comparison [43]. Aerosol infection using the Madison chamber (Glas-Col) at approximately 10^5 CFU/mL for 20–30 minutes provides uniform infection dose simulating natural route of transmission. Two to four weeks after infection, the animals are randomly assigned into treatment and control groups. Compounds under evaluation are dosed via oral gavage at 10–50 mg/kg per day one to two times for 4–8 weeks according to the compound PK half-life evaluated in previous single-dose experiment [44].

Endpoint Measurements

At week 4 during treatment, some animals are euthanized, their lungs and spleens are homogenized, and the homogenate is plated onto Middlebrook 7H10 plus OADC agar. A $2 \log_{10}$ CFU reduction in lung tissues compared to non-treated control animals is regarded as an efficacy endpoint. For histopathological examination, lungs are fixed in 10% neutral formalin, embedded in paraffin, sectioned at 5 μ m, and stained with Haematoxylin and Eosin. The degree of granuloma formation is assessed on a 0 to 4 point scale (0 – no lesion; 4 – advanced disease with cavities) [45]. Oral acute toxicity test is conducted according to OECD Test Guideline 423 [46], where three mice get a single dose of 2000 mg/kg orally, and are monitored for 14 days. No animal deaths result in $LD_{50} > 2000$ mg/kg. Plasma pharmacokinetics are established after a single 50 mg/kg oral administration of the substance, followed by sampling of blood and tissues at 0.5, 1, 2, 4, 8, and 24 hours; compound levels are analyzed via LC-MS/MS [47].

Translational Challenges and Formulation Strategies

The In Vitro–In Vivo Disconnect

The major cause of failure of computationally screened phytochemical hits is molecular liability as opposed to target recognition failure. Many flavonoid glycosides and polyphenolics with good docking profiles tend to have molecular weight >500 Da, multiple H-bond acceptors, high TPSA values, and poor aqueous solubility, thus leading to reduced bioavailability [48]. In the case of quercetin, it is quickly converted by glucuronidation in the intestinal wall and first pass effect in the liver such that the concentration achieved does not get close to the *in vitro* IC_{50} for MtSK. For terpenoids and alkaloids, there are similar problems of being cleared in the cytochrome P450 3A4 pathway before reaching the lungs. Another critical difficulty lies in the highly impermeable nature of the cell wall of the mycobacteria. The covalent peptidoglycan-arabinogalactan-mycolic acid layer that contains the highly hydrophobic outer layer of C70–C90 mycolic acids constitutes one of the most difficult permeability barriers encountered in nature [49]. A molecule that might exhibit strong inhibition of the shikimate enzyme in isolation in a biochemical assay may not attain sufficiently high intracellular concentrations to exhibit biological activity. The mycobacterial cell wall is also responsible for conferring resistance to many antibiotics on the Mtb. Lastly, the computational screening studies published in the literature in this area vary widely in quality. A great many publications have proposed potential hits on the basis of AutoDockVina binding energy scores without MD proof, without comparison to a crystallographically proven positive control, without adequate protein preparation, and without any subsequent experimentation [50]. There is no agreement at present regarding what value for ΔG would be considered a good hit, what proof of MD binding stability would be sufficient, or even what kind of negative result would be appropriate.

Liposomal Encapsulation

Liposomes are spherical vesicles composed of phospholipid bilayers, which contain hydrophobic drugs within their bilayers and hydrophilic drugs within their aqueous core. In pulmonary TB, liposomes have pharmacological superiority because lung macrophages actively engulf liposomes – ensuring that drug is delivered precisely to the specific intracellular milieu in which Mtb resides [51]. In addition, the phospholipid layer reduces the first-pass effect via the CYP450 pathway in comparison to free drugs, leading to enhanced bioavailability. The preparation of



Nikhil Ranjan Behera *et al.*,

liposomes involves dissolution of the drug in ethanol, followed by mixing with egg phosphatidylcholine in a 1:5 drug-to-lipid molar ratio; formation of a lipid film via rotary evaporation, hydration with PBS, and size reduction by extrusion using 200-nm polycarbonate filters. The resulting product is assessed for its particle size via dynamic light scattering, encapsulation efficiency via HPLC, and stability over three months under 4°C.

Polymeric Nanoparticle Formulation

PLGA nanoparticles are biodegradable, FDA approved drug delivery systems where both size (50-500nm, depending on the polymer concentration and conditions used) and drug release rate (ranging from hours to weeks and controlled by lactic/glycolic acid ratios) can be individually optimized [52]. The particles are prepared using the double emulsion/solvent evaporation method or nanoprecipitation and evaluated for encapsulation efficiency and drug release profile in PBS or synthetic lung fluid at 37°C. Mannose modification on the surface ensures drug delivery through interaction with the mannose receptor on the macrophages. In addition, liposomal and PLGA systems overcome oral bioavailability issue by using inhalation or intravenous administration to the lungs.

Structure-Activity Relationship Guided Optimisation

Natural product hits do not constitute optimum lead drug molecules. Interaction maps from molecular docking show the functional groups involved in interactions with the target and those that protrude into the solvent environment and are pharmacokinetically unnecessary. These latter functional groups may be modified without interfering with binding; hydroxylation or addition of a carboxylic acid group enhances water solubility; replacement of unstable functional groups decreases clearance; removal of non-interacting ring systems reduces molecular size. Resynthesized analogues undergo docking and biological tests to construct a structure activity relationship (SAR). The pharmacophore from the active natural product serves as the basis for designing simpler drug-like synthetic analogues. This semi-synthesis approach, characterized traditionally by the transformation of artemisinin into artesunate for the treatment of malaria, offers promise for producing molecules with both natural product target interaction properties and drug-like pharmacokinetics [53].

Future Directions: Pan-Shikimate Inhibition and Ai-Driven Discovery

What makes the shikimate pathway as a drug target highly strategically distinct lies in the possibility of pan-shikimate pathway inhibition, which refers to finding inhibitors of two or more enzymes of the shikimate pathway. The fact that the enzymes involved in the pathway share shikimate as their common substrate/product pair (i.e., SDH produces shikimate and SK uses it up) suggests that an inhibitor molecule having a similar structure to shikimate could be used effectively to inhibit several active sites [54]. If an inhibitor capable of blocking MtSK and MtEPSPS at the same time were to be found, Mtb would need to develop resistive mutations on two separate gene loci to avoid being inhibited – a much more genetically difficult task than developing one such mutation. Computation-based pan-shikimate pathway screening entails dockings of the filtered phytochemical database against the seven enzymes simultaneously and compounds with high multi-target binding scores instead of binding affinity to individual enzymes will be ranked higher. Compounds that perform well in multiple targets will be considered for experimental verification using isobologram analysis to verify synergy and multiple target inhibition in live bacteria [55].

Artificial intelligence and machine learning are starting to revolutionize the process of natural product-based drug discovery at an early stage. Graph neural networks (GNNs) are able to predict binding affinities of ligands and proteins with MM-GBSA precision, making the screening of all 17,967 molecules in the IMPPAT 2.0 library against all seven shikimate enzymes a matter of hours, not weeks [56]. Generative methods such as variational autoencoders and diffusion-based molecular generation models can generate new structural analogs with improved pharmacological properties of the top hits, thus automating one cycle of lead optimization. Of course, this approach does not eliminate the need for biological experiments; it just speeds up their preparation. If one looks down the road towards the translation process, one can see that the leads derived from phyto compounds would not be used as drugs themselves, but rather as a source of ideas for synthetic chemistry. For instance, the active components of pharmacophore, which were derived from a flavonoid inhibitor of MtSK, could be incorporated in a smaller



**Nikhil Ranjan Behera et al.,**

molecule structure that would be better absorbed when taken orally and would still engage the biological target. PK/PD modelling, in combination with the analysis of patent landscape carried out at the early stage of the program, would increase the efficiency of the whole process.

CONCLUSIONS

Shikimate pathway of *Mycobacterium tuberculosis* is arguably the most robust and structurally accessible drug target axis in the anti-TB drug discovery space. All seven enzymes of the shikimate pathway are individually essential, structurally resolved, biochemically characterized and absolutely not expressed in the human host. However, it is the least experimentally investigated pathway compared to the Mtb cell wall or RNA polymerase. The recently constructed IMPPAT 2.0 database containing 17,967 structurally diverse phytochemicals derived from Indian medicinal plants systematically annotated for ADMET properties offers a compound library to fill this gap. The proposed pipeline consisting of ADMET filtration, AutoDockVina docking, GROMACS molecular dynamics, surrogate testing using *M. smegmatis* and BCG models, THP-1 cytotoxicity analysis and staged in vivo evaluation is reproducible, scalable and can be executed by an academic laboratory in the absence of specialized infrastructure at most stages. The isoorientin is shown as an example of the validated hit (MtSK $\Delta G = -9.4$ kcal/mol; MM-PBSA = -8.2 kcal/mol; *M. smegmatis* MIC₉₀ = 8.2 μ M; SI >12). What is required at this stage is not further computational screening rounds with increasing length of hit lists but careful refinement of the best leads via experimental validation starting from IC₅₀ of the enzyme activity, followed by MIC in vitro and efficacy in vivo. The pan-shikimate inhibition, AI-assisted screening, and semi-synthesis driven by SAR are the three most promising routes that can be employed to move natural product-based inhibitors of the shikimate pathway to become clinical candidates for MDR-TB.

List of Abbreviations

ADMET: absorption, distribution, metabolism, excretion, toxicity; ATP: adenosine triphosphate; BCG: bacille Calmette-Guérin; BPaLM: bedaquiline–pretomanid–linezolid–moxifloxacin; CC₅₀: cytotoxic concentration reducing viability by 50%; CFU: colony-forming units; CS: chorismate synthase; DAH7PS: 3-deoxy-D-arabino-heptulosonate-7-phosphate synthase; DAHP: 3-deoxy-D-arabino-heptulosonate-7-phosphate; DHQ: 3-dehydroquininate; DHQS: 3-dehydroquininate synthase; DHQase: 3-dehydroquininate dehydratase; DHS: 3-dehydroshikimate; E4P: erythrose-4-phosphate; EGCG: epigallocatechin gallate; EPSPS: 5-enolpyruvylshikimate-3-phosphate synthase; FMN: flavin mononucleotide; GAFF2: General AMBER Force Field 2; GNN: graph neural network; GROMACS: GROMingMachine for Chemical Simulations; IAEC: Institutional Animal Ethics Committee; IMPPAT: Indian Medicinal Plants, Phytochemistry and Therapeutics; LC-MS/MS: liquid chromatography–tandem mass spectrometry; LID: ligand-induced domain; MDR-TB: multidrug-resistant tuberculosis; MIC: minimum inhibitory concentration; MM-GBSA: molecular mechanics generalised Born surface area; MM-PBSA: molecular mechanics Poisson-Boltzmann surface area; Mtb: *Mycobacterium tuberculosis*; MtSK: *M. tuberculosis* shikimate kinase; NAD(P)H: nicotinamide adenine dinucleotide phosphate (reduced); NMP: nucleoside monophosphate; OECD: Organisation for Economic Co-operation and Development; PDB: Protein Data Bank; PEP: phosphoenolpyruvate; PK: pharmacokinetics; PLGA: poly(lactic-co-glycolic acid); RMSD: root mean square deviation; RMSF: root mean square fluctuation; SAR: structure-activity relationship; SBDD: structure-based drug design; SDH: shikimate dehydrogenase; SI: selectivity index; SK: shikimate kinase; TB: tuberculosis; THP-1: human acute monocytic leukemia cell line; TPSA: topological polar surface area; WHO: World Health Organization; XDR-TB: extensively drug-resistant tuberculosis.

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Not applicable. This is a review article and involves no original animal or human participant research.





Nikhil Ranjan Behera et al.,

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Competing Interests

The authors declare no conflict of interest, financial or otherwise.

Author Contributions

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Declaration on Language Assistance

The standard of the English language in this article has been enhanced using LLMs, namely Llama-3.3-70B-GGML. The authors bear full responsibility for the contents of this publication and have conducted a comprehensive examination, made improvements, and refined all language generated by the LLM to ensure precise alignment with the original research outcomes and interpretations.

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Table 1: ProTox 3.0 toxicity classification framework used for compound filtering

Class	Predicted LD ₅₀ (mg/kg)	Interpretation
1	< 5	Highly toxic – exclude
2	5–50	Toxic – exclude
3	50–300	Moderately toxic – exclude
4	300–2000	Harmful – borderline; include with caution
5	2000–20000	Slightly harmful – include
6	> 20000	Negligible toxicity – include

Only compounds classified as Class 4 or above (LD₅₀ >300 mg/kg) advance in the pipeline.

Table 2: Protein Data Bank entries and structural features of the seven *Mtb* shikimate pathway enzymes

Enzyme	Gene	PDB Entry	Structural Features
DAH7PS	aroG/F	2B7O / 1QR7	TIM barrel, tetramer; allosteric pocket
DHQS	aroB	2YBQ / 1DQS	Bilobal dimer; NAD ⁺ tightly bound
DHQase	aroD	2XB8 / 1DHQ	Dodecamer (Type II); Schiff-base Lys



Nikhil Ranjan Behera *et al.*,

SDH	aroE	2NPX / 1SDH	Rossmann fold; NADPH cofactor
SK	aroK	2IYQ	NMP kinase; CORE/LID/SB domains
EPSPS	aroA	2BJB / 1G3R	Two-domain α/β ; glyphosate site
CS	aroC	1QXO / 1CSM	FMN-dependent homodimer

PDB = Protein Data Bank; TIM = triosephosphate isomerase; NMP = nucleoside monophosphate; FMN = flavin mononucleotide.

Table 3: Representative natural product and synthetic inhibitors of MtSK and related shikimate pathway enzymes

Compound	Class	IC ₅₀ / Ki / ΔG	MIC (H37Rv)	Inhibition Type	Reference
Manzamine A	Marine alkaloid	1.3 μM (Ki)	2.0 $\mu g/mL$	Slow-binding noncompetitive	Simithy et al., 2018
Benzothiazole derivative	Heterocyclic	0.8 μM (IC ₅₀)	ND	Allosteric (LID domain)	Mehra et al., 2016
Alpha-linolenic acid	Fatty acid	~50 μM (MtSK)	ND	Competitive (shikimate site)	Masoko et al., 2016
Isoorientin	C-glycoside flavone	9.4 kcal/mol (ΔG)	ND	Predicted (P-loop, Arg57)	Present study
Narirutin	Flavanone glycoside	In silico hit	ND	Predicted competitive	Sahu et al., 2020
EGCG	Polyphenol	~30 μM (DHQase)	ND	Type IIDHQase inhibitor	Reviewed herein

ND = not determined; ΔG = AutoDockVina binding free energy; MIC = minimum inhibitory concentration; EGCG = epigallocatechin gallate.

Table 4: AutoDockVina binding affinities (ΔG , kcal/mol) of representative natural product hits against all seven shikimate enzymes. Values meeting advancement thresholds are in bold.

Compound	DAH7PS	DHQS	DHQase	SDH	SK (2IYQ)	EPSPS	CS
Isoorientin	-7.4	-6.8	-7.1	-7.6	-9.4*	-7.2	-6.5
Orobanchol	-7.1	-7.6	-6.8	-8.9	-7.2	-9.1	-6.7
Stigol	-7.7	-8.1	-7.4	-6.3	-8.8	-8.7	-8.3

* Meets tightened MtSK threshold of -9.0 kcal/mol. TBD = to be determined upon completion of screening cascade.





Nikhil Ranjan Behera et al.,

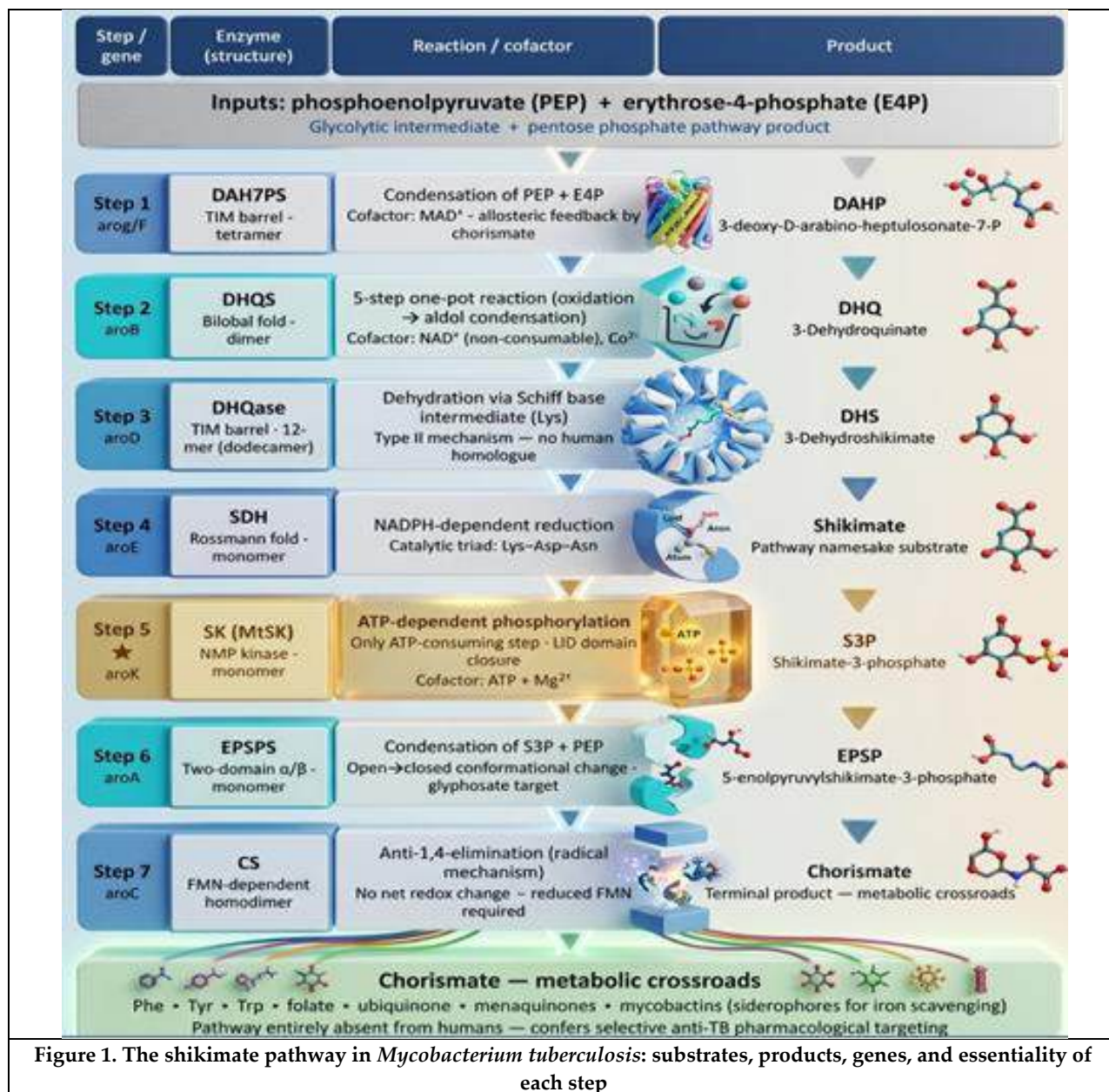


Figure 1. The shikimate pathway in *Mycobacterium tuberculosis*: substrates, products, genes, and essentiality of each step



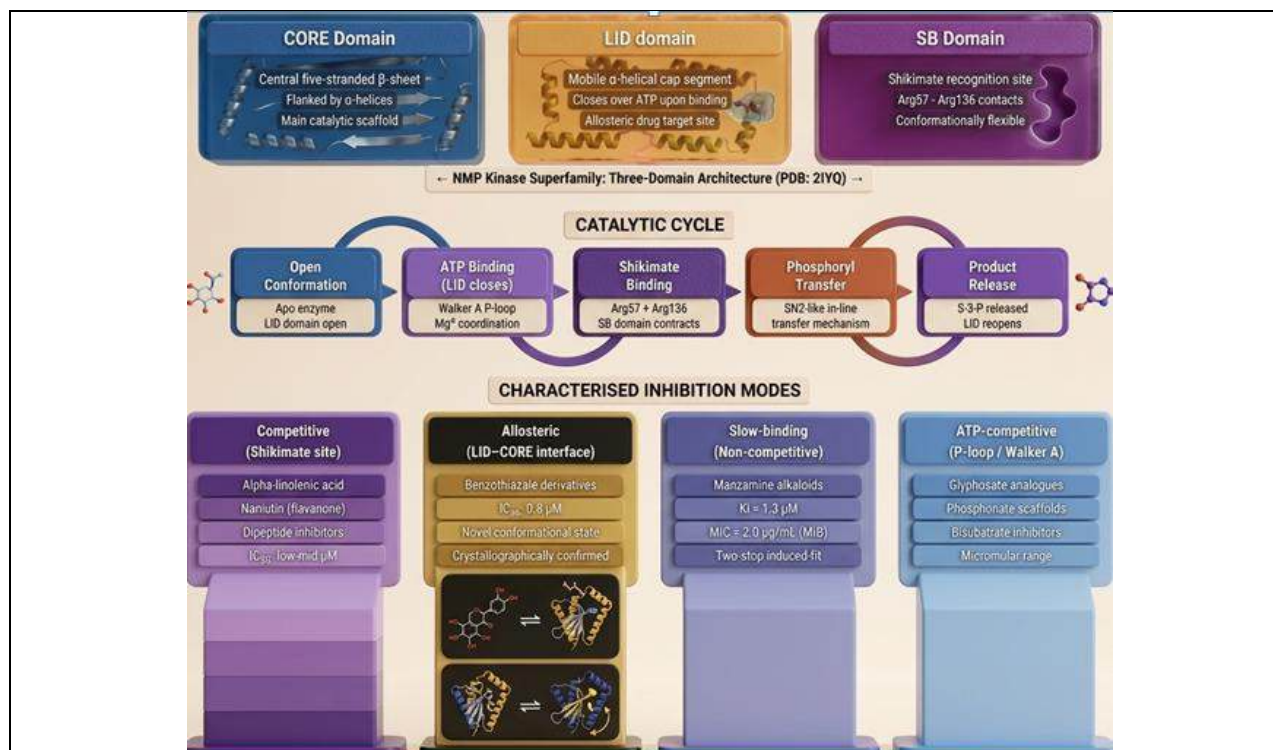
Nikhil Ranjan Behera *et al.*,

Figure 2. MtSK domain architecture, catalytic cycle, and inhibition modes



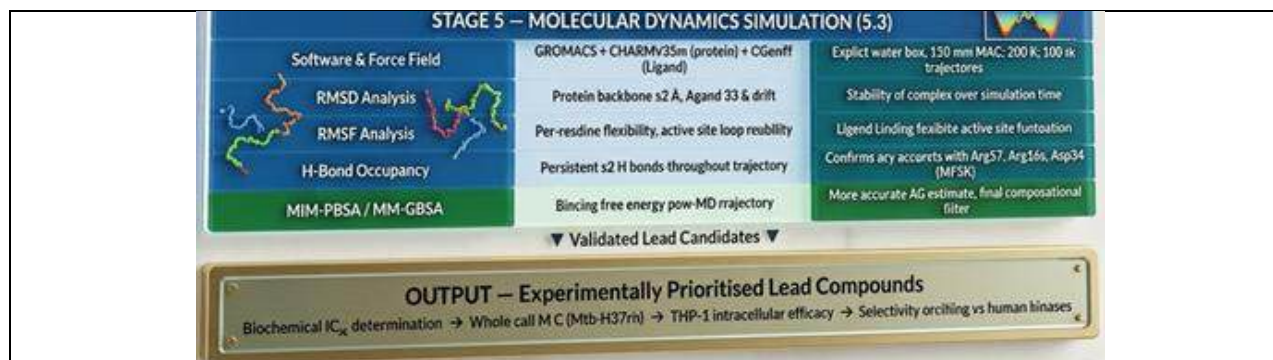
Nikhil Ranjan Behera *et al.*,

Figure 3. Multi-stage virtual screening pipeline: compound numbers at each filtration step

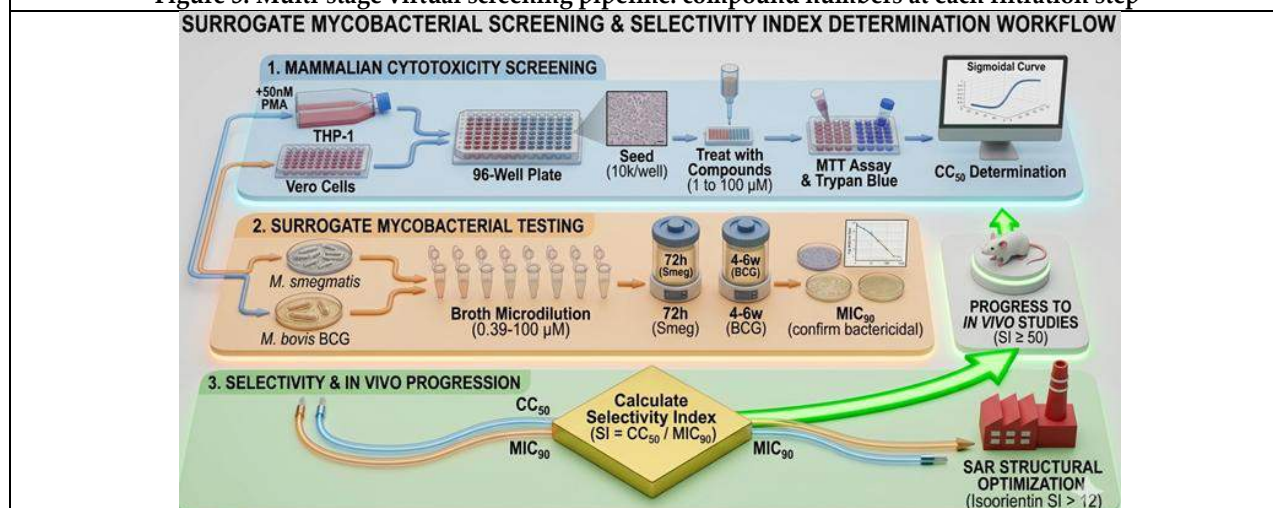
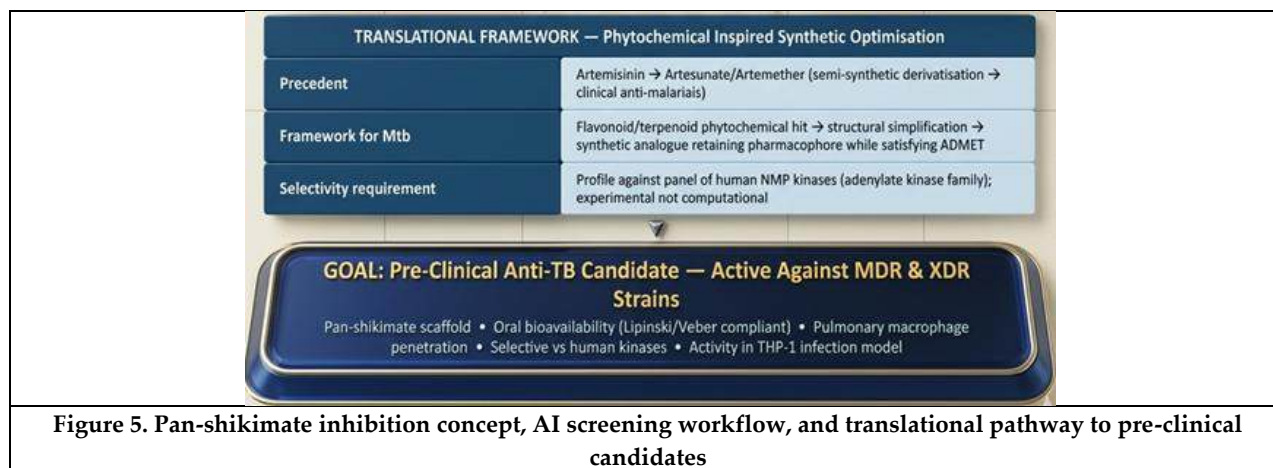


Figure 4. In vitro cytotoxicity and antimycobacterial testing results for representative hits



Nikhil Ranjan Behera *et al.*,

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RESEARCH ARTICLE

PBTA-S : A Priority -based Task Allocation and Scheduling Mechanism using Multi-Level Fog Node Categorization for Healthcare Monitoring

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ABSTRACT

Fog-enabled healthcare monitoring generates a continuous stream of patient-related tasks near IoT devices, where some requests are routine updates while others represent emergency alerts that demand immediate processing. Sending every task to a distant cloud can introduce unnecessary latency and congestion, which may delay critical decisions and reduce service reliability during peak arrivals. To address this challenge, this paper presents PBTA-S, a priority-based task allocation and scheduling mechanism for fog computing that improves responsiveness through multi-level fog node categorization. Incoming monitoring tasks are first classified into three urgency groups (critical, medium, and routine start/end) and then mapped to three fog resource tiers that reflect different processing capabilities and response roles. Each tier maintains an independent priority queue, and tasks are executed using a scheduling key that favors urgency and timeliness while maintaining fair ordering within the same class. When a preferred fog tier becomes overloaded, PBTA-S activates a controlled spillover strategy that shifts tasks to the nearest suitable tier based on predicted waiting time, preventing urgent workloads from being trapped in long queues. Simulation-driven evaluation under fog computing assumptions demonstrates that the proposed mechanism achieves faster service for critical tasks by reducing waiting and response time, while also lowering SLA/deadline violations under high-load conditions. Overall, PBTA-S shows that combining priority-aware allocation, tiered fog resource pooling, queue-based scheduling, and overload-aware spillover offers a practical and effective approach for mixed-criticality healthcare workloads in fog environments.

Keywords: Fog computing, IoT healthcare, Priority scheduling, Resource allocation, Latency, SLA violations, Edge computing





INTRODUCTION

The rapid adoption of IoT-based healthcare monitoring has enabled continuous tracking of patient conditions through wearable sensors and smart medical devices. These systems generate a steady flow of data and service requests such as vital-sign updates, medication reminders, trend analysis, and emergency alerts [1]. However, not all tasks produced in healthcare environments carry the same urgency. A sudden drop in oxygen saturation, abnormal heart rate, or fall detection event requires immediate processing and response, whereas routine monitoring updates can tolerate minor delays [2][3]. In such mixed-criticality environments, the ability to process urgent tasks quickly becomes essential to support timely clinical intervention and to maintain the reliability of health monitoring services [4]. Although cloud platforms offer high storage and computational scalability, relying only on centralized cloud processing is often unsuitable for latency-sensitive healthcare workloads. When all tasks are forwarded to the cloud, the system may experience increased communication delay, bandwidth overhead, and service disruption during network congestion or burst traffic conditions [5]. Fog computing addresses this limitation by bringing computation closer to where the data is produced, enabling faster decisions at the network edge. Yet, fog environments introduce their own challenges because fog nodes are distributed, heterogeneous, and resource-constrained when compared to centralized datacenters [6]. This makes task scheduling and resource allocation a critical concern, especially when urgent healthcare tasks compete with routine requests. Therefore, there is a strong need for a fog-oriented scheduling mechanism that can prioritize critical workloads, utilize fog resources efficiently, and reduce waiting time and SLA violations under varying load conditions [7].

To address these challenges, this paper proposes PBTA-S, a Priority-Based Task Allocation and Scheduling mechanism designed for fog computing in IoT healthcare environments. The proposed approach first evaluates each incoming healthcare task based on its urgency and assigns it to one of three priority classes, ensuring that emergency-like requests are never treated the same as routine monitoring updates. Next, fog resources are organized into multi-level fog node categories that represent different processing strengths and operational roles, and each category maintains a dedicated priority queue for structured task handling. PBTA-S then schedules tasks using a queue-driven allocation strategy that balances urgency and timeliness, while continuously tracking waiting time to avoid prolonged delays. When a preferred fog tier becomes saturated, an overload-aware spillover policy shifts tasks to the nearest suitable fog tier based on waiting-time thresholds, ensuring stable service continuity during traffic bursts. Through this design, PBTA-S improves fog-level responsiveness, reduces overall processing delay for critical tasks, and strengthens SLA adherence for mixed-priority healthcare workloads at the edge.

The key contributions of this paper are as follows:

- Fog-based priority classification for healthcare tasks: A structured three-level priority model is introduced to differentiate critical, moderate, and routine healthcare requests, enabling urgency-aware decision making at the fog layer.
- Multi-level fog node categorization (resource-tiering): Fog nodes are grouped into capability-aware categories so that high-priority tasks are directed toward suitable fog resources rather than competing randomly across heterogeneous nodes.
- Queue-driven scheduling mechanism for mixed-critical workloads: Independent queues are maintained for each fog tier, ensuring systematic ordering and improved service discipline, especially during continuous task arrivals.
- Overload-aware spillover strategy for stable fog operation: A waiting-time threshold policy triggers-controlled spillover between fog tiers, preventing critical tasks from being trapped in long queues when preferred nodes are overloaded.
- Improved fog performance in delay-sensitive monitoring scenarios: The proposed PBTA-S approach enhances response time for urgent tasks and reduces SLA/deadline violations under high-load conditions, demonstrating suitability for real-time healthcare monitoring at the edge.



**Ramesh Kannan and George Dharma Prakash Raj****Related Work**

Healthcare monitoring workloads typically involve heterogeneous tasks with different urgency levels, where delays in processing critical alerts can degrade response quality. To address this, Elshahed et al. [8] proposed a fog-computing-based healthcare monitoring framework that aims to support real-time patient notification with low latency. It collects vital signs such as body temperature, heart rate, and blood pressure using wearable sensors and processes the data closer to the patient through a fog gateway layer, instead of relying only on distant cloud servers. The system performs local filtering and analysis and triggers instant alerts to doctors/caregivers when abnormal readings cross threshold limits, while also supporting medication or diet reminders. Machine learning is used to assist decision-making for identifying critical conditions, and the cloud layer is mainly used for long-term storage and future analysis. The authors also emphasize handling healthcare constraints such as network failures via temporary local storage and propose security measures like encryption/watermarking along with compression for efficient cloud storage. Overall, the work highlights how fog computing improves QoS in healthcare by reducing delay and enabling timely intervention in emergency situations.

A closely related direction is to explicitly classify healthcare tasks by importance and align them with resource categories. Aladwaniet al. [9] proposed a fog-computing scheduling approach where IoT healthcare tasks are first classified into multiple importance classes based on patient status, and then assigned using a strategy that also considers VM categorization. This is particularly relevant because it moves beyond pure timing objectives to incorporate task criticality into placement decisions in a fog setting. This method is conceptually aligned with our work because it supports the idea that task classes should be mapped to VM classes, which naturally fits multi-level VM categorization. However, many studies still leave room for a more explicit end-to-end pipeline that separates (i) classification, (ii) category-level allocation, (iii) queue-level scheduling, and (iv) overload spillover policies as clearly defined mechanism components.

Healthcare monitoring is also characterized by dynamic context changes, especially in IoT scenarios where patients or devices can move across coverage zones. Abdelmoneem et al. [10] studied healthcare task scheduling across cloud and fog while considering mobility, proposing policies that aim to distribute tasks dynamically between fog and cloud nodes and reduce missed tasks under movement. These results reinforce that real deployments benefit from mechanisms that (a) remain responsive under changing loads and (b) adapt their allocation decisions when preferred resources are not immediately available. Such observations provide strong support for introducing overload-aware spillover rules (from a preferred category to an adjacent category) in scheduling mechanisms designed for mixed-criticality healthcare tasks.

Some scheduling research derives task priority from multiple criteria rather than a single indicator, typically using decision-making techniques. Ghafari and Mansouri[11] proposed a fog scheduling approach where Analytic Hierarchy Process (AHP) is used to determine task priority as part of a broader optimization method, targeting improved energy/time/cost behavior. Similarly, Hosseini et al. [12] used a fuzzy-AHP-assisted approach (PQFAHP) to manage cost-latency trade-offs in mobile fog computing, emphasizing that practical schedulers often need to handle multiple objectives and heterogeneous tasks with different priorities. El-Natat et al. [13] proposed a deadline-aware fog resource allocation approach for IoT healthcare monitoring, motivated by the need to handle time-sensitive medical workloads with low latency and fewer delays in comparison to cloud-only processing. Their work introduces a Task Classification algorithm based on Deadline and Criticality (TCDC), which explicitly prioritizes critical healthcare tasks first while also respecting each task's deadline constraints for real-time responsiveness. The authors evaluate TCDC in a fog environment and report improvements in key QoS indicators such as makespan and resource utilization, showing notable overall performance gains over baseline methods. The study also reinforces that deadline handling is a dominant QoS factor in fog-based healthcare scheduling, since many monitoring tasks must be completed within strict timing bounds to avoid unsafe delays. Overall, this work supports the view that combining criticality-aware prioritization with deadline-driven scheduling is essential for fog healthcare systems that must deliver fast and reliable responses under dynamic IoT workloads.



**Ramesh Kannan and George Dharma Prakash Raj**

Sharif et al. [14] proposed a priority-based task scheduling and resource allocation (PTS-RA) mechanism for healthcare monitoring in a mobile edge computing (MEC) setting, where tasks are assigned priorities based on emergency levels derived from wearable-device data. Their method jointly decides whether each task should be processed locally (hospital workstations) or offloaded to the cloud, aiming to reduce overall processing time and bandwidth cost while ensuring emergency tasks run first. They evaluate the approach against existing methods using metrics such as average latency, execution time, network usage, CPU utilization, and energy consumption, reporting improved handling of latency-sensitive tasks. This work supports the importance of priority-aware scheduling in healthcare environments; however, it primarily focuses on edge-cloud offloading decisions rather than structuring cloud execution resources into multi-level VM categories based on expertise. Sirjaniet al. [15] proposed a deadline-aware fog scheduling framework for IoT applications where timely responses are critical. Their approach (RIGEO) dynamically classifies fog nodes into low-traffic and high-traffic groups based on observed network conditions, then applies different scheduling strategies to match deadline urgency: low-deadline tasks are scheduled on low-traffic nodes using an Improved Golden Eagle Optimization (IGEO) method (a discretized meta-heuristic enhanced with genetic operators), while high-deadline tasks on high-traffic nodes are handled using reinforcement learning (RL) for robust decision-making under congestion. The authors report that this hybrid design improves key QoS outcomes, including response time, deadline violation behavior, and energy consumption, compared with several existing methods. In the context of PBTA-S, this study is relevant because it demonstrates how resource categorization plus urgency-aware scheduling can improve timeliness under heterogeneous and dynamic loads, although its categorization is traffic/deadline-driven rather than expertise-tier VM pools.

Choppara and Lokesh [16] proposed a fog-computing scheduling framework for time-sensitive healthcare scenarios where sending all data to the cloud introduces avoidable latency. Their key idea is to use deep reinforcement learning (DRL) to learn dynamic task allocation decisions across nearby fog nodes so that urgent workloads are processed closer to where IoT health data is generated. They validate the approach using SimPy-based simulations that emulate multiple healthcare settings (e.g., continuous monitoring and emergency response), and show that the learned policy adapts to changing system states and resource demand. Reported outcomes indicate noticeable gains over traditional scheduling: up to 30% reduction in makespan / completion time, about 40% reduction in operational latency, and around 25% improvement in fault tolerance, highlighting the suitability of DRL for real-time fog-assisted healthcare processing. The study also positions the same DRL-based scheduler as transferable to other real-time domains, suggesting broader usefulness beyond healthcare.

Several studies have explored task scheduling and resource allocation in fog computing to support latency-sensitive IoT applications such as healthcare monitoring, smart cities, and industrial automation. In general, existing fog scheduling methods aim to reduce execution delay by placing computation closer to the data source, while also balancing workload across distributed fog nodes. Common approaches include heuristic-based scheduling, load-aware allocation, deadline-based processing, and energy-efficient placement strategies. However, many of these solutions treat incoming tasks in a mostly uniform manner or provide limited support for mixed-priority scenarios where emergency requests must be handled ahead of routine monitoring tasks. In healthcare contexts, this limitation can lead to avoidable delays because critical events may still experience long waiting times during burst traffic or fog node contention. Moreover, fog environments are naturally heterogeneous, and a scheduling policy that ignores capability variation across fog nodes may cause inefficient resource usage and SLA violations. These gaps highlight the need for a scheduling mechanism that combines priority-awareness, fog resource tiering, and overload handling, while remaining practical and adaptable for real-time healthcare workloads.

Problem Formulation

This section defines and formalizes the proposed priority-based task allocation and scheduling problem in a fog computing environment by specifying the task model, fog resource (fog node) model, decision variables, constraints, and optimization objectives. The formulation reflects an IoT-enabled healthcare monitoring scenario, where patient-





Ramesh Kannan and George Dharma Prakash Raj

related tasks arrive dynamically at the edge and must be processed under strict urgency requirements and heterogeneous fog resource capabilities.

Problem Definition

Fog-assisted healthcare monitoring generates a continuous stream of service requests such as routine vital-sign updates, follow-up processing, and start/end assessments. However, these tasks are not equally critical in real-time healthcare operations. Some tasks may indicate abnormal clinical conditions and require immediate processing, whereas routine monitoring tasks can tolerate small delays. If scheduling is performed using a simple first-come-first-served approach or without urgency awareness, critical healthcare tasks may experience unacceptable waiting time, leading to delayed response actions and potential SLA violations. In fog computing, computation is intentionally placed closer to IoT devices to reduce network delay and support faster decision-making. At the same time, fog environments are distributed and heterogeneous, meaning that fog nodes differ in processing capability, availability, and operational role. Therefore, fog resources cannot be treated as identical units. Instead, fog nodes can be logically categorized into multiple capability tiers (for example, high-capability fog nodes, mid-tier fog nodes, and lightweight fog gateways), similar to how healthcare workflows depend on different levels of clinical expertise. Hence, the problem is to allocate each incoming healthcare task to an appropriate fog node category and schedule the execution order within each category such that high-priority tasks receive faster service while maintaining overall system stability. The objectives are to minimize waiting time and end-to-end response time (especially for critical tasks), reduce SLA/deadline violations, maintain good throughput and fog resource utilization, and handle congestion effectively through a controlled spillover strategy when the preferred fog tier becomes overloaded.

Task Model

In the proposed fog-based healthcare monitoring system, a task represents a processing request generated by IoT devices or health monitoring applications, such as periodic vital-sign updates, event-triggered alerts, medical follow-up processing, and start/end assessment requests. Tasks arrive dynamically at the fog gateway and are characterized by a set of attributes that influence allocation and scheduling decisions.

Let the stream of incoming tasks be represented as:

$$T = \{t_1, t_2, \dots, t_n\}$$

Each task t_i is described using the following parameters:

- Arrival time (a_i): time when the task reaches the fog gateway.
- Computation demand (l_i): workload length in million instructions (MI).
- Deadline (optional) (d_i): acceptable completion time for SLA compliance.
- Priority class (p_i): urgency level assigned to the task.
- Service type (s_i): healthcare operation type (routine update, follow-up, start/end assessment, emergency event).

Since fog-based healthcare workloads contain mixed criticality, the incoming tasks are grouped into three priority levels:

- P_1 : High-priority/critical tasks (emergency or risk-indicating events)
- P_2 : Medium-priority tasks (follow-ups and clinically important requests)
- P_3 : Low-priority tasks (routine monitoring and start/end assessments)

This classification ensures that urgent healthcare events are processed ahead of delay-tolerant workloads in the fog layer.

Fog Resource (Fog Node) Model

Fog computing resources consist of geographically distributed nodes deployed closer to IoT devices, such as healthcare gateways, edge servers, and micro data centers. Unlike centralized cloud environments, fog nodes are heterogeneous in terms of processing capacity, memory availability, and service responsiveness. Therefore, fog nodes must be modeled as capability-aware resources rather than identical units.

Let the set of available fog nodes be:





Ramesh Kannan and George Dharma Prakash Raj

$$F = \{f_1, f_2, \dots, f_m\}$$

Each fog node f_j is characterized by:

- Processing capability (C_j): CPU speed measured in MIPS
- Available memory (M_j) and storage (S_j)
- Current load (U_j): utilization/queue occupancy
- Execution queue (Q_j): local task queue at the fog node
- Network latency (L_j): communication delay between IoT source and the fog node (fog-layer delay)

To enable structured allocation, the fog nodes are grouped into three capability tiers:

- FN_1 (High-capability fog tier): suitable for urgent and time-critical tasks
- FN_2 (Mid-tier fog): suitable for moderate tasks and general processing
- FN_3 (Lightweight fog/gateway tier): suitable for routine and background processing

This categorization supports efficient task matching in PBTA-S, where resource tiers serve a role similar to expertise levels in healthcare workflows.

Decision Variables

The task allocation and scheduling problem is defined using decision variables that represent task-to-fog-node mapping and execution ordering within each fog tier.

Allocation Variable

Let:

$$x_{ij} = \begin{cases} 1 & \text{if task } t_i \text{ is assigned to fog node } f_j \\ 0 & \text{otherwise} \end{cases}$$

Fog Tier Selection

Each task is first mapped to a fog tier based on its priority:

$$\text{tier}(t_i) \in \{FN_1, FN_2, FN_3\}$$

Scheduling Order Variable

Let o_i represent the execution position of task t_i inside its tier queue. PBTA-S ensures priority ordering while maintaining stable ordering within the same class.

Constraints

The fog-based scheduling process is subject to the following constraints:

C1: Unique Assignment Constraint

Each task must be assigned to exactly one fog node:

$$\sum_{j=1}^m x_{ij} = 1, \forall t_i \in T \quad (1)$$

C2: Fog Node Capacity Constraint

A fog node can only handle tasks within its available processing and memory limits:

$$\text{load}(f_j) \leq \text{capacity}(f_j)$$

C3: Priority-to-Tier Mapping Constraint

Urgent tasks should be directed to higher-capability fog tiers unless congestion forces spillover:

- $P_1 \rightarrow FN_1$
- $P_2 \rightarrow FN_2$
- $P_3 \rightarrow FN_3$

C4: Waiting-Time Threshold / Spillover Constraint

If the predicted waiting time of a task in the preferred tier exceeds a predefined threshold θ , the task can be shifted to the nearest available fog tier:

$$W_i > \theta \Rightarrow \text{spillover}(t_i)$$

This constraint prevents excessive delay for higher-priority tasks under overload conditions.





Ramesh Kannan and George Dharma Prakash Raj

C5: Deadline / SLA Constraint (Optional)

For tasks with deadlines, completion must ideally occur within:

$$c_i \leq d_i$$

where c_i is the completion time.

Objective Functions

The proposed PBTA-S mechanism is formulated to perform priority-based task allocation and scheduling for healthcare monitoring using multi-level fog node categorization in a fog computing environment. The optimization goal is to ensure that critical healthcare tasks receive faster and more reliable service by reducing waiting time and end-to-end response time, while also minimizing SLA/deadline violations, improving throughput, and maintaining balanced fog resource utilization under dynamic workload conditions.

Objective 1: Minimize End-to-End Response Time

Fog response time includes both processing and fog-layer communication delay:

$$\min \sum_{i=1}^n R T_i \quad (2)$$

where

$$R T_i = (\text{execution time}) + (\text{waiting time}) + (\text{fog network delay})$$

Objective 2: Minimize Waiting Time for Critical Tasks

PBTA-S prioritizes reducing queue waiting delay particularly for P_1 :

$$\min \sum_{t_i \in P_1} W_i \quad (3)$$

Objective 3: Minimize SLA/Deadline Violations

The number of tasks missing their SLA target should be minimized:

$$\min \sum_{i=1}^n V_i \quad (4)$$

where $V_i = 1$ if task t_i violates deadline/SLA, else 0.

Objective 4: Maximize Throughput

The number of successfully completed tasks per unit time should be maximized:

$$\max \text{Throughput}$$

Objective 5: Maintain Balanced Fog Utilization

PBTA-S aims to prevent overload on specific fog tiers and promote stable utilization:

$$\min \max_j (U_j)$$

The proposed fog-based scheduling model considers an online task arrival scenario, where healthcare-monitoring requests are generated continuously by IoT devices and enter the fog layer dynamically over time rather than being available as a fixed batch. Once a task is allocated to a selected fog node and execution begins, preemption is not performed, meaning the task runs until completion. This design choice avoids runtime overheads such as state checkpointing, context switching, and task migration, which may be costly in distributed fog environments. Nevertheless, the framework can be extended to support preemptive handling for emergency tasks when strict clinical urgency demands immediate execution. Each fog node is assumed to follow a single-task execution model, where only one task is processed at a time (i.e., node-level concurrency is set to 1) to ensure clear scheduling behavior and reproducible evaluation. Finally, spillover across fog tiers is permitted only under congestion, and assigning a task outside its preferred fog category is treated as a controlled and limited exception. During performance evaluation, such spillover events can be explicitly measured using a penalty factor or reported as a tier-violation rate, reflecting the practical trade-off between strict capability alignment and timely response requirements for critical healthcare tasks.





Ramesh Kannan and George Dharma Prakash Raj

Proposed PBTA-S Model

This section presents the proposed Priority-Based Task Allocation and Scheduling (PBTA-S) mechanism tailored for fog-assisted IoT healthcare monitoring. The model is designed to process mixed-criticality tasks (critical, moderate, routine) in a distributed fog environment by combining priority-aware task classification, multi-level fog node categorization, tier-specific queue management, and an overload-aware spillover strategy. The key intention of PBTA-S is to minimize waiting and response time for high-priority healthcare tasks while maintaining stable fog resource utilization under fluctuating arrivals. The proposed PBTA-S framework is designed to support latency-sensitive healthcare monitoring by performing priority-aware task allocation and scheduling within a fog computing environment. Incoming tasks generated by IoT devices are first analyzed and classified into urgency levels, after which they are mapped to capability-aware fog tiers to ensure appropriate resource matching. PBTA-S then organizes tasks into tier-specific queues and dispatches them to the least-loaded fog nodes for execution under a non-preemptive model. To maintain stable performance during burst arrivals, the framework further incorporates an overload-aware spillover mechanism that redirects tasks only when the expected waiting time in the preferred tier exceeds a predefined threshold. As shown in Figure 1, this architecture enables structured handling of mixed-criticality workloads while improving responsiveness for high-priority healthcare tasks.

Figure 1 illustrates the overall workflow of the proposed PBTA-S framework deployed in a fog computing environment for IoT-enabled healthcare monitoring. Healthcare tasks generated from edge devices are received at the fog gateway and dynamically processed in an online manner. Each incoming task is classified into a priority level (P1, P2, or P3) based on clinical urgency and then mapped to an appropriate fog resource tier (FN1, FN2, or FN3) that reflects different capability levels. PBTA-S maintains independent priority queues for each fog tier, enabling structured and urgency-aware task ordering. The fog scheduler selects tasks from the queues and dispatches them to the least-loaded fog node within the preferred tier using a non-preemptive execution model. During congestion, the framework activates a controlled spillover mechanism based on waiting-time thresholds to prevent critical tasks from being blocked by long queues. The monitoring and feedback module continuously records performance measures such as waiting time, end-to-end response time, SLA violations, fog-tier utilization, and tier-violation rate, which supports stable scheduling decisions under varying workload conditions.

Overview of the PBTA-S Fog Workflow

In a fog-based healthcare monitoring environment, tasks are generated continuously from IoT devices and reach the fog layer through a gateway or edge controller. PBTA-S processes each task through the following stages:

- **Task Arrival at Fog Gateway**

Incoming healthcare tasks are received dynamically and stored temporarily at the gateway for classification.

- **Priority Classification (P1, P2, P3)**

Each task is assigned a priority label based on urgency:

- P1: critical / emergency / risk-indicating tasks
- P2: moderate tasks (follow-up, high clinical relevance)
- P3: routine monitoring and start/end assessments

- **Fog Node Tier Mapping (FN1, FN2, FN3)**

Fog nodes are grouped into capability tiers:

- FN1: high-capability fog tier (closest micro-datacenter / hospital edge server)
- FN2: mid-tier fog (regional edge server / MEC node)
- FN3: lightweight fog (gateway-class resources)

- **Tier-Specific Queue Assignment (Q1, Q2, Q3)**

Tasks are inserted into the queue corresponding to their fog tier.

- **Fog Node Selection and Dispatch**

PBTA-S selects the **least-loaded node** inside the chosen tier and dispatches the task for execution.

- **Overload-Aware Spillover (Congestion Handling)**





If the predicted waiting time for a task exceeds a threshold, PBTA-S triggers controlled spillover to a suitable adjacent tier to avoid long delays.

PBTA-S Fog Scheduling Components

Priority Analyzer

The priority analyzer identifies the urgency level of each healthcare task using predefined rules. For example:

- abnormal sensor patterns or alarm conditions → P1
- follow-up tasks and escalations → P2
- periodic monitoring / start-end logs → P3

This ensures urgent tasks are always positioned ahead of routine workloads in the fog layer.

Fog Tier Categorizer

Fog nodes are categorized into tiers based on resource capability (CPU speed, memory, historical performance, and reliability). The categorization helps PBTA-S ensure that critical workloads are processed on stronger fog resources.

Tier policy:

- P1 tasks are mapped to FN1
- P2 tasks are mapped to FN2
- P3 tasks are mapped to FN3

Queue Manager (Tier-Specific Queues)

PBTA-S maintains independent queues at the fog controller:

- $Q_1 \rightarrow$ tasks mapped to FN1
- $Q_2 \rightarrow$ tasks mapped to FN2
- $Q_3 \rightarrow$ tasks mapped to FN3

Within each queue, tasks are ordered based on scheduling keys such as:

- higher urgency first
- earlier arrival first (within same priority)
- deadline sensitivity (optional)

Fog Dispatcher and Node Selection

When a task reaches the head of a queue, PBTA-S selects a fog node from the corresponding tier using a **least-loaded selection rule**, such as:

- lowest current utilization
- shortest queue length
- earliest expected availability

This improves load balance and prevents certain fog nodes from becoming bottlenecks.

Congestion Controller and Spillover Strategy

Fog resources may experience burst workloads, especially in healthcare environments during abnormal monitoring conditions. PBTA-S introduces a controlled spillover strategy:

- If the estimated waiting time in the preferred tier exceeds a threshold θ , the task may be redirected to the nearest feasible tier.
- Spillover is applied as an exception and not as a default.

Example spillover rules:

- FN1 overloaded → redirect some tasks to FN2 (for continuity)
- FN2 overloaded → redirect to FN3 (for non-critical tasks)
- FN3 overloaded → redirect to FN2 (if capacity exists)

This prevents urgent tasks from being stuck in long queues during fog congestion.





PBTA-S Fog Algorithm

Algorithm: PBTA-S for Fog Task Allocation and Scheduling

Input:

Task stream T , fog node sets FN_1, FN_2, FN_3 , queues Q_1, Q_2, Q_3 , waiting threshold θ

Output:

Priority-aware task execution with minimized delay and controlled spillover

Steps:

1. When a task t_i arrives at the fog gateway, extract attributes (a_i, l_i, p_i)
2. Assign task priority class:
 - If critical $\rightarrow P_1$
 - Else if moderate $\rightarrow P_2$
 - Else $\rightarrow P_3$
3. Map the task to fog tier:
 - $P_1 \rightarrow FN_1$
 - $P_2 \rightarrow FN_2$
 - $P_3 \rightarrow FN_3$
4. Insert task into the corresponding queue Q_k
5. While queue Q_k is not empty:
 - Select the head task t_i
 - Estimate waiting time W_i in preferred tier
 - If $W_i \leq \theta$
Dispatch t_i to the least-loaded fog node in the preferred tier
 - Else
Activate spillover and dispatch t_i to the nearest feasible tier with minimum predicted waiting
6. Execute task non-preemptively to completion at the selected fog node
7. Update node status and queue statistics (waiting time, completion time, violations)

Handling Spillover and Tier-Violation Rate

Since PBTA-S is designed for fog environments where capability alignment matters, spillover must be treated as a controlled exception. To quantify spillover impact, a tier-violation rate (TVR) can be computed as:

$$TVR = \frac{\text{Number of tasks executed outside preferred tier}}{\text{Total tasks executed}} \quad (5)$$

A lower TVR indicates stricter alignment between task urgency and fog tier capability, while a slightly higher TVR may be acceptable under heavy congestion to protect urgent tasks from long delays.

Optionally, a spillover penalty can be added in performance evaluation:

$$Penalty(t_i) = \begin{cases} 0 & \text{if executed in preferred tier} \\ \lambda & \text{if executed in non-preferred tier} \end{cases}$$

where λ reflects the operational cost or reduced suitability of executing tasks outside the optimal tier.

Time Complexity Discussion

The computational cost of PBTA-S primarily depends on:

- task classification: $O(1)$ per task
- queue insertion: $O(\log m)$ if priority queue is used, else $O(1)$ for FIFO
- node selection inside tier: $O(m)$ for scanning nodes, or $O(\log m)$ using a heap structure

Therefore, PBTA-S remains efficient and suitable for real-time fog scheduling where tasks arrive continuously.





Experimental Setup and Evaluation

This section describes the experimental setup and evaluation methodology used to validate the performance of the proposed PBTA-S framework using the CloudSim simulator. Although PBTA-S is motivated by fog-assisted healthcare monitoring, its tiered scheduling behavior can be effectively modeled in CloudSim by representing fog capability tiers as heterogeneous VM pools and implementing the scheduling logic at the broker level. The key objective of PBTA-S is to improve the responsiveness of mixed-criticality healthcare monitoring workloads by prioritizing urgent tasks, reducing queueing delay, and controlling congestion through spillover. The experimental evaluation compares PBTA-S against baseline scheduling strategies under varying workload intensities to quantify improvements in waiting time, response time, SLA/deadline violations, throughput, and VM utilization. The experiments are conducted using CloudSim, a widely used simulation toolkit for modeling cloud datacenters, virtualized resources, and task scheduling policies. CloudSim enables controlled evaluation of resource allocation algorithms by allowing configuration of datacenter hosts, VM provisioning, Cloudlet execution, and broker-driven task assignment. In this study, CloudSim is used to emulate a healthcare computing environment in which tasks arrive dynamically over time and must be scheduled efficiently on heterogeneous VM resources. By introducing multi-level VM categorization and queue-based dispatching in the broker, the proposed PBTA-S framework can be evaluated under reproducible conditions with consistent workload patterns and resource configurations.

Cloud Sim Infrastructure Configuration and Load Modeling

To emulate heterogeneous fog/edge capability within a CloudSim environment, the simulation is configured with three VM categories representing high-, mid-, and lightweight execution tiers, so that tasks can be allocated based on urgency and resource suitability. A typical VM configuration follows three capability pools: **C1** for critical tasks, **C2** for medium tasks, and **C3** for routine tasks, with compute and memory ranges summarized in Table 1 (e.g., C1: 2–4 VMs with 8000–12000 MIPS; C2: 3–6 VMs with 4000–8000 MIPS; C3: 4–8 VMs with 1000–4000 MIPS, with corresponding RAM ranges).

Incoming healthcare tasks are modeled as Cloudlets characterized by arrival time, computation length (MI), and an optional deadline/SLA target. To reflect mixed-criticality monitoring, tasks are grouped into P1/P2/P3 classes (P1: critical abnormal events; P2: clinically significant follow-ups; P3: routine/start-end updates), and Cloudlets arrive dynamically in an online mode under three workload intensities—light, medium, and heavy (bursty)—with typical proportions of P1: 15–25%, P2: 25–35%, P3: 40–60%. PBTA-S is implemented through a custom CloudSim broker that tags Cloudlets by priority, maps them to VM categories (C1/C2/C3), inserts them into independent category-specific queues (served in the order $Q1 > Q2 > Q3$), and dispatches tasks to the least-loaded VM in the preferred category; spillover is activated only when predicted waiting exceeds a threshold, and spillover events are logged for tier-violation analysis.

Load definition (utilization ratio): In this work, “load” is defined using the offered-load (utilization) ratio ρ , which measures how strongly the incoming workload stresses the available VM capacity. Specifically,

$$\rho = \frac{\lambda \cdot \mathbb{E}[L]}{\mu_{tot}} \quad (6)$$

where λ is the Cloudlet arrival rate (tasks/s), $\mathbb{E}[L]$ is the mean Cloudlet computation demand (MI), and μ_{tot} is the total VM service capacity (MI/s). In CloudSim terms, μ_{tot} is computed from VM speeds as:

$$\mu_{tot} = \sum_{j=1}^m (MIP S_j \times PE_j) \quad (7)$$

(when each VM has one processing element, $PE_j = 1$, then $\mu_{tot} = \sum MIP S_j$). We categorize workload intensity using the following cut-offs:

- Light load: $0 < \rho \leq 0.4$
- Medium load: $0.4 < \rho \leq 0.8$





- Heavy (bursty) load: $0.8 < \rho \leq 1.2$

where $\rho > 1$ represents temporary overload (arrivals exceed service capacity), leading to queue buildup and congestion—precisely the condition under which spillover becomes beneficial. These load definitions correspond to the “light/medium/heavy” settings used to report response time and other QoS outcomes in the results tables (e.g., Table 2).

RESULTS AND DISCUSSION

This section evaluates the performance of the proposed PBTA-S framework using the Cloud Sim simulator and compares it with baseline approaches including FCFS, Priority Scheduling (PS), Load-Based Scheduling (LB), and Tiered Scheduling without Spillover (TS) under light, medium, and heavy workload conditions. The evaluation focuses on key indicators for mixed-criticality healthcare workloads, such as average response time, average waiting time (especially for P1 tasks), SLA/deadline violation rate, throughput, VM utilization balance across categories, and the category-violation rate introduced by spillover. As shown in Table 2, response time trends remain similar across methods under light load due to minimal queueing, but differences become significant as demand increases; FCFS performs worst due to its non-priority behavior, PS improves responsiveness through urgency ordering, LB improves balancing without strong urgency guarantees, and TS benefits from tiering but loses flexibility under overload, whereas PBTA-S consistently achieves the lowest response time, with the largest improvement under heavy load.

For critical healthcare tasks, reducing queue delay is essential, and Table 3 shows that PBTA-S achieves the minimum waiting time for P1 emergency tasks across all load levels by prioritizing Q1, selecting the least-loaded VM within the high-capability category, and using spillover only when congestion would otherwise cause excessive delay. SLA and deadline compliance is a key QoS requirement in time-sensitive healthcare processing. As reported in Table 4, PBTA-S produces the lowest violation rates under all workload intensities, particularly during heavy-load conditions, indicating that urgency-driven scheduling combined with controlled spillover improves deadline adherence more effectively than FCFS, PS, LB, and strict tiering without spillover. In terms of system stability, throughput comparisons in Table 5 indicate that all methods perform similarly under light load, whereas PBTA-S maintains the highest throughput under medium and heavy loads by avoiding category-level bottlenecks through least-load dispatching and preserving continuity during overload via spillover. Resource efficiency is validated through utilization balance across VM categories. Under heavy load, Table 6 shows that FCFS and PS tend to overload high-capability VMs while leaving lower tiers underused, and TS can saturate the top tier due to strict mapping. PBTA-S achieves a more balanced utilization profile across categories by selecting the least-loaded VM within each tier and activating spillover only when required, thereby improving both responsiveness and stability.

Finally, PBTA-S introduces spillover as a controlled exception to prevent excessive delays when the preferred category is congested. As presented in Table 7, TS reports a category-violation rate of 0% due to strict tier enforcement, while PBTA-S shows a small CVR/TVR because it permits limited redirection to maintain responsiveness. This slight category deviation is justified by the substantial reductions achieved in response time and SLA violations under heavy load, and TVR is computed only for tier-aware methods since non-tiered baselines do not define preferred execution categories. The Cloud Sim-based evaluation confirms that PBTA-S consistently delivers the best performance across light, medium, and heavy-load scenarios by achieving the lowest response time, minimizing waiting delay for critical tasks, reducing SLA violations, maintaining higher throughput under burst arrivals, and balancing VM utilization across categories. The controlled spillover mechanism introduces only limited category violations while providing strong congestion resilience, demonstrating that combining urgency-driven prioritization with multi-level VM categorization and overload-aware scheduling is an effective strategy for time-sensitive healthcare monitoring workloads.





Ramesh Kannan and George Dharma Prakash Raj

CONCLUSION AND FUTURE WORK

This paper proposed PBTA-S, a priority-based task allocation and scheduling framework for IoT-enabled healthcare monitoring, where tasks are generated continuously and must be processed within strict time constraints under mixed-criticality conditions. PBTA-S classifies healthcare tasks into three urgency levels and maps them to heterogeneous VM categories representing different capability tiers, enabling category-specific queue scheduling and least-load VM dispatching to improve responsiveness under dynamic workloads. A controlled spillover mechanism is activated only during congestion to prevent critical tasks from being delayed by long queues, thereby reducing waiting time and overall response time while lowering SLA/deadline violations during burst arrivals, without compromising throughput and utilization stability across VM categories. Future work will focus on enhancing task prioritization using clinical severity scoring derived from real sensor patterns and patient context, improving spillover decisions through adaptive thresholds driven by runtime feedback or lightweight learning models, and extending the framework with energy-aware scheduling for efficient resource usage. Additional improvements include incorporating privacy-aware processing and secure data handling, strengthening fault tolerance under VM or host failures, enabling mobility-aware execution for dynamic monitoring environments, and integrating fairness-aware mechanisms that provide bounded delays for non-critical workloads while maintaining strict urgency guarantees for emergency healthcare tasks.

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Ramesh Kannan and George Dharma Prakash Raj

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Table 1. Simulation Configuration

VM Category	VMs (Count)	CPU (MIPS)	RAM (MB)	Intended Role
C1	2–4	8000–12000	8192–16384	Critical tasks (P1)
C2	3–6	4000–8000	4096–8192	Medium tasks (P2)
C3	4–8	1000–4000	1024–4096	Routine tasks (P3)

Table 2. Average Response Time (ms)

Method	Light Load	Medium Load	Heavy Load
FCFS	190	410	860
PS	165	360	740
LB	175	335	710
TS (no spillover)	155	310	690
PBTA-S (Proposed)	130	260	520

Table 3. Average Waiting Time for P1 Tasks (ms)

Method	Light Load	Medium Load	Heavy Load
FCFS	85	210	540
PS	65	165	420
LB	70	150	390
TS (no spillover)	55	135	360
PBTA-S (Proposed)	35	95	210

Table 4. SLA/Deadline Violation Rate (%)

Method	Light Load	Medium Load	Heavy Load
FCFS	2.9	8.4	19.6
PS	2.2	6.7	15.2
LB	2.1	6.0	13.8
TS (no spillover)	1.8	5.4	12.9
PBTA-S (Proposed)	1.1	3.5	7.2





Ramesh Kannan and George Dharma Prakash Raj

Table 5. Completed Tasks per Simulation Interval

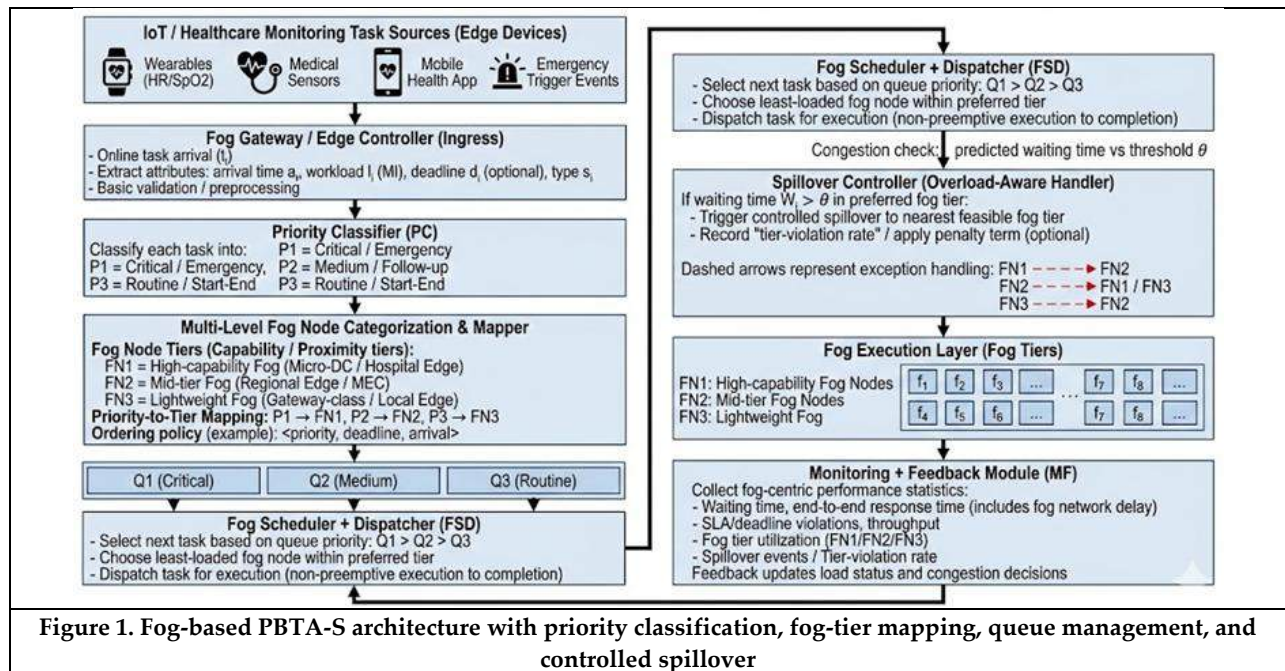
Method	Light Load	Medium Load	Heavy Load
FCFS	980	920	780
PS	990	940	810
LB	995	955	835
TS (no spillover)	1000	960	850
PBTA-S (Proposed)	1005	975	900

Table 6. Average VM Utilization (%) by Category – Heavy Load

Method	C1 Util.	C2 Util.	C3 Util.
FCFS	92	66	40
PS	90	70	44
LB	86	78	58
TS (no spillover)	95	62	38
PBTA-S (Proposed)	88	74	55

Table 7. Category-Violation Rate (CVR/TVR %) – Heavy Load

Method	CVR / TVR (%)
TS (no spillover)	0.0
PBTA-S (Proposed)	6.8



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A Systematic Review of *Pramana Sharir* : Bridging Classical Ayurvedic Anthropometry With Contemporary Measurement Science

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ABSTRACT

In the world of *Ayurveda*, there is a highly individualized approach to measuring the human body known as *Pramana Sharir*. Rather than relying on universal, standardized units like inches or centimeters, it uses the patient's own anatomy—specifically, their finger width (*Swa-Angula Pramana*)—as the core metric. However, there has been a noticeable lack of systematic research connecting these ancient anatomical rules to modern scientific standards. This review was built to tackle four main goals: (1) break down the foundational concepts of *Pramana Sharir* from authoritative classical texts, (2) see how these ancient metrics map onto modern measurement systems, (3) figure out if there is actual, solid evidence for using this in medicine and forensics today, and (4) pinpoint exactly where more research is desperately needed. We combed through major databases (PubMed, Scopus, and Google Scholar) for studies published between 2010 and 2025. We also analyzed authoritative English translations of heavy-hitting classical texts like the *Charaka Samhita* and *Sushruta Samhita* [1,2]. Following PRISMA 2020 guidelines, two independent reviewers vetted the study selection and evaluated the risk of bias using JBI Critical Appraisal Tools. We started with 847 records and narrowed it down to 32 solid studies. The ancient texts outline a highly detailed system for measuring both linear length (using finger-breadths, or *Anguli*) and volumetric capacity (using cupped hands, or *Anjali*). Historically, having perfectly balanced body proportions was thought to guarantee a longer, healthier life. Today, we found these measurements being





Tanya Singh et al.,

applied in surgical planning, pharmacology, and forensic identification. While the evidence moderately supports the physical measurement applications, there is very little proof validating the historical claims about lifespan. *Pramana Sharir* offers a fascinating, custom-tailored framework for evaluating the human body, with moderate scientific evidence backing up its practical measurement applications. That being said, we need long-term modern studies before this system can be fully integrated into everyday clinical practice.

Keywords: *Pramana Sharir*, *Ayurvedic* anthropometry, *Swa-Angula Pramana*, systematic review, PRISMA, personalized medicine

INTRODUCTION

Ayurveda looks at human health as a complete package—a balancing act of mind, body, and spirit. On the physical side of things, *Pramana Sharir* is the traditional method used to measure and evaluate the body's structure. If we break down the Sanskrit, "*Pramana*" essentially means a valid way to gain knowledge, while "*Sharir*" refers to the physical form itself. While plenty of ancient cultures had their own ways of measuring people, *Ayurveda* did something entirely unique: it used the individual's own body as the ruler. By using the width of a person's own finger (known as *Anguli Pramana* or *Swa-Angula*), *Ayurvedic* practitioners acknowledged that everyone has a unique physical constitution that demands a customized assessment.

Classical Foundations

The foundational authorities of ancient *Ayurveda*—including *Charaka*, *Sushruta*, and *Vagbhata*—wrote extensively about these measurement protocols. *Charaka* actually made it a core part of his ten-step patient examination to figure out a person's physical strength and overall prognosis¹. *Sushruta* took the concept even further, arguing that a perfectly proportioned body directly correlated with enhanced longevity, establishing a direct link between structural harmony and a long life [2].

Contemporary Context

Fast forward to today, and modern anthropometry traces its roots back to 19th-century criminal identification, eventually expanding into clinical medicine, ergonomics, and sports science [10,13,14]. Today, clinical practice relies heavily on standardized metrics like Body Mass Index (BMI), Waist-to-Hip Ratio (WHR), and Body Surface Area (BSA) to figure out nutritional health and stratify disease risk [15].

Rationale and Research Question

There is a massive gap when it comes to systematically blending these ancient, personalized rules of *Ayurvedic* anthropometry with modern empirical validation. This review asks a straightforward question: What is the current scientific evidence backing up the principles and practical applications of *Pramana Sharir* today?

Objectives

- Systematically pull the core rules of *Pramana Sharir* from classical *Ayurvedic* texts.
- Establish how these ancient measurement concepts compare to modern approaches.
- Critically judge the quality of evidence supporting their use in clinical and forensic settings.
- Highlight significant gaps where further scientific investigation is needed.





MATERIALS AND METHODS

Study Design: To ensure our methodology was rigorous and completely transparent, this systematic review strictly followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines.

Search Strategy: On January 15, 2025, we ran deep, comprehensive searches across major electronic databases. We blended traditional keywords (like "Pramana Sharir" and "Anguli Pramana") with modern scientific terms (like "forensic anthropology" and "body composition") to cast a wide net across PubMed, Scopus, and Google Scholar.

Study Selection Process: Using Rayyan QCRI software, two independent reviewers filtered the retrieved records. They first screened titles and abstracts, then pulled the full texts of potentially eligible studies for a much closer look. The two reviewers had a remarkably strong agreement rate (Cohen's kappa = 0.87), and any minor disagreements were settled through discussion.

Sources of Information

Classical Ayurvedic Literature

We analyzed primary texts including the *Charaka Samhita*, *Sushruta Samhita*, *Ashtanga Hridaya*, *Ashtanga Sangraha*, *Kashyapa Samhita*, *Bhela Samhita*, *Sarangadhara Samhita*, and *Vangasena Samhita* [1-8].

Modern Scientific Sources

We gathered recent papers on forensics, clinical anatomy, and anthropometry from databases like PubMed, Scopus, and Google Scholar. The search was locked strictly to English-language articles published between 2010 and 2025.

Inclusion and Exclusion Criteria

Data Extraction

Two reviewers independently pulled the data using a standardized extraction form. For the modern studies, we grabbed details on the authors, study design, demographics, measurement tools, and the hard numbers (like p-values and correlation coefficients). For the classical texts, we extracted all relevant passages regarding *Anguli Pramana*, *Anjali Pramana*, and body proportionality.

Quality Assessment (Risk of Bias)

The reviewers judged the methodological quality of the included studies using JBI Critical Appraisal Checklists tailored to the specific study designs (such as cross-sectional or cadaveric studies). Studies scoring 80% or higher were graded "High quality," 60–79% earned a "Moderate" grade, and anything below 60% was marked "Low."

Data Synthesis

Because the studies varied wildly in their setups, outcome measures, and populations, a meta-analysis was impossible. Instead, we wrote a narrative synthesis organized around three main themes: classical measurement principles, ancient body proportions, and the modern scientific validation of those concepts.

RESULTS

Study Selection Results

Our initial database searches kicked back 847 records. After scrubbing the duplicates and screening the abstracts, we closely reviewed 74 full-text articles. We ended up rejecting 42 of them (mostly because they lacked original data or measurable proportions), leaving us with 32 solid studies for the final analysis—comprising 9 text analyses and 23 modern validation studies.

PRISMA 2020 Flow Diagram

Records identified (n = 847)





Tanya Singh et al.,

PubMed (n = 312)
Scopus (n = 289)
Google Scholar (n = 246)
↓
Records after duplicates removed (n = 563)
↓
Records screened (n = 563) → Records excluded (n = 489)
↓
Full-text articles assessed (n = 74) → Full-text excluded (n = 42)
• No original data (n = 18)
• No measurable proportions (n = 15)
• Non-English (n = 6)
• Pre-2010 (n = 3)
↓
Studies included in review (n = 32)
Classical text analyses (n = 9)
Modern validation studies (n = 23)

Conceptual Basis of *Pramana Sharir*

Meaning of *Pramana*: In *Ayurvedic* philosophy, *Pramana* is the valid acquisition of knowledge. *Charaka* laid out four primary ways to get it: reliable authority (*Aptopadesha*), direct observation (*Pratyaksha*), inference (*Anumana*), and logical reasoning (*Yukti*) [1]. Systems of Measurement in *Ayurveda*: P Classical texts mention several regional measurement systems. Generally, they categorize measurements into three buckets: linear length, physical weight, and liquid volume [9].

Anguli Pramana vs. *Anjali Pramana*

Concept of *Sama Sharir*: If a person's arm span perfectly matches their total body height, they possess what is called *Sama Sharir*[2]. Historically, individuals with this perfect proportion were believed to have a longer lifespan, greater physical strength, and rock-solid immunity.

DISCUSSION

Summary of Principal Findings

Our deep dive into the 32 studies shows that *Pramana Sharir* is a highly sophisticated, individualized framework for measuring the body. Using a person's own finger width as a base unit actually mirrors modern practices that use ratios to account for different body sizes across varying populations. We found these measurements being practically applied in everything from forensic identification to customized surgical planning.

Alignment with Allometry

This self-referential system aligns perfectly with the biological principle of allometry—the study of how body proportions shift and change with overall size [23]. This suggests that *Ayurveda* figured out centuries ago that biological ratios matter far more than absolute numbers.

Comparison with Modern Anthropometry

Certainty of Evidence Assessment

Using GRADE (Grading of Recommendations Assessment, Development and Evaluation) principles adapted for narrative synthesis, we assigned the following certainty grades to major claims:





Tanya Singh et al.,

Strengths and Weaknesses of the Review**Strengths**

The brilliance of this system is that it doesn't require external tools—the patient is the measuring stick. It seamlessly links physical size to functional health, and its direct clinical and forensic applications are highly relevant today.

Weaknesses (including language bias)

The biggest flaw is how difficult it is to standardize what a "finger width" actually means across different research clinics. Additionally, by restricting our search strictly to English papers published between 2010 and 2025, we likely missed older, foundational studies as well as valuable research published in Indian languages like Hindi or Sanskrit.

Future Research Directions

To really pull *Pramana Sharir* into the 21st century, researchers need to use MRI and 3D imaging to map these ancient external measurements onto actual internal anatomy. We also need massive demographic databases to establish what normal *Ayurvedic* proportions look like across different ethnic groups today. Finally, longitudinal studies are essential to see if these proportions actually do predict a longer lifespan.

CONCLUSION

Pramana Sharir is a fiercely individualized way of looking at human anatomy that traces its roots back to classical *Ayurvedic* medicine. Instead of relying on universal averages, it uses the patient's own body as the baseline, offering a customized approach that pairs beautifully with standardized modern metrics. In forensics, it can improve regression formulas, and in clinical settings, it can add a personalized layer to conventional risk assessments. While we definitely need more large-scale research to validate the bolder historical claims, this system has immense potential to make our understanding of human health much more holistic and personalized.

Conflict of Interest Statement

The authors declare no conflict of interest.

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Tanya Singh et al.,

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Tanya Singh et al.,

Table 1: Inclusion and Exclusion Criteria

Parameter	Inclusion Criteria	Exclusion Criteria
Classical Texts	Sanskrit commentaries with English translations available	Texts lacking commentary or reliable translation
Modern Literature	Peer-reviewed original research, systematic reviews, narrative reviews	Conference abstracts, unpublished data, non-English publications
Publication Date	Classical: any edition; Modern: 2010-2025	Modern literature pre-2010
Subject Relevance	Direct relevance to Pramana Sharir or anthropometric measurement	No measurable body proportion data presented

Table 2: Quality Assessment Grades

Reference	Study Design	JB1 Score	Quality Grade
Mehta & Joshi (2021) ²⁰	Cadaveric validation	8/10	High
Kumar & Singh (2023) ²⁹	3D photogrammetry validation	7/10	Moderate
Sharma et al. (2021) ²⁷	Cross-sectional	6/10	Moderate
Singh et al. (2020) ¹⁶	Cross-sectional	7/10	Moderate
Rao & Reddy (2020) ²⁶	Cross-sectional	4/10	Low
Patel & Thakur (2022) ¹⁸	Pilot cross-sectional	5/10	Low

Table 3: Comparison of Anguli and Anjali Pramana

Feature	Anguli Pramana	Anjali Pramana
Measurement Type	Linear dimensions	Volumetric capacity
Unit Basis	Individual finger breadth	Capacity of joined palms
Clinical Applications	Height assessment, surgical instrument sizing, Marma point localization	Body fluid quantification (Rasa, Rakta, etc.)
Example	84 Angula for total body height	9 Anjali for Rasa

Table 4: Ideal Body Height in Classical Texts

Text	Height(Angula)	Special Condition
Charaka Samhita ¹	84	Measured standing erect, feet together, heel to crown
SushrutaSamhita ²	120	Standing with arms extended vertically overhead, fingertip to fingertip
Ashtanga Hridaya ³	3.5× forearm length	Height derived by multiplying forearm length (elbow to middle fingertip) by 3.5

Table 5: Clinical Applications of Pramana Sharir

Specialty	Application	Example
Shalya Tantra (Surgery) ²	Surgical instrument design	Needles: 2 Angula (delicate), 3 Angula (muscular)
Marma Therapy ³³	Vital point localization	Measurement-based identification
Panchakarma ³	Basti instrument length	Bastinetra: ~12 Angula
Dravyaguna ⁴	Plant morphology description	Standardized references
Rasashastra ⁸	Pharmaceutical dimensions	Material sizing
Forensic Medicine ^{16,17,19,20}	Stature from partial remains	Height from nose/ear/foot length



Tanya Singh *et al.*,**Table 6: Ayurvedic vs Modern Anthropometry Comparison**

Aspect	Ayurvedic System	Modern System
Philosophical Basis	Personalized, holistic (physical-functional integration)	Standardized, primarily physical
Unit of Measurement	<i>Swa-Angula Pramana</i> (individual finger breadth)	Standardized units (cm, inches, kg)
Key Indices	Total height (84-120 Angula), <i>Sama Sharir</i>	BMI, WHR, skinfold thickness
Clinical Use	Prognosis (<i>Ayushya Pramana</i>), surgical planning	Nutritional assessment, disease risk
Forensic Use	Height from nose/ear length	Population-specific regression formulas
Knowledge Source	<i>Aptopadesha + Pratyaksha</i>	<i>Pratyaksha + Yukti</i> (statistical)

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Homoeopathic Approach in Pseudo - Surgical Conditions with Special Reference to Adenoid Hypertrophy: A Literature - Based Review

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ABSTRACT

Adenoid hypertrophy is a common paediatric condition and a major cause of upper airway obstruction in children. It may lead to nasal obstruction, mouth breathing, recurrent infections, sleep disturbances, and impaired quality of life. Although adenoidectomy is commonly recommended in severe cases, adenoid hypertrophy is considered a pseudo-surgical condition in which conservative management may be beneficial. This literature-based review highlights the anatomy, etiopathogenesis, clinical features, diagnosis, and conventional management of adenoid hypertrophy, with special emphasis on the homoeopathic perspective. Homoeopathy approaches the condition as a manifestation of an underlying constitutional disturbance and aims to restore health through individualized treatment. The review suggests a potential role for homoeopathy in the management of adenoid hypertrophy while pointing out the need for further scientific research to strengthen the existing evidence base.

Keywords: Adenoid Hypertrophy; Homoeopathy; Pseudo-Surgical Conditions; Paediatric Airway Obstruction; Individualized Treatment

INTRODUCTION

Pseudo-surgical diseases are clinical conditions for which surgical treatment is often recommended when significant local pathological changes are present. However, surgery is not necessary for all of these conditions, as many can be



**Atmaja Joshi and Hima Mehta**

effectively managed with conservative, non-surgical therapies. They commonly present with chronic, relapsing, or structural abnormalities, giving an impression of requiring surgical treatment when satisfactory results can be obtained with other modes of management. Some common examples are adenoid hypertrophy, chronic tonsillitis, renal calculi, warts, corns, ovarian cysts and fistula-in-ano. Pseudo-surgical conditions emphasize the need to consider more conservative treatment options before surgery.[1] Waldeyer's lymphatic ring is a circular arrangement of lymphoid tissue located in the pharynx and comprises four components: the adenoid (nasopharyngeal tonsil), tubal tonsils, palatine tonsils, and lingual tonsils. The adenoid is centrally situated in the nasopharynx, attached to the junction of the roof and posterior wall, lying posterosuperior to the posterior nasal apertures (choanae) and adjacent to lymphoid tissue surrounding the nasopharyngeal openings of the eustachian tubes.[1] Owing to its strategic anatomical position, the adenoid plays a crucial role in mucosal immune defence during early childhood.[2]

The adenoid was first anatomically described in 1661 by Conrad Victor Schneider. Subsequently, in 1868, Wilhelm Meyer introduced the term "adenoid vegetations" and described them as soft, tumour-like masses occupying the nasopharyngeal space above the soft tissue palate.[3] Meyer was also the first to establish the association between adenoid enlargement and clinical features such as nasal obstruction, mouth breathing, snoring, and hearing impairment, and he advocated surgical removal as a treatment modality.[4] Adenoid hypertrophy (AH) refers to the pathological enlargement of adenoid tissue and may occur with or without associated acute or chronic infection.[5] Radiologically, the normal adenoid thickness is approximately 6.2 mm, whereas hypertrophied adenoids measure around 11.6 mm. An adenoid size exceeding 12 mm is considered abnormal in children aged 2–10 years.[6] Adenoid hypertrophy is the most common cause of upper airway obstruction in children and adolescents. Although it represents a physiological response to increased immunological activity in early life, persistent enlargement may result in significant morbidity. Clinical manifestations include mouth breathing, nasal obstruction, recurrent upper respiratory tract infections, speech disturbances, asthma, and obstructive sleep apnea. Chronic upper airway obstruction may further lead to altered craniofacial growth, sleep disturbances, behavioural problems, impaired cognitive development, and reduced scholastic performance.[7]

A recent systematic review and meta-analysis reported the prevalence of adenoid hypertrophy among children and adolescents to be approximately 34.46%, emphasizing its substantial public health burden.[7] Conventional management of adenoid hypertrophy includes antibiotics and intranasal corticosteroids, while surgical adenoidectomy is recommended in severe or refractory cases. However, surgical intervention is associated with potential complications, including haemorrhage, infection, recurrence, anaesthetic risks, and adverse effects on quality of life. [8,9,10] Homoeopathy adopts a holistic and individualized approach intended to stimulating the body's self-regulatory mechanisms and restoring constitutional balance. Preliminary clinical observations and published case reports suggest that individualized homoeopathic treatment may help reduce symptoms of adenoid hypertrophy, improve nasal airflow, and decrease the frequency of recurrent infections, and potentially prevent the need for surgical intervention. Nevertheless, despite encouraging outcomes reported in isolated case studies, high-quality scientific evidence remains limited. An article titled "Evidence-Based Study of Enlarged Adenoid Treated with Homoeopathic Medicine: A Case Report" draws attention to the need for subsequent observational studies and well-designed randomized controlled trials to establish the efficacy of homoeopathy in the management of enlarged adenoids.[11]

Etiopathogenesis

Adenoid hypertrophy is a multifactorial disorder resulting from persistent antigenic stimulation and chronic inflammation of adenoid tissue. While adenoidal enlargement is a normal physiological process during childhood, infectious, allergic, and environmental elements may contribute to excessive lymphoid hyperplasia, leading to pathological enlargement and associated clinical manifestations. The principal factors involved in its etiopathogenesis are outlined below.



**Physiological Factors:**

Physiological enlargement of the adenoid is a normal developmental phenomenon that occurs during childhood as part of the maturation of the immune system. Continuous exposure to environmental antigens stimulates lymphoid tissue growth, and the adenoid typically reaches its maximum size between 5 and 7 years of age. This enlargement is generally transient and undergoes gradual involution after puberty. However, prolonged or excessive antigenic stimulation may contribute to pathological hypertrophy. [5,12]

Infectious Factors:

Recurrent and persistent upper respiratory tract infections are important contributors to adenoid hypertrophy. Both bacterial and viral pathogens can trigger chronic inflammation and sustained immune activation in the adenoid tissue, leading to lymphoid hyperplasia and tissue enlargement. Chronic infection may manifest clinically as nasal obstruction, mouth breathing, hyponasal speech, and eustachian tube dysfunction, thereby increasing the risk of middle-ear disorders and recurrent respiratory illnesses. [5,12]

Allergic Factors:

Allergic diseases, particularly allergic rhinitis, play a significant role in the development of adenoid hypertrophy. Persistent exposure to allergens induces IgE-mediated inflammatory responses that promote mucosal oedema and lymphoid tissue proliferation. As a result, children with allergic rhinitis are more likely to develop enlarged adenoids and experience more severe symptoms than their non-allergic counterparts. [13,14]

Other Contributing Factors:

Additional factors such as gastroesophageal reflux disease (GERD) and passive exposure to tobacco smoke have been associated with adenoid hypertrophy. Reflux of gastric contents into the nasopharynx can cause chronic irritation and inflammation of the adenoid tissue, while second-hand smoke impairs mucociliary function and perpetuates inflammatory changes. Both factors may cause persistent adenoid enlargement and its associated clinical manifestations. [15,16]

Clinical Manifestations

The clinical manifestations of adenoid hypertrophy are determined not only by the absolute size of the adenoidal tissue but also by its relationship to the available nasopharyngeal space. Hypertrophied and infected adenoids may produce a wide spectrum of nasal, aural, and general systemic symptoms.[17]

Nasal Symptoms

- Nasal Obstruction and Congestion: These are the commonest symptoms. This leads to mouth breathing and snoring.
- Nasal Discharge: It is partly due to choanal obstruction, as normal nasal secretions cannot drain into the nasopharynx, and partly due to associated rhinitis and recurrent pharyngitis.
- Sinusitis: Chronic maxillary sinusitis is commonly associated with adenoids. It is due to persistent nasal discharge and infection.
- Epistaxis: When adenoids are acutely inflamed, epistaxis can occur with nose blowing.
- Voice Change: Toneless, with nasal quality lost due to nasal obstruction.

Tubal Symptoms

- Tubal Obstruction: An adenoid mass blocks the Eustachian tube, leading to a retracted tympanic membrane and conductive hearing loss.
- Recurrent attacks of acute otitis media may occur due to the spread of infection via the eustachian tube.
- Serous Otitis Media: The waxing and waning signs of adenoids cause intermittent eustachian tube obstruction with intermittent hearing loss.



**Atmaja Joshi and Hima Mehta****General Symptoms**

- Adenoid Facies: In long-standing cases, children may develop a characteristic facial appearance known as "adenoid facies." This is characterised by a dull facial expression, pinched nostrils, persistent mouth opening, a narrow maxillary arch, a shortened or retracted upper lip, and protruding upper incisors. These features result from chronic nasal obstruction and habitual mouth breathing associated with adenoid hypertrophy.
- Difficult breathing, obstructive sleep apnoea syndrome.

Diagnosis of Adenoid Hypertrophy

A thorough history and detailed physical examination are often sufficient to diagnose adenoid hypertrophy. Physical examination of children with adenoid hypertrophy commonly reveals habitual mouth breathing, parted lips, and a dull facial expression. In chronic cases, characteristic features of adenoid facies, including an open-mouth posture, elongated face, high-arched palate, dental malocclusion, and facial muscle hypotonia, may be present. Anterior rhinoscopy may demonstrate nasal cavity narrowing, mucosal congestion, and mucopurulent discharge, while pale, oedematous mucosa may suggest associated allergic rhinitis. Oral examination may show persistent mouth breathing, a high-arched palate, dental abnormalities, and postnasal drip. Enlarged, mobile, non-tender upper deep cervical lymph nodes may be palpable due to chronic infection. Although the adenoid is not usually visible on routine examination, it may occasionally be observed behind the soft palate, and digital palpation can reveal a soft, lobulated nasopharyngeal mass consistent with adenoid tissue.[18] The diagnosis of adenoid hypertrophy is primarily based on radiological and endoscopic evaluation. Lateral X-ray of the nasopharynx is a simple, cost-effective screening tool that demonstrates adenoidal enlargement and the degree of nasopharyngeal airway obstruction. Flexible nasopharyngoscopy is considered the gold standard, allowing direct visualization of the adenoids and precise evaluation of choanal obstruction. Computed tomography (CT) and magnetic resonance imaging (MRI) provide detailed anatomical evaluation and are generally reserved for selected cases, as they are not routinely indicated in the assessment of adenoid hypertrophy.[19]

Generalized Management of Adenoid Hypertrophy

The management of adenoid hypertrophy aims to relieve upper airway obstruction, prevent complications such as otitis media with effusion, and control recurrent nasopharyngeal infections.[20] Initial treatment is primarily conservative and includes intranasal corticosteroids, which have been shown to improve nasal airflow and reduce adenoid size. Supportive measures such as saline nasal irrigation, management of associated allergic rhinitis, and appropriate antibiotic use for acute bacterial infections may also be beneficial. Observation and periodic follow-up are appropriate for children with mild symptoms and no significant functional impairment. [21,22,23] Surgical intervention in the form of adenoidectomy is reserved for patients who do not respond adequately to medical management or who present with significant nasal obstruction, obstructive sleep apnea, recurrent adenoiditis, chronic otitis media with effusion, or chronic rhinosinusitis. The decision for surgery should be based on a comprehensive clinical evaluation and appropriate diagnostic investigations. [24,25]

Homoeopathic Management of Adenoid Hypertrophy

According to the Hahnemannian concept of disease, so-called local diseases are not simply confined to a particular part of the body but are expressions of a general morbid disturbance of the vital force. Samuel Hahnemann classified local diseases into two broad categories.[26] 1. Local Diseases of Internal Origin (Pseudo-local or Pseudo-surgical Diseases) 2. Local Diseases of External Origin (True Surgical Diseases) [26] Local diseases of internal origin do not arise independently at the affected part but originate from an internal derangement of the vital force, of which the local affection is only a manifestation. Although such diseases appear to be confined to a particular organ or tissue, they represent a general dynamic disturbance of the organism as a whole. As stated by Samuel Hahnemann in the Organon of Medicine:

Aphorism 185: "Those diseases which are called local do not arise from local causes, but depend upon an internal disease, of which the local affection is only a manifestation." [26] **Aphorism 186** further clarifies: "The local symptoms disappear only when the internal disease is cured."



**Atmaja Joshi and Hima Mehta**

In Aphorism 186, he distinctly states: “Those local maladies which arise solely from external causes, such as injuries, wounds, fractures, dislocations, burns, etc., must be treated by surgical or mechanical aid.” According to Hahnemann, surgical intervention is essential in local diseases arising from definite external causes; however, when no external impediment to cure is present, the appropriate and rational approach is constitutional Homoeopathic treatment.[26] In Hahnemann’s Lesser Writings, he also condemns unnecessary surgery, calling it “mutilation” if carried out without addressing the internal disease.[27] Some of the medicines which are indicated in cases of Adenoid hypertrophy are as follows:[28,29,30,31]

Agraphis nutans

Adenoids, throat deafness. Catarrhal conditions, obstruction of nostrils; Throat and ear troubles with tendency to free discharge from mucous membranes. Clarke of London regarded this remedy as a leading one in cases of adenoids. It was first used by Cooper in 1892. Deafness and deaf-mutism, breathes with mouth open. It is essentially a catarrhal remedy and acts specially on the glands connected with the nasal cavity, and its use generally avoids adenoid surgeon.

Calcarea iodata

Enlarged adenoids, tonsils filled with little crypts and pockets. In cases which are febrile in nature. Flabby children subjects to cold, discharges profuse and yellow, profuse sweat.

Sanguinarinum nitricum

Nose feels obstructed, enlarged turbinates at beginning of hypertrophic process. Post-nasal secretions adherent to nasopharynx, swallowing difficult. Secretion scanty, tendency to dryness.

Baryta carbonica:

Awakes with dry mouth. Catarrh of posterior nares, with frequent epistaxis. Reverberation on blowing nose. Especially in children who have recurring attacks of acute tonsillitis. Tendency to suppuration of glands. Delayed physical and mental development. Chilly patient, greatly sensitive to cold, have offensive foot sweat, timid, lack of self-confidence, Thinking of complaints makes them worse, better in open air

Calcarea carbonica: Scrofulous constitutions who take cold easily with increased mucoid secretions. Stoppage of nose, with fetid, yellow discharge. Swelling of tonsils and submaxillary glands. Chilly patient, takes cold easily, fat, fair, flabby, distended abdomen, pale, weak, easily tired, head sweat profusely while sleeping, sour smelling discharges, longing for fresh air, desire for eggs and indigestible things, sweets, aversion to meat and milk, fearful, slow, sluggish, feels better when constipated.

Calcarea phosphorica: Adenoid growth. Defective hearing. Suffocative cough; better lying down. Highly Recommended by Cooper, Clifton and many others as a most effective remedy in adenoid vegetations. It corresponds to dyscrasia, which permits adenoids growths or tonsillar enlargement. Anaemic children who are peevish, delicate, tall, thin with dirty brownish skin, have cold extremities and feeble digestion, all the symptoms are worse from any change of weather, craving for salted or smoked meat.

Iodium

Adenoid vegetations. Nose stopped up; larynx feels constricted. Eustachian deafness. Rapid metabolism, loss of flesh with great appetite, great debility, feels always too hot, slightest efforts induces perspiration, better after eating.

Tuberculinum

Persistent, offensive otorrhoea. Perforation in membrane tympani, with ragged edges. Sensation of suffocation, even with plenty of fresh air. Enlarged tonsils. Takes cold very easily, tall, lean, thin, narrow-chested, active and precocious mentally but weak physically, emaciated with good appetite, obstinate and disobedient children, irritable,



**Atmaja Joshi and Hima Mehta**

desire to use foul language. Adenoids are greatly benefited and often times cured by a weakly dose of Tuberculinum or preferably Bacillinum.

Medorrhinum

Coldness of tip. Posterior nares obstructed. Chronic nasal and pharyngeal catarrhs. Can inhale but cannot exhale. Nose dirty, tonsils enlarged, thick yellow mucus from nostrils; lips thickened from mouth breathing. Hot patients, takes cold easily, chronic catarrhal conditions in children, children pale, dwarfed and stunted in growth, mentally dull and weak, difficult concentration, desire salt, sweets, ice, acids, oranges, sour things.

Kalium iodatum

Nasal catarrh, involving frontal sinuses. Stuffiness and dryness of nose, without discharge. Profuse, cool, greenish, non-irritating discharges. Accumulation of very tenacious mucus in nostrils. Offensive odour from mouth. Glandular swellings or atrophy, diffused sensitiveness of glands, discharges are copious, watery, acrid, salty, thick, green or foul, weakness, emaciation.

Hydrastis Canadensis

It is the First remedy to be tried, when the mucous membrane is yellow and there is excessive enlargement. Dr. Lambrechts, of Antwerp, uses a tampon of Hydrastis and glycerine 1:6, introduced deep into each nostril, having child make inspirations. Lymphoid hypertrophy, mucous yellowish in colour, post-nasal catarrh.

Cistus Canadensis

In scrofulous or arthritic individuals who have adenoid vegetations. There is extreme sensitiveness to cold air, this is characteristic. Sensation of heat and dryness in the throat so intense that the patient must drink to relieve. Cold air inhaled causes distress in the throat, the 6th potency seems to succeed the best. Cistus has a special affinity for the nasopharynx.

CONCLUSION

Adenoid hypertrophy is a common paediatric disorder that frequently leads to upper airway obstruction and associated functional impairments, often prompting consideration of surgical intervention. Homoeopathy views this condition as a manifestation of an underlying constitutional imbalance rather than merely a localized pathological process. Through individualized remedy selection based on the totality of symptoms, homoeopathic treatment aims to stimulate the body's self-regulatory mechanisms and restore overall health. Clinical observations and published case reports suggest that homoeopathy may play a beneficial role in reducing symptoms, improving quality of life, and potentially lessening the need for surgical intervention in selected cases. Nevertheless, further well-designed clinical studies are essential to strengthen the evidence base and establish the role of homoeopathy in the management of adenoid hypertrophy as a pseudo-surgical condition.

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Atmaja Joshi and Hima Mehta

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RESEARCH ARTICLE

Surgical Orthodontics in Contemporary Practice: Diagnosis, Planning and Treatment Outcomes

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ABSTRACT

Surgical orthodontics or orthodontic-orthognathic treatment is a multidisciplinary treatment used for the management of severe dentofacial deformities not correctable by conventional orthodontic treatment alone. This approach re-establishes skeletal harmony, functional occlusion and facial esthetics, by combining the orthodontic therapy with the orthognathic surgery. Advancements in diagnostic imaging, 3-dimensional treatment planning, virtual surgical simulation, and rigid fixation techniques in the past several decades have greatly enhanced the accuracy and predictability of treatment. Success depends on correct diagnosis, judicious treatment planning, adequate presurgical orthodontic preparation, precise surgical execution and judicious postsurgical orthodontic finishing. A review of current concepts of surgical orthodontics including evolution, indications, diagnostic evaluation, cephalometric assessment, treatment sequence, stability, complications and recent advances in technology is presented.

Keywords: Surgical orthodontics, orthognathic surgery, dentofacial deformity, presurgical orthodontics, cephalometric analysis, orthognathic treatment.

INTRODUCTION

The scope of surgical orthodontics has increased considerably over the years. The early orthodontic treatment focused mainly on the correction of dental malpositions and functional occlusion. The treatment objectives have now evolved from dental alignment to include a better understanding of facial esthetics, skeletal harmony and patient



**Rubal Chandna et al.,**

quality of life [1]. Today the emphasis is on achieving a balanced relationship between the facial skeleton, dentition and soft tissues in the restoration of function and aesthetics. Surgical orthodontics is a multidisciplinary approach that combines orthodontic treatment with orthognathic surgery to manage dentofacial deformities that cannot be corrected by orthodontics alone. It is indicated for patients with significant skeletal discrepancies involving the maxilla, mandible, or both jaws in the sagittal, vertical, or transverse planes, where surgical correction is necessary to achieve optimal functional and esthetic outcomes. The modern era of orthognathic surgery began with the pioneering work of Trauner and Obwegeser in 1957, who presented new techniques that revolutionized the treatment of mandibular deformities. Bell, Epker, Wolford, and other investigators have improved maxillary surgery, Le Fort osteotomies, and fixation techniques, making orthognathic surgery safer and more predictable [2]. Advancements in surgical techniques, imaging technologies, and treatment planning have made surgical orthodontics a reliable treatment modality that enhances facial esthetics, functional occlusion, airway dimensions, and overall patient well-being today.

Indications for Surgical Orthodontic Treatment

Skeletal discrepancies that are not amenable to adequate correction with conventional orthodontic treatment or growth modification alone are indications for surgical orthodontics. The decision to perform surgical treatment depends on several factors: the severity of the skeletal deformity, the degree of functional impairment, the patient's esthetic concerns and his expectations from the results of treatment [3]. A complete clinical assessment and a personalized treatment plan are important to determine whether a combined orthodontic-surgical approach is the best option.

Common indications include:**Sagittal discrepancies**

- Skeletal Class II due to mandibular deficiency
- Skeletal Class III due to mandibular prognathism or maxillary deficiency
- Severe maxillary protrusion/retrusion and excessive overjet.

Vertical discrepancies

- Anterior open bite
- Skeletal deep bite
- Vertical maxillary excess or deficiency
- Long-face syndrome

Transverse discrepancies

- Maxillary constriction
- Skeletal crossbite
- Facial asymmetry
- Occlusal cant

Craniofacial deformities

- Cleft lip and palate
- Hemifacial microsomia
- Treacher Collins syndrome
- Pierre Robin sequence
- Other craniofacial syndromes

Proffit's Envelope of Discrepancy continues to be a vital guide to the decision of when skeletal discrepancies require orthognathic surgery and not orthodontics alone.



**Rubal Chandna et al.,****Objectives of Orthognathic Treatment [4]**

The objectives of orthognathic treatment are:

- Function – Achieve normal masticatory, speech, respiratory, swallowing, and temporomandibular joint function.
- Esthetics – Enhance facial harmony, profile, smile esthetics, and adjacent soft tissue balance.
- Stability – Provide predictable long term stability with proper diagnosis, judicious orthodontic preparation, precise surgical correction and favorable soft tissue adaptation.

Diagnosis and Treatment Planning

Careful diagnosis and treatment planning are critical to success in surgical orthodontic treatment. Orthodontists and oral and maxillofacial surgeons should approach cases from a team perspective to optimize functional and esthetic results. Restorative and periodontal specialists should be consulted when necessary.

Patient evaluation

A comprehensive patient evaluation should include the chief complaint, medical and dental history, periodontal status, previous orthodontic treatment, and patient psychological acceptance of treatment. Systemic problems that may negatively impact treatment results should be addressed and patient expectations clarified.

Clinical Facial Analysis

Clinical facial analysis should be a part of the treatment planning records and should include symmetry of the face, facial midlines, facial proportions, lip relationships (competence/incompetence), smile characteristics (relationships, lip breakup, gingival show), facial profile convexity, jaw prominence, chin vertical and horizontal positions, and overall soft tissue balance.[5,6] Because the success of orthognathic surgery is determined primarily by the improvement of facial appearance, diligent soft tissue analysis is essential.

Temporomandibular joint assessment

The temporomandibular joint should be assessed in all orthognathic patients. Joint sounds, range of mandibular movement, functional deviation, pain, and muscle tenderness should be recorded. If the patient has an underlying disease process such as internal derangement, degenerative joint disease, condylar resorption, or inflammatory disease process, it should be noted and treated prior to orthognathic surgery. [7]

Radiographic and Imaging Assessment

Radiographic evaluation plays a vital role in the diagnosis and treatment planning of surgical orthodontic patients. Routine investigations include lateral and posteroanterior cephalograms, panoramic radiographs, and periapical radiographs to assess skeletal and dental relationships. Cone Beam Computed Tomography (CBCT) [8] has further enhanced diagnosis by providing three-dimensional visualization of the craniofacial skeleton, airway, temporomandibular joints (TMJs), root morphology, and facial asymmetry, thereby improving the accuracy of diagnosis and virtual surgical planning.

Cephalometric Evaluation

Cephalometric analysis remains an indispensable component of orthognathic treatment planning, providing valuable information about the underlying skeletal relationships and growth pattern.

- **Sagittal analysis:** Measurements such as SNA, SNB, ANB, Wits appraisal, and facial convexity angle are used to assess the anteroposterior relationship between the maxilla and mandible.
- **Vertical analysis:** Parameters including lower anterior facial height, mandibular plane angle, Jarabak ratio, Sassouni analysis, McNamara analysis, and COGS analysis help evaluate vertical facial proportions and skeletal growth patterns. [9]
- **Transverse analysis:** Grummons[10] and Ricketts posteroanterior analyses [11] are useful for assessing facial asymmetry, transverse skeletal discrepancies, midline deviations, occlusal cant, and maxillary constriction.



**Rubal Chandna et al.,****Presurgical Orthodontics**

Presurgical orthodontics plays a crucial role in orthognathic treatment. It prepares the teeth and supporting structures for surgical correction. The main goal is to position the teeth properly over their underlying bones. This process helps eliminate dentoalveolar compensations and creates a suitable dental relationship for accurate surgical jaw repositioning. Unlike traditional orthodontic treatment, which often hides skeletal issues, presurgical orthodontics aims to reveal the actual skeletal deformity. This approach allows for complete surgical corrections. [12]

Objectives of Presurgical Orthodontics

The key goals of presurgical orthodontic treatment are to:

- Align and level the dental arches.
- Correct tooth rotations and fix arch length discrepancies.
- Set the ideal angle and torque for incisors.
- Remove dentoalveolar compensations.
- Coordinate the shapes of the upper and lower arches.
- Achieve the best intercuspation after surgery.
- Use stable, full-dimension surgical archwires before the operation.

Orthodontic Decompensation

Dental compensations may be counterproductive to the underlying skeletal discrepancies, and may be responsible for maintaining a functional occlusion in the presence of the deformity. These compensations need to be removed before orthognathic surgery, to allow accurate jaw repositioning. If orthodontic decompensation is corrected, the true skeletal discrepancy will be uncovered and total correction of this discrepancy can be achieved with surgery, providing a better functional and esthetic correction. [12]

Sagittal Decompensation

Typically, the mandibular incisors are proclined and the maxillary incisors are retroclined, as an adaptation in patients with skeletal Class II malocclusion. In contrast, skeletal Class III malocclusion is usually associated with retroclined mandibular incisors and proclined maxillary incisors. The goal of presurgical orthodontics is to eliminate these compensations by moving the incisors into their optimum position in relation to the basal bone and revealing the true skeletal discrepancy for the correct surgical correction. [13]

Vertical Decompensation

Dentoalveolar compensations may be present as a vertical pattern in patients with a deep bite or anterior open bite, and may mask any underlying skeletal imbalance. The primary goal of presurgical orthodontics is to remove these compensations mindfully along with the lower anterior facial height, incisor display and overall facial proportions, so that the planned surgical movements result in optimal function and esthetic smile.

Transverse Decompensation

Transverse decompensation focuses on correcting the dental compensations associated with skeletal crossbites and transverse jaw discrepancies. Teeth are aligned over their supporting alveolar bone to establish proper maxillary and mandibular arch coordination, thereby facilitating accurate correction of the transverse skeletal deformity during surgery. [14]

Presurgical Considerations in Deep-Bite Cases

Management of deep-bite patients requires careful evaluation of the lower anterior facial height and the curve of Spee. In patients with a reduced lower facial height, complete leveling of the curve of Spee before surgery may not be desirable. Preserving the curve allows mandibular advancement surgery to increase lower facial height and improve facial balance. Final leveling of the occlusion can then be completed during the postsurgical orthodontic phase, contributing to improved occlusal stability and facial esthetics. [15]



**Rubal Chandna et al.,****Presurgical Considerations in Open-Bite Cases**

Patients with anterior open bite generally require extensive orthodontic decompensation before surgery to expose the underlying skeletal discrepancy. In skeletal Class III open-bite cases, the mandibular incisors are proclined and the maxillary incisors are uprighed to establish an adequate reverse overjet. Mandibular incisors are uprighed in skeletal Class II open bite patients, and maxillary incisors are properly compensated, although this may lead to an increase in overjet before surgery. This decompensation is crucial to obtain correct skeletal correction and postoperative occlusion stability. [16]

Arch Coordination

Proper coordination of the maxillary and mandibular arches is a fundamental requirement for successful orthognathic surgery. Well-coordinated arches facilitate accurate jaw repositioning and contribute to optimal postoperative intercuspation.

Arch Width Coordination

The transverse dimensions of the maxillary and mandibular arches should be compatible in the planned postsurgical position. Severe transverse skeletal discrepancies may require surgically assisted maxillary expansion or segmental osteotomies, whereas minor transverse discrepancies can often be corrected through conventional orthodontic treatment.

Arch Form Coordination

Presurgical orthodontics should also establish compatible arch forms to ensure stable postoperative occlusion. Skeletal Class II malocclusions commonly present with tapered maxillary arches, while skeletal Class III malocclusions are frequently associated with tapered mandibular arches. Appropriate arch coordination promotes ideal intercuspation and enhances long-term treatment stability. [17]

Appliance Selection and Surgical Preparation

Preadjusted edgewise appliances are generally preferred during presurgical orthodontics because they provide excellent control of tooth movement and sufficient stability during surgery. Before the surgical procedure, full-dimension rectangular stainless-steel archwires [18] are placed to stabilize the dentition and facilitate accurate jaw positioning during orthognathic surgery.

Common stabilizing archwires include

- 0.017 × 0.025-inch stainless steel in 0.018-inch slot appliances.
- 0.021 × 0.025-inch stainless steel or TMA in 0.022-inch slot appliances.
- 0.019 × 0.025-inch stainless steel in selected 0.022-inch systems.

Kobayashi or surgical hooks are incorporated into the archwires to facilitate intermaxillary fixation.

Presurgical Records and Final Planning

Once presurgical orthodontic treatment has been completed, comprehensive pretreatment records are obtained to finalize the surgical plan. These records typically include lateral and posteroanterior cephalograms, panoramic radiographs, study casts, and intraoral and extraoral photographs. Together, they are used for cephalometric prediction tracing, model surgery, virtual surgical planning, and the fabrication of surgical splints, ensuring accurate transfer of the treatment plan to the operating room.

Clinical Significance

Presurgical orthodontics forms the cornerstone of successful orthognathic treatment. Precise surgical correction is founded on careful alignment of the dentition, elimination of dental compensations, coordination of the dental arches and stabilisation of the occlusion. This systematic preparation not only enhances facial esthetics and functional occlusion but also is a critical step towards achieving long-term stability and predictable treatment outcomes.



**Rubal Chandna et al.,****Orthognathic Surgical Procedures**

The goal of orthognathic surgery is to move one or more of the maxilla, mandible, chin or other anatomical structures (through surgery) to provide skeletal stability, establish a functional and aesthetically appropriate relationship between the upper and lower teeth, and optimize the esthetics and function of the overall system. The exact procedure to be used will depend on the type of dentofacial deformity that is to be corrected and the treatment objectives that are defined during the planning phase of treatment (before a final treatment plan is decided upon).

Maxillary Surgery**Le Fort I Osteotomy**

The Le Fort I osteotomy is the most commonly performed maxillary procedure and allows movement of the maxilla in all three planes of space. [19]

Applications include:

- Maxillary advancement or setback
- Superior or inferior repositioning
- Correction of asymmetry
- Closure of anterior open bite
- Segmental expansion

Segmental Maxillary Osteotomy

Segmental osteotomies are used to correct arch width discrepancies, open bites, dental protrusion, and localized transverse deficiencies.

Mandibular Surgery Bilateral Sagittal Split Osteotomy (BSSO) [20] BSSO is the most frequently used procedure in the mandible, and is indicated for advancement, setback and correction of asymmetry. It promotes predictable healing, rigid fixation and good stability. Vertical Ramus Osteotomy [21] This procedure is mainly used for mandibular setback in selected patients but usually requires prolonged intermaxillary fixation. Genioplasty [22] Genioplasty is commonly performed to improve facial balance and includes advancement, reduction, vertical augmentation or reduction, and correction of chin asymmetry. Bimaxillary Surgery [23] For severe dentofacial deformities, surgery may be necessary on both jaws at the same time. It offers better facial harmony, corrected occlusion, better airway results and long-term stability over single jaw procedures.

Surgical Prediction and Treatment Planning

Careful surgical prediction and treatment planning is mandatory for accurate and predictable results in orthognathic surgery. Prediction tracing assists the clinician in predicting the changes in the facial profile, evaluating the expected occlusal relationship, determining the need for dental extractions, and assessing the need for adjunctive procedures like genioplasty. It also allows the orthodontist to communicate with the surgeon in the treatment planning stage. While traditional cephalometric prediction tracing continues to be a useful diagnostic tool, it has been largely replaced by computer-assisted simulations, three-dimensional virtual surgical planning, and digital treatment workflows that are more accurate, improve surgical precision, and improve the predictability of overall treatment.²⁴

Postsurgical Orthodontics [25]

Following surgery, orthodontic treatment is required to refine the occlusion and ensure long-term stability.

Objectives

- Settling of occlusion
- Closure of residual spaces
- Refinement of intercuspation
- Correction of minor tooth discrepancies
- Establishment of ideal overjet and overbite



**Rubal Chandna et al.,**

Postsurgical orthodontics is generally shorter than presurgical treatment because the major skeletal correction has already been achieved.

Retention

Retention protocols may include:

- Fixed lingual retainers
- Removable retainers
- Vacuum-formed retainers

Retention is particularly important in patients with severe skeletal discrepancies and those treated for open bite deformities. [26]

Stability and Relapse [27]

Long-term stability is one of the most important determinants of treatment success.

Factors Influencing Stability**Surgical Factors**

- Magnitude of skeletal movement
- Direction of movement
- Fixation technique
- Surgical accuracy

Orthodontic Factors

- Adequate decompensation
- Proper arch coordination
- Occlusal finishing

Biological Factors

- Muscular adaptation
- Soft tissue tension
- Growth potential
- Temporomandibular joint health

Stability of Common Procedures

Highly stable procedures:

- Maxillary impaction
- Mandibular advancement (moderate movements)
- Genioplasty

Moderately stable procedures:

- Maxillary advancement
- Bimaxillary surgery

Less stable procedures:

- Mandibular setback
- Vertical maxillary elongation
- Open bite correction involving extrusion

Complications of Orthognathic Surgery

Orthognathic surgery is a well-established and predictable procedure with a high success rate. However, as any major surgical intervention, it is associated with the risk of complications in the intraoperative, early postoperative and late postoperative periods. Careful patient selection, meticulous surgical technique and appropriate postoperative care are important in minimizing these risks. Intraoperative complications may include excessive bleeding, adverse fractures, damage to the inferior alveolar or other sensory nerve, and inadvertent trauma to



**Rubal Chandna et al.,**

adjacent teeth or surrounding anatomical structures. Good preoperative planning and accurate surgical technique contribute to reduce the incidence of these complications. [28]

Early post-operative complications include infection, facial oedema, haematoma formation, temporary airway compromise and post-operative malocclusion. Most of these problems are amenable to appropriate medications, supportive care and close clinical follow-up during the healing phase.

Late postoperative complications include skeletal relapse, persistent sensory disturbances, temporomandibular joint (TMJ) dysfunction, condylar resorption, and hardware-related complications such as loosening or failure of fixation plates and screws. Regular postoperative monitoring, timely intervention when necessary, and long-term follow-up are necessary to maintain treatment stability and achieve favorable functional and esthetic outcomes. [29]

Recent Advances in Surgical Orthodontics

Technological innovations continue to transform orthognathic treatment.

Three-Dimensional Imaging [30]

CBCT provides accurate assessment of:

- Skeletal morphology
- Airway volume
- Facial asymmetry
- Root positions

Virtual Surgical Planning [32]

Digital workflows enable:

- Precise osteotomy planning
- Surgical simulations
- CAD/CAM splints
- Patient-specific fixation systems

Skeletal Anchorage Systems [33]

Temporary anchorage devices (TADs) have expanded the orthodontic envelope of discrepancy and reduced the need for surgery in selected patients.

Artificial Intelligencev [34]

Emerging applications include:

- Automated cephalometric landmark identification
- Surgical outcome prediction
- Treatment planning assistance
- Facial simulation models

Airway-Focused Treatment Planning [35]

The correlation between the jaw position and airway dimensions is increasingly demonstrated. In addition to the esthetic and occlusal benefits, orthognathic surgery is increasingly being used for the improvement of respiratory function and obstructive sleep apnea.

Future Perspectives

Digital technology and bio-materials are progressing at a rapid pace, shaping the future of surgical orthodontics. New innovations are expected to improve all aspects of treatment from diagnosis and planning to surgical execution and long term follow up. Likely future developments include artificial intelligence assisted diagnosis, fully digital treatment workflows, augmented reality guided surgical navigation, personalized treatment planning, advanced biomaterials with improved fixation systems and more accurate prediction of soft tissue changes following surgery.



**Rubal Chandna et al.,**

Further development of these technologies should further increase the accuracy of treatment, improve the efficiency of surgery, reduce the morbidity to the patient and ultimately predict better function and esthetic results with greater patient satisfaction. [36]

CONCLUSION

Surgical orthodontics is indicated for severe dentofacial deformities which cannot be corrected by orthodontic treatment alone. Successful outcomes depend on accurate diagnosis, comprehensive treatment planning, coordinated presurgical orthodontics, precise surgery, and meticulous postsurgical finishing. The precision and predictability of treatment have been greatly improved by recent advances in CBCT imaging, virtual surgical planning, skeletal anchorage, and digital workflows. The objective of modern orthognathic treatment is to improve occlusion, facial aesthetics, function, airway health, and quality of life. Continued technology will continue to make surgical orthodontic care more precise, more personalized and more successful in the long term.

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Rubal Chandna et al.,

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Loops in Orthodontics: A Comprehensive Clinical and Biomechanical Review

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ABSTRACT

Loops in orthodontics constitute one of the most biomechanically sophisticated and clinically versatile tools available to the orthodontist. Rooted in the principles of frictionless mechanics, loop springs deliver controlled, predictable force systems characterised by well-defined moment-to-force (M/F) ratios that offer superior command over tooth movement type compared to conventional sliding mechanics. This review addresses the fundamental biomechanics governing loop design, including the principles of centre of resistance, centre of rotation, M/F ratio, load-deflection rate (LDR), springiness, pre activation bends, and anchorage control. Basic designs including the vertical loop, horizontal loop, omega loop, helical loop, Bull loop, and resilient vertical loop are examined for their historical origin, wire specifications, and biomechanical properties. Advanced designs like the T-loop, asymmetric T-loop, Opus loop, PG retraction spring, K-SIR arch, mushroom loop, MEAW, snail loop, and ballista spring are evaluated in depth. Clinical applications, Complications, risk management, and contemporary directions including CAD/CAM loop fabrication and skeletal anchorage integration are discussed, together with a critical appraisal of friction versus frictionless mechanics.

Keywords: Loops in orthodontics, frictionless mechanics, T-loop, moment-to-force ratio, space closure, beta-titanium, load-deflection rate, MEAW





INTRODUCTION

Space closure is one of the most biomechanically difficult stages of orthodontic treatment. While sliding mechanics dominated clinical practice for decades, its basic requirement on bracket archwire contact creates resistance to movement that includes torque expression, force prediction, and anchor control through friction, binding and notching. These limitations forced a fundamental revaluation of the management of extraction areas, which ultimately led to the creation and improvement of frictionless loop mechanics as a more regulated option. By applying force through specially designed springs, loop based mechanics completely eliminates frictional losses. This enables the clinician to purpose engineered springs, allowing the clinician to precisely control the moment to force ratio that determines whether a tooth tips, translates, or undergoes root movement. The development of beta-titanium alloy, which offers better spring back and lower load- deflection rates than stainless steel, which completely expanded the therapeutic utility of loops. Loop geometry has evolved over time to handle the four dimensions of force control, i.e. distribution, direction, amplitude, and duration ranging from early helical and tear drop loops to complex designs like the T-loop, Opus loop, and K-SIR arch. In current modern and orthodontics, a variety of loop designs are used, analysing their optimal size, activation protocols, and clinical uses.[1]

Basic Principles of Orthodontic Tooth Movement

In simple terms, orthodontic tooth movement is a physiological process that is started by applying precise mechanical stresses to the teeth. When a tooth is subjected to force, the periodontal ligament (PDL) and alveolar bone experience a pressure-tension phenomena that results in osteoclastic resorption on the pressure side and osteoblastic opposition on the tension side. The histology foundation of orthodontic tooth movement is this linked cellular response. Hooke's Law, which states that deflection is proportional to the applied force within the elastic limit; Newton's Third Law, which requires equal and opposite reaction forces and requires careful anchorage planning; and the principle of equilibrium, which demands that all forces and moments in the system be taken into account in order to achieve, predictable tooth movement, are the three guiding principles of physics that support appliance design. The guiding archwire in loop mechanics is replaced by specifically designed springs, which must independently produce the proper forces, moments, and moment to force ratios. In relation to sliding mechanics, where friction between the bracket and archwire produces unexpected variation, loop mechanics delivers stable force systems with far greater precision because no force is lost due to frictional resistance at a bracket wire interface. The theoretical point at which a single force results in pure translation without any rotating component is known as the Center of Resistance (CR). This is roughly around the mid-root level for single-rooted teeth and close to the furcation for multirooted teeth. Due to the fact that orthodontic forces applied at the bracket from the CR all at once produce a force and a moment, the latter of which is equal to the force multiplied by the distance measured perpendicular between the CR and the line of force ($M = F \times d$). [2]

The point around which a tooth really rotates under a specific force system is known as the Center of Rotation (CoR), and it moves according with the Moment-to-Force ratio (M/F ratio). The key biomechanical factor that affects the kind of tooth movement produced is this ratio:

- M/F ratio of 5:1 (Uncontrolled tipping where crown moves and root displaces in the opposite direction)
- M/F ratio of ~7:1 (Controlled tipping where crown moves and root apex remains relatively stationary)
- M/F ratio of ~10:1 (Translation or bodily movement where crown and root move equally)
- M/F ratio of >12-14:1 (Root movement or torque where root apex displaces while crown remains stable)

The ability of loop mechanics to produce a certain M/F ratio through control of loop shape, preactivation bends, loop centricity, and wire material is what makes it preferable to slide mechanics.

Load-Deflection Rate, Springiness, and Wire Properties

The most important feature of any loop spring is the Load-Deflection Rate (LDR) which is the force change per unit of activation. A high acceptable working load, a wide range of action, and a low LDR are characteristics of an ideal loop. A low LDR reduces the danger of PDL hyalinization and root resorption by assuring that light, biologically



**Vanshika Pasricha et al.,**

appropriate, and continuous pressures are maintained across the whole activation range. The LDR is significantly affected by loop geometry. To lower the LDR and increase the activation range, loop height can be increased, a helical component can be added at the apex, wire length can be increased, or combination horizontal and vertical designs can be used. Finite element technique evaluations of existing designs have verified that the snail loop, which maximizes wire length inside a compact spiral shape, produces the lowest LDR. Equally important is wire material. The favored material for the majority of complex loop designs is beta-titanium (TMA), which was first introduced by Burstone and Goldberg in 1980. It has a modulus of elasticity that is about one-third that of stainless steel (~60–80 GPa versus ~200 GPa), offering superior springback, lower LDR, and greater resiliency. While stainless steel is still suitable for simpler loop designs requiring more rigidity despite its significant stiffness, cobalt-chromium alloys (Elgiloy) offer intermediate mechanical properties. The square of a wire's yield strength divided by its modulus of elasticity determines its resilience. This explains why TMA wire has a high yield strength and a low modulus, which is the ideal combination for loop designs.[3]

Preactivation Bends and Anchorage Control

Preactivation, also known as gable bends, are angular bends inserted into a loop's legs before it is inserted. This creates a stored moment that enhances the loop's geometric moment and increases the total M/F ratio to levels that are clinically effective. Even a well designed loop may not achieve the M/F ratio required for translation in absence of adequate preactivation, leading to uncontrolled tipping, anterior flaring, and deepening of the overbite. To enable en-masse anterior retraction, gable bends of about 30° are frequently positioned at either bracket end in TMA T-loop. In loop mechanics, the varying M/F ratios provided to the anchoring and active units control anchorage. While positioning the loop closer to the posterior (beta) bracket increases the beta moment and thereby reinforces posterior anchorage by creating a distal root tipping tendency at the molar, positioning the loop off center toward the anterior (alpha) bracket increases the alpha moment and decreases the beta moment. Three categories of differential space closure are represented by this direct relationship between loop position and anchorage response: Group A (maximum anchorage), Group B (moderate anchorage), and Group C (minimum anchorage), all of which can be attained by specially adjusting loop symmetry and preactivation geometry. [4]

Classification of Loops

Orthodontic loops may be classified according to several criteria, each reflecting a distinct aspect of their design or intended function.

- **Based on Configuration**

Geometrically terms, loops can be divided into three categories: horizontal loops (like the boot loop), vertical loops, and combination designs that combine aspects of both, like the T-loop.

- **Based on Clinical Purpose**

Loops have three main functions: space closure and retraction during the mid phase of treatment, leveling and alignment during the initial phase, and multifunctional applications where a single loop design simultaneously tackles many biomechanical objectives.

- **Based on the Presence of a Helical Component**

Loops may be further classified into two categories: those containing one or more helical bulbous loop, and those without a helix, like the simple vertical loop. By adding a helix, the effective wire length within the inter-bracket span is increased, which lowers the load deflection rate and expands the activation range without raising the appliance's overall height.

- **Based on Function**

Finally, loops are differentiated by the direction of tooth movement they produce: closing loops are used in extraction space closure because they supply forces that approximate teeth, whereas opening loops create forces that move teeth apart. [5]

Since a single design may fall into more than one category based on how it is activated and used clinically, this broader classification highlights the variety of loop mechanics.



Vanshika Pasricha *et al.*,**Various Loop Designs in Orthodontics****Vertical Loop (Figure 1)**

The vertical loop is the oldest loop design that was officially entered into the edgewise appliance system. It was first used in 1933 by Dr. Robert H.W. Strang, who made it from 0.016-inch stainless steel wire that was positioned perpendicular to the archwire along the occlusogingival axis and had a standard height of 6mm. It comes in two different forms: closed vertical loop, which is primarily used for space closure and the open vertical loop, which maximizes efficiency for space opening by leg compression activation. The closed version has a wider activation range than its open counterpart due to the Bauschinger effect. According to Burstone's analysis, the vertical loop yields a force deflection rate of 437 g/mm and an M/F ratio of merely 2.2 mm at 6 mm height, which is insufficient for controlled tipping or bodily translation. While helices with more than three turns provide declining biomechanical returns, increasing the loop height gradually improves the M/F ratio and adding a helical element at the apex further lowers the load deflection rate. The loop's main clinical benefit is obtained during the leveling and alignment stage, where its simple manufacturing and mild force delivery are very helpful.[6] The double vertical loop, S-loop, omega loop, horizontal loop, torquing loop, and box loop are variations of vertical loop.

Horizontal Loop (Figure 2)

According to Morris M. Stoner's 1960 description of its basic form, the horizontal loop is also known as the boot loop. When compared to the vertical loop, the loop body is positioned in the horizontal (occlusal) plane since its active legs run parallel to the archwire. Only the occlusogingival and labio-lingual planes are activated in this position; the mesiodistal plane is not included. Its main biomechanical benefit is that it reduces force in the vertical plane, allowing for instantaneous bracket engagement in teeth that are significantly intruded or extruded without using excessive force. When the double horizontal loop is placed to a single tooth, with one loop shaped to elevate and the other to depress, it works especially well for bite opening. In this manner, the tooth can be vertically repositioned or tipped under control. Although it has been described, contouring the loop against the gingival surface to produce root torque is not always successful in clinical settings.[7]

Omega Loop (Figure 3)

Morris M. Stoner first described the omega loop in 1960. It was called after Greek letter omega and was a variation of the open vertical loop. Its unique characteristic is a broadly curved, rounded apical portion made of 0.016-inch or 0.018-inch stainless steel, reduces the fracture tendency that marks the acute apex of the simple vertical loop by distributing bending stress more evenly across the loops arc. Clinically, it has two main functions: it acts as a molar stop, preventing unintentional distal sliding of the archwire from the molar tube, and it acts as a physical root thrust to the final tooth in the arch.[8]

Helical Loop (Figure 4)

In order to reduce the load-deflection rate and increase the activation range, the helical loop increases wire length inside the interbracket span by including one or more coiled elements at the apex or along the legs of a vertical loop. It functions as three combined springs, including the coil and two cantilever arms. The arms of loop provide directional control, whereas wire cross-section, modulus, diameter, number of coils, and arm length affect force magnitude. Helices at the loop's reflex point typically have 1.5-2.5 turns; lateral extension coils at right-angle bends improve flexibility and keep nearby teeth from tipping. Despite being advantageous and biomechanically mild, these loops are typically heavy and prone to deformation. The bulbous helical variation is utilized for mandibular anchoring preparation and molar uprighting. It is generated at the terminal end of a 0.020 × 0.025-inch archwire to a height of 7.5 mm with a lingually coiled helix.⁹

Bull Loop (Squashed Vertical Loop) (Figure 5)

The Bull loop is essentially a flattened (squashed) vertical closed loop made of 0.0215 × 0.025-inch stainless steel wire. It was first introduced by Dr. Harry L. Bull in 1951 for anterior retraction in Class II, Division 1 treatment. Instead of squeezing the legs, it is actuated by opening and extending them. For the upper arch, the standard measurements are 7 mm in height with 18 mm and 22 mm of wire distal and mesial to the loop, respectively; for the lower arch, they are



**Vanshika Pasricha et al.,**

5 mm in height with 20 mm distal and 28 mm mesial. One significant biomechanical benefit of using Class II elastics is that the closing loops prevent mandibular incisor proclination, which would otherwise be caused by Class II elastic forces. In order to accommodate Class II elastic engagement. [10]

T-Loop (Figure 6)

The most researched and clinically used advanced loop in modern orthodontics, the T-loop was first presented by Dr. Charles J. Burstone in 1962 and is named for its characteristic T-shaped profile. The addition of a horizontal apical segment to the vertical legs dramatically increases wire length, elevates the M/F ratio, and enables the delivery of biologically optimal forces for anterior retraction and space closure. [11] The standard Burstone T-loop is fabricated from 0.017 × 0.025-inch TMA wire with a height of approximately 7 mm and an apical horizontal length of 10 mm.¹²The preactivated T-loops achieve approximately 10:1M/F ratios consistent with translational tooth movement. The M/F ratio is manipulated by loop centricity within the inter bracket span. The anterior placement of loop increases the alpha moment (Group A anchorage), while posterior placement increases the beta moment (Group C mechanics).¹³Preactivation through gable bends that is typically ~ 10.5° at the alpha end and 25-35° at the beta end for Group A retraction to introduce a stored moment that supplements the geometric M/F ratio. Importantly, the T-loop is self correcting: as the tooth moves and the loop deactivates, the force decreases by 30% while the moment decreases by only ~18%, causing the M/F ratio to rise progressively and leading to a desirable shift from tipping through translation to root up righting. Trial activation before insertion is essential to stabilize the force system and confirm no permanent deformation occurs. The T-loop is used for individual canine retraction, en-masse anterior retraction, molar up righting, and space closure in Group A, B, and C anchorage mechanics. [14]

Asymmetric T-Loop: Described by James Hilger in 1992, the asymmetric T-loop is fabricated from 0.016 × 0.022-inch or 0.019 × 0.025-inch TMA with a 2 mm anterior loop, 5 mm posterior loop, a 5 mm vertical step, and an exaggerated reverse curve of Spee. [15] This deliberate geometric asymmetric loop has shorter mesial arm generating a higher alpha moment, longer distal arm generating a lower beta moment which creates the conditions for simultaneous bite opening and anterior retraction. Activation is performed by compressing the short mesial loop and opening the long distal loop, making it particularly suited to deep bite extraction cases. [16]

Tear-Drop Loop (Figure 7)

The teardrop loop, attributed to R.G. Alexander, is characterised by its broad, rounded ovoid apical configuration, which lies between the simple vertical loop and the more complex T-loop. It is available in heights from 6 to 8 mm, with the specific height selected based on desired force and activation range. Research has established that the 6 mm teardrop loop activated by 0.5 mm delivers 2.51 N which is close to the ideal 2.60 N for mandibular incisor movement while the 8mm loop activated by 1.0 mm delivers 2.77 N. Finite element analysis by Rao et al. confirmed that the teardrop loop exhibits a higher LDR than both the Opus and snail loops, limiting its activation range. Its main drawback is limited torque control; it is mostly used in physiologically impaired individuals where conservative force levels are important and in two-step retraction techniques where light canine tipping forces are needed. [17]

Opus Loop

Raymond E. Siatkowski first proposed it in 1977. The Opus loop is one of the most systematically designed orthodontic appliance in history. Siatkowski applied Castigliano's theorem from structural mechanics to derive equations relating the M/F ratio to specific loop geometry, allowing analytical rather than empirical optimization of the design. The ascending legs are inclined at 70° to the bracket plane, the apical horizontal leg measures 10 mm, and the overall dimensions are 10 mm height, 10 mm length, and 0.5 mm corner radius. The ascending legs must be no more than 1 mm apart at the apex. Fabricated from 0.016 × 0.022-inch SS, 0.018 × 0.025-inch SS, or 0.017 × 0.025-inch TMA wire, the Opus loop delivers an M/F consistently within the range of 8.0-9.1 mm without requiring any preactivation bends which has a advantage over the T-loop that simplifies clinical use and eliminates preactivation errors. [18]



**Mushroom Loop (Figure 8)**

Introduced by Drs. Flavio Uribe and Ravindra Nanda in 2003, the mushroom loop is a prefabricated retraction archwire system with a broad, domed apical profile resembling a mushroom cap which is fabricated from 0.017 × 0.025-inch beta-III Connecticut new TMA archwire. The loop retains its shape despite repeated activation without distortion, and the broad apical arrangement optimizes wire length without the vertical height that frequently results in gingival impingement with T-loops. To maintain a constant translational M/F ratio during each activation interval, pre-activation of the 3 mm leg separation is done anteriorly prior to insertion; reactivation is postponed until 3 mm of space closure has taken place. In Class II, Division 2 situations where careful incisor torque preservation is required, it is especially well suited for en masse retraction. [19]

Delta Loop (Keyhole Loop) (Figure 9)

The delta loop, introduced by Burstone as a compact triangular alternative, is named for its resemblance to the Greek letter delta. It is characterized by a wedged shaped structure, which is broader at the apex that tapers toward the archwire base, results in a lower LDR than conventional vertical loops. It can be made with or without a helical element at the top; the helical version lowers the LDR even more at the expense of a little higher volume. Patients with shallow buccal vestibules, where the height of a typical T-loop or Opus loop would invade soft tissue, are its main indication. It requires exact replication of the triangular shape and is sensitive to technique. [20]

Double Keyhole Loop (DKL)

Keyhole Loop (DKL) which was first used in Roth straight wire mechanics by John Parker of Alameda, California, is made of 0.019 × 0.025-inch rectangular stainless steel archwire and comprises of two symmetrical keyhole shaped loops positioned bilaterally near the canines on each side of the arch. Each loop has a height of 7 mm; the distance between the two loops on the same side is approximately 8 mm. The combined vertical and horizontal geometry creates a champagne bottle appearance. Three prerequisites for DKL insertion are mandatory: an anterior sector free of diastema and consolidated with ligature wire; well aligned arches with torque fully expressed. Two activation modes are described as: distal fraction of the arch from behind the molar tube (not exceeding 1 mm per appointment) or retro-ligature activation using a ligature wire tied between the molar tube hook and the distal DKL loop. Its primary advantage is the integration of retraction into a full arch continuous wire that simultaneously expresses bracket-prescribed torque and angulation. [20]

PG Retraction Spring (Figure 10)

Introduced by Poul Jessing of Denmark in 1985, the PG retraction spring is fabricated from 0.016 × 0.022-inch stainless steel wire. Its predominant active element is an ovoid double helix loop extending 10 mm apically with two closely spaced helical coils forming an ovoid configuration with a smaller occlusally positioned helix. Activation of 140-160 grams is obtained by separating the two helix sections by 1 mm distally, repeated every 4 weeks. Buccal segment leveling must be finished before insertion because the spring is especially made to withstand rotational and tipping tendencies during retraction but not to correct preexisting rotations or excessive canine inclinations. At 3 mm activation, the anterior M/F ratio is 7 mm; at 1 mm activation, it is 10.5 mm. A self-correcting sequence, biomechanically comparable to the self-correction of the T-loop, from controlled tipping at heavy activation toward near translation as the dog moves and the spring deactivates. After retraction, lingual elastics are used to treat minor residual canine rotations. [21]

K-SIR Arch

Developed by ,The K-SIR (Kalra Simultaneous Intrusion and Retraction) arch developed by Dr. Varun Kalra and published in 1998, addresses anterior deep-bite correction and retraction simultaneously within a single continuous archwire. Closed U-loops measuring 7 mm in height and 2 mm in width are integrated at extraction sites using three distinct bend configurations: a 90° centered V-bend at each U-loop that generates equal retraction and intrusion moments; an off-centered 60° V-bend with the shorter arm near the molar tube that increases clockwise moment on the first molar for posterior anchorage augmentation; and a 20° anti-rotation bend that counteracts mesiolingual rotation of the buccal segments. Prior to insertion, trial activation stabilizes the intensity of the V-bend and lessens





stress accumulation. It is most beneficially applied in Group A extraction cases with deep overbite where sequential bite-opening prior to retraction would otherwise extend treatment duration. [22]

Snail Loop (Figure 11)

Introduced by Dr. Pavankumar Vibhute in 2004, the snail loop is fashioned from 0.017 x 0.025-inch stainless steel wire by bending a simple omega loop into a progressively tightening spiral. The resulting nested configuration consists of an outer loop of 8 mm height and 6 mm width and an inner loop of 6 mm height and 3 mm width. The spiral geometry maximizes total wire length within the available vestibular space to a degree unmatched by any other commonly used loop design. Finite element analysis by Rao et al. (2013) confirmed that the snail loop achieves the lowest LDR of any tested design - lower than both the Opus loop and the tear-drop loop - conferring the most constant force delivery and widest activation range before reactivation. Vibhute et al. (2008) reported twice the activation potential of a stainless steel omega loop before permanent deformation. This also minimizes soft tissue irritation. The main drawback is the difficulty of manufacturing; chairside manufacture is technically challenging because to the spiral geometry, which necessitates exact sequential bending with the right pliers. [23]

Rectangular Loop (R-Loop)

The rectangular loop, described by Drs. Vittorio Cassiafesta and Birte Melsen, is a versatile design fabricated primarily in TMA wire whose weldability permits joining segments of different cross-sections - typically 0.018-inch or 0.017 x 0.025-inch, providing a large working range. Being inserted and held at both ends, it constitutes a statically indeterminate force system. It is the most versatile design for correcting single-tooth discrepancies in all three planes of space that is first, second, and third order corrections, while transferring undesirable reciprocal forces to the anchorage unit. In the context of segmented arch mechanics, Marcotte described the R-loop specifically for intrusion, extrusion, and rotation correction of individual canines and premolars, which can simultaneously superimpose a rotational couple on a translatory force. [24]

Cherry Loop

Described by PerettaRedento in 2002, the cherry loop is constructed from 0.017 x 0.025-inch stainless steel wire. A largediameter round loop of 8 mm width is bent using a Rouland plier to a height of 8-9 mm, opening 3-4 mm at the occlusal end to prevent stress concentration and deformation. It is specifically designed for molar protraction and is placed at one-half distances within the extraction space when moving molars mesially. Its rounded largediameter apical configuration distributes bending stress and reduces fracture tendency, while the wide occlusal opening provides a comfortable activation range for the relatively heavy forces required to protract molars. [25]

Bulbous Helical Loop (Figure 12)

A variant within the helical loop category, the bulbous helical loop is fabricated at the terminal end of a 0.020 x 0.025-inch archwire to a height of 7.5 mm, positioned flush against the second-order bends with the helix wound toward the lingual surface during fabrication. Before insertion, it is activated by opening approximately 1 mm, generating a distalizing force on the posterior teeth. It is employed during mandibular anchorage preparation counteracting the mesializing tendency of the posterior anchorage unit during anterior retraction and also serves for molar uprighting. Its helical element provides the biomechanically gentle, continuous light forces characteristic of all helical designs.

Ricketts Canine Retraction Spring

Described by Robert Ricketts in 1976, the Ricketts canine retraction spring is a double vertical helical T-closing loop with extended crossed arms, fabricated in the maxillary arch from 0.016 x 0.022-inch stainless steel wire of 70 mm length. It generates a canine retraction force of approximately 50 g for 1 mm of activation, with 3-4 mm of activation required for individual canine retraction. The mandibular version is more compact, fabricated from 0.016 x 0.016-inch blue Elgiloy, generating 75 g per millimeter of activation with 2-3 mm of activation needed. The double helical configuration provides a combination of spring gentleness and torque resistance that makes it suited to sequential two-step retraction protocols within bioprogressive therapy.



**Ballista Spring**

Introduced by Harry Jacoby in 1979, the ballista spring is a purpose-designed appliance for managing palatally impacted maxillary canines. It consists of two parts: a horizontal arm inserted into both the headgear and edgewise molar tubes and ligated to the first premolar bracket as a hinge axis, which stores torsional energy when deflected; and a vertical arm bent at 90° terminating in a small loop for ligature or elastic attachment. The spring delivers 60-100 g (0.016-inch wire) or 120-150 g (0.018-inch wire) of controlled vertical and palatal force to the impacted tooth. Its key advantage is adjustability: inclining, lengthening, or shortening the vertical arm modifies the direction of traction that is anteroposterior, toward the midline, or toward the arch, allowing precise navigation of the canine away from adjacent root apices before eruption. [26]

Multiloop Edgewise Archwire (MEAW)

Introduced by Dr. Young H. Kim in 1967, the MEAW consists of L-shaped horizontal loops at each interproximal contact from distal of the lateral incisors to the mesial of the second molars — five loops per side. Wire specifications are 0.016 × 0.022-inch SS for a 0.018-inch slot and 0.018 × 0.022-inch SS for a 0.022-inch slot. The MEAW is 2.5-3 times longer than a standard archwire, reducing forces to approximately one-fifth of a standard wire and delivering consistent low-level continuous forces. Each loop has four components: a horizontal loop (reducing vertical force and enabling vertical tooth control); a vertical leg/breaker (ensuring individual tooth independence); a loop base (where tip-back and torque activations are incorporated); and a horizontal archwire portion (inserted into the bracket slot, kept straight). Progressive tip-back bends of 3-5° at each loop base from first premolars to second molars with a total of 15-20° to upright the posterior teeth, reconstruct the occlusal plane, and create conditions for anterior extrusion. Vertical elastics drive anterior teeth into the open bite space. [27,28]

Mollenhauer Aligning Auxiliary

The Mollenhauer aligning auxiliary is an adjunct spring typically 0.016-inch round stainless steel or TMA which is applied to a single tooth requiring individual correction during the aligning and leveling phase, without disturbing the continuous main archwire. While the main archwire continues to level the remaining dentition, it is engaged in or tied over the bracket of the misaligned teeth. With the primary archwire acting as the anchorage reference, this segmented technique reduces reciprocal effects on neighbouring teeth while applying a precisely specified correction force to a single severely rotated, pointed, or lingually displaced tooth. Palatally misplaced lateral incisors, severely rotated premolars, and pointed canines that would require excessively high archwire forces if treated with a continuous wire alone are the most prevalent conditions for which it is employed.

Begg's Torquing Auxiliary (Torquing Loop)

After the crown-tipping retraction of Stages 1 and 2, Begg's torquing auxiliary, a compressed vertical loop, is utilized in Stage 3 of the Begg light-wire differential force procedure to provide lingual root torque to the anterior teeth. It is made of 0.014-inch or 0.016-inch stainless steel wire and is shaped to press against the incisor crown's gingival surface. This creates a lingual force at the archwire and a labial force at the bracket, creating a couple that pushes root apices lingually to achieve the proper axial inclination. An M/F ratio of >12:1 is required for pure root movement. In the Begg framework, the torquing auxiliary completes the sequential movement to correct final incisor inclination without requiring bracket archwire torque expression by compensating for the torque that is accrued during the initial uncontrolled tipping retraction phases.[29]

Complications and Risk Management in Loop Mechanics

Inadequate loop design, activation, or clinical monitoring can lead to a number of issues despite the biomechanical benefits of frictionless loop mechanics.

- The most frequent negative effect of loop mechanics is anchorage loss. Unintentional molar mesialization can be caused by inadequate posterior consolidation, incorrect loop orientation, or excessive activation. Transpalatal arches, Nance holding appliances, or temporary anchorage devices (TADs) in conjunction with suitable differential M/F ratios at the beta bracket end to increase posterior resistance are necessary for prevention.



Vanshika Pasricha *et al.*,

- When the M/F ratio supplied at the bracket is inadequate for the intended tooth movement, **uncontrolled tipping** and torque loss occur. Anterior flaring, bite deepening, and loss of incisor torque can result from improper loop height, insufficient preactivation bends, or over-activation beyond the elastic limit.
- Prolonged treatment duration, jiggling movements from recurrent over-activation, and high activation pressures all worsen **root resorption**. The biological optimum of 35–60 g for tipping and 70–120 g for body movement must be maintained, and loop activation shouldn't go over 1 mm every session.
- When stainless steel loops are repeatedly activated and deactivated, there is a risk of **wire fatigue and loop distortion**. TMA is the material of choice for advanced retraction springs since it resists fatigue far better than stainless steel. Regular monitoring at intervals of four to six weeks is necessary to quickly identify patient distortion of loops between sessions.
- **Soft Tissue** near bulky or tall loops that press against the buccal mucosa or alveolar gingiva cause irritation. The Opus loop, snail loop, and mushroom loop are simple designs that reduce vestibular projection and improve patient comfort, especially for those with shallow vestibules.
- The box loop and other complicated loop designs are known to be hampered by **plaque accumulation**. Before and during loop therapy, patients need to be reminded to practice good dental hygiene in order to avoid iatrogenic periodontal problems. [30]
- Unlike sliding mechanics, **fabrication errors** offer a special risk in loop mechanics. Large-scale, irreversible faults in tooth movement can result from even small flaws in loop geometry, gable bend angulation, or loop centricity.

Friction Versus Frictionless Mechanics

The management of orthodontic extraction space is broadly classified in two mechanical philosophies: frictional (sliding) mechanics and frictionless (loop) mechanics, each carrying distinct biomechanical characteristics, clinical advantages, and inherent limitations. In sliding mechanics, the bracket moves along a continuous, to move the teeth. Despite being commonly used in pre adjusted edgewise systems, it is operationally simple. Resistance to sliding, a multi factorial phenomenon of friction, binding, and notching at the bracket archwire contact reported by Kusy and Whitley, counteracts its simplicity. Resistance increases dramatically after the contact angle beyond a critical threshold, resulting in unpredictable force delivery, anchoring loss, uncontrollable anterior tipping, and overbite deepening. Elastomeric chains, which are frequently utilized as the activating component, require periodic reactivation and compromise force consistency because they lose 50–70% of their initial force within the first day of application due to water and saliva absorption. [31]

The bracket-wire sliding contact is completely eliminated in frictionless mechanics loop springs in place of the continuous archwire as the force-generating component. The spring produces more consistent, predictable, and controllable tooth movement by independently delivering the necessary moment-to-force ratio in three dimensions. Through differential M/F ratios, independent control over anterior and posterior tooth segments, and the capacity to achieve complex movements like translation, root torque, simultaneous intrusion, and retraction—all of which sliding mechanics cannot consistently duplicate the clinician is able to precisely regulate anchorage. Tipping, translation, and root movement are the three closure that both systems go through. On the other hand, a well-designed loop appliance consistently maintains a larger activation range, a more steady force delivery, and a more constant center of rotation. [32] Neither strategy is always better. Anchorage requirements, necessary tooth movements, clinical knowledge, and the particular biomechanical goals of each patient continue to decide the best option.

CONCLUSION

Loop mechanics is used for initial alignment or later space closure, it is essential for regulated orthodontic tooth movement. No single loop design is universally better, as several assessments confirm; instead, the right choice relies on the particular biomechanical demand, anchorage needs, and clinical situation. Since even small design flaws can result in unpredictable tooth movement, effective loop therapy depends on a solid understanding of wire





characteristics, activation range, and moment-to-force ratios. While more recent configurations continue to enhance anchorage conservation and patient comfort, historical contributions by Strang, Stoner, Burstone, and other innovators have gradually improved loop shape to balance flexibility with sufficient force delivery. Orthodontic space closure should ultimately be customized, guided by careful diagnostic and treatment planning as opposed to a set technique. Neither friction nor frictionless mechanics is universally superior; the optimal strategy remains individually determined by the diagnosis, anchorage demands, required tooth movements, and clinical expertise of the treating orthodontist.

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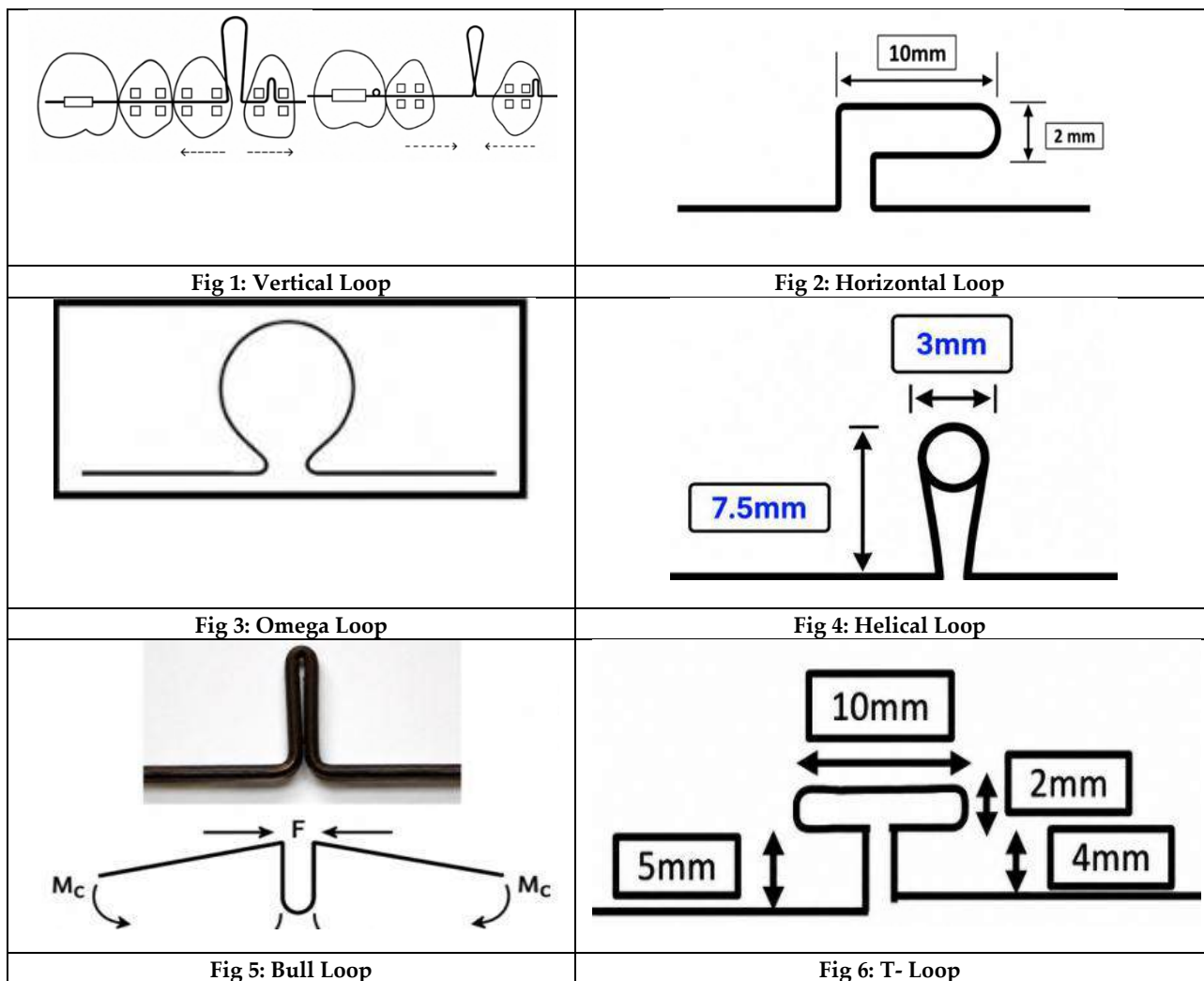




Fig 7: Tear drop Loop

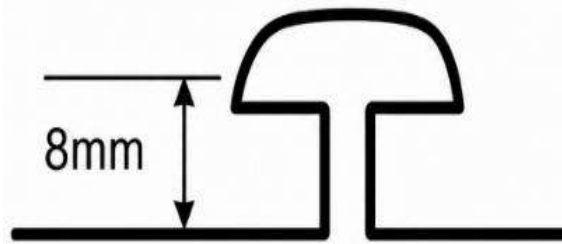


Fig 8: Mushroom Loop

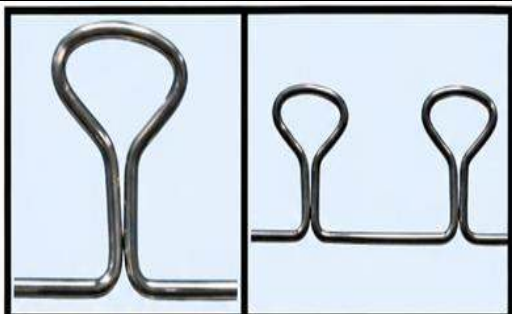


Fig 9: Delta Loop

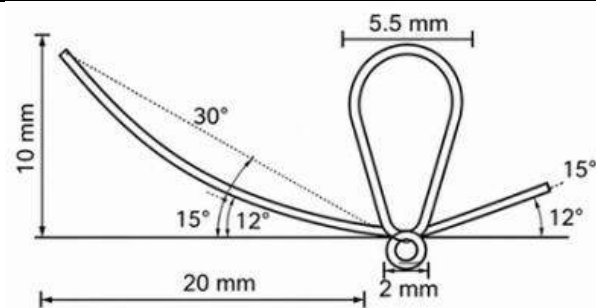


Fig 10: PG Retraction Spring

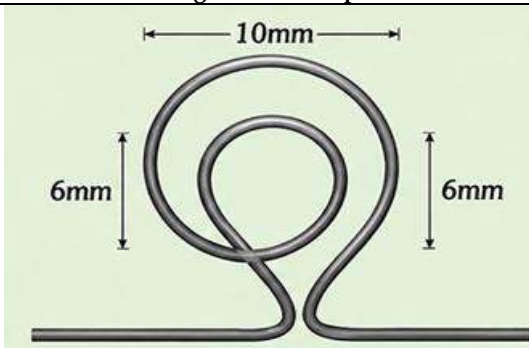


Fig 11: Snail Loop

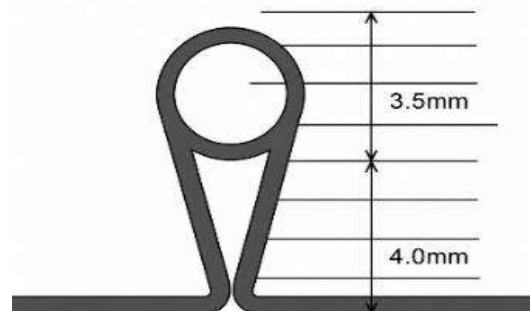


Fig 12: Bulbous Helical Loop

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RESEARCH ARTICLE

A Retrospective Clinical Study on Reducing Hyperuricemia in Gout Patients with Homoeopathic Medicines

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ABSTRACT

Gout was first described by Hippocrates. The term 'gout' is derived from Latin word 'gutta' and French word gote, both meaning a drop of liquid. A long time ago, people believed that gout was caused by drops of thick body fluids flowing from the blood into the joints. Gout is a type of joint disease that causes inflammation. It happens when there is too much uric acid in the blood. Uric acid forms sharp crystals called monosodium urate, which build up in the joints and cause swelling and pain. In the last twenty years, the number of people with gout has doubled, mostly because of changes in what people eat.

Keywords: Gout, Homoeopathy, Hyperuricemia, Purine-rich diet, Serum uric acid.

INTRODUCTION

Gout is a type of inflammatory arthritis caused by high levels of urate in the blood, which stays elevated for a long time. This leads to too much urate in the body, making crystals of monosodium urate form and build up in the joints. Gout is a metabolic illness that mainly affects women who have gone through menopause and middle-aged to



**Isaac Jose and Rakesh N Gohel**

elderly males. Gout affects 1-4% of the general population, but in people over 80, that number can jump to 10% in males and 6% in women. Men experience the condition two to four times more frequently than women do worldwide. The biomarker for gout is hyperuricemia.

MATERIALS AND METHODS

A single arm, retrospective, non-randomised observational study which was conducted on 30 patients with the diagnosis of gout at the outpatient department of White Memorial Homoeo Medical college and Hospital, Attor over a duration of six months. Each patient was provided with a patient information sheet in local vernacular Tamil language detailing the objectives, methods, risks and benefits of participating and confidentiality issues. Before enrolment, written informed consent was taken from the patients.

- **Outcome assessment:** Outcome measurements of this study were done based on laboratory investigations of serum uric acid level in gout patients.
- **Intervention and Follow - Up:** All patients received suitable homeopathic medicines that were tailored to their individual needs according to homeopathic guidelines. They were also given advice on improving their lifestyle by making changes to their diet and daily routine. If needed, their cases were carefully reviewed and organized using the repertory method. The treatment was repeated as necessary based on each patient's specific needs. Each patient was treated for six months, and follow-up visits were scheduled at least once a month or more frequently if needed by the patient.
- **Statistical analysis;** Before and after treatment assessment were done and hypothesis was analyzed by paired 't' test.

RESULT

From this study it was found that men (53%) are more commonly affected than females (47%), the affected age group between 36-40 years were commonly affected (60%), the most commonly affected site is right side (68%). Patients coming from high socioeconomic status (46.7%) were likely to develop the disease. The Group of people who consumes non-vegetarian foods (83.4%) were commonly affected, the affected group is people who had addictions to alcohol and smoking (23%). The medicine *Lycopodium Clavatum* were prescribed for (26.7%) cases, potency selected for majority of cases were 200 (90%) and 27 cases (90%) were prescribed with 200th potency, with an average reduction of 1.22mg/dl was noted and 3 cases (10%) were prescribed with 30th potency, with an average reduction of 1.03mg/dl in the levels of serum uric acid. 83% of cases had marked improvement in the reduction of serum uric acid level after the homeopathic treatment. The mean uric acid level before the treatment was 7.28mg/dl, and after the treatment, it decreased to 6.07 mg/dl. The standard deviations were .331 before the treatment and .622 after the treatment, show that uric acid levels were more variable after the treatment. The standard error means were .060 before the treatment and .114 after the treatment indicate the precision of the sample means. The mean difference between the two time points is 1.210 mg/dl, with a t-value of 11.171 and a p-value of .000, indicating this difference is highly statistically significant. The 95% confidence interval (0.988 to 1.432) suggests we are 95% confident that the true mean decrease lies within this range. In simple terms, the intervention significantly reduced uric acid levels in the subjects. Since p value < 0.05, null hypothesis is rejected. The t-test value is 11.171, which is greater than table value, the null hypothesis is rejected. We can conclude that the Homoeopathic medicines are effective in reducing hyperuricemia in gout patients.





Isaac Jose and Rakesh N Gohel

DISCUSSION

Early onset gout, majority affected between 36-40 years of age suggesting lifestyle and dietary influences. There is higher prevalence in males which is consistent with known epidemiology of gout. Right side involvement is the most common which is due to gout presentation in the first MTP joint. High socioeconomic group is mostly affected due to more exposure to purine rich foods, sedentary lifestyle and alcohol consumption. Non vegetarians are commonly affected, reinforcing the role of purine rich food in increasing serum uric acid levels. Addictions such as alcohol consumption and smoking are associated with increase in the levels of serum uric acid. Lycopodium clavatum is the most used medicine. The observations were analyzed using paired “t” test. It showed that there is significant change in before and after values of serum uric levels after treatment.

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RESEARCH ARTICLE

Effectiveness of Nordic Walking on Pulmonary Function, Functional Capacity and Quality of Life in Individuals with Chronic Obstructive Pulmonary Disease: Study Protocol

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ABSTRACT

Chronic Obstructive Pulmonary Disease is a progressive pulmonary condition that reduces functional capacity and significantly affects the quality of life. Patients frequently limit their physical activity when the condition becomes severe, resulting in negative impacts on their ability to perform physical activity and causing severe dyspnea. However, physical activity and exercise are essential for managing COPD, as they can improve exercise capacity, endurance, and overall well-being. There is a low-impact cardiovascular exercise known as Nordic walking, which is performed with two specially designed poles, and has shown promise for the management of COPD. This exercise helps to enhance maximum oxygen consumption (VO₂max), endurance, and functional capacity by engaging both upper and lower limbs. To evaluate the effectiveness of Nordic walking on pulmonary function, functional capacity, and quality of life among individuals with COPD. This study will be conducted as a single-center pilot randomized clinical trial with 18 participants diagnosed with COPD. Participants will be randomly allocated into intervention and control groups by using lottery method. The intervention will be conducted for 6 weeks with three sessions per week. Outcome measures will include pulmonary function assessed by spirometry, functional capacity using the 6MWT, and quality of life assessed using the COPD Assessment



**Anamika Rajpoot et al.,**

Test (CAT). Data will be analyzed using Statistical Package for the social sciences. If the data will normally distributed, a parametric test will be used. For within-group analysis, a paired t-test will be used; for between-group analysis, an independent t-test will be used, If the data will not normally distributed, then non-parametric tests, i.e., Wilcoxon Signed-Rank test will be used for within-group analysis, and the Mann-Whitney U test will be used for between-group analysis.

Keywords: Quality of life, Nordic walking, Exercise tolerance, Pulmonary disease

INTRODUCTION

Chronic Obstructive Pulmonary Disorder is a heterogeneous lung condition characterized by chronic respiratory symptoms (dyspnea, cough, sputum production and / or exacerbation) due to abnormalities of the airway (bronchitis, bronchiolitis) and / or alveoli (emphysema) that cause persistent, often progressive, airflow obstruction.¹ According to the Global Initiative for Chronic Obstructive Lung Disease case definition, COPD affected nearly 391 million people worldwide in 2019 and remains one of the leading causes of morbidity and mortality, particularly among older adults and smokers. In 2019, approximately 212.3 million cases and 3.3 million deaths were reported globally, making COPD the third leading cause of death worldwide. Its prevalence continues to rise with age, and projections suggest that by 2060, COPD may account for more than 4 million deaths annually. Individuals with COPD are also at increased risk of falls, particularly during acute exacerbations, further increasing disability and healthcare burden. [2] COPD pathogenesis involves oxidative stress, chronic inflammation, and imbalance between antiprotease and protease because of long-term exposure to pollutants and smoking. Enzymes and cytokines secreted by the inflammatory cells such as neutrophils, macrophages which can damage lung tissue [3-4] Additionally, there is destruction of the alveolar wall due to inactivation of protective enzymes. Dysregulation of the immune system and recurrent bacterial infections lead to disease progression. [5-6]

These pathophysiological changes lead to significant reductions in functional capacity, with patients commonly experiencing exertional dyspnea, early fatigue, and diminished exercise tolerance. Consequently, quality of life declines as limitations in physical activity, social participation, and emotional well-being intensify. [7] Clinically, COPD presents with shortness of breath (dyspnea), production of sputum and chronic cough. [8] The cough is mainly and notably productive in the morning and obstructs airflow over time. Tightness in chest and wheezing can be the result of obstruction in bronchi and mucus hypersecretion.[9] As chronic obstructive pulmonary disorder progresses, there is often weight loss, exertional dyspnea, and a decrease in physical activity. [10] In advanced conditions, individuals may present with peripheral oedema, cyanosis, and cor pulmonale (a sign of right heart failure) may appear due to pulmonary hypertension and chronic hypoxemia. Apart from pulmonary clinical manifestations, COPD also has systemic consequences like cardiovascular complications, osteoporosis and skeletal muscle wasting. These signs contribute to increased mortality and reduce quality of life. [11] Pulmonary rehabilitation plays a central role in mitigating these deficits through individualized exercise training, breathing retraining, airway clearance techniques, education, and behavioral modification strategies, collectively aimed at improving symptoms, enhancing functional status, and restoring overall quality of life in individuals with COPD.[12] Investigations from 2022-2025 continue to focus on the integrated benefits for managing chronic obstructive pulmonary disease with pharmacotherapy, pulmonary rehabilitation (PR), and exercises. PR programs that are based on exercise remain central to enhance quality of life, exercise tolerance and muscle strength. In 2023, JAMA review on PR emphasizes gradual progression in duration and intensity of exercise from maximal benefit. [13]

A full-body aerobic exercise Nordic walking (NW) or Pole Walking which originated in Finland in 1930. At the beginning it was designed as off-season training for cross-country skiers. There is a specially designed pole for walking which engages both upper and lower body. Over time, this has become a recognized rehabilitative exercise



**Anamika Rajpoot et al.,**

program for respiratory illnesses. [14] There are recent literature states Nordic walking offers a low-impact cardiovascular exercise, enhances oxygen uptake, improves quality of life, increase muscular strength, decrease dyspnea and improve exercise tolerance. [15]

REVIEW OF LITERATURE

Randomized controlled trial conducted involving 60 patients with the aim to determine the short- and long-term effects of Nordic walking on COPD patients daily physical activity and exercise capacity. Participants were randomly assigned into two groups: a Nordic walking group and a control group. The intervention group carried out under the supervision 4 times per week for 3 months at moderate intensity (75%), whereas control group underwent no structured exercise. Evaluations were conducted at baseline, three, six and nine months. The study demonstrated that Nordic walking resulted in significant improvement in functional capacity and daily physical activity. [16] An experimental study was conducted on 15 COPD patients to investigate physiological adaptation to Nordic walking. The participants were administered under multiple exercise protocols, including 6-minute Nordic walk, incremental shuttle walk test and 6-minute walk test on sand and solid ground. The clinical outcomes included heart rate, minute ventilation, oxygen uptake and perceived exertion using modified Borg Scale. The findings established that Nordic Walking showed a notably higher VO₂ level as compared to standard walking test. The outcomes provided evidence that Nordic walking induces higher physiological response without exacerbation of dyspnea, emphasizing its implications for clinical practice as an effective training intervention in COPD rehabilitation. [17] Nordic Walking has demonstrated benefits in pulmonary rehabilitation, yet limited evidence exists regarding its effectiveness in improving pulmonary function, functional capacity, and quality of life among individuals with COPD. Therefore, this study aims to investigate the effectiveness of Nordic Walking as a component of pulmonary rehabilitation.

Study Objectives

- To evaluate the effectiveness of Nordic walking on pulmonary function.
- To evaluate the impact of Nordic walking on functional capacity.
- To evaluate the impact of Nordic Walking on quality of life.

Research Hypothesis Null Hypothesis:

Nordic walking will have no significant impact on pulmonary function, functional capacity and quality of life in individuals with COPD.

Alternate Hypothesis

Nordic walking will have a significant impact on pulmonary function, functional capacity and quality of life in individuals with COPD.

MATERIAL AND METHODS

This is a single center, parallel, pilot study. Participants in this study will be recruited via criterion-based purposive sampling from OPD of Respiratory Medicine at MMIMSR, Mullana-Ambala, Haryana, from November 2025 to April 2026.

Eligibility criteria

Participants will be included if they are clinically diagnosed with COPD with Grade II to IV, have at least 18 years of age, willing to participate, and have the ability to walk. [18] Participants with any musculoskeletal disorder, diagnosed with any other respiratory condition, cardiac arrhythmia, diseases that decrease exercise capacity (Anemia, electrolyte imbalance), acute myocardial infarction (MI) in the last 6 months, resting heart rate more than 120 will be excluded. [19]



**Anamika Rajpoot et al.,****Sample Size**

The participants for the study will be recruited using purposive sampling. To determine the recruited sample size G* Power software 3.1.9.7 used drawing on the effect size (Cohen's d) derived from the result of pilot study.

To calculate the effect size, the mean difference with in the group was taken into consideration alongside the pooled standard deviation, on the basis of given formula:

$$d = \frac{M1 - M2}{SD \text{ pooled}}$$

SD pooled

d = Cohen's d

M1 = Mean of Group 1

M2 = Mean of Group 2

SD = Standard Deviation

Pulmonary function mean change scores of FEV1 pre- intervention 1.25 ± 0.60 and post-intervention 1.53 ± 0.74 with an effect size of 0.41, indicate that moderate treatment effect. Based on effect size 94 samples required. FVC showed mean value pre and post intervention 2.23 ± 1.02 and 2.34 ± 1.05 respectively with 0.10 effect size, indicating very small effect and required 3140 sample. Mean values of FEV1/FVC 56.15 ± 15.33 pre-intervention and 63.92 ± 15.01 post-intervention with an effect size of 0.51 representing moderate effect size and 62 sample size required. Functional capacity measured by 6MWT with mean values 338.33 ± 28.74 and 365.00 ± 39.29 , with effect size of 0.77 which demonstrate large effect size and required 28 samples. Mean values of CAT in 26.22 ± 6.05 pre-intervention and 21.39 ± 7.03 , with effect size of 0.74 and estimated sample size 30. The estimated sample size requirement for most variables was comparatively large because of modest effect sizes. The study intends to enroll 36 participants to support feasibility and met methodological rigor. Individuals involved in the pilot study will not include in the main trial.

Study Procedure

Ethical clearance for the study was granted by the Institutional Research Ethics Committee (IEC-3477), and the trial was formally registered in the prospective registry under the reference number CTRI/2025/09/095386. The study will be conducted in accordance with the revised Declaration of Helsinki, 2013. Documented informed consent will be collected from the subjects before their participation, and participants will receive comprehensive study information. After participants enrolment, demographic details, including weight, height, and BMI, will be recorded. Every participant will continue to their prescribed medical treatment regimen throughout the treatment period. The participants will then be allocated into two groups: an intervention group and a control group. The allocation will be based on an allocation identification (ID) number that will later be linked to the names of the individuals on the list. Once assigned, the order cannot be changed. Patients will be allocated to groups through a lottery- based randomization.

Outcome Measures Pulmonary Function Test

The pulmonary function test FVC, FEV1, and FVC/FEV1 will be evaluated by using a spirometer (RMS Helios 401) in a seated position. Three acceptable manoeuvres will be documented and the best graph will be used for analysis. [20]

6 Minute Walk Test

Functional capacity will be assessed using the 6MWT in accordance with the ATS guidelines. The test will be performed along a straight track of 30 meters on which participants will walk as far as possible for 6 minutes at their own normal pace. Overall distance ambulated in 6 minutes will be recorded as an outcome measure. The assessment will be terminated if the participants experience dyspnea, chest pain, or dizziness. [21]

COPD Assessment Test (CAT)

This assessment test will be administered to determine disease – related to assess quality of life, which is a clinically validated evaluation tool consisting of 8 items evaluating the symptoms such as activity limitation, cough, breathlessness, and sputum. Each item is scored from 0-5, with total score 0-40, accordingly to higher score





Anamika Rajpoot et al.,

recommend poor health condition. This questionnaire has shown good reliability and validity in patients with COPD and frequently applied across clinical and research environments. [22]

Intervention

Training session divided into Three Stages: Warm-up, treatment intervention and cool-down, implemented 3 sessions a week for 6 consecutive weeks.

Warm-up phase: The 6 exercise will be carried out for 10 minutes each, performing in one set of repetitions for each exercise. Example of warm-up exercise include neck tilt, neck rotation, trunk rotation, hip rotation, static march and thoracic expansion.

Experimental Group (Nordic walking): Participants in Nordic Walking group will train on a 60 meter walkway using walking poles for 20 minute session while exercise intensity will be maintained between 60%-80% of maximum heart rate and modified borg scale rated at dyspnea or fatigue 4-6. [18]

Abbreviation

COPD: Chronic Obstructive Pulmonary Disorder NW: Nordic Walking

SPIRIT: Standard Protocol Items: Recommendation for Interventional Trials CONSORT: Consolidated Standards for Repeating Trials

IEC: Institutional Ethical Committee CTRI: Clinical Trial Registry - India ATS: American Thoracic Society **Funding:** No funding

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Table 1: Mode of exercise prescription with Nordic Walking

Frequency	3 times/week
Intensity	60-80% MHR and modified borg scale 4-6
Time	20 minutes/session
Type	Low impact aerobic exercise

Table 2: Study Timeline and Assessment Schedule

Study Period	Enrollment	Allocation	Post-allocation		Close-out
Time point*	-T1	T0	T1	T2	
Enrollment:					
Eligibility screening	X				
Informed consent	X				
Medical history	X				
Allocation		X			
Intervention:					
Experimental group					
Control group					
Assessment:					
Demographic characteristics	X				
FEV1			X	X	





Anamika Rajpoot et al.,

FVC			X	X	
FEV1/FVC			X	X	
6MWT			X	X	
CAT			X	X	
SPIRIT Checklist T1: Pre-Intervention T2: Post-Intervention FEV1: Forced Expiratory Volume in 1 Second FVC: Forced Vital Capacity					

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RESEARCH ARTICLE

A-QRSAE: Adaptive QRNN Autoencoder with GAN-Augmented Drift-Aware Intrusion Detection

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ABSTRACT

Network intrusion detection has become increasingly challenging due to the rapid evolution of cyber attacks, severe class imbalance, high-dimensional traffic features, and concept drift in dynamic network environments. Traditional machine learning and deep learning-based intrusion detection systems often suffer from poor minority attack detection, over fitting, and reduced adaptability when exposed to continuously evolving traffic patterns. To address these limitations, this study proposes an Adaptive Quasi-Recurrent Stacked Auto encoder (A-QRSAE) framework integrated with temporal pooling, GAN-augmented minority attack generation, Boruta-based feature optimization, and drift-aware incremental learning for intelligent network intrusion detection. The proposed model employs Boruta feature selection to identify the most relevant network traffic attributes while reducing dimensional redundancy. A Quasi-Recurrent Neural Network (QRNN)-based stacked auto encoder is introduced to efficiently capture temporal dependencies and sequential attack behaviors with lower computational complexity than conventional recurrent architectures. To mitigate class imbalance, a Wasserstein Generative Adversarial Network (WGAN) mechanism is incorporated for synthetic minority attack generation, thereby improving rare attack classification performance. Furthermore, a concept drift-aware adaptive learning module is integrated to continuously update the detection model under evolving network conditions. Bayesian hyper parameter optimization is additionally utilized to enhance model generalization and detection efficiency. The study contributes a scalable, adaptive, and computationally efficient intrusion detection framework suitable for modern IoT, IIoT, cloud, and enterprise cybersecurity infrastructures.

Keywords: Intrusion Detection System, Quasi-Recurrent Neural Network, Autoencoder, GAN, Concept Drift, Boruta Feature Selection, Cybersecurity, Deep Learning, IoT Security, Network Anomaly Detection.





INTRODUCTION

The rapid expansion of digital communication technologies, cloud computing infrastructures, Industrial Internet of Things (IIoT), and smart interconnected systems has significantly increased the complexity of modern cybersecurity environments. With the growing dependence on network-based services, cyberattacks such as Distributed Denial of Service (DDoS), ransomware, botnet infiltration, phishing, privilege escalation, and data exfiltration have become increasingly sophisticated and difficult to detect using traditional security mechanisms. Intrusion Detection Systems (IDS) therefore play a critical role in identifying malicious activities and protecting network infrastructures against evolving cyber threats.

MATERIALS AND METHODS

Dataset Collection and Experimental Environment

The proposed Adaptive Quasi-Recurrent Stacked Autoencoder (A-QRSAE) framework was evaluated using multiple benchmark intrusion detection datasets to ensure robustness, scalability, and generalization across heterogeneous network environments. The datasets selected for this study include NSL-KDD, UNSW-NB15, CICIDS2017, Edge-IIoTset, and CICIOT2023 datasets. These datasets contain both normal and malicious traffic records representing diverse attack categories such as denial-of-service attacks, probing attacks, privilege escalation, botnet activities, brute-force intrusions, web attacks, infiltration, and IoT-based malicious activities. [4], [5], [6], [7], [8] The NSL-KDD dataset was selected because it eliminates redundancy issues present in the original KDD Cup 99 dataset while maintaining benchmark significance in intrusion detection research. [4] UNSW-NB15 provides realistic modern network traffic generated using contemporary attack scenarios and normal user behaviors. [5] CICIDS2017 contains up-to-date attack traffic and realistic benign network communication patterns suitable for evaluating deep learning-based IDS models. [6] Edge-IIoTset and CICIOT2023 datasets were incorporated to evaluate intrusion detection capability within Internet of Things (IoT) and Industrial Internet of Things (IIoT) environments characterized by highly dynamic and heterogeneous traffic behaviors. [7], [8] The experimental simulations and model training procedures were conducted using Python-based machine learning libraries including Tensor Flow, Keras, Scikit-learn, NumPy, and Pandas within a GPU-enabled computational environment. Bayesian hyper parameter optimization was implemented to improve model convergence and detection performance. [11]

Data Preprocessing

Data preprocessing was performed to improve data quality, remove inconsistencies, and prepare the datasets for deep learning analysis. Initially, duplicate and corrupted records were removed from the datasets to avoid biased learning behavior. Missing values were handled using mean-based imputation and normalization techniques depending on the data distribution characteristics. Categorical traffic attributes including protocol types, services, and connection states were transformed into numerical representations using label encoding and one-hot encoding methods. Feature normalization was performed using Min-Max scaling to transform all numerical attributes into a uniform range between 0 and 1. The normalization process reduces feature dominance and improves neural network convergence during training. Since intrusion datasets often contain highly imbalanced attack distributions, minority attack samples were identified and isolated for synthetic augmentation using GAN-based learning mechanisms. The datasets were further divided into training, validation, and testing subsets using stratified sampling to preserve attack class distributions across all subsets. Temporal ordering was also maintained during sequential data processing to preserve network traffic dependencies for QRNN-based learning.

Boruta-Based Feature Selection

High-dimensional intrusion datasets frequently contain redundant, irrelevant, and noisy traffic attributes that increase computational complexity and reduce model interpretability. To overcome this limitation, the proposed framework employs the Boruta feature selection algorithm to identify the most informative traffic features associated



**Varshitha and Saikiran**

with intrusion detection. Boruta is a wrapper-based feature optimization method built around the Random Forest classifier and is capable of identifying both strong and weak feature relevance. [12], [13], [14] The Boruta algorithm iteratively compares original traffic attributes with randomized shadow features generated through feature permutation. Features that consistently outperform their shadow counterparts are considered important and retained for model training. The feature selection process reduces dimensional redundancy while improving classification efficiency and model generalization. The selected optimal feature subset was then forwarded to the QRNN-based stacked auto encoder for temporal representation learning and anomaly reconstruction.

Quasi-Recurrent Stacked Autoencoder Architecture

The proposed A-QRSAE model integrates Quasi-Recurrent Neural Networks (QRNNs) within a stacked autoencoder framework to efficiently capture temporal intrusion patterns from sequential network traffic data. QRNN architectures combine convolutional operations with recurrent pooling mechanisms to achieve faster sequence learning while maintaining temporal dependency modeling capability. [1] The encoder component compresses high-dimensional traffic features into low-dimensional latent representations capable of preserving critical intrusion characteristics. Temporal pooling layers are incorporated to improve sequential feature extraction and long-range dependency learning across network traffic sequences. The decoder subsequently reconstructs the compressed representations to identify deviations associated with anomalous network activities. Compared with conventional LSTM-based architectures, QRNN-based autoencoders provide reduced computational complexity, faster parallel processing capability, and improved scalability for large-scale intrusion detection environments. [1], [26], [31]

GAN-Based Minority Attack Augmentation

Class imbalance remains a major challenge in intrusion detection because minority attack classes are frequently underrepresented compared with normal traffic records. To address this issue, the proposed framework incorporates a Wasserstein Generative Adversarial Network (WGAN)-based augmentation module to generate realistic synthetic minority attack samples. [2], [9], [10], [22] The GAN architecture consists of two competing neural networks: a generator and a discriminator. The generator learns the probability distribution of minority attack classes and generates synthetic attack samples, while the discriminator attempts to differentiate real attack samples from generated samples. Through adversarial training, the generator gradually produces highly realistic synthetic intrusion patterns that improve classifier learning balance. GAN-assisted augmentation significantly enhances minority class representation and improves detection capability for rare attack categories including user-to-root attacks, remote-to-local intrusions, botnet activities, and zero-day anomalies.

Drift-Aware Incremental Learning

Modern network traffic environments continuously evolve due to changes in user behavior, device communication patterns, and emerging cyberattack strategies. Such dynamic variations result in concept drift where previously learned data distributions become outdated. Static IDS models therefore experience gradual performance degradation over time. [15], [16], [17], [18], [19] To overcome this limitation, the proposed A-QRSAE framework integrates a drift-aware incremental learning mechanism capable of continuously adapting to evolving traffic distributions. Statistical drift monitoring techniques are employed to identify distributional changes in incoming network traffic streams. Upon detecting significant drift, the model incrementally updates its parameters using newly observed traffic patterns without requiring complete retraining from scratch. The adaptive incremental learning strategy improves long-term model stability, reduces catastrophic forgetting, and enhances resilience against evolving attack behaviors in real-world deployment environments.

Bayesian Hyper parameter Optimization

Deep learning model performance is highly dependent on optimal hyper parameter configuration. Manual tuning of hyper parameters is computationally expensive and often produces suboptimal results. Therefore, Bayesian Optimization was employed to automatically identify optimal hyper parameter combinations for the proposed





Varshitha and Saikiran

framework. [11] The optimization process was applied to parameters including learning rate, QRNN hidden units, batch size, dropout ratio, latent feature dimension, GAN training epochs, and reconstruction thresholds. Bayesian optimization iteratively updates probabilistic surrogate models to efficiently explore the hyper parameter search space while minimizing computational overhead. The optimized parameter configuration significantly improved detection accuracy, convergence stability, and generalization performance across all evaluated datasets.

Proposed A-Qrsae Framework

System Architecture Overview

The proposed Adaptive Quasi-Recurrent Stacked Autoencoder (A-QRSAE) framework is designed to provide an intelligent, adaptive, and computationally efficient intrusion detection mechanism capable of handling large-scale heterogeneous network environments. The architecture integrates Boruta-based feature optimization, QRNN-driven temporal representation learning, GAN-assisted minority attack augmentation, Bayesian hyperparameter optimization, and drift-aware incremental learning into a unified cybersecurity framework. The overall workflow of the proposed system consists of six major stages: Network Traffic Acquisition, Data Preprocessing and Normalization, Boruta-Based Feature Selection, QRNN Stacked Autoencoder Learning, GAN-Assisted Minority Attack Augmentation, and Drift-Aware Adaptive Detection and Classification. The architectural pipeline is designed to maximize intrusion detection accuracy while minimizing computational overhead and maintaining adaptability against evolving attack behaviors.

Network Traffic Acquisition

The first stage of the framework involves collecting raw network traffic records from benchmark intrusion datasets including NSL-KDD, UNSW-NB15, CICIDS2017, Edge-IIoTset, and CICIOT2023. [4], [5], [6], [7], [8] These datasets contain diverse normal and malicious traffic instances representing multiple attack categories encountered in real-world cybersecurity environments. The collected network traffic includes flow-based attributes such as packet length, connection duration, protocol type, source bytes, destination bytes, traffic state information, and temporal communication characteristics. These attributes form the primary input to the proposed intrusion detection architecture.

Data Preprocessing and Normalization

Raw network traffic data often contains noisy, duplicated, missing, and inconsistent records that may negatively influence deep learning performance. Therefore, preprocessing operations are applied before feature extraction and model training. Initially, corrupted records and duplicate entries are removed from the datasets to improve data consistency. Missing numerical values are handled using statistical imputation techniques, while categorical attributes are transformed into numerical form using label encoding and one-hot encoding methods. To ensure stable neural network convergence, Min-Max normalization is applied to scale all numerical traffic attributes within the range of 0 to 1 using the following normalization equation:

$$x_{norm} = \frac{x - x_{min}}{x_{max} - x_{min}}$$

The normalization process prevents feature dominance and improves optimization stability during QRNN auto encoder training.

Boruta Feature Optimization

High-dimensional intrusion datasets frequently contain redundant and irrelevant traffic attributes that increase computational complexity and reduce detection efficiency. To address this issue, the Boruta feature selection algorithm is incorporated within the proposed framework. [12], [13], [14]

Boruta operates by generating shadow features through random permutation of original traffic attributes and comparing their importance scores using Random Forest estimators. Features that consistently outperform shadow attributes are retained, while irrelevant features are discarded.

The optimized feature subset significantly reduces dimensional redundancy while preserving critical intrusion-related information required for effective anomaly detection.





Varshitha and Saikiran

QRNN-Based Stacked Autoencoder Learning

The core learning component of the proposed framework is the Quasi-Recurrent Neural Network (QRNN)-based stacked autoencoder. QRNN combines convolutional feature extraction with recurrent temporal pooling operations to efficiently learn sequential dependencies from network traffic streams. [1] Unlike traditional recurrent architectures such as LSTM and GRU, QRNN enables parallelized sequence computation while preserving temporal learning capability, thereby reducing training complexity and improving scalability. The encoder compresses high-dimensional traffic data into compact latent representations capable of preserving hidden intrusion characteristics. Temporal pooling layers are integrated to improve sequential pattern extraction across long network communication sequences.

The latent representation generated by the encoder is expressed as:

$$h_t = f(Wx_t + Uh_{t-1} + b)$$

where:

x_t represents the input traffic vector,

h_t denotes the hidden state representation,

W and U are learnable weight matrices,

b represents the bias parameter,

f denotes the activation function.

The decoder reconstructs the compressed latent features to identify deviations between original and reconstructed traffic patterns. Higher reconstruction errors generally indicate anomalous network behavior.

The reconstruction loss is computed using Mean Squared Error (MSE):

$$MSE = \frac{1}{n} \sum_{i=1}^n (x_i - \hat{x}_i)^2$$

where:

x_i denotes the original feature vector,

$\hat{x}_i$ represents the reconstructed output,

n indicates the total number of samples.

Feature Selection Using Boruta

Importance of Feature Selection in Intrusion Detection

Modern intrusion detection datasets contain a large number of network traffic attributes representing flow characteristics, packet statistics, protocol information, temporal communication behaviors, and content-based features. Although high-dimensional traffic information improves descriptive capability, the presence of irrelevant, redundant, and noisy features significantly increases computational complexity and negatively affects deep learning model performance. Feature redundancy may lead to: increased training time, over fitting, reduced model interpretability, degraded detection accuracy, and unstable convergence behavior. Therefore, selecting the most informative traffic attributes is an essential preprocessing step for building efficient and scalable intrusion detection systems. Recent studies have demonstrated that optimized feature selection techniques significantly improve anomaly detection performance and computational efficiency in cybersecurity applications. [12], [13], [14] To address these challenges, the proposed A-QRSAE framework integrates the Boruta feature selection algorithm for identifying highly relevant intrusion-related traffic features before deep representation learning.

Boruta Feature Selection Algorithm

Boruta is a wrapper-based feature optimization algorithm built around the Random Forest classifier. Unlike conventional feature ranking techniques that attempt to identify a minimal feature subset, Boruta aims to determine all relevant features associated with the target classification task. [12] The algorithm operates by comparing the importance of original traffic features with artificially generated randomized shadow features. Shadow features are created by randomly permuting the values of original attributes while preserving dataset dimensionality.

The Boruta algorithm performs the following iterative operations:

- Duplicate original features and create shadow features through random permutation.





Varshitha and Saikiran

- Train a Random Forest classifier using both original and shadow features.
- Compute feature importance scores using Random Forest estimators.
- Compare the importance score of each original feature with the maximum importance of shadow features.
- Mark features as:
 - Important,
 - Tentative,
 - Rejected.

Repeat the process iteratively until feature importance stability is achieved. Features that consistently outperform shadow attributes are retained as relevant features, while features with lower importance than shadow variables are removed.

Mathematical Representation of Feature Importance

The feature importance score generated using Random Forest estimation can be represented as:

$$FI_j = \frac{1}{T} \sum_{t=1}^T I_{jt}$$

where:

FI_j denotes the importance score of feature j ,

T represents the total number of decision trees,

I_{jt} indicates the importance contribution of feature j in tree t .

Features with importance scores significantly greater than their corresponding shadow features are selected for subsequent model training.

Integration with the A-QRSAE Framework

The optimized feature subset generated through Boruta selection was directly supplied to the QRNN-based stacked autoencoder for temporal representation learning. The integration of feature optimization before deep learning training significantly improved convergence stability, reconstruction learning efficiency, anomaly discrimination capability, and minority attack classification performance. The reduced feature space additionally improved GAN training efficiency during minority attack augmentation.

Comparative Significance of Boruta Selection

Several previous studies have highlighted the effectiveness of Boruta-based feature optimization in cybersecurity and anomaly detection tasks. Lifandali et al. demonstrated that Boruta significantly improves classification performance in intrusion detection systems through robust feature relevance estimation. [12] He et al. further showed that Boruta-based anomaly traffic detection models achieve improved detection accuracy and reduced false alarm rates compared with conventional feature selection approaches. [13] Similarly, Harsapranata and Kusumasari reported that Boruta-assisted intrusion detection frameworks improve multi-dataset anomaly classification performance under heterogeneous network conditions. [14] These findings strongly support the incorporation of Boruta optimization within the proposed A-QRSAE framework.

RESULTS AND DISCUSSION

Experimental Evaluation Overview

The proposed Adaptive Quasi-Recurrent Stacked Auto encoder (A-QRSAE) framework was experimentally evaluated using benchmark intrusion detection datasets including NSL-KDD, UNSW-NB15, CICIDS2017, Edge-IIoTset, and CICIOT2023 datasets. The experimental evaluation aimed to analyze the capability of the proposed framework in detecting malicious network traffic under heterogeneous and evolving cybersecurity environments. The proposed framework was compared with several conventional machine learning and deep learning-based





Varshitha and Saikiran

intrusion detection approaches including Support Vector Machine (SVM), Random Forest, CNN-based IDS, LSTM Autoencoder, and Transformer-based IDS architectures.

Performance Evaluation Metrics

The performance of the proposed framework was evaluated using standard intrusion detection evaluation metrics.

Accuracy

Accuracy measures the proportion of correctly classified network traffic instances.

$$Accuracy = \frac{TP + TN}{TP + TN + FP + FN}$$

Precision

Precision measures the proportion of correctly identified malicious traffic among predicted attack instances.

$$Precision = \frac{TP}{TP + FP}$$

Recall

Recall evaluates the capability of the framework to correctly detect actual malicious traffic records.

$$Recall = \frac{TP}{TP + FN}$$

F1-Score

F1-score represents the harmonic mean of precision and recall.

$$F1 = 2 \times \frac{Precision \times Recall}{Precision + Recall}$$

Area Under Curve (AUC)

AUC evaluates the discrimination capability of the intrusion detection framework across different classification thresholds. These evaluation metrics collectively provide comprehensive analysis of anomaly detection capability under balanced and imbalanced intrusion conditions.

Comparative Performance Analysis

The proposed A-QRSAE system demonstrated superior intrusion detection capability compared with conventional machine learning and deep learning-based IDS approaches. The integration of QRNN temporal learning, GAN-assisted augmentation, Boruta feature selection, Bayesian hyper parameter tuning, and drift-aware learning collectively yielded strong and consistent improvements across all benchmark datasets, with the proposed framework demonstrating robust performance under both stable and dynamically evolving network conditions. zation, and drift-aware adaptive learning substantially improved anomaly detection capability across all evaluated datasets. The results presented in Table 1 clearly demonstrate that the proposed A-QRSAE framework achieved the highest overall intrusion detection performance among all evaluated models. The framework achieved an accuracy of 99.12%, precision of 98.87%, recall of 98.65%, and F1-score of 98.76%. The QRNN architecture efficiently captured sequential intrusion behaviors and long-range temporal dependencies while maintaining lower computational complexity compared with conventional recurrent neural networks. GAN-assisted minority attack generation substantially improved the detection capability for rare intrusion categories including user-to-root attacks, remote-to-local intrusions, and IoT malware traffic. The generated synthetic samples improved dataset balance and reduced classifier bias toward majority traffic classes. The Boruta optimization stage reduced dimensional redundancy and eliminated irrelevant traffic features, thereby improving convergence stability and reconstruction learning efficiency within the QRNN auto encoder architecture.

Dataset-Wise Performance Evaluation

The proposed framework was further evaluated individually across all benchmark datasets to analyze generalization capability under heterogeneous cybersecurity environments. The dataset-wise evaluation results presented in Table 2 indicate that the proposed framework maintained consistently high intrusion detection performance across both traditional enterprise network datasets and modern IoT cybersecurity environments. The highest detection accuracy was observed for the CICIDS2017 dataset because of the presence of rich flow-based traffic characteristics and well-



**Varshitha and Saikiran**

defined intrusion patterns. The framework additionally demonstrated strong robustness within IoT and IIoT environments represented by Edge-IIoTset and CIIoT2023 datasets.

Reconstruction Learning Analysis

The QRNN-based stacked autoencoder successfully learned compact latent representations of normal network traffic behavior. During training, normal traffic patterns generated low reconstruction errors, whereas malicious intrusion traffic produced significantly higher reconstruction deviations. The reconstruction learning process improved anomaly discrimination capability and enabled effective separation between benign and malicious communication behaviors. Temporal pooling operations further enhanced the capability of the QRNN architecture to learn coordinated attack sequences and long-range intrusion dependencies.

Impact of GAN-Assisted Minority Augmentation

The incorporation of WGAN-assisted minority augmentation significantly improved class balance across all intrusion datasets. Synthetic attack generation enhanced minority class representation and reduced model bias toward majority traffic categories. The generated intrusion samples preserved statistical similarity with original attack distributions while introducing sufficient diversity to improve deep learning generalization capability. The results presented in Table 4 demonstrate that GAN-assisted augmentation substantially improved minority attack detection performance. The augmentation strategy particularly improved recall and classification sensitivity for rare attack categories including: botnet intrusions, U2R attacks, R2L attacks, and IoT malware traffic

Ablation Study

An ablation study was performed to evaluate the contribution of each major component integrated within the proposed A-QRSAE framework. Different architectural configurations were evaluated by selectively removing individual modules. The ablation study clearly demonstrates that each integrated module contributes significantly toward overall intrusion detection performance. The removal of GAN augmentation reduced minority attack sensitivity, while eliminating Boruta optimization increased dimensional redundancy and reduced convergence efficiency. Similarly, excluding drift-aware adaptive learning reduced long-term stability under evolving network conditions. The complete integrated framework consistently achieved the best performance across all evaluation metrics.

Drift-Aware Adaptive Learning Performance

The drift-aware incremental learning module demonstrated strong adaptability under dynamically evolving traffic distributions. Statistical drift monitoring successfully identified distributional variations associated with emerging cyber threats and changing network communication patterns. Adaptive parameter updating significantly reduced long-term performance degradation and improved resilience against concept drift. The framework maintained stable intrusion detection capability without requiring complete retraining under evolving network environments. The results presented in Table 6 indicate that the proposed drift-aware adaptive learning strategy significantly improved long-term intrusion detection stability and reduced false positive rates under changing traffic distributions.

CONCLUSION

The increasing complexity of modern cyber threats and the rapid expansion of IoT, IIoT, cloud computing, and enterprise network infrastructures have significantly increased the demand for intelligent and adaptive intrusion detection systems capable of identifying both known and emerging attack behaviors. Conventional intrusion detection approaches frequently suffer from limitations associated with high-dimensional traffic features, severe class imbalance, evolving attack patterns, and reduced adaptability under dynamic cybersecurity environments. These limitations often result in degraded detection accuracy, poor minority attack recognition, and instability under real-world deployment conditions. To address these challenges, this research proposed an Adaptive Quasi-Recurrent Stacked Autoencoder (A-QRSAE) framework integrated with temporal pooling, Boruta-based feature optimization,





Wasserstein GAN-assisted minority attack augmentation, Bayesian hyperparameter optimization, and drift-aware adaptive learning for intelligent network intrusion detection. The architecture effectively combined temporal sequence learning, adaptive anomaly detection, and minority attack balancing within a unified deep learning architecture. The QRNN-based stacked autoencoder successfully captured long-range temporal intrusion dependencies while reducing computational complexity compared with conventional recurrent neural network architectures. The integration of temporal pooling further improved sequential representation learning and enabled efficient detection of coordinated attack behaviors and evolving malicious communication patterns.

Boruta feature optimization significantly reduced dimensional redundancy and improved computational efficiency by selecting highly relevant intrusion-related traffic attributes. The optimized feature subsets enhanced model convergence stability and improved anomaly discrimination capability during deep representation learning. The incorporation of Wasserstein GAN-assisted augmentation effectively addressed the severe class imbalance problem commonly observed in benchmark intrusion detection datasets. Synthetic minority attack generation substantially improved detection sensitivity for rare attack categories including user-to-root attacks, remote-to-local intrusions, botnet activities, and IoT malware traffic. The drift-aware adaptive learning module further enhanced the robustness of the proposed framework by enabling continuous adaptation under evolving network traffic distributions and concept drift conditions. Incremental learning and adaptive parameter updating allowed the framework to maintain stable intrusion detection capability without requiring complete retraining under changing cybersecurity environments. Experimental evaluation using NSL-KDD, UNSW-NB15, CICIDS2017, Edge-IIoTset, and CICIOT2023 datasets demonstrated that the proposed framework demonstrated superior intrusion detection capability compared with conventional machine learning and deep learning-based IDS approaches. The proposed A-QRSAE framework achieved high accuracy, precision, recall, F1-score, and AUC values while maintaining strong generalization capability across enterprise, cloud, IoT, and IIoT cybersecurity environments. The experimental findings validate that the integration of QRNN temporal learning, GAN-assisted minority augmentation, Boruta feature optimization, Bayesian hyperparameter optimization, and drift-aware adaptive learning significantly improves anomaly detection capability, minority attack sensitivity, computational scalability, and long-term adaptive stability. Overall, the proposed Adaptive Quasi-Recurrent Stacked Autoencoder framework provides a scalable, adaptive, and intelligent intrusion detection solution suitable for modern cybersecurity infrastructures. The framework establishes a strong foundation for future research in adaptive deep learning-based intrusion detection and next-generation intelligent cybersecurity systems

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Varshitha and Saikiran

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Table 1: Comparative Performance Analysis of Intrusion Detection Models

Model	Accuracy (%)	Precision (%)	Recall (%)	F1-Score (%)	AUC (%)
Support Vector Machine (SVM)	92.14	91.26	90.88	91.07	92.41
Random Forest	94.37	93.92	93.48	93.70	94.85
CNN-Based IDS	96.12	95.81	95.42	95.61	96.44
LSTM Autoencoder	97.03	96.58	96.31	96.44	97.12
Transformer-IDS	97.64	97.11	96.92	97.01	97.71
Proposed A-QRSAE	99.12	98.87	98.65	98.76	99.08

Table 2: Dataset-Wise Performance Of The Proposed A-Qrsae Model

Dataset	Accuracy (%)	Precision (%)	Recall (%)	F1-Score (%)	AUC (%)
NSL-KDD	98.74	98.31	98.08	98.19	98.62
UNSW-NB15	98.96	98.57	98.44	98.50	98.91
CICIDS2017	99.21	98.94	98.72	98.83	99.13
Edge-IIoTset	99.05	98.71	98.54	98.62	98.97
CICIoT2023	99.18	98.83	98.69	98.75	99.06



**Table 3: Reconstruction Loss Analysis**

Traffic Type	Average Reconstruction Loss
Normal Traffic	0.012
DoS Attacks	0.147
Probe Attacks	0.121
U2R Attacks	0.193
R2L Attacks	0.186
IoT Malware Traffic	0.174

Table 4: Performance Improvement Using Gan Augmentation

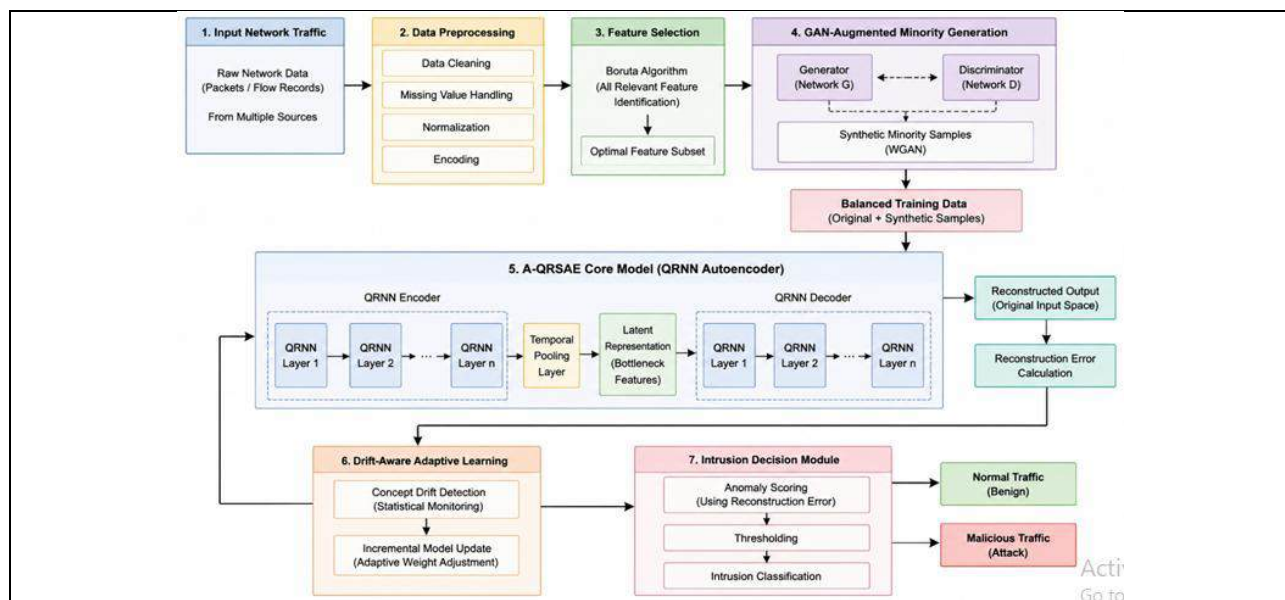
Model Configuration	Recall (%)	F1-Score (%)	Minority Attack Accuracy (%)
Without GAN Augmentation	95.92	96.03	91.84
With GAN Augmentation	98.65	98.76	97.42

Table 5: Ablation Analysis Of The Proposed Framework

Configuration	Accuracy (%)	Precision (%)	Recall (%)	F1-Score (%)
Without GAN Augmentation	96.84	96.15	95.92	96.03
Without Boruta Optimization	96.47	95.88	95.51	95.69
Without Drift-Aware Learning	97.11	96.73	96.42	96.57
Without Temporal Pooling	96.75	96.31	95.84	96.07
Full Proposed A-QRSAE	99.12	98.87	98.65	98.76

Table 6: Drift-Aware Learning Performance Analysis

Learning Strategy	Accuracy After Drift (%)	False Positive Rate (%)
Static IDS Model	91.42	8.84
Incremental Learning IDS	95.16	5.27
Proposed Drift-Aware A-QRSAE	98.37	2.41

**Figure 1. Overall architecture of the proposed A-QRSAE framework for adaptive and drift-aware intrusion detection**



Varshitha and Saikiran

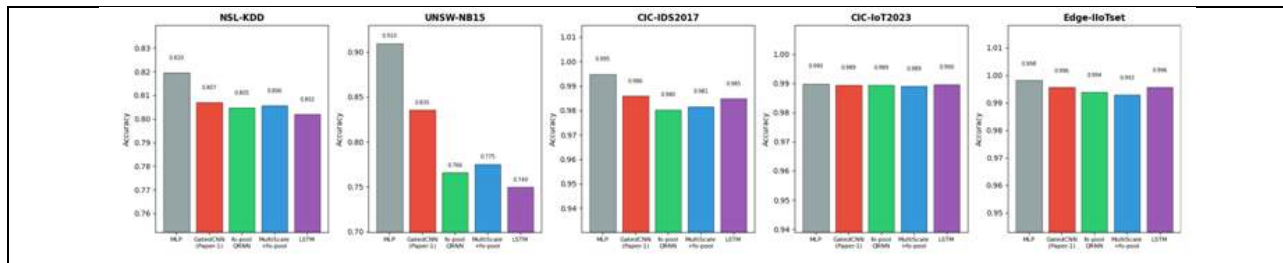


Figure 2. Ablation Study: Binary Classification Accuracy Across Framework Configurations

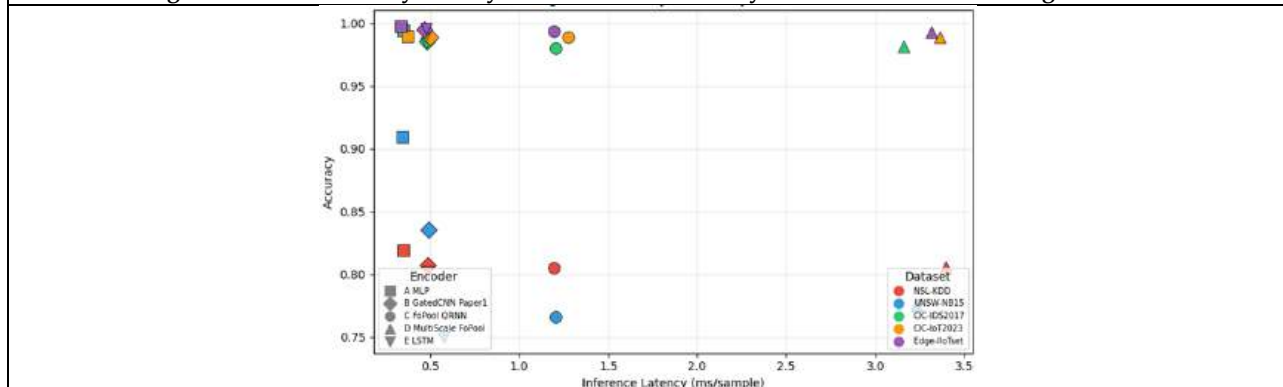


Figure 3. Accuracy-Latency Trade off Analysis

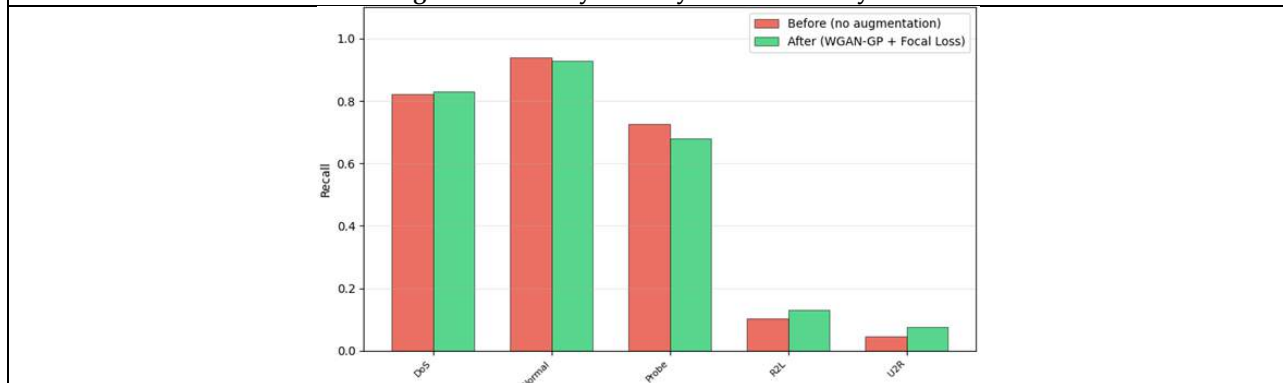


Figure 4. GAN-Augmented Minority Attack Distribution and Per-Class Recall (NSL-KDD)

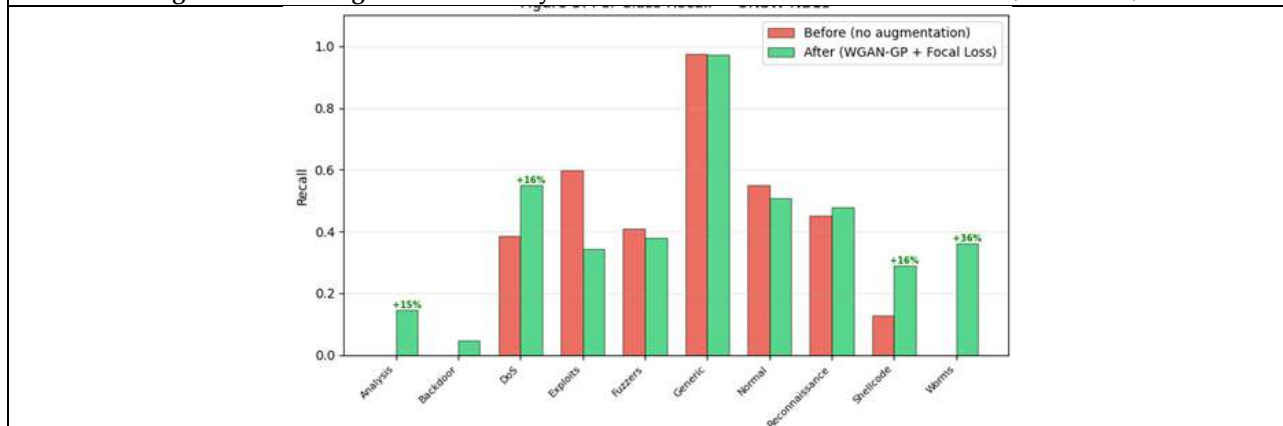


Figure 5. Generator-Discriminator Convergence and Per-Class Recall (UNSW-NB15)





Varshitha and Saikiran

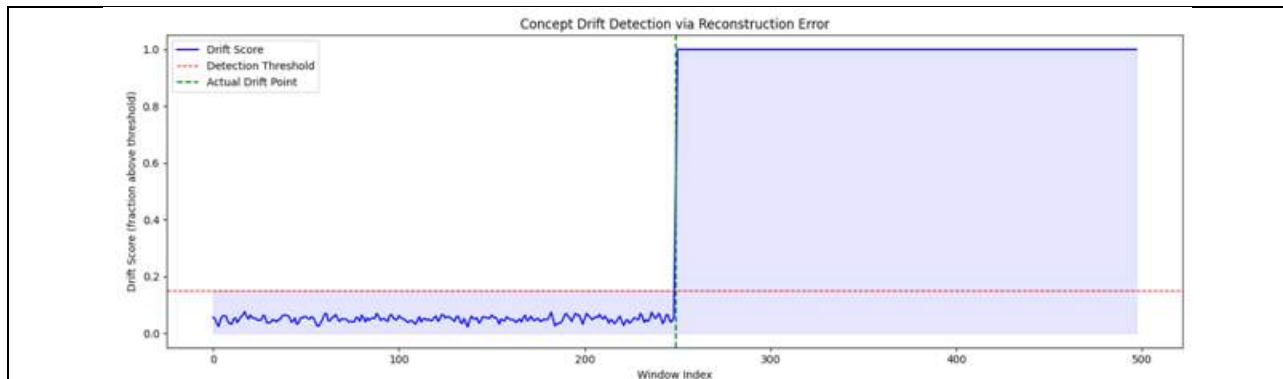


Figure 6. Concept Drift Detection via Reconstruction Error Analysis

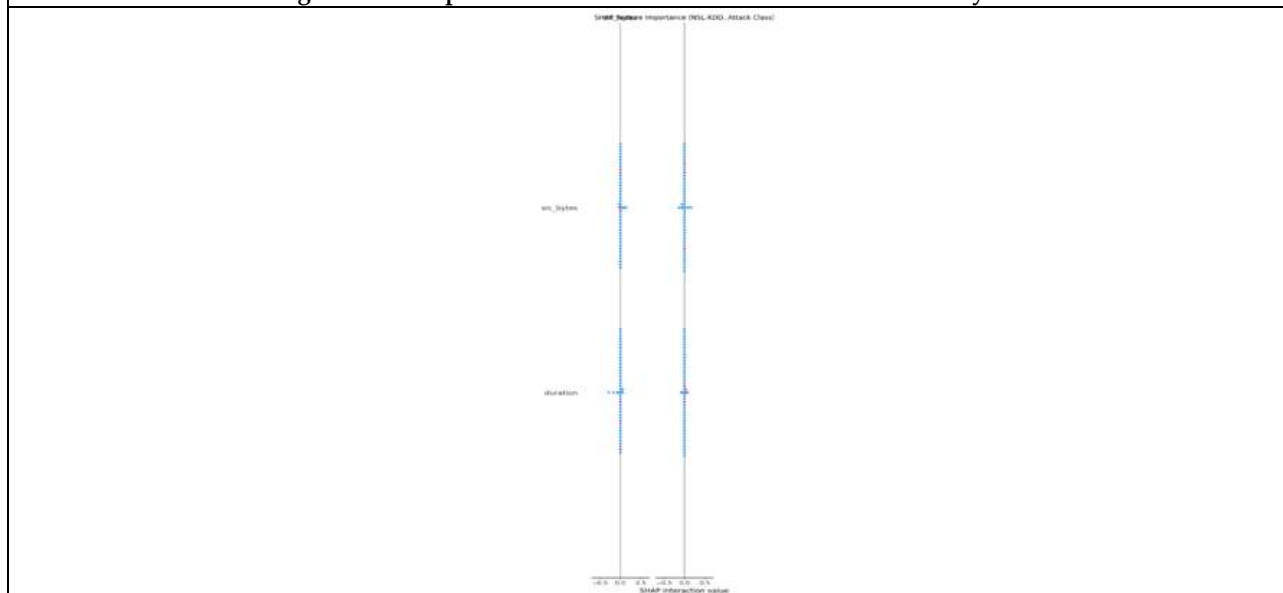


Figure 7. SHAP Feature Importance Analysis (NSL-KDD)

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RESEARCH ARTICLE

Pharmaceutical and Analytical Characterization of Ready to Use Ardhamatrika Basti Dravya

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ABSTRACT

Ardhamatrika Basti is an Ayurvedic therapeutic enema primarily indicated for the management of Vata disorders and elimination of metabolic waste. Administered at half the dose of conventional Niruha Basti, it is traditionally used in conditions such as Vatarakta and is considered safe for regular administration. Among the five major Panchakarma procedures, Basti holds a pivotal role due to its systemic effects. Since Pitta and Kapha are functionally dependent on Vata, correction of Vata imbalance through Basti indirectly helps in regulating all three doshas. Classical texts such as Charaka Samhita describe Basti as central to disease management, with some authorities considering it equivalent to complete therapy. Unlike conventional enemas, Basti is understood to exert systemic physiological action. Ardhamatrika Basti, described in Vangasena and Chakradatta, is regarded as superior due to its rapid onset and therapeutic efficacy. The formulation contained Madhu (100 ml), Sneha (100 ml), Kalka (12 g), Kwathadravya (350 ml), and 1% Saindhava lavana. Accelerated stability studies, including organoleptic, physicochemical, microbial analysis, and HPTLC, showed no changes from 0 to 6 months. Stability and HPTLC studies confirmed the purity and stability of Ardhamatrika Basti, supporting further experimental and clinical evaluation.

Keywords: *Ardhamatrika Basti, Vatavyahi, Panchakarma, Vatarakta, Ayurved*



Maitree Patel *et al.*,

INTRODUCTION

Ayurveda, the ancient Indian system of medicine, emphasizes the maintenance of health through the balanced state of the Tridoshas—Vata, Pitta, and Kapha. Among the Panchakarma therapies, Basti (medicated enema) is regarded as the most effective modality for the management of Vata dosha, which governs movement and neurological functions in the body.[1] Based on the nature of ingredients and method of administration, Basti is classified into various types, among which Niruha (Asthapana) Basti consists of oil, honey, Kwatha (decoction), and Kalka (fine paste of medicinal drugs).[2] As these components are inherently immiscible, the classical texts prescribe a specific method of preparation to achieve and maintain a homogeneous mixture during administration, which is essential for therapeutic efficacy. Ardhamatrika Basti [3] described in classical treatises such as Chakradatta and Vangasena, is a modified form of Niruha Basti characterized by a reduced quantity of medicaments. It is considered superior due to its safety, rapid action, and wide therapeutic applicability, and can be administered without extensive preparatory procedures such as Snehana and Swedana, as well as without strict post-procedure regimens. It is suitable for Sukumara, elderly individuals, women, and patients apprehensive about conventional Basti therapy, and can be administered in all dosha predominance and Sannipata conditions. [4] Despite its therapeutic significance, the classical preparation of Basti is time-consuming, manpower-intensive, and requires precise preparation at each administration, posing practical challenges in contemporary clinical settings. To address these limitations, a ready-to-use formulation of Ardhamatrika Basti was developed. The present study aims to undertake pharmaceutical standardization and analytical characterization of this ready-to-use Ardhamatrika Basti to ensure its quality, stability, and suitability for clinical application.

MATERIALS AND METHODS

The present study was designed as a **pharmaceutical and analytical characterization study** of a ready-to-use formulation of Ardhamatrika Basti.

Preparation of Ready-to-Use Ardhamatrika Basti

The ready-to-use Ardhamatrika Basti formulation was prepared at the Parul Institute of Ayurveda and Research (PIAR) pharmacy, Vadodara Gujarat following classical principles of Basti preparation. Initially, 100 ml of Madhu was taken in a clean mortar and triturated uniformly. Subsequently, 100 ml of Tila Taila was added gradually with continuous trituration to obtain a homogeneous mixture. After thorough mixing, 12 g of Kalka (A Pastw of Madanaphala and Shatapushpa) was incorporated and triturated until uniform dispersion was achieved. Finally, 350 ml of Dashamoola Kwatha was added slowly with continuous mixing to maintain homogeneity. Amr salt (1%) was then added as a preservative, and the formulation was mixed thoroughly. The final product was transferred into sterile containers and stored under appropriate conditions for further analysis.

Pharmacognostical Evaluation

Pharmacognostic evaluation of the finished Ardhamatrika Basti formulation was carried out, including organoleptic examination such as colour, odour, taste, and appearance. Microscopic examination of the formulation was also performed to assess the presence of characteristic features of the constituent drugs. The analysis was conducted at the PIAR pharmacy and Vasu Research Laboratory Vadodara, Gujarat.

Physicochemical Analysis

Physicochemical parameters of the Ardhamatrika Basti formulation were evaluated using standard procedures. Parameters such as **specific gravity** and **density** were determined to assess the physical consistency and stability of the formulation.



Maitree Patel *et al.*,**High-Performance Thin-Layer Chromatography (HPTLC)**

HPTLC analysis was carried out as per standard guidelines to develop a chromatographic fingerprint of the Ardhamatrika Basti formulation. The analysis was performed to assess chemical consistency and stability of the formulation over the study period.

Stability Study

An accelerated stability study was conducted to evaluate the stability of the ready-to-use Ardhamatrika Basti formulation. The formulation was assessed at predetermined intervals from **Day of preparation to 6th month** for organoleptic characteristics, physicochemical parameters, microbial load, and HPTLC profile to detect any changes over time.

OBSERVATION AND RESULT**Organoleptic Evaluation**

The organoleptic characteristics of Ardhamatrika Basti remained unchanged throughout the study period. The formulation exhibited a light brown colour and characteristic odour at both 0 month and 6 months, indicating no visible physical deterioration during storage entitled in Table 1.

Physicochemical Analysis

Physicochemical parameters of the formulation at 0 and 6 months are presented in Table 1. The pH of the formulation showed a marginal decrease from 5.67 to 5.48, which remained within an acceptable acidic range and did not indicate instability. Total solid content remained almost constant (89.27% to 89.46%), suggesting good compositional stability. A slight increase in specific gravity (1.015 to 1.019) and minimal variation in viscosity (4.43 to 4.24cP) was observed, which were not considered pharmaceutically significant. These findings indicate that the formulation maintained physical and chemical consistency over the 6-month storage period.

Microbiological Analysis

Microbiological evaluation revealed a marked improvement in microbial quality over the study period. The total microbial plate count reduced from 3449 cfu/g at 0 month to <10 cfu/g at 6 months, while yeast and mould counts, initially present at 720 cfu/g, were absent at 6 months. Pathogenic organisms including *Staphylococcus aureus*, *Salmonella* spp., *Pseudomonas aeruginosa*, and *Escherichia coli* were absent at both time points, confirming the microbial safety of the formulation.

HPTLC Fingerprint Analysis

HPTLC fingerprinting of Ardhamatrika Basti at 0 and 6 months demonstrated as shown in figure 1 are identical chromatographic profiles at 254 nm, 366 nm, and 540 nm. At 254 nm, nine spots with identical R_f values (0.17–0.82) were observed in both samples. Similarly, at 366 nm and 540 nm, the number of spots and their respective R_f values remained unchanged between the two time points.

The overlapping chromatographic fingerprints indicate no qualitative changes in chemical constituents, confirming the chemical stability and consistency of the formulation during the storage period.

DISCUSSION

The present study aimed at the development and analytical evaluation of an instant mix Ardhamatrika Basti using a standardized homogeneous preparation method. In this formulation, Makshika (Madhu), Sneha, Kalka, and Kwathadravya were combined systematically to ensure uniform mixing of immiscible components, followed by the addition of 0.1% Amr salt as a preservative.[5] This approach was adopted to address practical limitations associated with classical Basti preparation, which requires repeated fresh preparation, skilled manpower, and strict procedural



Maitree Patel *et al.*,

precision at each administration. Analytical evaluation revealed that key physicochemical parameters, including pH, viscosity, and total solid content of the instant mix Ardhmatrika Basti, were comparable to those of the routinely prepared classical formulation. These findings suggest that the instant mix retains essential pharmaceutical characteristics required for safe administration. Accelerated stability testing further demonstrated a shelf life of six months, with no significant changes observed in organoleptic, physicochemical, microbiological, or HPTLC parameters. Identical chromatographic fingerprints at 0 and 6 months confirmed the chemical stability and consistency of the formulation. From a practical perspective, the development of a ready-to-use Ardhmatrika Basti offers significant advantages for routine clinical practice. The instant mix formulation reduces preparation time, minimizes the need for trained manpower, and eliminates variability associated with repeated bedside preparation. This facilitates ease of administration in outpatient, inpatient, and institutional settings, thereby improving feasibility and standardization of Basti therapy in day-to-day practice. Classical Ayurvedic texts such as Vangasena and Chakradatta emphasize the suitability of Ardhmatrika Basti for repeated and even daily administration, particularly in chronic disorders, without compromising safety. The instant mix formulation aligns with these classical indications while adapting the therapy to contemporary healthcare environments. By simplifying preparation without altering the fundamental composition, this approach serves as a bridge between classical Basti principles and modern clinical workflow. Despite its wide classical indications, systematic scientific evaluation of Ardhmatrika Basti has remained limited. The present study contributes by providing pharmaceutical standardization, analytical validation, and stability data for a ready-to-use formulation. This lays a foundation for future clinical trials and facilitates broader acceptance of Basti therapy within integrative and evidence-based healthcare systems.

CONCLUSION

The instant mix Ardhmatrika Basti developed in this study demonstrated satisfactory analytical stability and quality over a six-month period. The standardized homogeneous preparation method reduces preparation time and manpower requirements, making the formulation easy to use and suitable for routine clinical practice. The findings support the feasibility of this ready-to-use formulation and provide a basis for further clinical evaluation.

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Table 1: Organoleptic, Physicochemical, and Microbiological Evaluation of Ardhmatrika Basti at 0 and 6 Months

Sr.No	Parameters	0 month	6 month
Organoleptic Analysis			
1	Description	Light brown coloured basti	Light brown coloured basti
2	Odour	Characteristic	Characteristic



Maitree Patel *et al.*,

Physico-chemical Analysis			
1	PH Analysis	5.67	5.48
2	Total Solid content	89.27%	89.46%
3	Specific Gravity	1.015 cp	1.019 cp
4	Viscosity by Ostwald	4.43 cp	4.24 cp
Microbiological analysis			
1	Total Microbial Plate Count	3449 cfu/g	< 10 cgu/g
2	Total Yeast & Mould Count	720 cfu/g	Absent
3	Staphylococcus aureus	Absent	Absent
4	Salmonella sp.	Absent	Absent
5	Pseudomonas aeruginosa	Absent	Absent
6	Escherichia coli	Absent	Absent

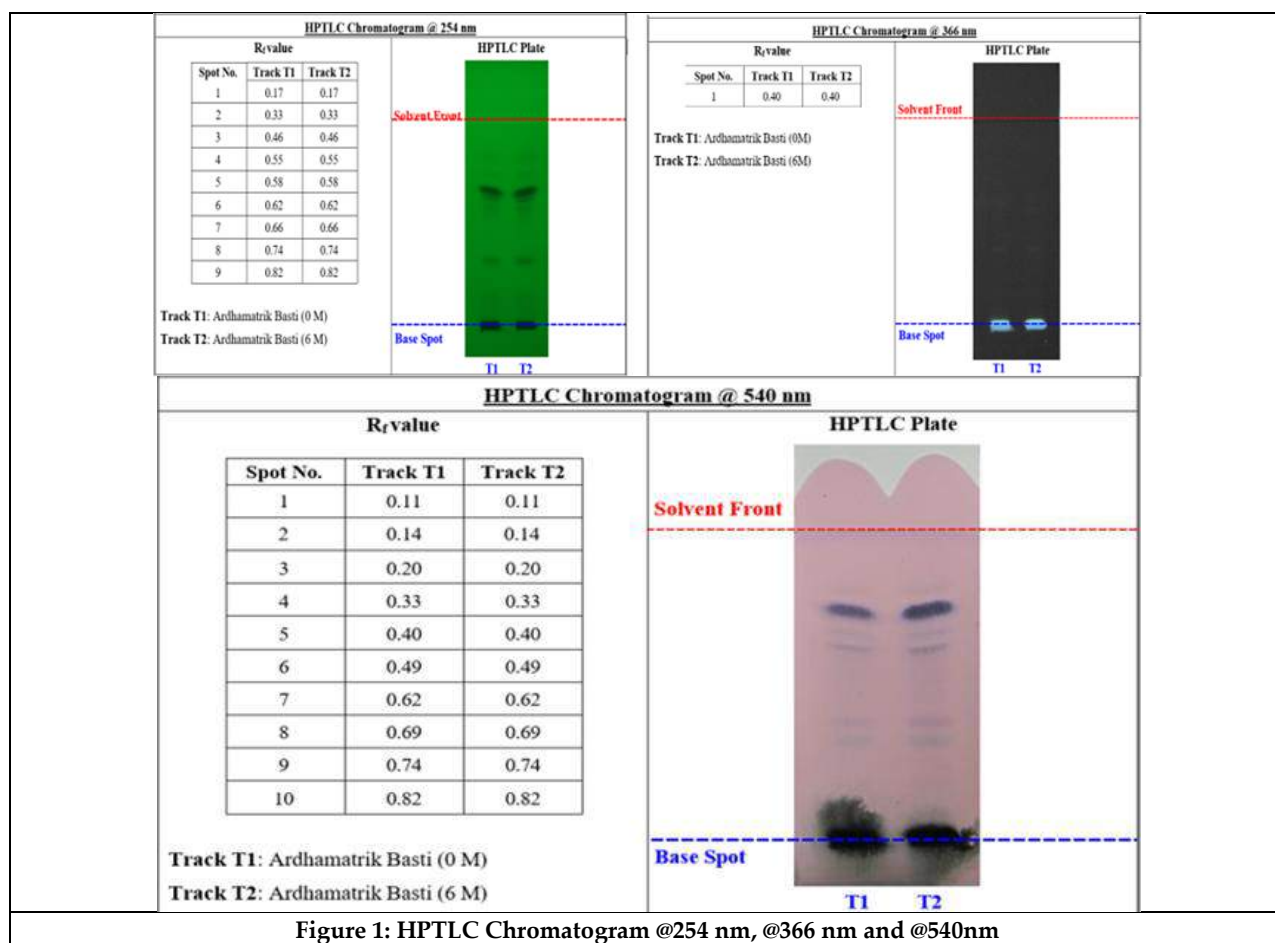


Figure 1: HPTLC Chromatogram @254 nm, @366 nm and @540nm

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RESEARCH ARTICLE

Urban - Industrial Air Quality Divergence in Eastern India: A Multi-City Assessment of PM_{2.5}, PM₁₀, SO₂, and NO₂ Across Ranchi, Dhanbad, and Jamshedpur, Jharkhand, India

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ABSTRACT

The air in the three main cities of Jharkhand- Ranchi (the state capital), Dhanbad (a coal belt town), and Jamshedpur (a steel town)- is polluted in different ways. Moreover, the polluted state of air reveals two different types of anthropogenic emission regimes, but both are disturbing at the same time. The study combines two years (2022–2023) of primary field observations in five land-use zones of Ranchi with secondary published data from CPCB, JSPCB and peer-reviewed papers for Dhanbad and Jamshedpur to develop the first systematic tri-city comparative air quality assessment for the region. The monthly concentrations of PM₁₀, PM_{2.5}, SO₂ and NO₂ alongwith eight meteorological parameters measured at Ranchi have been interpreted through a comparative framework placing the pollution at Ranchi alongwith Dhanbad, a coal dominated city and Jamshedpur, an industrially intensive city. Continuous monitoring of PM_{2.5} at Kantatoli road traffic intersection, Ranchi shows unregulated use of roads and construction of temporary and permanent infrastructures with non-measured impacts on air pollution. In 2022, PM_{2.5} reached a level at an annual mean of 111.44 µg/m³, which is 2.79 times the National Ambient Air Quality Standards (NAAQS) of India and 22.3 times the WHO 2021 guideline value of 5 µg/m³. PM₁₀ at all five sites exceeded NAAQS during both the years. The NAAQS permissible limit for NO₂ at Kantatoli during winter-inversion months for 24 hr is approaching at 5.9% per annum increase. Out of the three cities, Dhanbad exhibits the maximum burden of coal dust and SO₂ due to open-cast mining and thermal power activities, while Jamshedpur shows high particulate and metallic aerosol loading due to integrated steel-making. In Ranchi, with not much heavy industrial presence, the NO₂ value was highest due to the rapidly growing vehicular population. Between 2022 and 2023, Ranchi, urban sites experienced a continuous inter-annual deterioration trend despite greater monsoon rainfall in 2023. It is clear that the emissions growth is the primary driver behind this. The air quality buffering capacity of intact forest cover has



**Sandeep Prasad and Namita Lal**

been quantified and NAAQS and WHO compliance of all the three cities has been evaluated along with city-specific emission profiles policy prescriptions.

Keywords: Urban air quality; PM_{2.5}; PM₁₀; SO₂; NO₂; Ranchi; Dhanbad; Jamshedpur; Jharkhand; NAAQS; WHO guidelines; coal mining pollution; steel industry emissions; vehicular exhaust; meteorological determinants; seasonal variation; urban heat island; source apportionment; health risk assessment; eastern India; Tier-2 cities

INTRODUCTION

Ambient air pollution contributes to a staggering 1.67 million premature deaths in 2019 in India or nearly 18 per cent of total deaths in India. Gaubert et al's (2020) estimate might be the tip of the iceberg as more severe estimates exist. Long-term exposure to fine particulate matter (PM_{2.5}) and other gaseous pollutants causes various health impacts including chromosome damage (deoxyribonucleic acid or DNA damage and genetic mutations) and cardiovascular disease, among many others. According to Lelieveld et al. and Landrigan et al., children and elderly are most affected. The launch of the NCAP scheme in 2019 by India and its target of the 20%-30% reduction of PM_{2.5} and PM₁₀ concentration by 2024 in 122 non-attainment cities shows the seriousness of the regulations. Implementation shortcomings are serious especially in any cities not mentioned above which are not CPCB. Ranchi, Dhanbad, and Jamshedpur the three prime cities of Jharkhand host industrial and infrastructural activities of national economic importance but the ambient air quality literature of the region is sporadic, methodologically inconsistent and comparative. Dhanbad city which is in the Jharia coalfield and known as the coal capital of India has coking coal. It is one of the largest coalfields in Asia. Continuous dust, SO₂ and volatile organic compound (VOC) emissions from the opencast mining, underground fire, washeries and coal-based thermal power at this place are chronic (Singh et al., 2021; Patra et al., 2016). The headquarters of Tata Steel's flagship integrated steel plant, Jamshedpur has a clear metallic chemical signature. This suspended particulate matter has iron oxide, manganese, chromium, and other coke oven by-products which contribute to the known carcinogen polycyclic aromatic hydrocarbons (PAHs) in the urban airshed (Ghosh et al. (2019); Srivastava & Jain (2020)). The capital of the state, Ranchi does not host that kind of heavy industry but the vehicular fleet, construction activity, and mixed-use densifying are all growing at a decent clip that is generating out here a pollution profile that is different from neighbouring parts of India. Ranchi is dealing with pollution because of heavy traffic.

Multiple studies in the scientific literature show an increasing focus on Indian urban air quality, especially NCR Delhi, Indo-Gangetic Plain megacities, and coastal metropolitan cities. Located in Eastern India, the collection of small towns Jharkhand, Odisha and Chhattisgarh is represented by a modest work. In 2020, Kumar and colleagues conducted a research on PM_{2.5} source profiles in six Indian urban locations excluding Jharkhand. Gupta and others in 2021 documented PM variability seasonally in urban-industrial sites of Jharkhand at only one monitoring station. The objective of this study is to fill a knowledge gap that exists due to the absence of a multi-city comparative framework with a standardized methodology and contemporaneous weather characterization.

Three cities offer a unique natural experiment in identifying emission sources. By studying a traffic-urbanization dominant city, Ranchi, a coal extraction dominant city, Dhanbad and a metallurgical-industrial dominant city, Jamshedpur, the study intends to identify how the differences in source sector reflect differences in pollutant composition, seasonality and exposure to health effects. These cities belong to the same biogeographic region, same monsoonal system and same regulatory framework. Such comparison will have high importance for designing of targeted policies under NCAP, which require city-wise emission characterization instead of a common control intervention, action plans.

Research Objectives

- Assess the monthly and annual concentrations of PM₁₀, PM_{2.5}, SO₂, and NO₂ across five land-use zones of Ranchi for the year 2022–2023 using primary field measurements.



**Sandeep Prasad and Namita Lal**

- Create a comparative framework of air quality by integrating primary data from Ranchi with published secondary data from the monitoring networks of CPCB/JSPCB and peer-reviewed literature from Dhanbad and Jamshedpur.
- The goal is to identify the dominant anthropogenic emission sources causing spatial heterogeneity within Ranchi and sectoral difference across the three cities.
- This study aims to assess compliance with NAAQS (2009) and WHO Air Quality Guideline (2021) at all three cities, quantify magnitude and trend of exceedances
- Examine the meteorological factors like temperature, rainfall, humidity, wind speed and direction, solar radiation, and atmospheric pressure affecting the dispersion and accumulation of pollutants.
- Public health risk exposure characterization and formulation of pan-city differentiated evidence-based policy recommendations in pursuit of India's NCAP objectives.

New Features and Research Contribution

This research has important scientific contributions. To begin with, it offers the most extensive primary multi-parameter air quality dataset ever compiled for Ranchi to date over 24 months from five land-use zones with a temporal and spatial depth that is lacking in any previous literature from Jharkhand. Secondly, It builds a comparative tri-city framework of the three largest cities of Jharkhand which has not so far been published in any SCI-indexed journal, to the best knowledge of the authors. This state-of-the-art report expands the knowledge base on inter-annual variability in air quality, highlighting that Ranchi's air quality is adversely impacted by emission growth rather than meteorological variation. Use of the 2023 monsoon intensity as a natural control variable is significant and helps further our understanding of this problem. In addition, it provides quantified evidence for the air quality co-benefit of intact urban forest cover data that can be directly leveraged to inform green infrastructure investment decisions and decision-making around NCAP implementation. The research investigation fills a documented gap in the eastern Indian Tier-2 city air quality literature and provides the scientific baseline needed to monitor the effectiveness of future policy interventions across Jharkhand.

LITERATURE REVIEW**Air Pollution in Indian Tier-2 Cities and Eastern India**

The course of research on Indian air quality has been disproportionately influenced by the crises observable in National Capital Region Delhi, where $PM_{2.5}$ concentrations commonly go above $300 \mu g/m^3$ in winter episodes. (Mahato et al., 2020; Guo et al., 2020). Yet, data from CPCB's National Ambient Air Quality Monitoring Programme (NAMP) reveals that exceedances in the NAAQS standards are much more geographically widespread than the Delhi narrative suggests. In their 2019 study Balakrishnan et al. used satellite-derived $PM_{2.5}$ data to estimate that more than 660 million Indian live in places where annual $PM_{2.5}$ concentration exceed WHO guideline. Moreover, huge populations from Tier-2 and Tier-3 cities face $PM_{2.5}$ exposure level that are comparable to exceed those found in megacities. The monitoring infrastructure shortcoming in secondary Indian cities is a barrier to evidence-based action Singh et al. (2022) argue that the existing NAMP networks monitor less than 30% of exposure of India's urban population. The industrial geography of eastern India, which is mainly based on coal, steel, aluminum and heavy engineering in Jharkhand, Odisha and Chhattisgarh, generates pollution characteristics which are quite different from the crop burning and traffic of Indo-Gangetic Plain studies. Patra et al. (2016) undertook one of the early systematic evaluations of air quality in a coal mine area in the belt region of Jharia coalfield, and levels of PM_{10} were above $400 \mu g/m^3$ in areas near coal loading. Senior researchers Srivastava and Jain wrote a research work to identify the growth of particulate matter in Jamshedpur. In this research work, it has been stated that the major crustal and industrial trace elements of Srivastava & Jain are iron oxide, silica and aluminum. According to Ghosh et al. (2019), benzene and PAH concentrations were elevated in the vicinity of coke oven units of the industrial belt of eastern India. The studies point to the fact that industrial cities of East India face a qualitatively different type of pollution than elsewhere in the country.



**Weather factors of urban pollution in subtropical India.**

India's monsoon system is the primary meteorological factor influencing subcontinent-wide variations in the seasonality of urban air quality. Wet scavenging is the removal of pollutants by rain. During the Southwest Monsoon (June – September), a substantial fraction of both particulate matter and soluble gaseous pollutants are removed by wet scavenging. As SO₂ dissolves readily in rain, sulphurous and sulphuric acid are formed (Parashar et al., 2016). This happens due to the hygroscopic growth and below-cloud wet scavenging of accumulation-mode particles. Reduced mixing heights of boundary layer during winter (November to January) due to radiative cooling at the surface and weak convective circulation create the optimum conditions for maximum build-up of pollutants (Dey & Tripathi, 2007). The plateau regions of India, at latitudes of roughly 23° to 40°N, experience seasonal temperature inversions which trap emissions within shallow atmospheric layers. This phenomenon increases exposure levels, with the worst exposures occurring during the season when emissions are the highest (Kumar et al. 2016). The urban heat island (UHI) effect increases the dynamics in built areas. Research by Mohan et al. (2012) established the UHI intensities in Indian cities to be in the range of 2 – 5°C. This study also highlighted the role of the UHI-induced secondary circulations in mixing. This mixing can lead to the reduction of pollution concentrations in certain cases. However, it also gives rise to local convergence zones that enhance the pollutant concentrations downwind the area of strong flux of heat. In Indian monsoon circulation, the change in the wind direction from northerly in winter to south westerly in the monsoon impacts cross-city pollutant transport pathways in specific cities. This is especially true for the geographically proximate Ranchi–Dhanbad–Jamshedpur cluster. Here, industrial emissions from one city can impact the airshed of another city under specific wind regime as suggested by Yadav et al. (2019).

Vehicular Emissions and Traffic-Derived Pollution

The Indian traffic corridors' main source of NO₂, fine particulate matter especially its elemental carbon and organic carbon fractions and carbon monoxide is vehicular exhaust (Pant & Harrison, 2013; Goel & Kumar, 2015). Vehicle fleet in India has been increasing at about 8-12% annually in Tier-2 cities. For instance, two-wheelers have 60-70% share of the fleet mix in the cities such as Ranchi (CPCB 2021). These two-wheelers are mainly older carbureted two-wheelers with poor emission characteristic. Following the implementation of new emission rules BS-VI in April 2020, new vehicles now emit less NO_x and particulates per vehicle. However, the vast legacy fleet of pre-BS-VI vehicles continues to be the leader of actual on-road emissions. Anand et al. (2021) estimated that greater than 60% of vehicular PM_{2.5} emissions from Indian Tier-2 cities come from pre-BS-IV cars which form less than 35% of the fleet. Patterns of traffic congestion during the morning and evening. 07:00 – 10:00 hours and 17:00-20:00 hours, respectively, cause episodic concentration events. 24-hour NAAQS limits may be breached in rush hours only. They may comply with the National Ambient Air Quality Standards (NAAQS) standards on a daily or monthly average level.

Pollution through coal mining and thermal power at Dhanbad

Dhanbad occupies a central position in the Jharia coalfield. It has an estimated 3.5 billion tonnes of recoverable coking coal. Moreover, it has more than 100 operational collieries, 9 coal washeries and few coal-based thermal power stations (within 50-km radius) (Central Mine Planning and Design Institute, 2021). The Bokaro Thermal Power Station, Chandrapura Thermal Power Station, and the Tenughat thermal power station located in Dhanbad–Bokaro urban cluster emit thousands of tonnes of SO₂, fly ash every year. The activities of blasting, loading and hauling in open-cast coal mining cause high-intensity fugitive dust events. Such operations can increase PM₁₀ concentrations to a level higher than 500 µg/m³ in the proximate community of the mine (Patra et al. 2016). For more than a century, underground coal seam fires have been burning in the Jharia coalfield and they represent an essentially uncontrolled source of emission of CO, SO₂ and particulate carbon that is unique thus far in India (Singh et al., 2021). According to NAMP Data of CPCB, Dhanbad has been found to have SO₂ concentrations many times dating back 2012, close to, and exceeding NAAQS limits. Dhanbad, a highly polluted area, shows a sharp contrast with Ranchi and Jamshedpur. The concentrations of SO₂ are compliant with NAAQS in Ranchi and Jamshedpur.





Steel industry and metallurgical pollution in Jamshedpur

Tata Steel's integrated plant in Jamshedpur is one of Asia's biggest and oldest integrated plants with an installed capacity of more than 10 million tonnes per annum. Further, it is an emission complex consisting of sintering, blast furnace, basic oxygen furnace steelmaking, coke ovens, rolling mills and raw material handling. Each process unit has its own signature of pollutants. There are SO₂, NO_x, and metallic particulates from the sinter plants; CO and fine iron oxide particulates from the blast furnaces; PAHs, benzene, and H₂S from the coke ovens; and fugitive iron ore and coking coal dust from raw material handling (Ghosh et al., 2019; Srivastava & Jain, 2020). The water-soluble fraction of air PM_{2.5} from Jamshedpur shows higher iron and heavy metals (Mn, Cr, Pb) and lower organic carbon to elemental carbon ratios as compared to those from Ranchi and Dhanbad, indicating metallurgical source like steel production. Tata Steel has been at the forefront of environmental management for many decades. Its Meghalaya plant has a well-made use of perchlorate and all other necessary controls. Moreover, such massive investments, as well as the cumulative compliance status (JSPCB, 2022), pollution control keeps Jamshedpur measured NAAQS compliance status better than what its industrial intensity would suggest. Nevertheless, with recent fleet growth, NO₂ conformity reveals a more complex picture.

Health Burden of PM_{2.5} in South Asian Cities

In recent years, a lot of epidemiological evidence has come out linking long-term PM_{2.5} exposure to increased mortality and morbidity in South Asia. According to Balakrishnan et al. (2019), PM_{2.5} exposure was responsible for 980,000 deaths early in India in 2017. The major disease endpoints were chronic obstructive pulmonary disease (COPD) and ischemic heart disease. The World Health Organization (WHO) Comparative Risk Assessment framework indicates that there is a 6–7% rise in all-cause mortality with every 10 µg/m³ increase in long-term PM_{2.5} exposure (WHO, 2021). During the first three years of life, when the lungs are still developing, children develop respiratory diseases as a result of exposure to PM_{2.5} pollution. If the level of PM_{2.5} pollutants exceeds 35 µg/m³, research has shown measurable deficits in the development of lung function, which continue to exist through adulthood. (Landrigan et al, 2018) The disease burden attributed to these dose- response relationships for cities in Jharkhand, where annual PM_{2.5} means are in the range of 60–110 µg/m³ or more, is large and unquantified in any city-specific health impact assessment.

Study Area Description

Ranchi – The Rapidly Urbanizing State Capital

The Chota Nagpur Plateau is a tectonically stable crystalline basement. As such, it is a part of the Peninsular Shield of India. It is located in Ranchi (23°N, 85°E; elevation 651 m AMSL). The average elevation of the plateau produces a humid subtropical climate (KöppenCwa) cooler than the neighbouring Gangetic plains. The mean annual temperature of the region is around 24 °C. Annual rainfall is about 1,400–1,500 mm and concentrated in the Southwest Monsoon (June–September). Since its designation as the capital of the newly formed state of Jharkhand in 2000, Ranchi has been witnessing continuous demographic growth from approximately 0.85 million in 2001 to more than 1.4 million in the metropolitan area in 2023. Further it has witnessed unplanned extension of the city, significant infrastructure construction and exponential growth of the vehicular fleet. The focus of industrial activity is in the Tupudana, Namkum and Tatisilwai corridors where foundries, engineering works and small to medium manufacturing units are located. The busy commercial areas of Kantatoli, Albert Ekka Chowk, and Lalpur are the city's most heavily trafficked zones, which experience high-intensity pulses of mobile-source emissions due to congested vehicles as shown in map in Figure 1 below.

Dhanbad – The Coal Capital

Located in eastern Jharkhand at a distance of 130 km northeast of Ranchi is Dhanbad (23.8°N, 86.4°E; elevation 227 m AMSL). Dhanbad, located in Jharkhand, India, is called the coal capital of India. It is situated on the Jharia coalfield which contains the largest deposits of prime coking coal in India. The economy of the city, which has a population of around 1.2 million (Urban Agglomeration), is dominated by coal mining and coal processing, thermal power generation and auxiliary activities. Jharia coalfield has over 100 running collieries operated mainly by Bharat Coking Coal Limited (BCCCL), a Coal India Limited subsidiary with many illegal miners operating in the area Close



**Sandeep Prasad and Namita Lal**

proximity to Bokaro Steel City and several thermal power stations (like Chandrapura TPS, Bokaro TPS, Tenughat TPS) has created a multi-source airshed extending much beyond the administrative city limits. Dhanbad's lower elevation as compared to Ranchi and location in a more open terrain results in somewhat less trapping of terrain-driven pollution. Nevertheless, summer temperature inversions do create episodic accumulation events.

Jamshedpur – The Planned Steel City

Jamshedpur (22.8°N, 86.2°E; elevation 135 m AMSL) was established as a planned industrial township by Tata Steel (then Tata Iron and Steel Company) in 1907. It is located about 160 km south of Ranchi. Tata Steel Jamshedpur plant, which sprawls over 3000 acres, is one of the largest integrated steel production centres in Asia producing more than 10 million tonnes of crude steel. The metallurgical industrial complex of the city, comprising of blast furnaces, coke ovens, sinter plants, rolling mills, and extensive raw material (iron ore and coal) handling facilities, generates fugitive dust continuously. In addition to industrial emissions, Jamshedpur's population of about 1.3 million in the urban agglomeration has generated increasing vehicular traffic particularly in commercial areas beyond the controlled Tata Steel township boundary. The industrial environmental management system of Jamshedpur is relatively more sophisticated and provides better continuous emission monitoring data at main point sources as compared to Dhanbad's spread-out mining.

Ranchi Primary Monitoring Site Network

Five monitoring sites were selected in Ranchi using stratified purposive sampling to reflect the entire range of land-use conditions. These were Tupudana Industrial Area (Site 1: 23°15'56"N, 85°18'10"E), Kantatoli Traffic Hub (Site 2: 23°21'52"N, 85°20'43"E), Harmu Residential Zone (Site 3: 23°21'12"N, 85°18'30"E), Kanke Agricultural Area (Site 4: 23°27'19"N, 85°19'19"E) and Birsa Zoological Park Eco-Sensitive Area (Site 5: 23°27'48"N, 85°27'24"E). As this stratification allows for gradient analysis covering urban to rural and industry to green transects, it captures the entire range of emission environments in the city (Table 1).

MATERIALS AND METHODS**Primary Monitoring Methodology – Ranchi**

Using calibrated high-volume gravimetric air samplers that operate on USEPA equivalent methods (as per CFR 40 Part 50, Appendix J and L), particulate matter (PM₁₀ and PM_{2.5}) was measured through 24-hour integrated sampling at each site once every month. Weight of the filter samples was taken twice (before and after the sampling) on an analytical balance (sensitivity ±0.01 mg) in a temperature and humidity controlled environment. SO₂ was determined by West-Gaeke modified pararosaniline colourimetric method (IS: 5182 Part 2) and NO₂ was quantified by modified Jacob-Hochheiser sodium arsenitecolourimetric method (IS: 5182 Part 6), which are methods prescribed by CPCB SOPs. Weather stations, or automatic weather stations (AWS), are used to measure various meteorological parameters. These AWS were installed either at the site or in close proximity (within 10m) of the air quality monitoring stations. The complete period of the study was from January 2022 to December 2023, which generated 12 monthly mean observations from each parameter at each site.

Secondary Data Sources – Dhanbad and Jamshedpur

The secondary air quality data for Dhanbad and Jamshedpur has been compiled from the following published and institutional sources: (i) the annual reports of the NAMP of CPCB for the Jharkhand monitoring stations at Dhanbad and Jamshedpur; (ii) the Annual Environmental Status Reports and Air Quality Monitoring Reports of JSPCB for the years 2019–2023; (iii) the NCAP progress reports of the Jharkhand non-attainment cities; (iv) peer-reviewed studies published in journals from ScienceDirect, Springer and Elsevier containing Dhanbad and Jamshedpur air quality; and (v) satellite-derived PM_{2.5} datasets from MERRA-2 and GBD environmental pollution datasets. When multiple secondary sources gave overlapping data, which was available for the same year and the same station value, range values were given with the source citation and means were computed if the source value was three and above.



**Sandeep Prasad and Namita Lal****Statistical Methods**

The arithmetic mean, standard deviation (SD) and standard error of the mean (SE = SD/square root of n ; where $n = 12$ monthly observations) of all primary Ranchi data was worked out. SD quantifies month-to-month variation for each year, while SE indicates the accuracy of the year's mean estimate. To compare the various pollutants and the meteorological parameters at each site in Ranchi (temperature, rainfall, humidity, wind speed), Pearson correlation coefficients were determined for the individual pollutants. They were statistically assessed at $p < 0.05$ using a two-tailed t-test. Seasonal analysis was conducted on monsoon (June–September) and non-monsoon (October–May) period. The $PM_{2.5}/PM_{10}$ ratios were calculated for each site as a diagnostic of source attribution. Both absolute ($\mu g/m^3$) annual changes and proportional changes were expressed between 2022 and 2023.

Reference Framework for Regulation

All the concentrations which are measured are compared to annual and 24hour average of India's National Ambient Air Quality Standards (NAAQS, 2009) and WHO Global Air Quality Guidelines (2021) annual reference values (Table 2).

Meteorological Characterization of Ranchi**Temperature and Urban Heat Island**

Temperature data across Ranchi's five sites shows the four-phase seasonal architecture of the humid subtropical Chota Nagpur Plateau cool dry winter (January–February: 14.5–19.5°C) sharp pre-monsoon heat peak (April–May: 26.5–32°C) thermally moderated monsoon period (June–September: 24–29°C) post-monsoon transition (October–November: 20–25°C). Kantatoli (Site 2) recorded the maximum temperature which reached 31.5 °C in May 2023. This may be due to the effect of urban heat island, high impervious surface fraction and low albedo of road surface along with heat waste from vehicles. Birsa Zoological Park (Site 5) recorded temperatures 3–4 °C lower than those at Kantatoli in summers, indicating the forest canopy incorporates the ability of closed forests to buffer against temperature through evapo transpiration (Table 3). When comparing the months of 2023 with corresponding months of 2022, all sites show a consistent warming signal of 0.2–0.7°C, which is directionally consistent with documented trends of UHI intensification in fast-growing Indian cities.

Rainfall, Humidity, Wind Speed, and Solar Radiation

Rainfall during the monsoon season dominates the annual water cycle at all sites and contributes 75–80% of annual precipitation, i.e., during the months of June to September. In July 2023, rainfall was greater than the rainfall in 2022 universally over Ranchi. Kantatoli got 348 mm of rain (335 mm in 2022, +3.9%), Birsa Zoo 350 mm (340 mm, +2.9%), Harmu 345 mm (330 mm, +4.5%). Annual totals at all sites are higher by 6.5–7.3% from 2022 to 2023. This persistent higher rainfall in 2023 is scientifically important, as it sets up a natural experiment in which a higher wet scavenging efficiency should have produced lower 2023 pollutant concentrations compared to 2022. However, the opposite was observed at the urban sites, isolating emission growth as the controlling factor. The peak relative humidity from July to August was 85–91 %. Wind speed showed a seasonal trend. The wind is more in pre-monsoon months (12–15 km/h in May–June) due to thermal convective gradients. The wind becomes low (05–7 km/h) during calm winter months. Over the months of December to February, the wind direction were mostly from the north. This had changed to south westerly during the monsoon. This change in wind direction is a direct expression of the reversal of the Indian monsoon circulation. The cold winds from the north at winter time have the potential for cross-site pollutant transport from the Tupudana industrial zone northwards towards southern residential areas. Solar radiation was high (240–260 W/m²) during April–May at urban sites. It was lower (170–250 W/m²) at Birsa Zoo due to canopy light interception.

Air Quality Results and Analysis — Ranchi**Particulate Matter PM_{10}**

The concentration of PM_{10} has become more than the annual NAAQS standard of 60 $\mu g/m^3$ at all the five Ranchi sites in both years. This suggests that the PM pollution is not location-specific but a systemic one. The mean annual values ranged from 97.92 $\mu g/m^3$ (Birsa Zoo, 2020) to 109.62 $\mu g/m^3$ (Tupudana, 2020) which was 163% to 183% of NAAQS

117696



**Sandeep Prasad and Namita Lal**

limit and 553% to 631% of WHO limit (Table 4). The small standard deviations ($0.98\text{--}2.38\text{ }\mu\text{g}/\text{m}^3$) imply that the month-to-month concentrations of PM_{10} are relatively stable, which is quite unexpected due to the natural washout effect of the monsoon. Month-to-month data makes it evident that indeed monsoon months do show the lower range of values of PM_{10} (for instance, Tupudana August 2022 PM_{10} 106.5 versus January 2022 PM_{10} 114.2 $\mu\text{g}/\text{m}^3$). But the absolute magnitude of reduction is small compared to a high baseline. By construction activities, unpaved roads and dry soil, resuspension of crustal dust is causing a significant contribution to PM_{10} of Ranchi since crustal sources are washed away by wet deposition, which is much less as compared to combustion-generated aerosols.

Fine Particulate Matter $\text{PM}_{2.5}$ – The Critical Parameter

The $\text{PM}_{2.5}$ readings indicate the most disturbing air quality situation in the data set. In 2022, the annual mean at the Kantatoli Traffic Hub (Site 2) was $111.44\text{ }\mu\text{g}/\text{m}^3$, which was 279% of the NAAQS limit and a remarkable 2,229% of the WHO 2021 guideline value of $5\text{ }\mu\text{g}/\text{m}^3$. Tupudana Industrial Area (Site 1) recorded $99.34\text{ }\mu\text{g}/\text{m}^3$ which is 148% higher than NAAQS. The annual mean at even the cleanest site in the network, Birsa Zoological Park (Site 5), which is also the reference forest ecosystem, was $62.48\text{ }\mu\text{g}/\text{m}^3$, i.e., 56% above NAAQS. The findings suggest that regional background $\text{PM}_{2.5}$ loading thermal over the Chota Nagpur Plateau itself is too high to be health protective. This elevated loading which reflects the cumulative effect of regional coal burning, long-distance transport from the Pollution pool of the Indo-Gangetic Plain and biomass burning emissions from surrounding rural Hinterland. The relationship between $\text{PM}_{2.5}$ and PM_{10} indicates the source. At Sites 1 and 2, the value remained above 0.90 for 2022. This indicates that over 90 % of total particulate mass originates from combustion source fine particles. By international standards, this is exceptionally high (normal urban backgrounds typically range 0.50–0.70). They point to dominance of vehicular exhaust and industrial combustion aerosols. When measurements were taken at Site 5 (Birsa Zoo), the ratio dropped to around 0.64 which is more in line with background conditions. This is the case where a proportional mixture of crustal dust and other source contributions could be present. Seasonally, $\text{PM}_{2.5}$ was more variable than PM_{10} at all sites. At Kantatoli, in the month of August which is the monsoon minimum it was $101.5\text{ }\mu\text{g}/\text{m}^3$. Moreover, in the month of September it was $116.4\text{ }\mu\text{g}/\text{m}^3$. $116.4\text{ }\mu\text{g}/\text{m}^3$ is after-monsoon that is maximum due to several reasons including open burning and atmosphere imparting (fast) drying initiation. In 2023, $\text{PM}_{2.5}$ at Tupudana was 7–10 $\mu\text{g}/\text{m}^3$ higher in non-monsoon months than in 2022. This figure translates to 8–10% yearly worsening.

Sulfur Dioxide (SO_2)

At all Ranchi sites, SO_2 levels remained below the NAAQS annual ($50\text{ }\mu\text{g}/\text{m}^3$) and 24-hour ($80\text{ }\mu\text{g}/\text{m}^3$) limits in both years. This is currently the only pollutant in this dataset that is within limits (Table 6). Notable is the inter-annual trend: the year-on-year (YoY) increase of Tupudana was 11.2 per cent (26.76 to $29.76\text{ }\mu\text{g}/\text{m}^3$), which was the largest YoY increase of any pollutant-site combination. SO_2 is the most seasonal pollutant we measure. In December 2022, the monthly mean of sulphur dioxide (SO_2) at Tupudana (Site 1) was $34.86\text{ }\mu\text{g}/\text{m}^3$ while the monthly minimum was recorded at $11.22\text{ }\mu\text{g}/\text{m}^3$ in August. The seasonal range of approximately three times in concentrations is due to three factors operating together. First, the emissions from various sources in the area get trapped in a shallow boundary layer under thermal inversions during winters. Second, there is an increase in domestic burning of biomass for space heating (November–January). Third, there is the absence of wet scavenging during monsoons which otherwise removes SO_2 from the gas phase efficiently. In all sites, December values were higher than in 2022 in 2023 (Site 1: 36.86 vs. $34.86\text{ }\mu\text{g}/\text{m}^3$), as it appears to rise further.

Nitrogen Dioxide (NO_2)

In the dataset (Table 7), NO_2 poses the highest risk of a non-compliance. Kantatoli (Site 2) recorded monthly means of $69.17\text{ }\mu\text{g}/\text{m}^3$ in December, 2022 and $73.22\text{ }\mu\text{g}/\text{m}^3$ in December, 2023. These concentrations are much above the NAAQS annual standard of $40\text{ }\mu\text{g}/\text{m}^3$. The monthly means were approaching the NAAQS 24-hour standard of $80\text{ }\mu\text{g}/\text{m}^3$. Because these values are monthly means, instantaneous hourly peak concentrations during morning and evening rush hours in winter will almost certainly exceed the 24-hour limit on individual days. Tupudana (Site 1) showed a similar high value of $63.40\text{ }\mu\text{g}/\text{m}^3$ in December 2022, which rose to $67.11\text{ }\mu\text{g}/\text{m}^3$ in December 2023. At both sites there was a year on year increase of 5.9% in peak month NO_2 . If the vehicle population of Ranchi keeps



**Sandeep Prasad and Namita Lal**

increasing at the rate of 8-12% per year, Kantatoli is likely to exceed the NAAQS 24-hour standard as a monthly mean in 3-5 years under business as usual scenario. The seasonal cycle of NO_2 emerges from competing processes across the seasons. Primary emission accumulation occurs in winter when atmospheric mixing is lower and traffic activity is sustained. Photochemical transformation occurs in summer when NO_2 is consumed through the $\text{NO}_2 + \text{h}\nu \rightarrow \text{NO} + \text{O}$ reaction that initiates ozone. The strong winter dominance confirms that the effect of primary emissions is stronger than the impact of the photochemical sink during the cold months of Ranchi's. Kantatoli's monsoon minima ($24.39 \mu\text{g}/\text{m}^3$ in July 2022) reflect enhanced atmospheric mixing and reduced traffic during heavy rains and wet removal of soluble nitrogen species.

Comparative Air Quality Analysis: Ranchi, Dhanbad, and Jamshedpur**Comparative PM_{10} and $\text{PM}_{2.5}$ Profiles**

The variation in PM concentration profiles across three cities despite their similar seasonal and regulatory structure (Table 8) controlled by the monsoon. Dhanbad regularly records the highest levels of PM_{10} of the three cities, while CPCB NAMP as per data reports annual means of $130\text{--}180 \mu\text{g}/\text{m}^3$ at mining-adjacent stations in recent years exceedances of 217–300% of NAAQS (CPCB NAMP, 2021–2023; Singh et al., 2021). In Dhanbad, the PM loading is dominated by coarser particles. Therefore, the $\text{PM}_{2.5}/\text{PM}_{10}$ ratio at the monitoring stations is usually less than 0.55–0.65. This shows that open-cast activities and mine haul roads emit a considerable amount of crustal coal dust. The chemical composition of this dust differs from the fine particles. These fine particles are dominated by combustion which has a much heavier contribution in case of traffic sites at Ranchi. In Jamshedpur, the annual PM_{10} means observed at the CPCB-measured stations range from $90\text{--}140 \mu\text{g}/\text{m}^3$, reflecting mixed contributions from steel plant fugitive dust and raw material handling, along with growing vehicular traffic (JSPCB, 2022). The $\text{PM}_{2.5}$ and PM_{10} ratios at industrial sites in Jamshedpur are in the intermediate range (0.60–0.75). This indicates the presence of metallic industrial particulates and combustion aerosol in these sites.

 SO_2 as the Dhanbad Signature Pollutant

The most significant differentiating pollutant parameter in the three cities is SO_2 . The SO_2 burden in Ranchi is moderate and presently NAAQS-compliant. Small foundries, domestic burning of biomass, and limited use of fossil fuels in industries are the reasons responsible for this status. The elevated concentration of SO_2 in Jamshedpur is due to the sinter plant and coke oven operations in the Tata Steel complex. The concentration has nearly reached NAAQS limits during peak production and unfavourable meteorological conditions. The SO_2 profile of Dhanbad is qualitatively different. The burning of coal seam fires continuously at the surface results in uncontrolled emissions of sulfur dioxide which is not a part of any emission inventory and not controlled by any stack based control technology. During winter temperature inversions, the SO_2 from mine fires, thermal power stations, and coal combustion by industry and domestic users can build up at level concentrations that may violate NAAQS mainly by two processes near the mines (Singh et al., 2021). The spatial heterogeneity of SO_2 field at Dhanbad, where SO_2 concentrations are very high near mine fires and decreases to a low value at the central urban monitoring station, implies that the aggregated CPCB NAMP station data under estimates community exposure of populations adjacent to mines.

 NO_2 as the Ranchi Signature Pollutant

Ranchi's NO_2 profile is the most worrying among the three cities with respect to NAAQS limits. Winter monthlies in December and January at Kantatoli Traffic Hub are close to $80 \mu\text{g}/\text{m}^3$, the 24-hour standard. It is quite paradoxical as Dhanbad and Jamshedpur have a bigger industrial emission inventory. Ranchi's vehicular traffic density is high but it occupies a small area. Moreover, it lacks any facilities for dispersion in the atmosphere (few wide boulevards, limited green buffers, high building densities in commercial cores). Also, the weather effect of cooler winter temperature of the Chota Nagpur Plateau suppresses mixing height and concentrates NO_2 . Both Dhanbad and Jamshedpur witness increasing NO_2 concentrations in line with the growth of the vehicular fleet. However, compared to central monitoring stations (JSPCB, 2022; CPCB National Air Quality Monitoring Programme (NAMP), 2023) at Kantatoli Ranchi, the values have not yet reached that level. This observation has serious policy implications:



**Sandeep Prasad and Namita Lal**

traffic management of commercial corridor intersection in Ranchi deserves immediate priority intervention even before industrial controls are taken up.

Seasonal Variation Comparative Analysis

The three cities exhibit the canonical Indian monsoon seasonality: elevated pollution concentrations during winter (November–February) due to thermal inversions, reduced atmospheric mixing, and domestic heating combustion; rapid decline during June–July, when wet scavenging starts with monsoon rainfall; monsoon minima during July–August; and post-monsoon transition surge during September–October, when atmosphere dries and burning starts again. Yet, the seasonal variation extent differs systematically with city and pollutant. Dhanbad has the smallest seasonal PM_{10} amplitude because of its continuous coal mining throughout the year which leads to a near-constant baseline for coarse dust emissions. As such, neither the monsoon rains nor the winter inversions alter the condition very much. The $PM_{2.5}$ seasonal variation in Ranchi is stronger than of PM_{10} . This is consistent with emissions from combustion sources. Although such emissions are available throughout the year, they respond more strongly to meteorological trapping than by crustal dust. The industrial sectors of Jamshedpur, SO_2 , and metallic pm show varying degrees of seasonality. Due to their production schedule, steel companies show more effects of variability which are more prominent than Ranchi mostly dominated by vehicular capacity emissions. (Srivastava & Jain, 2020)

Integrated Source Attribution

Ranchi Site Pollution Hierarchy

Combining all four pollutants across both measurement years generates and a site hierarchy for Ranchi that was statistically robust (Table 9). Kantatoli (Site 2) is the most polluted site for all four pollutants. It is dominated by traffic emissions: peak NO_2 , highest $PM_{2.5}/PM_{10}$ ratio (> 0.93), and typical rush-hour concentration peaks unequivocally point to mobile sources as the main driver. Tupudana (Site 1) is second on the list where high SO_2 and peak $PM_{2.5}$ evidently fossil fuel combustion. Industrial processes metal foundries, smelters and thermal generators are typical to the Tupudana corridor. The SO_2 concentrations recorded during the study period were found to be below NAAQ standards. For instance, at Site 1, which is in proximity to industrial land, the SD was of little relevance.

Inter-City Source Differentiation

The trio of cities offers an excellent natural experiment on the type of industrial emissions. Ranchi exemplifies a model dominated by traffic and urbanization, which results in the patterns of pollution it exhibits. The higher than normal organic carbon content and elemental carbon content suggests diesel and petrol combustion while the higher ratio of NO_2 to NO_2 plus SO_2 indicates mobile source dominance. The formation of secondary inorganic aerosol (that is, nitrates and ammonium sulphates) by precursors of traffic emissions is also a result of the winter formation chemistry that takes place there. Dhanbad is the epitome of the coal extraction model as indicated by the elevated PM_{10} concentrations with a very high crustal fraction (Al, Si, Ca, Fe derived from coal overburden), an anomalously high ratio of SO_2 to NO_2 (which shows that it is not from vehicle emission, but due to the burning of sulphur-rich coal) and the unique signature of coal seam fire emissions (with combustive products at anomalously high temperatures including PAHs, CO, carbonaceous particulates). Jamshedpur serves as the metallurgical industry's prototype. The $PM_{2.5}$ is rich in iron, manganese, chromium and nickel due to steel manufacturing activities, elevated SO_2 resulting from sintering process, and PAH contamination from the coke oven. The targeted emission reduction strategies depend on these source signatures. If a PM filter mandate is effective in reducing PM due to vehicular source in Ranchi, it will have negligible impact on coal dust problem in Dhanbad. Controls that successfully regulate industrial stack emissions at Jamshedpur's point sources will not work at Ranchi. NCAP's city-specific action plans have to consider such source divergences rather than applying uniformly the generic nationwide emission reduction approach.

Public Health Risk Assessment

$PM_{2.5}$ Exposure and Mortality Burden

In Ranchi, all five locations surpassed the $40 \mu g/m^3$ $PM_{2.5}$ NAAQS by 56% to 179%. Therefore, the Government of India continues to fail to protect human health as no resident in Ranchi is breathing compliant air. As per WHO's

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**Sandeep Prasad and Namita Lal**

guideline of $5 \mu\text{g}/\text{m}^3$, every person in Ranchi is exposed to level whose average is over 12 times the limit. The WHO's dose-response function shows that any cause mortality can increase by 6-7% with an increase of $\text{PM}_{2.5}$ by $10 \mu\text{g}/\text{m}^3$. Overall, citizens of Kantatoli and Tupudana area are witnessing a high attributable mortality of $\text{PM}_{2.5}$ alone. In Dhanbad, the combination of high levels of $\text{PM}_{2.5}$ and SO_2 poses a breathing risk. Miners and peri-mine communities will be affected seriously. Epidemiological studies of Indian coal mine workers have indicated pneumoconiosis prevalence rates of 8–15% among populations with prolonged exposure (Patra et al., 2016).

 NO_2 and Respiratory Disease

The study site in Kantatoli has reported concentrations of NO_2 annually averaging around $45\text{--}50 \mu\text{g}/\text{m}^3$. NO_2 exposure at such concentrations has been linked to incidence of asthma, impaired growth of lung function in children and increased vulnerability to infections (WHO 2021; Landrigan et al. 2018). Because their lungs are still growing, children are at a greater risk. In addition, they spend more time outdoors than older individuals during the day, when NO_2 and $\text{PM}_{2.5}$ concentration levels from traffic are higher. Kantatoli is a major commercial transit hub bringing daily footfall from across Ranchi, although the populations exposed are not just those of the resident catchment of the monitoring station, estimates therefore indicate any working day 150,000–200,000 people are exposed to these concentrations.

Winter Cumulative Risk Period

The months between November and January posed the maximum cumulative health risk in all three cities when the peak concentrations of SO_2 , NO_2 and PM occur coincidentally. The atmospheric conditions at this time (low wind speeds (5-7 km/h at Ranchi), temperature inversions, reduced mixing heights) are also ideal for maximisation of personal exposure duration and intensity. The fact that there's no real-time public air quality index system available in all three cities means there's no system to allow residents to alter behavior during these hazardous times. Jharkhand cities do not have any school closure levels, any public advisories nor any emergency measures are active as in GRAP system in place in Delhi.

Children and Elderly Vulnerability

Children below five years and adults above 65 years are the most physiologically vulnerable groups in the population. When children are exposed to $\text{PM}_{2.5}$ levels of $35 \mu\text{g}/\text{m}^3$ or higher during critical developmental windows, they have impaired lung function that persists into adulthood (Landrigan et al. 2018). All five monitoring sites in Ranchi and the secondary data profiles for Dhanbad and Jamshedpur show annual mean $\text{PM}_{2.5}$ levels well above $60 \mu\text{g}/\text{m}^3$. Therefore, the entire child population of the Jharkhand urban cluster is chronically exposed above this level. Exposure to PM and NO_2 at observed concentrations substantially raises risk of cardiovascular incidences among the elderly, especially during the inflammatory response peak months of winter (Balakrishnan et al., 2019).

Policy Implications and Recommendations**Ranchi – Priority: Traffic Emission Control**

Since vehicular emission contributes the most to Ranchi's $\text{PM}_{2.5}$ and NO_2 burden, traffic management and fleet transition should be priority intervention points. Actions that are recommended contain. Speed up transitioning to CNG and electric vehicles for public transit along the Kantatoli commercial corridor. Aim for a 30% diesel fleet reduction within 3 years. During winter season (November-January) when NO_2 near NAAQS limit, implement odd-even vehicular restriction schemes or congestion pricing at Kantatoli during morning and evening peak hours. Implement remote-sensing pollution under control or PUC enforcement systems at major road entry points because consistent field studies show that vehicular emissions from Indian cities on average originate from 5-10% worst mechanical condition vehicles that account for 20-30% of total emissions. Set up specific cycling paths and pedestrian first priority streets to link up major residential areas with Kantatoli to ensure short-trip mode share from motorized vehicles to increase. Make installations of charging points for electric vehicles (EVs) mandatory for all commercial construction permit applications in the Kantatoli zone to accelerate private EV adoption.



**Sandeep Prasad and Namita Lal****Dhanbad – Priority: Coal Dust and Mine Fire Suppression**

Dhanbad's air quality challenge is structurally different and require coal sector specific solutions. Within five kilometers of residential areas, all currently running open cast collieries would be required to mandatorily install systems for suppression of fugitive dust (such as water sprinklers, enclosed conveyor belt, vegetative barriers, etc). The Mine Fire Control Programme of BCCL for Jharia coalfield should be sped up by action on seams which emit fire on surface in the residential areas. Require all coal washeries and all coal handling facilities having more than 1 million tonnes annual throughput to continuously monitor and publicly report emissions. Create and implement monitoring networks for PM_{2.5} and SO₂ at the village level in mine- adjacent communities. These communities are presently under-represented in the CPCB NAMP monitoring networks. The objective of this step is to assess exposure gradients near to the point sources.

Jamshedpur – Priority: Industrial PM and Metallic Aerosol Control

Jamshedpur has a comparatively well-developed industrial environmental management system on which to build. Ensure implementation of CEMS for all major stacks at Tata Steel Jamshedpur followed by real-time transmission of data to JSPCB and accessible to the public. Programs for baghouse and ESP upgrades of sinter plants, which have been historically the highest PM emission process unit in integrated steel plants, should be given highest priority for implementation. A 40 per cent reduction in sinter PM emissions is possible in five years. The health alert protocols should be activated when ambient concentrations of aerosol iron, manganese and chromium reach threshold concentrations. Ambient monitoring of metals should also be undertaken at residential stations located along the edge of the plant boundary. The vehicular NO₂ burden in Jamshedpur's expanding commercial zones is continuously worsening. Before NO₂ levels reach that of Ranchi's Kantatoli, Jamshedpur should be incentivized to transition to EVs. There should also be enforcement of Bharat Stage-VI.

Cross-City Governance Recommendations

Set up a Jharkhand state air quality monitoring network (JSAQMN) by deploying real-time sensor nodes in all these three cities with standard QA/QC protocols and dashboard for easy public access. We must urgently implement air quality index (JAI) along the lines of GRAP in the National capital but customizable to Jharkhand. Health advisory alerts in case of moderate to poor require monitoring. Monitoring if the health advisory of the JAAQI is active and whether it is going off in case of any moderate to poor air quality episodes. As part of the approval process for all industrial, major infrastructure and new township developments in all three cities, make air quality impact assessments mandatory. Include tree cover expansion targets in city master plans: a 20% increase in tree cover of Kantatoli and equivalent high-density zones within five years using native high-PM-interception species (*Ficus benghalensis*, *Terminalia arjuna*, *Mangifera indica*). Engage a joint Jharkhand health impact assessment that will quantify the attributable disease burden from PM_{2.5} and NO₂ exposure in all three cities to provide the economic case for policy investment.

CONCLUSIONS

The study provides the first multi-city air quality assessment of the three major cities or urban centres of Jharkhand, namely, Ranchi, Dhanbad and Jamshedpur, using systematic and comprehensive primary field data collected for Ranchi, secondary published data for the comparative cities and a scientific rationale and connecting pollution profiles to the source sector characteristics of the respective cities. The findings are clear on all fronts. In Ranchi, all five land-use zone monitoring sites showed PM₁₀ concentrations exceeding NAAQS in both years of measurement, with annual means of 97–110 µg/m³ against a standard of 60 µg/m³. Kantatoli recorded a PM_{2.5} exceedance of 111.44 µg/m³, which is 279% of NAAQS and 2,229% of the WHO guideline. The monitored air quality mostly reflected vehicular exhaust, and not heavy industry. At Kantatoli, under the business as usual scenario, the NO₂ 24 hour average monthly values during winter are close to the limit set by NAAQS. The annual rate of increase is 5.9% which is likely to exceed the limit set by NAAQS within the next 3-5 years. SO₂ is presently compliant at all Ranchi locations, but is showing annual rising trends of 5–11% at the industrial and traffic sites. The most crucial takeaway is



**Sandeep Prasad and Namita Lal**

that 2023 air quality turned worse than 2022 despite higher monsoon rainfall in 2023 – thus definitively proving that emission growth is the controlling driver, not interannual meteorological variability.

On comparison with Dhanbad, the quality of air of the two steel townships shows that there is much higher contribution of coal mining operations in Dhanbad as seen from the coarser particles that show up at Dhanbad. SO₂ appears to be the signature differentiating pollutant for Dhanbad. Coal seam fires represent an essentially uncontrolled emission source at Dhanbad that is unique to the Indian urban scene. The heavy metal aerosols present in the air of Jamshedpur come from the steel processing area. The NO₂ profile which is traffic dominated of Ranchi represents the most critical compliance failure of the three cities and surprisingly has a higher level of this pollutant than more industrial cities around. The ratio of PM_{2.5} to PM₁₀ in various cities reveals the nature of their air pollutants. This ratio was highest in Ranchi where traffic was heavy (greater than 0.90); and intermediate in certain industrial sites of Jamshedpur (0.60-0.75). Further, the ratio was lowest in Dhanbad with stations close to mining sites (0.50-0.65). This PM_{2.5} / PM₁₀ ratio across cities is expected to fingerprint the dominant source sector and hence guide the identification of targeted emission control strategies. The study confirms the effects of intact urban forest cover, that is, a 49 µg/m³ reduction in annual mean PM_{2.5} and 3-4°C reduction in summer temperatures in Ranchi. The monitoring site at Kantatoli in Ranchi is most urbanized and hence severely polluted with an annual mean PM_{2.5} of 111.44 µg/m³. The least polluted site is Birsa Zoological Park with PM_{2.5} of 62.48 µg/m³. The strongest locally grounded empirical evidence available for investing in urban forest expansion as an air quality and climate adaptation strategy. The cities of Jharkhand have the chance to fix their air quality before it get as bad as the most polluted megacities of India. This data is the basis for that action and the reason.

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Sandeep Prasad and Namita Lal

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Table 1: Ranchi Primary Monitoring Site Network Characteristics

Site	Name & Type	Latitude	Longitude	Primary Land Use	Key Emission Sources
1	Tupudana Industrial	23°15'56"N	85°18'10"E	Heavy Industry	Foundries, manufacturing, HGVs, fugitive dust
2	Kantatoli Traffic Hub	23°21'52"N	85°20'43"E	Commercial/Traffic	Vehicular exhaust, fuel combustion, road dust
3	Harmu Residential	23°21'12"N	85°18'30"E	Dense Residential	Biomass, LPG combustion, two-wheelers
4	Kanke Agricultural	23°27'19"N	85°19'19"E	Agricultural Peri-Urban	Biomass burning, minimal industry
5	Birsa Zoological Park	23°27'48"N	85°27'24"E	Forest/Eco-Sensitive	Natural background only

Table 2: Regulatory Reference Standards Applied in This Study

Pollutant	NAAQS Annual ($\mu\text{g}/\text{m}^3$)	NAAQS 24-hr ($\mu\text{g}/\text{m}^3$)	WHO Annual Guideline 2021 ($\mu\text{g}/\text{m}^3$)
PM ₁₀	60	100	15
PM _{2.5}	40	60	5
SO ₂	50	80	40
NO ₂	40	80	10

Table 3: Monthly Temperature Comparison Across Ranchi Sites, 2022 vs. 2023 (°C)

Site	Jan 2022	May 2022	Jul 2022	Jan 2023	May 2023	Jul 2023
Tupudana (S1)	15.5	29.8	25.5	16.0	30.2	25.8
Kantatoli (S2)	16.5	31.2	26.5	16.8	31.5	26.8
Harmu (S3)	16.2	30.5	26.0	16.7	31.0	26.2
Kanke (S4)	15.0	29.0	24.5	15.5	29.5	24.8
Birsa Zoo (S5)	14.5	28.8	24.0	15.0	29.0	24.2





Sandeep Prasad and Namita Lal

Table 4: PM₁₀ Annual Descriptive Statistics, Ranchi 2022–2023

Site	Mean 2022 (µg/m ³)	SD	SE	NAAQS Exceedance	WHO Exceedance	Mean 2023 (est.)
Tupudana S1	109.62	2.38	0.69	+83% (183%)	+631%	~112.1
Kantatoli S2	109.53	2.25	0.65	+83% (183%)	+630%	~111.8
Harmu S3	102.37	0.98	0.28	+71% (171%)	+582%	~104.2
Kanke S4	100.02	1.89	0.55	+67% (167%)	+567%	~101.9
Birsa Zoo S5	97.92	1.62	0.47	+63% (163%)	+553%	~99.1
NAAQS Limit	60	—	—	—	—	—

Table 5: PM_{2.5} Annual Statistics, Ranchi 2022–2023

Site	PM _{2.5} 2022(µg/m ³)	SD	PM _{2.5} 2023 (est.)	%Above NAAQS	PM _{2.5} /PM ₁₀ Ratio	Health Risk Level
Tupudana S1	99.34	2.41	~107.5	+148%	0.91	Very High
Kantatoli S2	111.44	5.17	~105.2	+179%	0.93	Severe
Harmu S3	72.30	3.26	~71.5	+81%	0.71	High
Kanke S4	64.25	2.14	~65.2	+61%	0.64	Moderate-High
Birsa Zoo S5	62.48	1.45	~63.5	+56%	0.64	Moderate
NAAQS Limit	40	—	—	—	—	—

Table 6: SO₂ Annual Statistics and Inter-Annual Trend, Ranchi 2022–2023

Site	Mean 2022 (µg/m ³)	SD	Mean 2023 (µg/m ³)	YoY Change	NAAQS Status
Tupudana S1	26.76	7.99	29.76	+11.2%	Compliant (59% of limit)
Kantatoli S2	27.19	6.71	28.76	+5.8%	Compliant (58% of limit)
Harmu S3	~15.9	~4.8	17.67	+11.1%	Compliant (35% of limit)
Kanke S4	~16.1	~4.1	17.76	+10.3%	Compliant (36% of limit)
Birsa Zoo S5	~14.8	~4.3	~15.2	+2.7%	Compliant (30% of limit)

Table 7: NO₂ Monthly Range, Winter Peaks, and Inter-Annual Trends, Ranchi 2022–2023

Site	Min Monthly 2022	Max Monthly 2022	Dec 2022	Dec 2023	YoY Δ (Dec)	Trend
Tupudana S1	31.30 (Jun)	63.40 (Dec)	63.40	67.11	+5.9%	↑ Rising
Kantatoli S2	24.39 (Jul)	69.17 (Dec)	69.17	73.22	+5.9%	↑ Rising (Critical)
Harmu S3	17.51 (Aug)	39.67 (May)	22.44	23.75	+5.8%	↑ Rising
Kanke S4	16.49 (Aug)	31.14 (Feb)	23.75	25.14	+5.8%	↑ Slight
Birsa Zoo S5	15.00 (Aug)	29.43 (Feb)	23.44	24.81	+5.8%	→ Stable

Table 8: Comparative Air Quality Profile – Ranchi, Dhanbad, and Jamshedpur (Most Recent Available Data)

Parameter	Ranchi (Primary, 2022)	Dhanbad (Secondary)*	Jamshedpur (Secondary)*	NAAQS Limit
PM ₁₀ Annual Mean (µg/m ³)	97–110 (5 sites)	130–180 [CPCB range]	90–140 [CPCB range]	60
PM _{2.5} Annual Mean (µg/m ³)	62–111 (5 sites)	70–120 [est. from lit.]	60–100 [CPCB/JSPCB]	40
SO ₂ Annual Mean (µg/m ³)	15–27 (5 sites)	25–55 [elevated]	15–35 [moderate]	50
NO ₂ Annual Mean	17–50 (5 sites)	18–38 [moderate]	20–45 [growing]	40





Sandeep Prasad and Namita Lal

($\mu\text{g}/\text{m}^3$)				
Dominant Pollutant	$\text{PM}_{2.5} + \text{NO}_2$	$\text{PM}_{10} + \text{SO}_2$	$\text{PM}_{10} + \text{PM}_{2.5}$	—
Primary Source Sector	Traffic + Mixed Industrial	Coal Mining + Thermal Power	Steel Industry + Traffic	—
$\text{PM}_{2.5}/\text{PM}_{10}$ Ratio (typical)	0.64–0.93	0.50–0.65	0.60–0.75	—

*Secondary data for Dhanbad and Jamshedpur derived from CPCB NAMP reports, JSPCB Annual Reports, and peer-reviewed literature.

Table 9: Integrated Site Pollution Hierarchy, Ranchi (2022 Annual Data)

Rank	Site	Primary Driver	$\text{PM}_{2.5}$ ($\mu\text{g}/\text{m}^3$)	NO_2 Max ($\mu\text{g}/\text{m}^3$)	SO_2 Mean	Risk Level
1 (Worst)	Kantatoli S2	Traffic & vehicles	111.44	69.17	27.19	Severe
2	Tupudana S1	Industry + traffic	99.34	63.40	26.76	Very High
3	Harmu S3	Residential + secondary	72.30	39.67	~15.9	High
4	Kanke S4	Agricultural + biomass	64.25	31.14	~16.1	Moderate
5 (Best)	Birsa Zoo S5	Forest background	62.48	29.43	~14.8	Low-Moderate

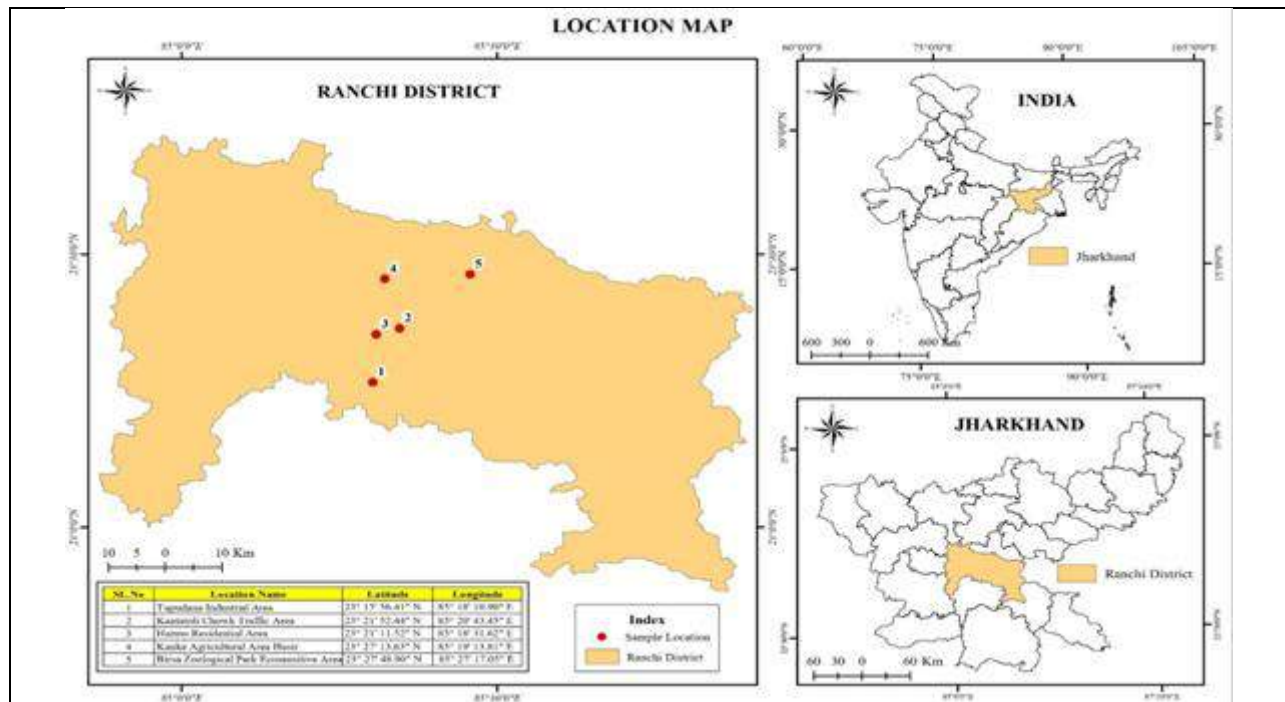


Fig 1: The map of Ranchi and adjoining areas, showing the selected monitoring sites.

(Source: QGIS Version 3.40)

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RESEARCH ARTICLE

Diaspora Identity and the Search for Belonging in the Golden Son (2015): A Critical Study of Cultural Conflict and Hybridity

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ABSTRACT

Diaspora literature plays a crucial role in understanding the complexities of identity and belonging in a globalized world. The *Golden Son* by Shilpi Somaya Gowda is a significant contribution to Indo-Canadian literature that explores the emotional and cultural struggles of migration. This research paper examines how the novel represents diaspora identity and the search for belonging through its central characters, particularly Anil and Leena. The narrative follows Anil, a young man who migrates from rural India to the United States in pursuit of professional success, while remaining deeply tied to his cultural roots. His journey reflects the tensions between tradition and modernity, as well as the psychological challenges of adapting to a new cultural environment. The novel portrays the experience of living “between two worlds,” highlighting issues such as cultural conflict, alienation, and identity crisis. This study adopts a thematic and analytical approach to examine how Gowda constructs diaspora identity as fluid and evolving. It also explores the role of family expectations, social norms, and personal ambition in shaping an individual’s sense of belonging. The paper argues that the novel presents belonging not as a fixed state but as a continuous process of negotiation between past and present, homeland and host land. By analyzing the novel, this research contributes to a deeper understanding of Indo-Canadian diaspora literature and its engagement with identity, migration, and cultural hybridity in the modern world.

Keywords: Diaspora, represents, identity, migration, and cultural hybridity





INTRODUCTION

In the contemporary globalized world, migration has become a defining feature of human experience, giving rise to what is commonly referred to as diaspora communities. The term “diaspora” denotes the dispersion of people from their original homeland to different geographical locations, often resulting in complex cultural, emotional, and psychological experiences. Literature emerging from such communities, particularly Indo-Canadian literature, provides deep insight into the struggles of identity and belonging faced by immigrants. Among the notable works in this field is *The Golden Son* by Shilpi Somaya Gowda, which vividly portrays the challenges of navigating between cultures. The novel centers on the life of Anil Patel, a young man from a rural Indian village who migrates to the United States to pursue a career in medicine. As the first member of his family to leave his homeland, Anil carries the weight of familial expectations and cultural values. His journey reflects the quintessential diasporic experience, characterized by the tension between maintaining one’s cultural identity and adapting to a new social environment. The narrative also juxtaposes Anil’s story with that of Leena, his childhood friend, who remains in India and faces her own struggles within traditional societal structures. One of the central themes of the novel is the concept of diaspora identity. Diasporic identity is inherently complex and fluid, shaped by the interaction between the culture of the homeland and that of the host country. Anil’s experiences in the United States illustrate this duality. On one hand, he embraces opportunities for professional growth and personal freedom; on the other hand, he grapples with feelings of alienation and cultural displacement. He is neither fully at home in America nor entirely connected to his roots in India, embodying what scholars describe as a “hyphenated identity.”

The novel also explores the idea of belonging, which is closely linked to identity. Belonging refers to the sense of acceptance and inclusion within a particular community or cultural context. For diasporic individuals like Anil, belonging is not easily attainable. His interactions in the United States reveal the subtle and overt challenges faced by immigrants, including cultural misunderstandings and social exclusion. At the same time, his periodic returns to India highlight a growing sense of distance from his own culture, further complicating his sense of belonging. Furthermore, the novel emphasizes the role of cultural conflict in shaping diaspora identity. Anil’s internal struggle is marked by the clash between traditional Indian values and the modern, individualistic ethos of Western society. This conflict is evident in various aspects of his life, including his relationships, career choices, and personal beliefs. The narrative demonstrates how these conflicting forces create a sense of instability and uncertainty, which is a defining feature of diasporic existence. Another important dimension of the novel is its exploration of cultural hybridity. Cultural hybridity refers to the blending of different cultural influences, resulting in new forms of identity. Anil’s character exemplifies this hybridity, as he gradually integrates elements of both Indian and American cultures into his identity. This process is neither linear nor straightforward; rather, it involves continuous negotiation and adaptation. The novel suggests that such hybridity is not a sign of loss but a creative and dynamic response to the challenges of migration. In addition to individual identity, the novel also addresses broader social and cultural issues, such as class differences, gender roles, and family expectations. Through the character of Leena, Gowda highlights the constraints faced by women in traditional Indian society, thereby providing a contrast to Anil’s experiences abroad. This dual narrative structure enriches the exploration of identity and belonging, offering multiple perspectives on the diasporic condition.

The significance of this study lies in its focus on how *The Golden Son* contributes to the discourse on diaspora identity and belonging. By analyzing the novel, this research aims to uncover the ways in which literature reflects and shapes our understanding of migration and cultural interaction. The novel not only portrays the struggles of diasporic individuals but also celebrates their resilience and adaptability. *The Golden Son* serves as a powerful exploration of diaspora identity and the search for belonging. Through its nuanced portrayal of characters and their experiences, the novel captures the complexities of living between cultures and highlights the ongoing process of identity formation in a globalized world.





LITERATURE REVIEW

The study of diaspora literature has become one of the most significant areas in contemporary literary criticism because it addresses questions of migration, identity, displacement, and belonging. Scholars have increasingly examined how diasporic narratives reflect the emotional and psychological consequences of living between cultures. In the context of Indian English fiction, diaspora writing often focuses on the negotiation between homeland values and host-country realities. The *Golden Son* by Shilpi Somaya Gowda contributes meaningfully to this literary tradition by portraying migration not merely as physical movement but as a deeply transformative psychological and cultural experience. A major theoretical foundation for diaspora studies comes from Stuart Hall, who argues that identity is not fixed but continuously formed through history, culture, and representation. Hall's understanding of cultural identity is highly relevant to *The Golden Son*, where Anil's sense of self changes through his movement from rural India to America. His identity is not stable; instead, it evolves as he encounters new cultural expectations, social relationships, and professional responsibilities. Hall suggests that diasporic identity exists through "becoming" rather than simply "being," and this concept is visible throughout Anil's experiences.

Similarly, Homi K. Bhabha introduces the idea of hybridity, which is central to understanding diasporic identity in the novel. Bhabha explains that when cultures interact, a "third space" emerges where new identities are formed. Anil occupies such a third space because he cannot fully belong either to traditional Indian village culture or to American urban life. His professional success in America does not eliminate his attachment to Indian family values. Instead, his life becomes a continuous negotiation between these cultural systems. Bhabha's theory helps explain why Anil's identity remains layered and unstable rather than resolved. The concept of "imagined belonging," often discussed in diaspora studies, also applies to the novel. Scholars note that migrants frequently idealize both homeland and hostland, yet remain emotionally unsettled in both spaces. Anil initially imagines America as a place of freedom and professional achievement. However, after arrival, he experiences loneliness, unfamiliarity, and subtle exclusion. His medical residency introduces him to competition and emotional distance that contrast sharply with village intimacy. This reflects what diaspora critics identify as the emotional contradiction of migration: opportunity often coexists with displacement.

Several scholars studying Indian diasporic fiction have emphasized that migration narratives often focus on male mobility while female characters remain tied to local structures. In *The Golden Son*, this pattern is challenged through the equally important narrative of Leena. While Anil moves geographically, Leena's struggle occurs within the confined social structures of rural India. Her marriage, domestic suffering, and limited freedom expose how gender shapes access to mobility. Critics of contemporary Indian fiction often argue that women's immobility can reveal social realities as powerfully as migration itself. Leena's character therefore broadens the novel's diasporic framework by showing that belonging is also contested within the homeland. Another important scholarly perspective comes from research on transnational family structures. Migration does not sever family obligations; instead, it often intensifies them. Anil's success abroad remains linked to expectations from his father, brother, and village community. Literary critics of transnational fiction argue that migrants often carry invisible emotional burdens because family identity continues across borders. This is evident when Anil must repeatedly evaluate whether personal ambition should outweigh responsibility toward home. The novel also aligns with studies of professional diaspora, particularly medical migration. Research on immigrant doctors often notes that professional success abroad does not automatically produce emotional security. In Anil's case, hospital life in America represents discipline, hierarchy, and distance. His role as a doctor creates social status but also isolation. The contrast between healing others and struggling internally gives the novel additional depth.

Comparatively, *The Golden Son* shares thematic concerns with *The Namesake* and *Interpreter of Maladies*, where diasporic identity is similarly shaped by cultural negotiation and emotional fragmentation. However, Gowda's novel differs because it maintains equal narrative attention on village India, thereby refusing to separate migration from homeland realities. Thus, existing scholarship on diaspora, hybridity, gender, and transnational identity provides a



**Bhavik Patel and Parthivkumar Patel**

strong framework for reading *The Golden Son*. The novel contributes uniquely by showing that belonging is not achieved through migration alone but remains a complex emotional process shaped by culture, memory, and moral responsibility.

Analysis of Diaspora Identity, Cultural Conflict, and Hybridity in the novel:**Cultural Conflict and Identity Crisis**

The Golden Son presents cultural conflict as one of the most central dimensions of diasporic existence. The novel demonstrates that migration is not only geographical movement but also psychological displacement. Through Anil Patel's journey from rural Gujarat to America, the narrative explores how individuals encounter conflicting cultural systems that challenge previously stable forms of identity. Before migration, Anil's life is shaped by family hierarchy, collective values, village familiarity, and clearly defined social roles. His identity is rooted in community recognition and family expectation. However, upon entering American society, these foundations become unstable because the new environment demands self-definition in unfamiliar terms. In rural India, Anil's achievements belong not only to himself but to his family and village. His educational success symbolizes collective aspiration. The title itself reflects this burden: the "golden son" is expected to fulfill family pride and social mobility. Yet America introduces a contrasting value system centered on individuality, personal choice, and emotional independence. This cultural difference creates internal conflict because Anil cannot easily detach himself from inherited expectations. His experience in hospital life intensifies this crisis.

In India, medicine represented service and social honor; in America, it also becomes institutional competition, technical discipline, and emotional detachment. He must learn not only medical systems but also social codes, communication styles, and professional behavior that differ from his upbringing. The struggle is subtle yet constant: language, humor, interpersonal distance, and decision-making all require adaptation. This conflict produces identity uncertainty. Anil gradually realizes that success abroad does not automatically create belonging. Though he gains professional opportunity, he also experiences emotional isolation. His loneliness in America reflects a common diasporic condition where migrants appear socially integrated but remain internally unsettled. He belongs neither fully to the host society nor completely to the homeland he left behind. The novel shows that identity crisis often emerges through ordinary moments rather than dramatic events. Anil's meals, conversations, clothing choices, and social relationships all become sites of negotiation. Even his silence often indicates uncertainty about how much of himself can be expressed within a new cultural setting. This demonstrates that diaspora identity is lived daily through repeated acts of adjustment. Importantly, cultural conflict is not presented as simple opposition between East and West. Gowda avoids binary stereotypes by showing complexity in both spaces. Rural India contains affection but also social rigidity; America offers opportunity but also alienation. Therefore, Anil's conflict is not about choosing one culture over another but about learning how to survive within both.

Hybridity and the Formation of a New Self

The concept of cultural hybridity is central to understanding Anil's transformation. Rather than abandoning one culture for another, he gradually develops a layered identity shaped by both Indian and American influences. Hybridity in the novel is not immediate; it develops slowly through discomfort, adaptation, and self-reflection. At the beginning of his migration, Anil attempts to preserve familiar values while functioning within foreign surroundings. He remains emotionally connected to family, village memory, and cultural discipline. Yet over time, his behavior changes. He becomes more professionally assertive, more individually reflective, and increasingly aware of personal choice. These changes do not erase his Indian identity; instead, they create a mixed cultural consciousness. His interactions with colleagues in America reveal this process. He learns how to participate in professional dialogue, interpret social independence, and understand emotional boundaries different from those in India. However, he often evaluates these experiences through inherited moral values. Thus, every new experience passes through both cultures simultaneously. Hybridity also appears in his emotional responses. He admires American efficiency and opportunity, yet he misses the intimacy of collective life. He values professional advancement but questions whether ambition alone creates fulfillment. This dual awareness reflects a hybrid identity in which both cultural systems remain active. The novel suggests that hybridity is creative but difficult. It offers expanded perspective but also



**Bhavik Patel and Parthivkumar Patel**

emotional instability. Migrants like Anil gain the ability to move between worlds, yet this mobility often produces uncertainty because no single cultural framework feels complete. Importantly, Gowda presents hybridity as dynamic rather than finished. Anil does not arrive at a final identity. Instead, he continues negotiating language, loyalty, ambition, and memory. His identity remains in process, which reflects modern diasporic experience accurately. This fluidity also affects his relationships. He begins to understand that emotional choices cannot be determined solely by family tradition or solely by Western independence. Both influence his decisions. Therefore, hybridity becomes not merely cultural blending but an ethical framework through which he interprets life.

Family Expectations and Diasporic Responsibility

Family remains one of the strongest forces shaping Anil's diasporic experience. Migration in the novel is never purely individual because Anil's departure carries collective meaning. His family invests emotional hope, social prestige, and future expectation in his success. His father's pride reflects traditional Indian family structures where the son's advancement symbolizes family progress. Education becomes a means of social mobility, and foreign success becomes proof of achievement. However, such pride also creates pressure. Anil cannot fail privately because his success belongs publicly to others. This creates what may be called diasporic responsibility. Even while physically distant, he remains emotionally accountable to family decisions, village needs, and moral obligations. Letters, memories, and expectations constantly connect him to home. Migration therefore expands responsibility rather than reducing it. The contrast between personal ambition and family duty becomes especially significant when Anil begins to evaluate his future. America offers stability and professional success, yet family ties continue to demand attention. The question becomes whether belonging is defined by where one succeeds or where one remains responsible. The novel shows that many migrants experience guilt alongside success. Professional advancement abroad often produces emotional distance from family realities at home. Anil's growing awareness of Leena's suffering intensifies this moral tension because he recognizes that his own mobility contrasts sharply with others' limitations. Family expectation also shapes emotional restraint. Anil cannot freely redefine himself because inherited values continue to regulate his decisions. Even in a new country, internalized family discipline remains powerful. Gowda therefore portrays migration as morally complex. Success abroad is not liberation from obligation; rather, it introduces new forms of ethical conflict.

Leena and Gendered Belonging

Leena's narrative is essential because it reveals that belonging is also shaped by gender. While Anil moves internationally, Leena remains within village structures where freedom is severely restricted. Her life exposes how women often experience immobility even within their own homeland. Leena's marriage demonstrates how traditional social systems regulate female identity. Her role is defined by domestic obedience, endurance, and silence. Unlike Anil, she has limited access to education, mobility, and self-determination. This contrast reveals unequal distribution of opportunity within the same cultural context. Her suffering is not only personal but structural. The expectations placed upon her reflect broader patriarchal systems in which women are valued primarily through family roles. Even when emotionally strong, her choices remain socially constrained. This creates another form of diaspora-like displacement: Leena experiences alienation within home itself. She belongs physically to the village yet lacks full emotional belonging because her individuality is suppressed. Thus, the novel suggests that displacement can occur without migration. Leena's presence also challenges male-centered migration narratives. If Anil represents outward mobility, Leena represents inward endurance. Together they reveal two forms of struggle shaped by social hierarchy. Her emotional resilience becomes one of the novel's strongest ethical elements. Gowda does not portray her merely as victimized; rather, she becomes morally central because her suffering exposes the limitations of traditional structures. Through Leena, the novel argues that belonging must include dignity and agency. Without these, physical presence in homeland does not create true belonging.

Memory, Return, and Emotional Homeland

An important aspect of diasporic identity in the novel is memory. For migrants, homeland often survives emotionally through remembered details rather than continuous presence. For Anil, village life remains active through memory even while he adapts abroad. Food, language, family voices, and childhood associations continue to shape his



**Bhavik Patel and Parthivkumar Patel**

internal world. These memories prevent complete assimilation because emotional belonging remains tied to earlier life experiences. However, return complicates memory. When Anil reconnects with India, he realizes that both he and home have changed. Homeland is no longer exactly as remembered. This creates a common diasporic realization: memory preserves a version of home that reality cannot fully restore. Thus, belonging becomes emotionally unstable. America offers present opportunity; India offers remembered attachment. Neither alone provides complete resolution. The novel suggests that home may ultimately become relational rather than geographical. Belonging exists where ethical responsibility, emotional truth, and human connection are sustained. This insight gives the novel its broader significance within diaspora literature: migration does not erase origin, and return does not fully restore identity. Instead, identity remains suspended between movement and memory.

Findings of the Study

The analysis of *The Golden Son* reveals that diaspora identity is presented as a dynamic and continuously negotiated process rather than a stable cultural condition. The novel demonstrates that migration does not simply involve geographical relocation; instead, it initiates profound psychological, emotional, and social transformations that affect how individuals understand themselves and their place in the world. Through the experiences of Anil Patel, the study finds that diasporic identity emerges through continuous interaction between inherited cultural values and newly encountered social realities. One of the most significant findings of this study is that cultural conflict remains central to diasporic identity formation. Anil's migration from rural India to the United States places him in an environment where traditional values are constantly challenged by modern Western practices. His professional life in America offers independence, opportunity, and social mobility, yet these advantages do not remove his attachment to family obligations and emotional memory. The study shows that this duality creates an internal conflict in which identity becomes divided between competing systems of belonging. Anil's life illustrates that migrants often exist in a condition of partial belonging, where neither homeland nor hostland fully satisfies emotional and cultural needs. A second important finding concerns the role of hybridity in identity construction.

The novel demonstrates that diasporic identity is not based on choosing one culture over another but on gradually integrating elements from both. Anil does not abandon his Indian identity after migration; rather, he adapts selectively while preserving emotional ties to his origins. His professional discipline, communication style, and social understanding evolve in America, but his moral judgments continue to reflect family values learned in India. This finding supports the argument that hybridity is not cultural loss but cultural transformation. The novel presents hybridity as a realistic response to migration, where individuals create new identities without completely dissolving earlier affiliations. The study also finds that family expectation remains one of the strongest forces shaping diasporic consciousness. Although Anil physically moves away from India, his family continues to influence his decisions through emotional responsibility and moral obligation. His educational success is treated not as private achievement but as collective family honor. This indicates that in many diasporic contexts, migration intensifies family responsibility rather than reducing it. The emotional burden of representing family ambition abroad becomes a defining feature of Anil's identity. Thus, the novel presents diaspora not as personal freedom alone but as responsibility carried across borders.

Another major finding concerns the contrast between mobility and immobility through the parallel narrative of Leena. While Anil experiences global movement, Leena remains within restrictive village structures shaped by gender inequality and patriarchal expectations. The study finds that her narrative broadens the meaning of displacement. Although she does not migrate internationally, she experiences emotional alienation within her own social environment. Her lack of autonomy in marriage and domestic life demonstrates that belonging cannot be understood simply through physical location. The novel therefore suggests that social exclusion within homeland may produce a condition similar to displacement. Gender emerges as a crucial factor in determining access to belonging. The study finds that male mobility and female restriction operate as parallel realities within the novel. Anil receives education, travel, and professional advancement, whereas Leena's possibilities are shaped by traditional domestic expectations. This contrast exposes unequal structures within society and highlights how belonging is differently experienced by men and women. Through Leena's suffering, the novel critiques social



**Bhavik Patel and Parthivkumar Patel**

systems that allow male advancement while limiting female agency. The study further reveals that memory functions as an important element in diasporic Identity. Even while adapting to American life, Anil remains emotionally connected to India through remembered experiences, family relationships, and village associations. However, the study finds that memory does not provide complete comfort because return to homeland also reveals change. The India he remembers is not identical to the India he later encounters. This creates emotional complexity in which homeland exists partly as memory and partly as transformed reality. Finally, the study finds that belonging in the novel is presented not as a final achievement but as an ongoing process of negotiation. Neither America nor India offers complete resolution for Anil. Instead, belonging develops through ethical responsibility, emotional connection, and self-awareness across multiple cultural spaces. The novel ultimately suggests that modern diasporic identity is fluid, relational, and unfinished.

CONCLUSION

The study of *The Golden Son* demonstrates that diaspora identity is a complex and evolving condition shaped by migration, cultural interaction, memory, and social responsibility. Through the parallel narratives of Anil and Leena, the novel offers a nuanced understanding of how belonging is negotiated within both global and local contexts. Rather than presenting migration as a simple movement toward success, the novel reveals the emotional and cultural tensions that accompany the experience of living between different worlds. Anil's journey from rural India to the United States represents the central diasporic struggle between opportunity and attachment. His movement across national boundaries provides access to education, professional recognition, and personal independence, yet it also generates emotional uncertainty and cultural conflict. The study has shown that his experiences reflect the broader condition of diasporic individuals who must continuously balance inherited traditions with new social realities. His identity does not become fully American, nor does it remain unchanged by migration. Instead, it develops through ongoing negotiation between multiple cultural influences.

A major conclusion of this study is that cultural hybridity in the novel functions as a realistic and necessary response to migration. Anil gradually incorporates aspects of both Indian and American life into his sense of self, creating an identity that is neither fixed and singular. This hybridity does not suggest loss of cultural roots; rather, it reveals the ability of individuals to adapt creatively while maintaining emotional continuity with their origins. The novel therefore presents hybridity as a dynamic process of identity formation in contemporary diasporic existence. The analysis also confirms that family remains central to diasporic belonging. Even after migration, Anil's choices remain deeply influenced by family expectations, moral obligation, and emotional memory. His success abroad cannot be separated from the collective hopes of his family and village. This indicates that diaspora often extends family structures across borders rather than weakening them. The emotional responsibility carried by migrants becomes one of the defining features of their identity.

Leena's narrative significantly strengthens the novel's exploration of belonging by showing that displacement is not limited to migration. Her struggles within traditional village structures reveal how gender inequality can produce forms of alienation even within one's homeland. Through her character, the novel critiques patriarchal systems that limit female autonomy and demonstrates that belonging must include dignity, agency, and emotional recognition. The study further concludes that belonging in *The Golden Son* is not presented as a final destination. Neither homeland nor hostland fully resolves the characters' internal conflicts. Instead, belonging emerges as a continuous process shaped by relationships, ethical choices, and emotional understanding. Memory, return, and social responsibility remain active forces in this process. The novel contributes significantly to contemporary diaspora literature by portraying identity as fluid, layered, and unfinished. The novel successfully captures the realities of modern migration while also highlighting the enduring importance of cultural roots, human connection, and moral responsibility in shaping the search for belonging.





Bhavik Patel and Parthivkumar Patel

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RESEARCH ARTICLE

Pharmaceutical Development and *In vitro* Antimicrobial Evaluation of Polyherbal Mouthwash Formulation

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ABSTRACT

Maintaining optimal oral hygiene is essential as the oral cavity serves as the primary portal to the respiratory and gastrointestinal systems. Standard chemical mouthwashes like chlorhexidine cause side effects including tooth staining and mucosal irritation, driving the clinical need for safe, chemical-free herbal alternatives. This study aimed to systematically execute the pharmaceutical development of an herbal mouthwash combining four classical Ayurveda plants recognized for oral cleansing: *Bakula* (*Mimusops elengi*), *Twaka* (*Cinnamomum zeylanicum*), *Musta* (*Cyperus rotundus*) and *Haritaki* (*Terminalia chebula*), while evaluating three processing methodologies. Authenticated botanicals were processed into three distinct dosage forms: an aqueous decoction (*Kwatha*), a hydro-distillate (*Arka*) and a simple steam distillate. *In vitro* antimicrobial screening was performed using the standardized disc diffusion technique against *Streptococci*, *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae*, measuring zones of inhibition (ZOI) after 24-hour incubation at 37°C. The *Arka* (hydro-distillate) formulation demonstrated superior and consistent efficacy, exhibiting a uniform zone of inhibition measuring 5.0 mm across all tested pathogens. Conversely, the steam distillate displayed minimal response (3.0 mm ZOI). The *Kwatha* formulation yielded poor sensitivity against *Streptococci* and *S. aureus* (3.0 mm ZOI) and failed to inhibit *E. coli* and *K. pneumoniae*. Hydro-distillation (*Arka*) effectively isolates and concentrates the volatile active principles of the selected plants. The resulting mouthwash represents a stable, safe and microbiologically



Kunal Gohil *et al.*,

active alternative to synthetic oral rinses, successfully validating the integration and modernization of traditional Ayurvedic drug-delivery platforms.

Keywords: Herbal mouthwash, *Arka* (hydro-distillate), Antimicrobial efficacy, *Mimusops elengi*, *Cinnamomum zeylanicum*, Zone of inhibition (ZOI).

INTRODUCTION

Ayurveda prescribes mainly about the two different aspects of human life that are, preserving the health of healthy persons (*Swasthasya Swasthya Rakshanam*) and curing diseased conditions (*Aturasya Vikara Prashamanam*). The mouth of human comprises multifaceted system and functions including chewing of food, awareness about the taste and helps in baseline metabolism. As the function of the oral cavity is the prime one to get the proper starting of digestion, maintaining its health and hygiene is mostly requires. Negligence in regular oral care creates a predisposition for biofilm accumulation, dental caries, gingivitis, and severe periodontal diseases, collectively classified under the domain of *Mukharogas* (oral cavity disorders) in classical Ayurveda texts.[1] Traditional Ayurveda scriptures describe daily regimens (*Dinacharya*) dedicated to preserving oral hygiene, with specific emphasis on *Gandusha* (the practice of filling the oral cavity completely with liquid to its maximum capacity without moving it) and *Kawala* (the retention of a fluid within the oral cavity accompanied by active gargling movements).[2] These procedures traditionally employ customized herbal decoctions, oils, or aqueous extracts designed to strengthen the periodontal ligament, eliminate debris, reduce halitosis, and exert direct antimicrobial action against oral pathogens. Contemporary pharmacological research verifies these practices, suggesting that plant-derived compounds—particularly those abundant in tannins, flavonoids, and essential oils—effectively disrupt microbial cellular integrity to prevent the formation of dental plaque.[3]

In modern dental therapy, the mechanical removal of plaque via brushing is frequently augmented by therapeutic mouthwashes.[4] Among the various chemical agents available, chlorhexidine gluconate (CHX) at a concentration of 0.2% remains the recognized gold standard due to its broad-spectrum bactericidal activity and high substantivity. However, prolonged use of synthetic chemical agents is limited by multiple clinical complications.[5] Patients frequently exhibit noticeable extrinsic brownish staining of teeth and dental restorations, significant alterations in gustatory perception (taste distortion), accelerated supragingival calculus deposition, and localized desquamation or irritation of the oral mucosa.[6] Furthermore, growing concerns regarding the development of microbial resistance to chlorhexidine emphasize the need to identify alternative antimicrobial compounds. To address these limitations, the Ayurveda provides different stable, compatible, natural substances, which are without alcohol and other harmful chemicals. This research selected mainly four herbal drugs as the lower number of ingredients facilitates lower cost of production. This directly benefited the consumer to maintain oral health at lower cost.

The four medicinal plants were selected based on predefined scientific criteria, including (i) documented use in Ayurveda literature for oral hygiene and management of *Mukharoga*, (ii) reported antimicrobial, anti-inflammatory and astringent properties relevant to oral health (iii) complementary phytochemical profiles capable of providing multitarget therapeutic effects and (iv) availability of authenticated pharmacognostic standards. Equal proportions (1:1:1:1, w/w) were adopted as a preliminary formulation strategy to ensure equal contribution of each ingredient and to eliminate formulation bias while comparing different pharmaceutical processing methods. Optimization of the formulation ratio was beyond the scope of the present study and permits future investigation. While traditional medicine relies on the fresh preparation of *Kwatha* (decoctions), modern consumer compliance requires stable, elegant and ready-to-use liquid formulations. This study describes the systematic pharmaceutical development of an herbal mouthwash, examining three distinct preparation methods—*Kwatha* (decoction), *Arka* (hydro-distillate), and Distillate (steam distillate)—and characterising their respective in-vitro antimicrobial behaviours against common oral and opportunistic pathogens.





MATERIALS AND METHODS

Selection of Herbal drugs

The selection of the four medicinal plants was based on both Ayurveda textual evidence and contemporary scientific literature, rather than on arbitrary selection. All four drugs are described in Ayurveda literature for maintaining oral hygiene or for treating diseases of the oral cavity. Instead of selecting multiple plants with similar actions, these four herbs were chosen because they provide complementary mechanisms relevant to oral health.

- **Bakula (*Mimusops elengi* L.):** It is used in classical text for its *Dantya* and *Dantadardhyakara* (strengthening teeth and gums) properties.[7] It has also potent astringent (*Kashaya*) properties, which help tighten the gingiva and manage bleeding gums.[8]
- **Twaka (*Cinnamomum zeylanicum* Blume):** It is commonly included in herbal tooth powders and mouth preparations along with other *katu*, *tikta*, and *kashaya* drugs for *Dantadhavana* practice.[9] This drug is more valuable for its rich content of volatile cinnamaldehyde, providing both excellent aroma and broad-spectrum antimicrobial properties.[10]
- **Musta (*Cyperus rotundus* L.):** In Ayurveda, *Musta* is used for *Mukharoga* and *Mukhashodhana* as a supportive anti-inflammatory, astringent and cleansing herb.[11][12]
- **Haritaki (*Terminalia chebula* Retz.):** A rich source of hydrolyzable tannins, hydrolyzable phenolics and gallic acid, which provide high antioxidant and tissue-binding properties.[13]. *Haritaki* is mentioned in *Mukharoga* treatment through *GomutraHaritaki*. [14]

Procurement and Authentication of Herbal Drugs

The raw herbal components—bark of *Mimusops elengi* (*Bakula*) & *Cinnamomum zeylanicum* (*Twaka*), rhizomes of *Cyperus rotundus* (*Musta*) and pericarp of the fruit of *Terminalia chebula* (*Haritaki*)—were procured from the institutional pharmacy storehouse of the Gujarat Ayurved University, Jamnagar (Figure 1 & Table 1). The raw drugs underwent rigorous macroscopic and microscopic validation at the Pharmacognosy Laboratory of the Institute for Post Graduate Teaching & Research in Ayurveda (IPGT&RA), confirming their identity against standard monograph benchmarks. The raw ingredients were cleared of foreign matter, shade-dried under controlled conditions and mechanically pulverized into a coarse powder suitable for pharmaceutical extraction.

Pharmaceutical Processing and Formulation Development

To evaluate the impact of extraction physics on the therapeutic profile of the polyherbal mixture, three separate pharmaceutical dosage forms were developed. The four ingredients *Bakula*, *Twaka*, *Musta* and *Haritaki* were combined in equal ratios (1:1:1:1 by weight) for all preparation batches. The objective of the present work was to identify the most suitable extraction process, so the equal proportion was adopted as an initial formulation strategy for the following scientific reasons:

- To ensure that each medicinal plant contributed equally during pharmaceutical processing and antimicrobial evaluation.
- To avoid introducing formulation bias arising from unequal quantities when the primary objective was to compare the pharmaceutical preparation methods (*Kwatha*, *Arka*, and *Distillate*), rather than to optimize the herbal composition.
- Equal proportions are commonly used in preliminary polyherbal formulation development to facilitate reproducibility and simplify interpretation of pharmacological outcomes before undertaking dose-optimization studies.

Preparation of Polyherbal *Kwatha* (Decoction)

Procedure

The mixture of the ingredients was allowed to soak in water overnight to facilitate proper penetration of water into the material. *Kwatha* is prepared by boiling one part of herb with sixteen parts of water in an open vessel on mild fire till it reduces to one eighth of original quantity. [15] The hot liquid was filtered through a multi-layered muslin cloth



**Kunal Gohil et al.,**

and allowed to cool to ambient temperature. (Table 1, Figure 2) Total 3 batches of *Kwatha* were prepared. The size of batch 1 was 400ml, size of batch 2 is 560 ml; while it is 800ml in batch 3. As *Kwatha* has very short shelf life, it was prepared in different quantities as per the need. (Table 2). The experiments were carried out in three different batches to ensure the proportion of the *Kwatha* including the same ingredients shown in table 1 and 2.

Precaution

To prepare *Kwatha*, powder taken must be coarse. If the powder is too fine, it passes through the cloth during filtration. Secondly, when the powder is too fine it settles down at the bottom of the vessel during the process leading to charring and carbonization. Earthen or chemically inactive vessels such as stainless steel must be used. The drug particularly hard in nature must be well soaked and boiling should be carried out on moderate heat. While boiling, vessel should be kept open. Constant observation and continuous stirring. For the preparation of *Kwatha*, de-ionized water or R.O. water should be used. During the preparation, aseptic conditions should be maintained.

Preparation of Polyherbal Arka (Hydro-distillate):**Procedure**

All the ingredients were made into coarse powder form. The coarse powder of all ingredients were taken and mixed in specific amount. Water was added in double of the total volume of mixture and kept as it is till it absorbs the water and becomes soft. Against same amount of water was added and kept in sunlight for 8 praharas and in moonlight for 8 praharas i.e. total 48 hrs. Then it was transferred to *Arka Patana Yantra* i.e. distillation assembly and *Arka* was collected till *Gat Rasatva*. Few porcelain pieces were added to check excessive bubbling during the procedure. [16] The three different batches of the *Arka* were prepared for the ensuring of the quality. The formula shown in the table 5.

Precaution

The coarsely powdered drugs should be used. *Arka* should be preserved in an air tight container and placed in a cool and dry place to check the evaporation of volatile principles.

Preparation of Polyherbal Distillate (Steam Distillate Variant):**Procedure**

A specific distillation apparatus was used. All the ingredients were coarsely powdered and mixed in specific amount as per mentioned in the table 10. The mixture was soaked in sufficient amount of water for overnight. Next day, 10 times of water was added to the mixture and transferred to the distillation assembly and heating was started. Few porcelain pieces were added to the assembly flask to check excessive bubbling during the procedure. The vapor produced, was cooled through condenser. Due to this principle the resulting aqueous distillate was collected in the closed sterile container. Distillate was collected up to 60% of the initial volume for the pure product. [17] This distillate was prepared in triplicate manner shown in table 10.

Precaution

The coarsely powdered drugs should be used. Only 60% of the distillate should be collected because the remaining part may not contain any volatile principle. Distillate should be preserved in an air tight container and placed in a cool and dry place to check the evaporation of volatile principles.

Standardization of finished products

Standardization of the prepared three different formulations *Kwatha*, *Arka* and *Distillate* were evaluated for the quality control checks by the following basic parameters and procedures.

A) Organoleptic Characters: The different organoleptic characters like color, odor and taste were observed of all the three products i.e. *Kwatha*, *Arka* and *Distillate*.

B) Physicochemical parameters:

Determination of pH: The pH value of an aqueous liquid may be defined as the common logarithm of the reciprocal of the hydrogen ion concentration expressed in g per lt. Although this definition provides a useful practical means for the quantitative indication of the acidity or alkalinity of a solution, it is less satisfactory from a strictly theoretical



Kunal Gohil *et al.*,

point of view. Procedure: The pH of the samples were determined by potentiometer or pH meter. Calibrate the apparatus using standard buffer solutions and then immerse the electrodes in the sample to be examined and measure the pH by putting the combined electrode into the samples of *Kwatha*, *Arka* & Distillate. [18]

Determination of Specific gravity at room temperature: The specific gravity of a liquid is the weight of a given volume of the liquid at 25°C (unless otherwise specified) compared with the weight of an equal volume of water at the same temperature. Procedure: Previously dried and weighed specific gravity bottle was taken and firstly filled with sample and weighed. Then washed with distilled water and kept in oven for drying. Again filled with the distilled water and weighed at room temperature. [19]

$$\text{Specific Gravity} = \frac{\text{Weight of sample at Specific Temp}}{\text{Weight of water at Same Temp}}$$

Determination of Total solid content [20]: 25 ml of the sample was taken in previously dried and weighed evaporating dish and was heated on hot water bath. After complete evaporation of the liquid, it was kept in hot air oven at 110°C till constant weight of the residue obtained. The total solid content was calculated with reference to volume taken and expressed in terms of % w/v. [20]

$$\text{Total Solid Content} = \frac{\text{Weight of Residue}}{\text{Volume of sample}} \times 100 \% \text{ w/v}$$

In Vitro Antimicrobial Screening Protocol

To establish the comparative therapeutic potential of the developed polyherbal formulations, the antimicrobial activities of the three mouthwash variants—*Kwatha*, *Arka*, and Distillate—were systematically evaluated using the standardized Kirby-Bauer Disc Diffusion Method, chosen for its reliability in quantifying the susceptibility of microorganisms to complex herbal extracts. The assay targeted prevalent oral pathogens, including two Gram +ve bacterial species (*Staphylococcus aureus* and *Streptococci*), two Gram –ve bacterial species (*Escherichia coli* and *Klebsiella*) to determine the respective zones of inhibition for each formulation. [21]

Microbial Panel Selection

The microbial panel was selected to include representative Gram-positive and Gram-negative bacteria associated with oral and opportunistic infections. *Streptococci* spp. were selected because they are primary colonizers of dental plaque and important etiological agents of dental caries. *Staphylococcus aureus* was included as a clinically relevant opportunistic oral pathogen, while *Escherichia coli* and *Klebsiella pneumoniae* were selected as representative Gram-negative organisms to evaluate the broad-spectrum antibacterial activity of the formulation. [22] This preliminary study was designed to compare different pharmaceutical preparation methods rather than to evaluate activity against the complete oral microbiome. These selected strains were cultured on Sheep blood agar media for 18 to 24 hours at 37 °C to ensure optimal physiological state before inoculation, after which bacterial suspensions were prepared using sterile water. [23] Turbidity was subsequently adjusted to a 0.5 McFarland standard to ensure consistent inoculum density. [24] The standardized suspensions were then evenly distributed onto Milar Hilton agar plates to facilitate the precise measurement of zones of inhibition. [25]

Culture Media Preparation and Inoculation

Müller-Hinton Agar (MHA) media was prepared, autoclaved at 121°C at 15 psi pressure for 20 minutes, and poured into sterile Petri dishes under a laminar airflow workbench to achieve a uniform depth of approximately 4.0 mm. The agar plates were allowed to solidify completely. Bacterial strains were adjusted to a standard inoculum density matching the 0.5 McFarland turbidity standard (sim 1.5 × 10⁸) using sterile normal saline. The optimized

117719



Kunal Gohil *et al.*,

bacterial suspensions were seeded uniformly across the agar surfaces using sterile cotton swabs to establish homogeneous lawns.

Disc Impregnation and Incubation

Sterile, blank filter paper discs (6.0 mm diameter, Whatman No. 1) were impregnated with exactly 80 ml of the respective undiluted polyherbal mouthwash preparations. Utilizing sterile forceps, the treated discs were positioned precisely onto the inoculated agar surfaces with uniform spacing. The plates were left undisturbed for 30 minutes at room temperature to allow complete outward radial diffusion of the extracted phytochemicals into the surrounding agar matrix. The culture plates were then incubated in an upright position within a digital incubator maintained at 37 degree for a continuous duration of 24 hours. Following incubation, the plates were inspected for clearing, and the absolute Zones of Inhibition (ZOI) were measured diagonally using digital Verniercallipers. Each assay run was performed in triplicate to ensure reproducibility.

RESULTS

Organoleptic and Physical Characteristics

The three formulation pathways produced distinctive physical properties, reflecting their differing physicochemical profiles.

Kwatha

Three batches of *Kwatha* were prepared to evaluate process reproducibility using equal proportions (1 part each) of *Bakula*, *Twaka*, *Haritaki*, and *Musta* (Table 1). Total combined drug volumes for Batches I, II, and III were 200 ml, 280 ml, and 400 ml, respectively. These were mixed with corresponding water volumes of 1600 ml, 2240 ml, and 3200 ml (Table 4).

Physical Observations

Consistent physical transformations were observed across all batches during the heating process shown in table 2 and 3. Initial Stage: The mixture began as a light brown liquid at room temperatures of 28°C to 30°C. Heating Phase: Progressive heating led to surface frothing, which intensified as temperatures rose. Maturation: As temperatures peaked between 108°C and 110°C, the liquid darkened, drug particles settled and steady evaporation occurred. Duration: The time required to reach the final evaporation stage was proportional to the batch volume, taking 90 minutes for Batches I and II, and 180 minutes for the larger Batch III. (Table 3 & Figure 2) Final Yield To achieve standard concentration, boiling was sustained until the liquid was reduced to 1/4th of the initial water volume. This standard reduction principle yielded 400 ml, 560 ml, and 800 ml of final *Kwatha* for Batches I, II, and III, respectively. The linear correlation between initial volumes and final yields confirms that this extraction method is highly reproducible and scalable across different batch sizes (Table 4).

Arka

The preparation of *Arka* was executed across three independent batches utilizing equal proportions (1 part each) of *Bakula*, *Twaka*, *Haritaki*, and *Musta* (Table 1). The raw material quantities were scaled progressively, using 20 ml, 50 ml, and 70 ml per ingredient for Batches I, II, and III, respectively. Water was introduced at a 2-part ratio, corresponding to 160+160 ml for Batch I, 400+400 ml for Batch II, and 560+560 ml for Batch III (Table 5)

Physical Observations

A highly consistent pattern of physical and thermal transitions was observed across all three experimental batches during the *Arka* process. Initial Stage: The starting mixture across all batches presented as a dark brown liquid containing coarsely powdered drugs, recorded at temperatures ranging from 26°C to 32°C. Thermal Transition: As the actual temperature increased (reaching 47°C in Batch I, 64°C in Batch II, and 60°C in Batch III), slight frothing became visible on the liquid surface. *Arka* Initiation: The collection of *Arka* commenced concurrently with the onset of



Kunal Gohil *et al.*,

bubbling, which occurred at temperatures of 60°C (Batch I), 70°C (Batch II), and 68°C (Batch III). Peak Distillation (*Arka*): Marked bubbling was maintained as internal temperatures rose further into the 72°C–80°C range. Process Completion: The process concluded upon reaching *Gat Rasatva*, with final collection temperatures peaking at 78°C for Batch I, 80°C for Batch II, and 82°C for Batch III. The total duration required for complete extraction scaled with the batch volume, taking 50 minutes for Batch I, 1 hour 40 minutes for Batch II, and 2 hours 30 minutes for Batch III (Table 6, 7, 8 & Figure 2). Final Yield The initial *Arka* volumes were recorded at 160 ml for Batch I, 400 ml for Batch II, and 560 ml for Batch III. Following the completion of the distillation cycle, the final recovered volumes of clear *Arka* were 105 ml, 248 ml, and 351 ml, respectively. These outcomes demonstrate a highly linear and reproducible distillation yield relative to the scale of the starting volume (Table 9).

Distillate:

The distillation process involved four primary ingredients—*Bakula*, *Twaka*, *Musta*, and *Haritaki*—combined in equal proportions of 1 part each alongside 10 parts of water (Table 1). For Batch I, 35 ml of each raw drug was mixed with 1400 ml of water. For the remaining two batch setups, 20 ml of each drug was combined with 800 ml of water (Table 10).

Physical Observations

Initial State: The baseline mixture across all three batches appeared as a brownish coloured liquid containing coarsely powdered drugs, recorded at temperatures between 26°C and 30°C. Frothing Phase: Initial thermal application triggered frothing at 60°C in Batch I and Batch II, and at 63°C in Batch III. This was followed by increased frothing and minor bubbling as temperatures rose. Collection Onset: Distillate collection began at an actual temperature of 67°C for Batch I and at 70°C for both Batch II and Batch III. Peak Distillation: Extended heating maintained marked bubbling across a temperature spectrum of 70°C to 85°C across the tracking periods. Process Termination: Heating was systematically terminated for all batches precisely when 60% of the distillate had been collected. This final collection stage was achieved at an actual temperature of 85°C for Batch I, 83°C for Batch II and 80°C for Batch III. (Table 11, 12, 13 and Figure 2) Final Yield Batch I began with an initial volume of 1400 ml and concluded with a final distillate volume of 840 ml. The subsequent batch columns started with an initial volume of 800 ml and both yielded a final distillate volume of 480 ml (Table 14). The final volumes confirm a highly uniform and mathematically consistent 60% recovery rate relative to the starting liquid volume across all experimental batches.

Standardization of finished products

Kwatha

The *Kwatha* presented as a brown-colored preparation with a characteristic odor and a distinctly pungent and astringent taste. Quantitative analysis yielded a pH of 3.62, a specific gravity of 1.0288, and a total solid content of 6.306% w/v.

Arka

The *Arka* presented as a transparent liquid characterized by a pleasant odor and a sweet yet pungent taste. Quantitative physico-chemical evaluation yielded a pH of 4.30, a specific gravity of 1.0102 w/w, and a trace total solid content of 0.004% w/v.

Distillate

The organoleptic evaluation of the distillate revealed it to be a transparent formulation characterized by a pleasant odor and a predominantly sweet and pungent taste. Further physico-chemical analysis was conducted to evaluate the quality of the distillate. The results yielded a pH value of 4.56, a specific gravity of 0.9964 w/w, and a negligible total solid content of 0.003% w/v.

Antimicrobial Profile

The experimental measurements for the zones of inhibition produced by the three distinct pharmaceutical mouthwash forms against the targeted microbial strains are presented in Table 15. The data indicates that the Polyherbal *Arka* formulation demonstrated the most consistent performance, establishing a uniform 5.0 mm zone of

117721





inhibition across all four bacterial strains, including both Gram-positive and Gram-negative organisms. The Polyherbal Distillate showed weak activity, producing a minimal 3.0 mm zone of inhibition across the entire panel. The Polyherbal *Kwatha* achieved only a 3.0 mm zone against *Streptococci* and *Staphylococcus aureus*, while failing to show any inhibitory activity against *Escherichia coli* and *Klebsiella pneumoniae*, indicating complete resistance under these experimental conditions. (Graph 1 and Table 15).

DISCUSSION

The comparative experimental data highlights the significant influence of pharmaceutical processing methodologies on the antimicrobial properties of polyherbal formulations. While identical ratios of *Mimusops elengi*, *Cinnamomum zeylanicum*, *Cyperus rotundus* and *Terminalia chebula* were maintained across all batches, their antibacterial efficacy varied markedly depending on the preparation method (*Arka*, *Kwatha*, or Distillate). The superior and consistent performance of the *Arka* preparation can be attributed to the physics of hydro-distillation. This process effectively co-distills volatile lipophilic secondary metabolites alongside water vapour. Elements such as cinnamaldehyde from *Cinnamomum zeylanicum* and specific terpenoid fractions from *Mimusops elengi* and *Cyperus rotundus* are vaporized and condensed into a stable, homogenous aqueous phase.[26] These volatile compounds can disrupt bacterial cytoplasmic membranes, increase permeability and induce cell death. In contrast, simple steam distillation (*Distillate*) appeared less effective at extracting these active fractions under the parameters tested, resulting in lower antimicrobial activity (3.0 mm ZOI). The poor in-vitro performance of the *Kwatha* (decoction) requires careful analysis. In classical Ayurveda clinical practice, *Kwatha* is routinely prescribed for use in a warm or hot state (*Sukhoshna*). This elevated temperature maintains the solubility of large, complex compounds such as the polyphenols and condensed tannins found in *Terminalia chebula* and *Mimusops elengi*. However, standard in-vitro disk diffusion assays are conducted at room temperature, causing the *Kwatha* to cool during plate application. This cooling can precipitate dense macromolecular complexes, reducing their outward radial diffusion through the agar matrix and resulting in a smaller or absent zone of inhibition. This finding highlights a methodological limitation of standard agar diffusion tests for evaluating dense, non-volatile traditional extracts.

To properly contextualize these findings within contemporary dental medicine, it is valuable to evaluate them alongside global clinical data. Worldwide, 0.2% chlorhexidine gluconate remains the gold standard for chemical plaque control due to its strong cationic binding to oral surfaces. However, its long-term use is restricted by tooth discoloration, taste alterations, and mucosal desquamation. Recent global clinical trials confirm that natural polyherbal mouthwashes can offer comparable clinical efficacy without these adverse effects.[27] For example, a randomized clinical trial by demonstrated that an all-natural polyherbal mouthwash significantly reduced full-mouth plaque and bleeding scores over a three-month period, demonstrating therapeutic efficacy comparable to standard protocols while maintaining high safety profiles.[28] Indeed, the transition toward such biocompatible formulations is increasingly motivated by the clinical imperative to mitigate the common side effects associated with chlorhexidine-based regimens [29]. Furthermore, *Triphala* has emerged as a particularly viable alternative, demonstrating a lack of adverse side effects and superior patient satisfaction in comparison to the metallic taste and soreness frequently reported by chlorhexidine users. [30] Similarly, a randomized non-inferiority clinical trial demonstrated that traditional herbal mouthwashes can match chlorhexidine in reducing anti-gingivitis and anti-plaque indices[31], as well as significantly lowering bacterial counts of key periodontal pathogens like *Porphyromonas gingivalis*. [32] Clinical evaluations also confirmed that natural preparations function effectively as adjuvants to scaling, showing a significant reduction in aerobic colony-forming units (CFUs) comparable to chemical counter-agents.[33]

Furthermore, recent advances in molecular sequencing have revealed insights into how mouthwashes affect the broader oral microbiome. A randomized, double-blind trial utilized 16S rRNA sequencing to compare the effects of chlorhexidine against a polyherbal formulation.[34] The study revealed that while chlorhexidine possesses strong antimicrobial activity, its formulation can disrupt microbial diversity, leading to dysbiosis and an increase in



**Kunal Gohil et al.,**

potentially opportunistic genera. In contrast, the polyherbal formulation selectively modulated the oral ecosystem, preserving overall microbial diversity and stability while achieving high patient acceptability in taste, comfort and moisture retention. This aligns with findings high patient satisfaction and clinical efficacy for alternative herbal mouth rinses.[35]The *Arka* formulation demonstrated reliable antimicrobial activity; however, its effects on the oral microbiome remain to be established through dedicated microbiome studies.

Limitations of the Study

The present study evaluated the antimicrobial activity of the developed polyherbal mouthwash against a representative panel of bacterial species and therefore does not encompass the full diversity of the oral microbiome. Important oral pathogens such as *Streptococcus mutans*, *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, *Aggregatibacter actinomycetemcomitans* and *Candida albicans* were not included. Moreover, the observed zones of inhibition were lower than those generally reported for chlorhexidine, which is expected because polyherbal formulations contain complex phytochemical mixtures with limited agar diffusion compared with purified synthetic antiseptics. The primary objective of this investigation was to compare different Ayurvedic pharmaceutical processing methods rather than to establish equivalence with chlorhexidine. Therefore, further studies involving MIC/MBC determination, biofilm and time-kill assays, phytochemical standardization, and well-designed in vivo clinical trials are required to comprehensively establish the antimicrobial efficacy and clinical applicability of the developed polyherbal mouthwash.

CONCLUSION

The present study successfully validates the pharmaceutical development of an Ayurveda polyherbal mouthwash, demonstrating that the chosen extraction methodology significantly dictates the final antimicrobial efficacy of the mixture. By comparatively evaluating three distinct traditional preparations, this research establishes that the hydro-distilled *Arka* formulation is the superior dosage form for modern dental applications. The *Arka* formulation delivered a uniform broad-spectrum antimicrobial response (producing a consistent 5.0 mm zone of inhibition) against both Gram-positive (*Streptococci*, *S. aureus*) and Gram-negative (*E. coli*, *K. pneumoniae*) pathogens. The traditional aqueous *Kwatha* displayed limited to no efficacy under standardized room-temperature testing conditions, highlighting a critical diffusion limitation of dense macromolecular complexes when not administered in their classical warm (*Sukhoshna*) state. This research supports the modernization of traditional Ayurveda pharmacology into ready-to-use platforms. Future in-vivo clinical trials and comprehensive microbiome sequencing are highly recommended to further establish the therapeutic equivalence and long-term ecological benefits of this *Arka* mouthwash in routine clinical dental practice.

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Table 1: The following table shows the ingredients, part used for the preparation of Kwatha, Arka and Distillate

Sr.No.	Drugs	LatinNames	Part used
1	Bakula	<i>Mimusopselengi</i> Linn.	Bark
2	Twaka	<i>Cinnamomum</i> <i>Zeylanicum</i> Breyn.	Bark
3	Musta	<i>Cyperusrotundus</i> Linn.	Rhizome
4	Haritaki	<i>Terminaliachebula</i> Retz.	Pericarp

Table2: Quantities of the ingredients used in each batch for Kwatha

Sr. No.	Name of ingredients	Proportion	Quantity Taken		
			Batch I	Batch II	Batch III
			ml	ml	ml
1	Bakula	1Part	50	70	100
2	Twaka	1Part	50	70	100
3	Haritaki	1Part	50	70	100
4	Musta	1Part	50	70	100

Table 3: Observations of Batch I, II and III of Kwatha

Duration (min)	Batch I	Batch II	Batch-III	Observations
	Temp (°c)	Temp(°c)	Temp (°c)	
0	28	30	28	Light brown colored liquid.
15	35	38	--	Light brown colored liquid with slight fro thing on surface.
30	56	62	35	More fro thing was observed.
55	78	76		Color darken sand drug starts settling down.
60	--	--	59	More fro thing was observed.





Kunal Gohil et al.,

70	94	97	--	Color darkens and evaporation started.
90	110	108	70	Color darkens and evaporation continued.
120	--	--	82	Color darkens and evaporation started.
150	--	--	94	Color darkens and evaporation continued.
180	--	--	109	Color darkens and evaporation continued.

Table4: Results & final yield obtained in each batch of *Kwatha*

Batch	I	II	III
Vol.ofdrugs	200ml	280ml	400ml
Quantity of water	1600ml	2240ml	3200ml
Reduced to	1/4 th	1/4 th	1/4 th
Final yield	400ml	560ml	800ml

Table5: Ingredients and their proportions used in each batch for *Arka*

Sr. No.	Name of ingredients	Proportion	Quantity Taken		
			Batch I	Batch II	Batch III
			ml	ml	ml
1	<i>Bakula</i>	1Part	20	50	70
2	<i>Twaka</i>	1Part	20	50	70
3	<i>Haritaki</i>	1Part	20	50	70
4	<i>Musta</i>	1Part	20	50	70
5	Water	2Parts	160+160	400+400	560+560

Table 6: Observation table for Batch I of *Arka*

Sr. No.	Time	Temp.		Observation
		Set	Actual	Batch I
1	3.30	65°C	32°C	Dark brown colored liquid containing coarsely powdered drugs.
2	3.40	65°C	47°C	Slightly frothing was seen.
3	3.50	65°C	60°C	Bubbling started and <i>Arka</i> was started collecting.
4	4.00	70°C	72°C	Marked bubbling.
5	4.10	70°C	75°C	Marked bubbling.
6	4.20	70°C	78°C	Gat rasatva and <i>Arka</i> was collected.

Table 7: Observation table for the Batch II of *Arka*

Sr. No.	Time	Temp.		Observation
		Set	Actual	Batch II
1	3.00	65°C	32°C	Dark brown colored liquid containing coarsely powdered drugs.
2	3.30	65°C	64°C	Slightly frothing was seen.
3	3.45	65°C	70°C	Bubbling started and <i>Arka</i> was started collecting.
4	4.00	70°C	75°C	Marked bubbling.
5	4.30	70°C	75°C	Marked bubbling.
6	4.40	70°C	80°C	Gat rasatva and <i>Arka</i> was collected.





Kunal Gohil et al.,

Table 8: Observation table for the Batch III of *Arka*

Sr. No.	Time	Temp.		Observation
		Set	Actual	Batch III
1	9.30	65°C	26°C	Dark brown colored liquid containing coarsely powdered drugs.
2	10.00	65°C	60°C	Slightly frothing was seen.
3	10.30	65°C	68°C	Bubbling started and <i>Arka</i> was started collecting.
4	11.00	70°C	74°C	Marked bubbling.
5	11.30	70°C	80°C	Marked bubbling.
6	12.00	70°C	82°C	Gat rasatva and <i>Arka</i> was collected.

Table 9: Results & final yield of Batch I, II and III of *Arka*

	Batch I	Batch II	Batch III
Initial volume	160 ml	400 ml	560ml
Final volume	105 ml	248 ml	351 ml

Table 10: Ingredients and their proportions used in each batch for Distillate

Sr. No.	Name of ingredients	Proportion	Quantity Taken		
			Batch I	Batch III	Batch III
1	<i>Bakula</i>	1Part	35ml	20ml	20ml
2	<i>Twaka</i>	1Part	35ml	20ml	20ml
3	<i>Musta</i>	1Part	35ml	20ml	20ml
4	<i>Haritaki</i>	1Part	35ml	20ml	20ml
5	Water	10Parts	1400ml	800ml	800ml

Table11: Observation table for the Batch I of Distillate

Sr. No.	Time	Temp.		Observation
		Set	Actual	Batch I
1	9.30	65°C	26°C	Brownish colored liquid containing coarsely powdered drugs.
2	10.00	65°C	60°C	Frothing started.
3	10.30	65°C	67°C	Frothing increased, little bit of bubbling was seen and distillate started collecting.
4	11.00	65°C	72°C	Marked bubbling.
5	11.30	65°C	75°C	Marked bubbling.
6	12.00	70°C	75°C	Marked bubbling.
7	12.30	70°C	78°C	Marked bubbling.
8	1.00	70°C	80°C	Marked bubbling.
9	1.30	70°C	85°C	Marked bubbling.
10	2.00	70°C	85°C	60% of distillate was collected and heating was stopped.

Table12: Observation table for the Batch II of Distillate

Sr. No.	Time	Temp.		Observation
		Set	Actual	Batch II
1	10.00	65°C	27°C	Brownish colored liquid containing coarsely powdered drugs.
2	10.30	65°C	60°C	Frothing started.
3	11.00	65°C	65°C	Frothing increased and little bit of bubbling was seen.
4	11.35	65°C	70°C	Distillate started Collecting.
5	12.00	70°C	70°C	Marked bubbling.
6	12.30	70°C	75°C	Marked bubbling.



Kunal Gohil *et al.*,

7	1.00	70°C	80°C	Marked bubbling.
8	1.30	70°C	83°C	Marked bubbling.
9	2.00	70°C	83°C	Marked bubbling.
10	2.05	70°C	83°C	60% of distillate was collected and heating was Stopped.

Table13: Observation table for the Batch III of Distillate

Sr. No.	Time	Temp.		Observation
		Set	Actual	
				Batch III
1	2.00	65°C	30°C	Brownish colored liquid containing coarsely powdered drugs.
2	2.30	65°C	63°C	Frothing started.
3	3.00	65°C	65°C	Frothing increased and little bit of bubbling was seen.
4	3.35	65°C	70°C	Distillate started collecting.
5	4.00	70°C	70°C	Marked bubbling.
6	4.30	70°C	75°C	Marked bubbling.
7	5.00	70°C	75°C	Marked bubbling.
8	5.30	70°C	80°C	Marked bubbling.
9	6.00	70°C	80°C	Marked bubbling.
10	6.15	70°C	80°C	60% of distillate was collected and heating was stopped.

Table14: Results & final yields of Batch I, II and III of Distillate

	Batch I	Batch II	Batch III
Initial volume	1400ml	800ml	800ml
Final volume	840ml	480ml	480ml

Table 15: Comparative In-Vitro Antimicrobial Zones of Inhibition (mm)

Experimental Formulation Form	<i>Streptococci</i> (mm)	<i>Staphylococcus aureus</i> (mm)	<i>Escherichia coli</i> (mm)	<i>Klebsiella pneumoniae</i> (mm)
<i>Kwatha</i>	3.0 (Poor)	3.0 (Poor)	0.0 (Resistant)	0.0 (Resistant)
<i>Arka</i>	5.0 (Moderate)	5.0 (Moderate)	5.0 (Moderate)	5.0 (Moderate)
<i>Distillate</i>	3.0 (Poor)	3.0 (Poor)	3.0 (Poor)	3.0 (Poor)

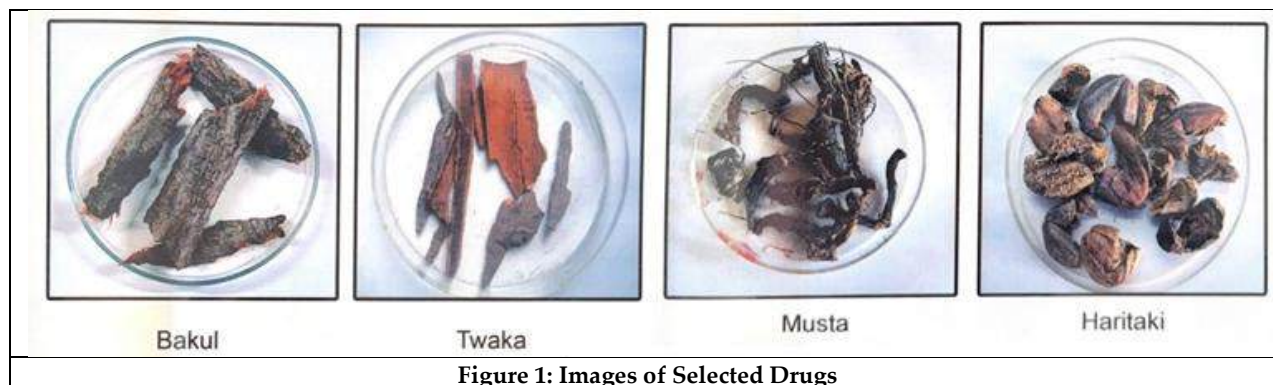


Figure 1: Images of Selected Drugs



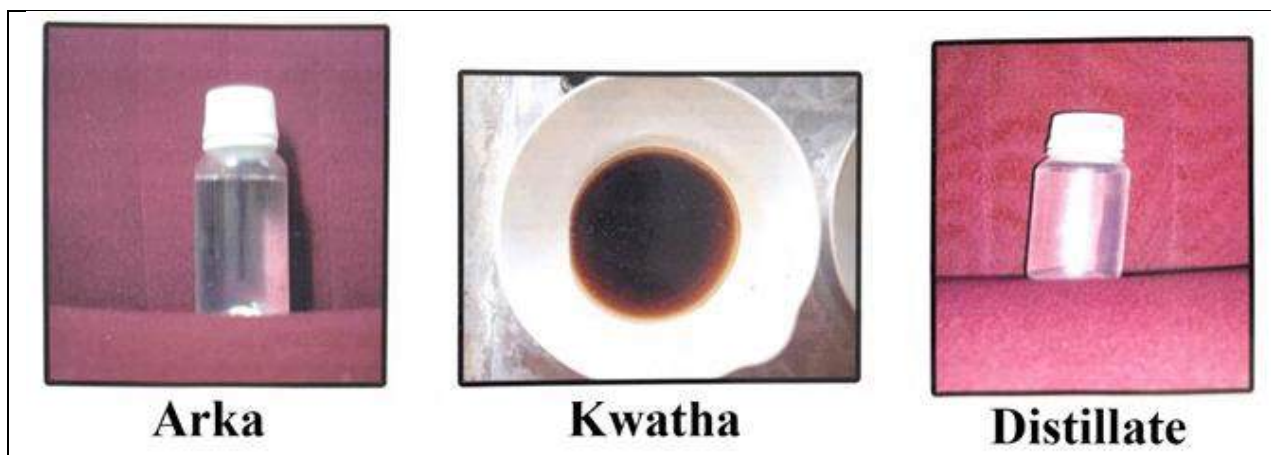
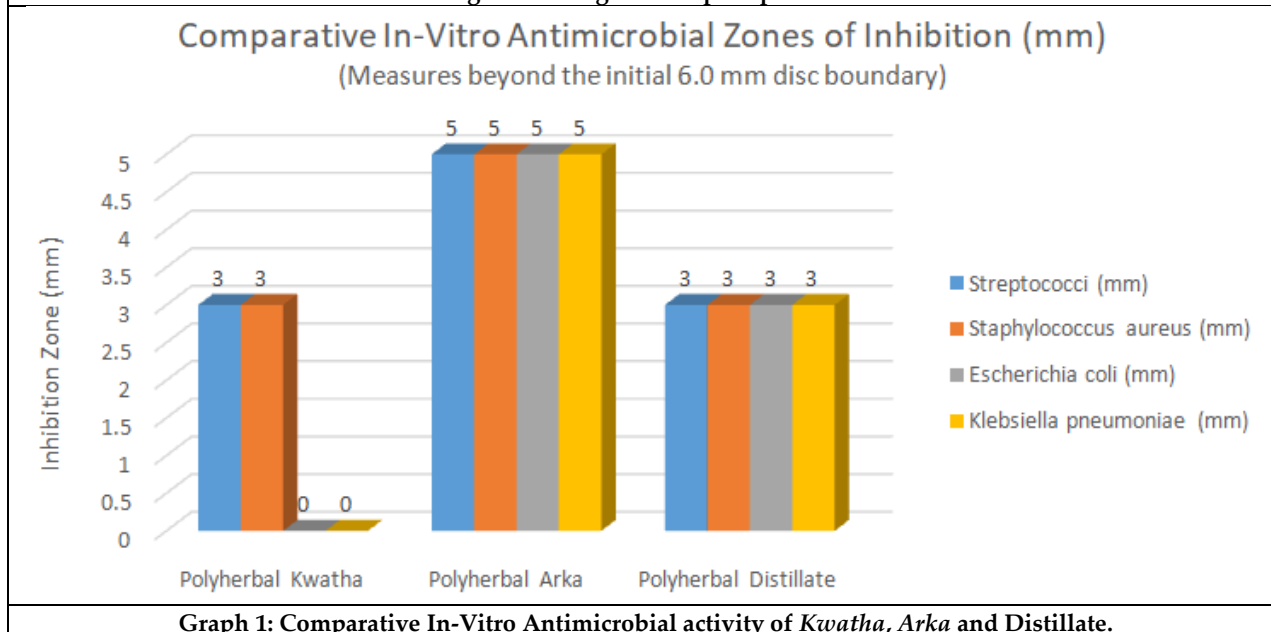
Kunal Gohil *et al.*,

Figure 2 : Image of Preaped product



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Evaluating the Efficacy of Multidetector CT Virtual Hysteroscopy in Diagnosing Female Infertility: A Systematic Review Study

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ABSTRACT

Infertility is experienced by 10–15% of couples, and female factors cause half of them. Uterine pathology, tubal pathology, and conditions such as endometriosis are frequent etiologies. Conventional imaging methods, although good, are invasive and expensive. Multi detector CT virtual hysteroscopy (MDCT-VH) provides a less invasive, high-fidelity 3D imaging option with the possibility of diagnosing uterine and tubal pathology. The purpose of this review is to assess the validity and clinical value of MDCT-VH in diagnosing infertility in women. More specifically, it determines the sensitivity and specificity of MDCT-VH in the diagnosis of tubal pathology, reviews its advantages in overall imaging of the female reproductive system, and compares its diagnostic capability with conventional imaging techniques such as ultrasound and MRI. A PRISMA-informed systematic review was done applying Scopus, PubMed, and Web of Science studies (2010–2025), and MDCT-VH's efficacy in diagnosing infertility disorders. MDCT-VH has high diagnostic efficiency, with sensitivity ranging up to 99.2% and specificity ranging up to 98%. MDCT-VH is complete reproductive imaging, usually superior to conventional methods such as HSG, and supplements MRI, ultrasound, and hysteroscopy in depicting tubal, uterine, and cervical pathology. MDCT-VH is a cost-effective diagnostic option for female infertility, particularly for tubal and high-resolution anatomical imaging. Although it is supplementary to other techniques, it enhances diagnostic accuracy and supports the management of infertility.

Keywords: Female infertility, Multi detector CT, Virtual hysteroscopy, Diagnostic accuracy, Uterine abnormalities, Non-invasive imaging.





INTRODUCTION

Infertility is present in 10–15% of couples worldwide, and female etiology accounts for 50% of the cases [1,2]. Infertility not only adversely affects conception but also imposes heavy psychological, emotional, and social burdens [3]. Infertility treatment is costly, and couples undergo many medical examinations, fertility treatments, and assisted reproductive technologies (ART), most of which are costly and not readily available. Proper diagnosis and management rely heavily on a complete knowledge of female causes of infertility. Uterine, tubal, and peritoneal pathology are frequent reasons for female infertility, with unique pathologies that interfere with conception or maintenance of pregnancy [2,4]. Accurate and early diagnosis is important in order to maximize fertility results. Uterine pathology is responsible for 10–15% of infertility [2,4] and can be congenital, such as Müllerian anomalies (e.g., septate, bicornuate uteri), or acquired, such as fibroids, adenomyosis, endometrial polyps, and intrauterine adhesions (Asherman's syndrome) [3]. Submucosal fibroids and endometrial polyps may interfere with implantation and result in infertility [5]. Imaging modalities like transvaginal ultrasonography, saline infusion sonography (SIS), MRI, and hysteroscopy are usually employed in the identification of uterine abnormalities. Tubal factor infertility due to conditions such as pelvic inflammatory disease (PID) or endometriosis accounts for 25–35% of infertility [2]. Tubal impairment, e.g., scarring or PID occlusion, may be the cause of failure of fertilization. Testing of tubal function using methods such as hysterosalpingography (HSG), laparoscopy, and CT virtual hysterosalpingography (CT-VHSG) is important in diagnosis [6,7]. Peritoneal factor infertility, in many cases linked to endometriosis or pelvic adhesions, is more difficult to establish by conventional imaging [2,4]. Laparoscopy is still the 'gold standard' for the diagnosis of these conditions, though MRI and MDCT-based virtual hysterosalpingography can generate valuable additional information [4]. Female infertility is assessed with a variety of imaging modalities, such as ultrasonography, SIS, HSG, MRI, and laparoscopy. These methods, employed together, give an overall evaluation of the uterine, tubal, and peritoneal causes [3]. MDCT, particularly employed for virtual hysterosalpingography, provides a non-invasive, high-resolution 3D imaging option over traditional techniques. It permits examination of both uterine and tubal anatomy and is possibly more comfortable and faster for patients, providing useful information that may minimize the necessity for further invasive procedures [7,8].

Aim and Objectives

The aim of this study is to evaluate the efficacy of multidetector CT virtual hysteroscopy (MDCT-VH) in diagnosing female infertility through a systematic review. Specifically, the objectives include assessing the sensitivity and specificity of MDCT-VH in detecting tubal diseases associated with infertility, evaluating its benefits in providing comprehensive imaging of the female reproductive system, and comparing its diagnostic outcomes with conventional imaging techniques such as ultrasound and MRI in infertility cases.

MATERIALS AND METHODS

A retrospective observational study was carried out at the Department of Radio-Imaging Technology, SGT University, Gurugram, Haryana, to evaluate the efficiency of multi detector CT virtual hysteroscopy (MDCT-VH) in the diagnosis of female infertility. The study evaluated 50 peer-reviewed research articles published in English language from January to May 2025.

Selection Criteria

Inclusion criteria

The study evaluated the use of MDCT-VH in diagnosing female infertility by reviewing published, peer-reviewed articles from Scopus, PubMed, and UGC Care Journals. Only human studies that provided specific diagnostic criteria for female infertility were included in the analysis.



Sparsh Gurung *et al.*,**Exclusion criteria**

The study excluded non-English articles, animal studies, and conference abstracts, as well as studies with unclear diagnostic criteria or outcomes. Duplicate publications and those with inadequate data for analysis were also excluded from the review.

Statistical Analysis

Statistical examination was done following PRISMA principles. A random effects model was applied to estimate MDCT-VH's sensitivity, specificity, and predictive values combined. Comparative accuracy against conventional imaging methods was calculated where appropriate, and heterogeneity was measured using the I^2 statistic.

RESULTS

Multi detector CT virtual hysteroscopy (MDCT-VH) has been systematically reviewed for its effectiveness in diagnosing female infertility, particularly its sensitivity and specificity in detecting tubal pathologies, its ability to provide comprehensive anatomical views, and its diagnostic performance compared to traditional imaging methods.

MDCT-VH's Diagnostic Accuracy in Tubal Pathology

MDCT-VH and related CT methods demonstrate high diagnostic accuracy for assessing tubal patency and identifying pathologies. A meta-analysis reported CT-VHSG with a combined sensitivity of 0.992 and specificity of 0.98[10]. MDCT also showed excellent sensitivity (100%) and specificity (96.7%) for tubal block detection, comparable to hysterosalpingography (HSG)[11]. Another study found 128-MDCT VHSG 100% sensitive and 85.71% specific for fallopian tube pathology, with strong predictive values[12]. MDCT-VH compares favorably with HSG[13]. HSG has shown varying performance, with a reported 91.3% sensitivity and 88.9% specificity for tubal patency relative to laparoscopy[14]. However, HSG can have high sensitivity (90.91%) but lower specificity (77.78%) for tubal disease, sometimes requiring laparoscopy for confirmation [15]. An individual patient data meta-analysis found HSG's sensitivity and specificity for any tubal pathology to be 53% and 87% respectively, and for bilateral tubal pathology, 46% and 95%, with lower sensitivity in women without tubal disease risk factors¹⁶. These inconsistencies highlight MDCT-VH's more reliable diagnostic accuracy¹⁰.

Advantages of MDCT-VH's Comprehensive Views

MDCT-VH offers a thorough assessment of the female reproductive system, providing detailed anatomical information beyond tubal patency. It improves diagnostic confidence via magnetic-resonance virtual hysterosalpingography (MR-VHSG) and computerized tomographic virtual hysterosalpingography (CT-VHSG) [17]. Its 2D, 3D, and virtual endoscopic imaging capabilities allow for a comprehensive examination of the cervix, fallopian tubes, uterine wall, uterine cavity, and para-uterine pelvic structures, a significant strength in infertility workup [18]. Sixty-four-row VHSG demonstrated excellent diagnostic accuracy for evaluating the uterus and female reproductive system. In a study of 2,500 patients, MDCT-VH accurately assessed internal female genital organs, detecting cervical abnormalities, uterine cavity pathology (e.g., polyps, myomas, synechiae, malformations, adenomyosis), and tubal obstruction, with a low mean radiation dose of 0.94 [19]. MDCT-VH's precise characterization of pathological processes makes it a valuable second-line diagnostic method, providing extensive information to gynecologists for improved therapeutic decisions [17,18].

MDCT-VH's Comparative Diagnostic Performance

MDCT-VH often outperforms and complements conventional imaging techniques like ultrasound (US) and MRI. It causes less patient discomfort and has a lower mean effective radiation dose compared to typical HSG. MDCT can also diagnose adenomyosis and ovarian tumors missed by HSG, though its inability to visualize pelvic adhesions due to lacking delayed imaging is a limitation HSG partially overcomes [11]. Virtual multislice CT hysterosalpingogram (VHSG) showed comparable diagnostic precision to multi parametric MRI-HSG for ovarian and extrauterine abnormalities, uterine morphology, and tubal patency, with high specificity (95.2%) and sensitivity (97.7%)²⁰. This

117732





Sparsh Gurung et al.,

suggests MDCT-VH is as effective as sophisticated MRI for various infertility conditions. Similarly, conventional HSG and MR HSG show comparable diagnostic value for tubal patency, supporting the efficacy of non-radiation-based technologies [21]. While MDCT-VH offers comprehensive views, other modalities excel in specific areas. TVS is preferred for myometrial pathology, HSG for tubal pathologies, and hysteroscopy (HSC) for endometrial pathologies [22]. TVS is more appropriate for myometrial and endometrial lesions, while HSG is better for obliterative uterine cavity disorders [23]. A multimodal approach is therefore often optimal, with MDCT-VH complementing other techniques. Advanced CT, such as virtual monoenergetic imaging (VMI) from dual-energy computed tomography (DECT), shows promise for uterine pathologies beyond infertility, demonstrating greater sensitivity for myometrial invasion than standard CT and MRI in endometrial cancer [24].

General Efficacy and Role of Hysteroscopy in Infertility Diagnosis

Hysteroscopy is crucial for diagnosing female infertility and is often considered the gold standard for direct viewing and precise treatment of intrauterine lesions[25,26]. It effectively identifies and manages disorders such as uterine septum, intrauterine adhesions, endometrial polyps, and submucous myomas [26,27]. Hysteroscopy consistently demonstrates superiority over other techniques for uterine cavity evaluation. It identified incidental findings in 15.38% of cases not seen with HSG, leading to recommendations for its routine use in infertile patients [28]. Saline infusion sonohysterography (SIS) showed 81.2% sensitivity, 86.9% specificity, and 86.5% accuracy compared to hysteroscopy, suggesting it as an alternative when hysteroscopy is unavailable[29]. However, hysteroscopy remains the gold standard for diagnosing submucosal myomas, despite moderate agreement with hysterosonography[30]. TVS, HSG, and hysteroscopy are complementary, but hysteroscopy more accurately diagnoses and treats small intrauterine lesions[31]. It is 30% more effective than ultrasound in identifying uterine anomalies, even in patients with normal ultrasound reports[32], and is superior to histopathology for detecting intracavitary lesions[33]. Hysteroscopy significantly impacts fertility outcomes, particularly with ART. Evidence indicates it increases clinical pregnancy rate (CPR) and live birth rate (LBR) in infertile women, especially those with previous implantation failure[34]. Diagnostic hysteroscopy before the first IVF cycle may optimize the uterine environment[35]. Outpatient hysteroscopy can increase live birth and clinical pregnancy rates among infertile women on ART, even with normal initial imaging[36]. Specifically, hysteroscopic polypectomy improved IVF outcomes in patients with unexplained infertility and a history of unsuccessful IVF cycles[37].

Hysterolaparoscopy is highly effective, providing both a summary of pelvic reproductive anatomy and close-up images, often detecting correctable abnormalities overlooked by other modalities[38]. It is valuable for diagnosing and managing tuboperitoneal and intrauterine pathologies [39,40] and leads to better pregnancy outcomes in female infertility assessment and management[41]. Specific pathologies addressed in studies include elusive uterine lesions, morphological abnormalities, adenomyosis, and chronic endometritis, which negatively affect fertility[42]. Hysteroscopy shows good specificity and accuracy for diagnosing chronic endometritis [43]. Chromohysteroscopy significantly improves endometrial pathology diagnosis accuracy (92%) over routine hysteroscopy (58%) [44]. In terms of patient experience and safety, MDCT causes considerably less discomfort than HSG [11]. Pain during hysteroscopy with local anesthetic is generally low, with cervical canal passage being the most painful phase[45]. Cervical ripening techniques, such as vaginal estradiol and misoprostol pretreatment, minimize complications in operative hysteroscopy[46,47]. Office hysteroscopy successfully treats cervical stenosis in 98.5% of cases, significantly minimizing failed procedure [48]. A previous history of hysteroscopy does not adversely affect subsequent pregnancy outcomes like miscarriage or preterm delivery [49]. Literature also explores less frequent or specialized uses. MDCT venography aids in diagnosing pelvic congestion syndrome [50]. Traditional MRI is compared with 3D ultrasound for predicting ablation rates in uterine fibroids [51]. Hysteroscopy is compared with histopathological results in PCOS-related infertility [52]. Ultrasound and MRI characterize uterine cavities in women with unusual bleeding and extragenital pathology [53]. Hysteroscopy is key for diagnosing female genital tuberculosis, with high pregnancy outcomes after treatment for latent endometrial tuberculosis [54–56].

Diagnostic methods continue to evolve. Disposable digital hysteroscopes are useful and efficient for outpatient hysteroscopy [57]. Negative intrauterine contrast-enhanced ultrasonography and multimodal hysterosalpingo-



**Sparsh Gurung et al.,**

contrast sonography enhance diagnostic efficiency for uterine lesions and tubal patency [58]. Recent outpatient tubal patency techniques with high negative predictive values are proposed as first-line options [59].

DISCUSSION

This systematic review evaluated the role of multi detector CT virtual hysteroscopy (MDCT-VH) in diagnosing female infertility, with a focus on its ability to identify tubal pathology, provide high-resolution imaging, and compare its effectiveness with traditional diagnostic techniques such as MRI, transvaginal ultrasonography (TVS), and hysterosalpingography (HSG). MDCT-VH has been recognized as a valuable non-invasive tool, offering superior anatomical visualization and enhancing patient comfort [60]. Tubal disease is a major contributor to infertility, and precise identification of conditions like tubal obstructions, hydrosalpinx, and pelvic adhesions is crucial for guiding treatment. MDCT-VH has demonstrated high sensitivity and specificity in detecting tubal pathologies, performing on par with or better than conventional HSG [11,60]. Unlike HSG, which carries risks of patient discomfort and poor visualization of pelvic structures, MDCT-VH provides a clear assessment of the fallopian tubes and pelvic environment in a single session, avoiding invasive procedures [61]. Moreover, it allows the detection of extratubal pathologies, such as pelvic adhesions or endometriosis, which can also impact fertility [60].

MDCT-VH offers a significant advantage with its ability to produce high-resolution, multi-planar, and 3D images of the entire reproductive system, including the uterine cavity, cervix, and surrounding pelvic structures [17]. The technique's virtual hysteroscopic images provide a clear view of uterine anomalies, such as polyps, fibroids, and adhesions, which are often missed by less sensitive imaging methods [42]. This comprehensive evaluation of both intra-uterine and extra-uterine structures is particularly beneficial for complex infertility cases [17]. Despite its many benefits, MDCT-VH is not a replacement for other diagnostic modalities but rather a complement. Hysteroscopy remains the gold standard for visualizing and treating intrauterine pathology due to its ability to provide direct visibility and enable immediate intervention [61]. Transvaginal ultrasound, with its non-invasive nature and ability to assess ovarian reserve and myometrial pathology, remains indispensable, especially in low-cost settings [17]. MRI is essential for assessing complex pelvic conditions like deep infiltrating endometriosis, where its superior soft tissue resolution is critical [11]. Integrating MDCT-VH into the diagnostic pathway alongside these established techniques provides a holistic approach to infertility diagnosis, addressing its multifactorial nature [60]. MDCT-VH is also advantageous from a patient-centered perspective, as it is less invasive and more comfortable compared to HSG and hysteroscopy, which involve cervical instrumentation and can be quite uncomfortable [42,60]. Patients generally accept MDCT-VH due to its rapid, non-anesthetic nature, reducing anxiety during the infertility workup [42]. Additionally, advances in multidetector CT technology have significantly reduced radiation exposure, addressing previous concerns about ionizing radiation in reproductive-age women, though adherence to radiation safety protocols remains critical [17].

However, MDCT-VH does have limitations. Despite advancements in reducing radiation doses, it still involves ionizing radiation, which poses a risk, particularly for patients requiring multiple imaging sessions during infertility evaluations [42]. Furthermore, MDCT-VH lacks therapeutic capabilities, necessitating additional procedures if abnormalities are detected. Cost and accessibility may limit its use in resource-constrained settings, and the variability in operator expertise and imaging protocols highlights the need for standardization to ensure diagnostic accuracy [11]. Overcoming these challenges through improved technical training and protocol optimization will be essential for wider clinical adoption [11]. Future research should focus on refining MDCT-VH protocols to further reduce radiation doses and improve image quality. The development of consensus guidelines and reporting standards will enhance comparability across studies and clinical practices. Additionally, integrating artificial intelligence for automated image interpretation could enhance diagnostic precision and reduce operator dependency. Long-term studies evaluating the impact of MDCT-VH on fertility outcomes and cost-effectiveness are needed to establish its place in infertility treatment [42].





CONCLUSION

This review highlights multidetector computed tomography virtual hysteroscopy (MDCT-VH) as a very powerful, less invasive diagnostic tool for female infertility. It yields better tubal abnormality detection, including obstruction and hydrosalpinx, than conventional hysterosalpingography (HSG) without HSG's limitations such as patient discomfort and restricted pelvic visualization. MDCT-VH provides excellent imaging of both intrauterine and extra-uterine pathology, facilitating better diagnosis and treatment planning. Nevertheless, MDCT-VH must supplement, rather than substitute, other techniques such as hysteroscopy, MRI, and TVS. Although its radiation burden has decreased with advances in technology, issues of exposure, expense, and standardization protocols persist. Future studies must aim to maximize imaging protocols, minimize radiation, and incorporate artificial intelligence. In conclusion, MDCT-VH improves infertility diagnosis and treatment, providing accurate anatomical views and increased patient comfort when combined with conventional modalities.

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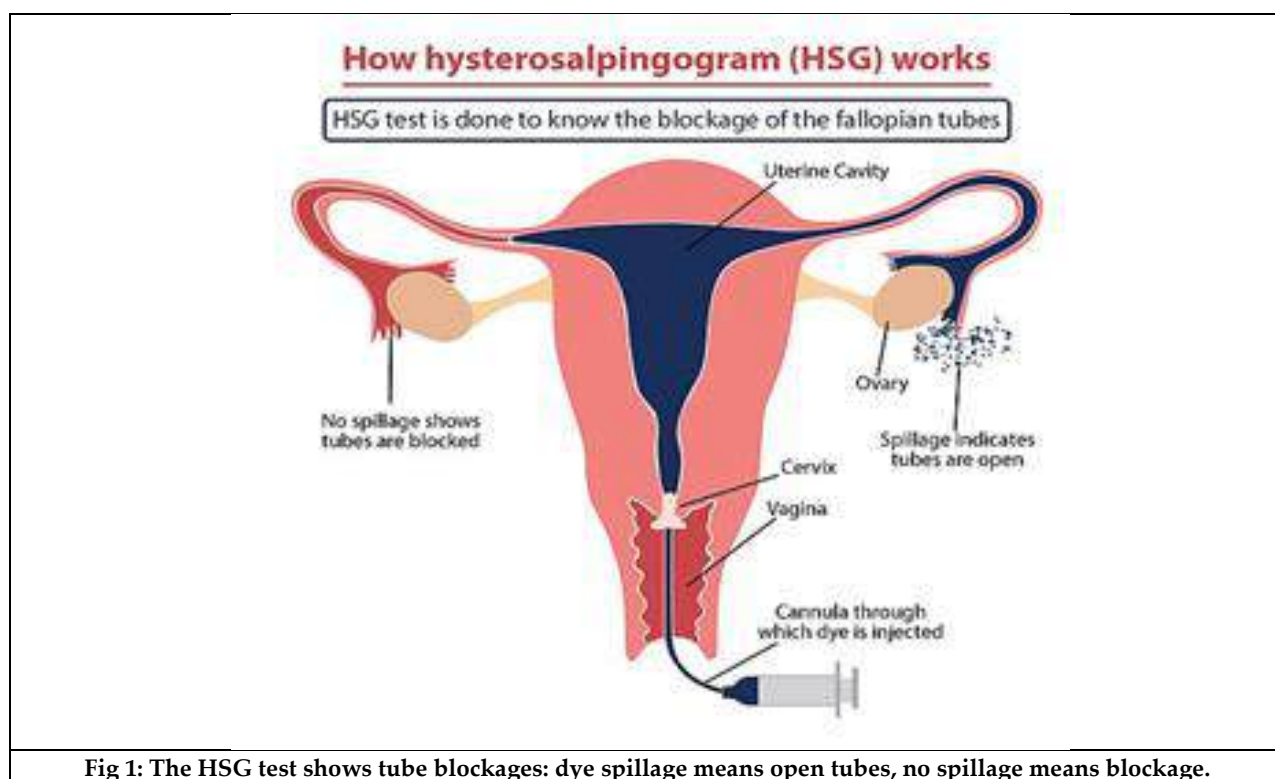
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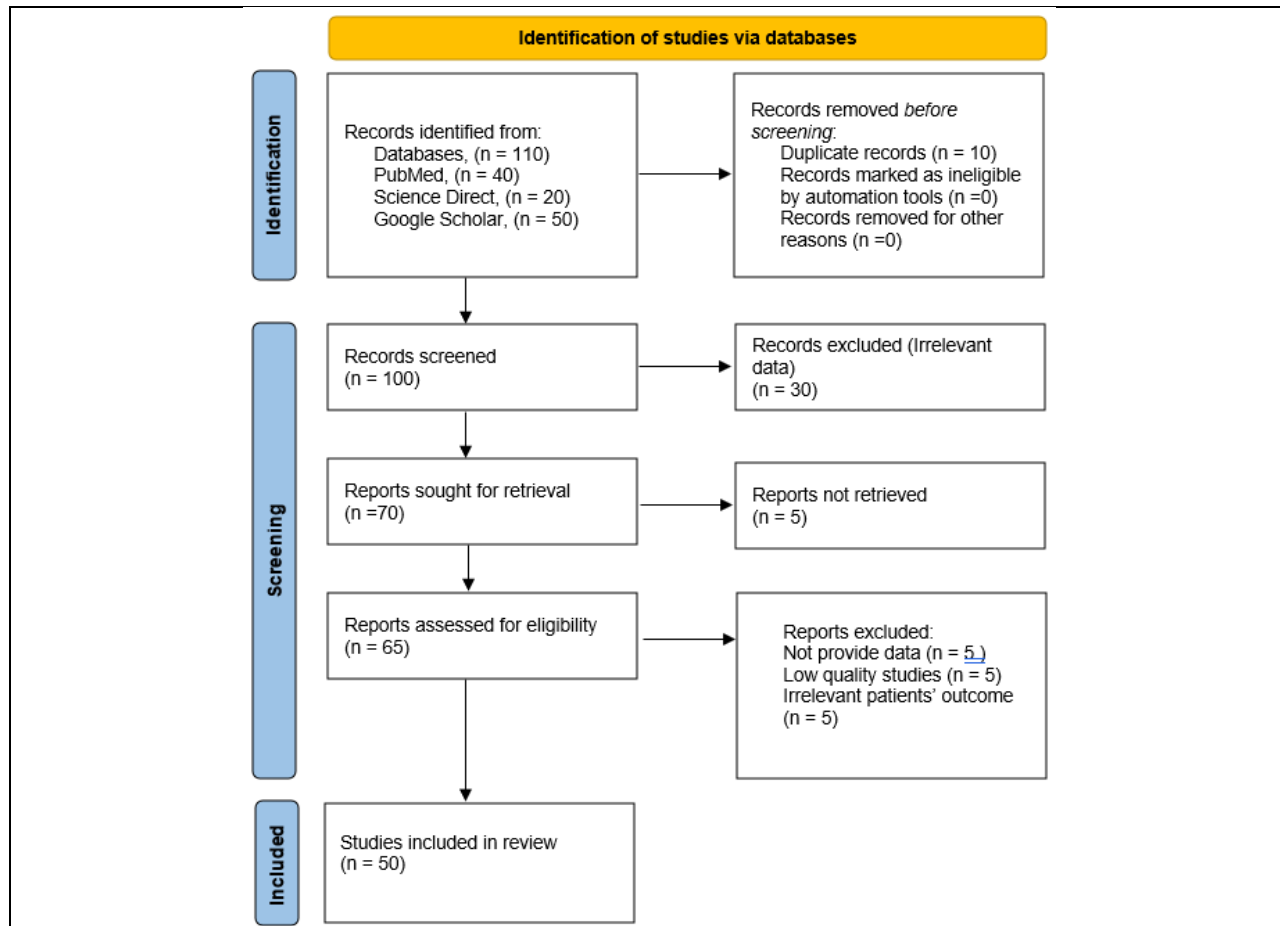


Fig 2: Figure shows the Prisma distribution of 110 original research papers. Out of these we included 50 papers in our study because they had useful information. The whole process is shown in the figure.

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RESEARCH ARTICLE

Experimental Study on Self - Curing Properties of Concrete with Polyethylene Glycol

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ABSTRACT

As water becomes a scarce material every day, there is an urgent need to explore ways to save water in concrete production and buildings. Curing of concrete is the maintenance of satisfactory moisture content of concrete in its initial stage to develop the desired properties. However, good healing is not always practical in many cases. Hardening of concrete plays an important role in the formation of the microstructure and pore structure of concrete and thus improves its durability and performance. Considering this importance, efforts have been made to develop self-hardening concrete using self-hardening water-soluble polyethylene glycol as granite and lightweight aggregate. The purpose of this research is to find out the strength and durability properties of concrete using water-soluble polyethylene glycol as a self-hardening agent. The task of self-hardening substances is to reduce the evaporation of water from the concrete, and thus they increase the water resistance of concrete compared to conventionally hardened concrete. The use of self-hardening additives is very important from the point of view that saving water is necessary every day (3m³ of water is used in the building for every cubic meter of concrete, most of which is used for stoning). In this work, the compressive and fracture strengths



**Prakashkumar et al.,**

of concrete containing self-hardening substances are investigated and compared with those of conventionally hardened concrete. In this experimental study, concrete cast with polyethylene glycol as a self-hardening agent was found to be stronger than concrete obtained by spray or immersion curing. In this study, the effect of an additive (PEG 400) on compressive strength, crack tensile strength and fracture layer was investigated for M₂₀ by varying the percentage of PEG by weight of cement between 0% and 2%. It was found that PEG 400 can help in self-healing, providing similar strength to conventional healing. It was also found that 1% PEG 400 cement was optimal for M₂₀ grade concretes to achieve maximum strength without compromising workability.

Keywords: PEG (Polyethylene Glycol), M₂₀Grade Concrete, PEG 400, W/C Ratio, Hardening Properties.

INTRODUCTION

Curing is the process of maintaining proper moisture content particularly within 28 days to promote optimum cement hydration immediately after placement. Curing plays a major role in developing the concrete microstructure and pore structure. Good curing is not practically possible in most of the cases. The self-curing concrete means that no external curing required for concrete.

General

Curing is the name given to the procedures used for promoting the hydration of the cement, and consists of a control of temperature and of moisture movement from and into the concrete. Curing allows continuous hydration of cement and consequently continuous gain in the strength, once curing stops strength gain of the concrete also stops. Proper moisture conditions are critical because the hydration of the cement virtually ceases when the relative humidity with in the capillaries drops below 80%. Proper curing of concrete structures is important to meet performance and durability requirements. In conventional curing this is achieved by external curing applied after mixing, placing and finishing. Self-curing or internal curing is a technique that can be used to provide additional moisture in concrete for more effective hydration of cement and reduced self-desiccation. When concrete is exposed to the environment evaporation of water takes place and loss of moisture will reduce the initial water cement ratio which will result in the incomplete hydration of the cement and hence lowering the quality of the concrete.

Various factors such as wind velocity, relative humidity, atmospheric temperature, water cement ratio of the mix and type of the cement used in the mix. Evaporation in the initial stage leads to plastic shrinkage cracking and at the final stage of setting it leads to drying shrinkage cracking. Curing temperature is one of the major factors that affect the strength development rate. At elevated temperature ordinary concrete losses its strength due to the formation of the cracks between two thermally incompatible ingredients, cement paste and aggregates. Concrete is the most widely used building material. It has the characteristic of being formed into desired shape most conveniently. It is an artificial material consisting of ingredients such as cement, fine aggregates, coarse aggregates and water. Aggregates are the major ingredients of concrete. Any construction activity requires several materials such as concrete, steel bricks, stone, glass clay, mud, wood and so on. For its sustainability and adaptability with respect to the changing environment the concrete must be such that it can converse resources protected environment, economize and lead to proper utilization of energy. Plain concrete possesses a very low tensile strength, limited ductility, and little resistance cracking. Internal micro cracks are inherently present in the concrete and its poor tensile strength is due to the propagation of such micro cracks, eventually leading to brittle failure of the concrete.

The most widely accepted remedy to this flexural weakness of concrete is the conventional reinforcement with high strength steel. Also, the reinforcement placing and efficient compaction of RCC is very difficult if the concrete is of low workability especially in the case of concrete. In plain concrete and similar brittle materials, structural cracks (micro- cracks) develop even before loading, particularly due to drying shrinkage or other causes of volume changes.





curing of concrete is important to ensure they meet their performance and intended durability requirements. Internal Curing (IC) is a very promising technique that can provide additional moisture in concrete for a more effective hydration of the cement. During hydration of cement, empty pores are created within the cement paste, leading to a reduction in its internal relative humidity and cause cracks to develop at the early-age. This situation is intensified in HPC which can be solved by using the self- curing agents like 'super-absorbent polymers' they can retain the moisture for a long period of time. The ACI-308 Code states that Internal Curing refers to the process by which the hydration of cement occurs because of the availability of additional internal water that is not part of the mixing water. Conventionally, curing concrete means creating conditions such that water is not lost from the surface i.e., curing is taken to happen from the outside to inside. In contrast, internal curing is allowing for curing from the inside to outside through the internal reservoirs (superabsorbent polymers) created. Internal curing "is often also referred as Self-curing".

Concrete

Concrete is a composite construction material composed of cement (commonly Portland cement) and other cementitious materials such as fly ash and slag cement, aggregate (generally a coarse aggregate made of gravels or crushed rocks such as limestone, or granite, plus a fine aggregate such as sand), water, and chemical admixtures. Concrete solidifies and hardens after mixing with water and placement due to a chemical process known as hydration. The water reacts with the cement, which bonds the other components together, eventually creating a robust stone-like material. Concrete is used to make pavements, pipe, architectural structures, foundations, and motorways/roads, bridges/overpasses, parking structures, brick/block walls and footings for gates, fences and poles. Concrete is used more than any other manmade material in the world. As of 2006, about 7.5 cubic kilometres of concrete are made each year more than one cubic meter for every person on Earth.

Self Curing:

Internal curing refers to the process by which the hydration of cement occurs because of the availability of additional internal water that is not part of the mixing water. Benefits of internal curing include increased hydration and strength development, reduced autogenous shrinkage and cracking, reduced permeability and increased durability. Internal curing distributes extra water throughout the entire microstructure, thus maintaining saturation of the cement paste during hydration. However, good curing is not always practical and often neglected in many cases. Conventional concrete was found that water soluble alcohols can be used as self-curing agents in concrete. The use of self-curing admixtures is very important from the point of view that water resource's are getting valuable every day. Self-curing or internal curing is a technique that can be used to provide additional moisture in concrete for more effective hydration of cement and reduced self - desiccation. The benefits of self-curing admixtures is more significant in desert areas where water is not adequately available.

Definition of Self-Curing

The ACI-308 Code states that self-curing refers to the process by which the hydration of cement occurs because of the availability of additional internal water that is not part of the mixing Water. Conventionally, curing concrete means creating conditions such that water is not lost from the surface i.e., curing is taken to happen from the outside to inside'. In contrast, self-curing is allowing for curing from the inside to outside through the internal reservoirs (in the form of saturated Lightweight fine aggregates, superabsorbent polymers, or saturated wood fibers) created. Self-curing is often also referred as Internal-curing.

Potential Materials For Self Curing

The following materials can provide internal water reservoirs

- Lightweight Aggregate (natural and synthetic, expanded shale),
- LWS Sand (Water absorption =17 %)
- LWA 19mm Coarse (Water absorption = 20%)



**Prakashkumar et al.,**

- Super-absorbent Polymers (SAP) (60-300 mm size)
- SRA (Shrinkage Reducing Admixture) (propylene glycol type i.e. polyethylene-glycol) Wood powder

Chemicals To Achieve Self-Curing

Some specific water-soluble chemicals added during the mixing can reduce water evaporation from and within the set concrete, making it self-curing. The chemicals should have abilities to reduce evaporation from solution and to improve water retention in ordinary Portland cement matrix.

Water Required For Self-Curing

It depends upon chemical and autogenous shrinkages expected during hydration reactions. Types of Shrinkage Drying Shrinkages may occur at early ages or at later ages over a longer period; different types of shrinkages may be identified as: Drying shrinkage, autogenous shrinkage, thermal shrinkage, and carbonation shrinkage.

Reason For Chemical Shrinkage

Chemical shrinkage is an internal volume reduction due to the absolute volume of the hydration Products being less than that of the reactants (cement and water). For example: Hydration of tricalcium silicate:

**Need For Self-Curing**

When the mineral admixtures react completely in a blended cement system, their demand for curing water (external or internal) can be much greater than that in a conventional ordinary Portland cement concrete. When this water is not readily available, due to Depercolation of the capillary porosity, for example, significant autogenous deformation and (early-age) cracking may result. Due to the chemical shrinkage occurring during cement hydration, empty pores are created within the cement paste, leading to a reduction in its internal relative humidity and also to shrinkage which may cause early-age cracking. This situation is intensified in HPC (compared to conventional concrete) due to its generally higher cement content, reduced water/cement (w/c) ratio and the pozzolanic mineral admixtures (fly ash, silica fume). The empty pores created during self-desiccation induce shrinkage stresses and also influence the kinetics of cement hydration process, limiting the final degree of hydration. The strength achieved by IC could be more than that possible under saturated curing conditions. Often specially in HPC, it is not easily possible to provide curing water from the top surface at the rate required to satisfy the on-going chemical shrinkage, due to the extreme.

Necessity of Self Curing

Conventionally, curing concrete means creating conditions such that water is not lost from the surface i.e., curing is taken to happen from the outside to inside. In contrast internal curing is allowing for curing from the inside to outside. Self-curing is also referred as internal curing.

Self-Curing Concrete

The concept of curing and recognition of its contribution to obtain desirable properties of concrete is not novel. This technique has been adopted to maintain moisture and temperature conditions in a freshly placed cementitious mixture to allow hydraulic cement hydration and pozzolanic reactions to occur so that the potential properties of the mixture may develop. In earlier stage though it has not received proper attention but nowadays it is found to be one of major concern in the study of concrete performance. However the concept of self-curing has been developed and now been in practice to eliminate some problems occurred in case of traditional curing. According to the definition provided in American Concrete Institute (ACI) Terminology guide self-curing is supplying water throughout a freshly placed cementitious mixture using reservoirs, via pre-wetted lightweight aggregates, that readily release water as needed for hydration or to replace moisture lost through evaporation or self-desiccation (American Concrete Institute.2010).

The principal contribution of self-curing results in the reduction of permeability that develops from a significant extension in the time of curing. Self-curing reduces plastic shrinkage cracking and settlement. Also a life-cycle cost





Prakashkumar *et al.*,

reduction was estimated when internally cured high performance concrete is used instead of normal concrete. So far different materials has been self-curing such as Super Absorbent Polymers (SAP), Wood Powder, crushed return concrete aggregates, rewetted Light Weight Aggregates (LWA) expanded shale, clays, and slates.

Improvements To Concrete Due To Self-Curing:

- Reduces autogenous cracking
- Reduces permeability
- Protects reinforcing steel
- Increases mortar strength
- Provides greater durability
- Higher early age flexural strength
- Higher early age compressive strength
- Higher modulus of elasticity

Need And Scope of Study

Curing of concrete is maintaining satisfactory moisture content in concrete during its early stages in order to develop the desired properties. However good curing is not always practical in many cases. The aim of this investigation is to evaluate the use of water-soluble polymeric glycol as self-curing agents. The use of self-curing admixture curing admixtures is very important from the point of view that the water resources are getting valuable every day. The benefit of self-curing admixtures is more significant in desert areas where water is not adequately available. In this study the mechanical properties of self-curing at different percentages of poly ethylene glycol will be evaluated and compared with conventional concrete specimen. Scope of the study is to identify the effect of Polyethylene Glycol (PEG) on strength characteristics of self-curing concrete and also to evaluate influence of poly ethylene glycol on mechanical properties which are experimentally investigated.

Mechanism And Significance of Self Curing Concrete

Continuous evaporation of moisture takes place from an exposed surface due to the difference in chemical potentials (Free energy) between the vapour and liquid phases. The polymers added in the mix mainly form hydrogen bonds with water molecules and reduce the chemical potential of the molecules which in turn reduces the vapour pressure, thus reducing the rate of evaporation from the surface. When the mineral admixtures react completely in a blended cement system, their demand for curing water (external or internal) can be much greater than that in a conventional ordinary Portland cement concrete. When this water is not readily available, significant autogenous deformation and (early-age) cracking may result. Due to the chemical shrinkage occurring during cement hydration, empty pores are created within the cement paste, leading to a reduction in its internal relative humidity and also to shrinkage which may cause early-age cracking.

Advantages of Self-Curing Concrete:

- Reduces autogenously cracking.
- self-curing
- Reduce the permeability.
- Increases mortar strength and early age strength sufficient to withstand strain.
- Greater utilization of cement.
- Lower Maintenances.

Advantages of Internal Curing

- Internal curing (IC) is a method to provide the water to hydrate all the cement, accomplishing what the mixing water alone cannot do. In low w/c ratio mixes (under 0.43 and increasingly those below 0.40) absorptive





lightweight aggregate, replacing some of the sand, provides water that is desorbed into the mortar fraction (paste) to be used as additional curing water. The cement, not hydrated by low amount of mixing water, will have more water available to it

- IC provides water to keep the relative humidity (RH) high, keeping self-desiccation from occurring.
- IC eliminates largely autogenous shrinkage.
- IC maintains the strengths of mortar/concrete at the early age (12 to 72 hrs.) above the level where internally & externally induced strains can cause cracking.
- IC can make up for some of the deficiencies of external curing, both human related (critical period when curing is required is the first 12 to 72 hours) and hydration related (because hydration products clog the passageways needed for the fluid curing water to travel to the cement particles thirsting for water).

Following factors establish the dynamics of water movement to the unhydrated cement particles:

- Thirst for water by the hydrating cement particles is very intense,
- Capillary action of the pores in the concrete is very strong, and
- Water in the properly distributed particles of LWA (fine) is very fluid.

Polyethylene Glycol

Introduction

Polyethylene Glycol (PEG) is family of water-soluble linear polymers formed by the additional reaction of Ethylene Oxide (EO) with Monoethylene Glycols (MEG) or Ethylene Glycol. The generalized formula for polyethylene glycol is: $H-(O-CH_2-CH_2)_n-OH$. It is a polyether compound with many applications from industrial manufacturing to medicine. PEG is also known as **Polyethylene Oxide (PEO)** or **Polyoxy Ethylene (POE)**, depending on its molecular weight. There are many grades of PEGs that represents them by their average molecular weight. For example, PEG 4000 consists of a distribution of polymers of varying molecular weight with an average of 4000. Polyethylene glycols are available in average molecular weight ranging from 200 to 8000 this wide range of products provides flexibility in choosing properties to meet the requirements of many different applications. One common feature of PEG appears to be the water-soluble nature. Poly ethylene glycol is non-toxic, odorless, neutral, lubricating, non-volatile, non – irritating and is used in a variety of pharmaceuticals. The most common which is mono-functional methyl ether PEG (Methoxy polyethylene glycol), abbreviated Mpeg. PEGs are also available with difference geometrics. Branched PEGs have 3 to 10 PEG chains emanating from a central core group. Star PEGs have 10-100 PEG chains emanating from a central core group. Comb PEGs have melting points vary depending on the formula weight of the polymer. PEG or PEO has the following structure: $HO-(CH_2-CH_2-O)_n-H$.

The Most Important Physical Property

Depend on molecular weight the wide range of the physical property such as solubility, hygroscopic, vapour pressure, melting or freezing point and viscosity are variable:

- **Solubility:** Increasing the molecular weight of PEGs results in decreasing solubility in water & solvents. PEGs are also soluble in many polar organic solvents such as acetone, alcohols.
- **Hygroscopic:** PEGs are hygroscopic, it means that they attract and retain moisture from the atmosphere. Hygroscopic decreases as molecular weight increases.
- **Viscosity:** PEGs can be considered Newtonian fluids, so the kinematic viscosity of PEGs decrease as temperature increases.
- **Stability:** PEGs have low volatility and are thermally stable for limited period of time below 300°C and without O_2 . Depending on their average molecular weight, the polyethylene glycol may be liquid or solid. PEG grades: 200,300,400,600 in liquid form PEG 1000,1500 soft solid(white) and PEG 2000,3000,4000,6000,8000 hard solid (white).





Prakashkumar et al.,

Applications

Pharmaceuticals: PEG grades meet requirements of USP/NF and BP and widely use in pharmaceutical formulations. As solvent, water soluble, binder, lubricant, plasticizer and use in ointment base, tablet coating, gelatin capsule, liquid oral medications.

Cosmetics: As the water soluble, odourless, neutral, hygroscopic, lubricant, plasticizer, on-volatile, no irritating properties. The PEGs are used in wide range of cosmetics and personal care products such as creams, lotions, sticks, cakes, powders and jellies.

Household products: Soaps, detergents, polishes & cleaners are main applicant of poly ethylene glycols, because these products are water soluble & inert, with low volatility and low toxicity.

Ceramics and tiles: As the plasticizer, lubricity, binders & carrier properties. PEGs are widely used in ceramic and tile industry.

Adhesives and textile: As the plasticizer, lubricants, softeners, antistatic agents & conditional agents. PEGs are used in adhesive & textile industry.

Other applications

- Agriculture as water solubility & solubilize for organic insecticides
- Paper, printing and inks as softener, humectants, solvents, lubricants & carriers
- Paints and coating as intermediates for alkyd & polyester resins.

Handling and storage: PEG product is only slightly toxic & safe for use in domestic cleaning product, when handling product, recommended that use safety goggles, PVC gloves & apron.

Available Forms and Nomenclature:

PEG, PEO, and POE refer to an oligomer or polymer of ethylene oxide. The three names are chemically synonymous, but historically PEG is preferred in the biomedical field, whereas PEO is more prevalent in the field of polymer chemistry. Because different applications require different polymer chain lengths, PEG has tended to refer to oligomers and polymers with a molecular mass below 20,000 g/mol, PEO to polymers with a molecular mass above 20,000 g/mol, and POE to a polymer of any molecular mass. PEGs are prepared by polymerization of ethylene oxide and are commercially available over a wide range of molecular weights from 300 g/mol to 10,000,000 g/mol. PEG and PEO are liquids or low-melting solids, depending on their molecular weights. While PEG and PEO with different molecular weights find use in different applications, and have different physical properties (e.g. viscosity) due to chain length effects, their chemical properties are nearly identical. Different forms of PEG are also available, depending on the initiator used for the polymerization process – the most common initiator is a monofunctional methyl ether PEG, or methoxypoly (ethylene glycol), abbreviated mPEG. Lower-molecular-weight PEGs are also available as purer oligomers, referred to as monodisperse, uniform, or discrete. Very high purity PEG has recently been shown to be crystalline, allowing determination of a crystal structure by x-ray diffraction.

Since purification and separation of pure oligomers is difficult, the price for this type of quality is often 10–1000 fold that of poly disperse PEG. PEGs are also available with different geometries. Comb PEGs have multiple PEG chains normally grafted onto a polymer backbone. The numbers that are often included in the names of PEGs indicate their average molecular weights (e.g. a PEG with $n = 9$ would have an average molecular weight of approximately 400 Daltons, and would be labelled PEG 400.) Most PEGs include molecules with a distribution of molecular weights (i.e. they are polydispersity). The size distribution can be characterized statistically by its weight average molecular weight (M_w) and its number average molecular weight (M_n), the ratio of which is called the polydispersity index (M_w/M_n). M_w and M_n can be measured by mass spectrometry. PEGylation is the act of covalently coupling a PEG structure to another larger molecule, for example, a therapeutic protein, which is then referred to as a PEGylated protein. PEGylated interferon alfa-2a or -2b are commonly used injectable treatments for hepatitis C infection. PEG is soluble in water, methanol, ethanol, acetonitrile, benzene, and dichloromethane, and is insoluble in diethyl ether and hexane. It is coupled to hydrophobic molecules to produce non-ionic surfactants. PEGs potentially contain toxic impurities, such as ethylene oxide and 1, 4-dioxane. Ethylene Glycol and its ethers are nephrotoxic if applied to damaged skin. Polyethylene glycol (PEG) and related polymers (PEG phospholipid constructs) are often sonicated when used in biomedical applications. However, as reported by Murali et al., PEG is very sensitive to son lytic

117747



**Prakashkumar et al.,**

degradation and PEG degradation products can be toxic to mammalian cells. It is, thus, imperative to assess potential PEG degradation to ensure that the final material does not contain undocumented contaminants that can introduce artefacts into experimental results. PEGs and methoxy polyethylene glycols are manufactured by Dow Chemical under the trade name Carbowax for industrial use, and Carbowax Sentry for food and pharmaceutical use. They vary in consistency from liquid to solid, depending on the molecular weight, as indicated by a number following the name. They are used commercially in numerous applications, including as surfactants, in foods, in cosmetics, in pharmaceuticals, in biomedicine, as dispersing agents, as solvents, in ointments, in suppository bases, as tablet excipients, and as laxatives. Some specific groups are lauromacrogols, nonoxynols, octoxynols, and poloxamers. Macrogol, used as a laxative, is a form of polyethylene glycol. The name may be followed by a number which represents the average molecular weight (e.g. Macrogol 3350, Macrogol 4000 or Macrogol 6000).

Experimental Investigations Working Methodology

CONCLUSION

Polymerization of ethylene oxide is an exothermic process. Overheating or contaminating ethylene oxide with catalysts such as alkalis or metal oxides can lead to runaway polymerization, which can end in an explosion after a few hours. Polyethylene oxide, or high-molecular weight polyethylene glycol, is synthesized by suspension polymerization. It is necessary to hold the growing polymer chain in solution in the course of the polycondensation process. The reaction is catalysed by magnesium, aluminium, or calcium-organ element compounds. To prevent coagulation of polymer chains from solution, chelating additives such as dimethylglyoxime are used. Alkaline catalysts such as sodium hydroxide (NaOH), potassium hydroxide (KOH), or sodium carbonate (Na₂CO₃) are used to prepare low-molecular-weight polyethylene glycol.

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Figure 1: Polyethylene Glycols

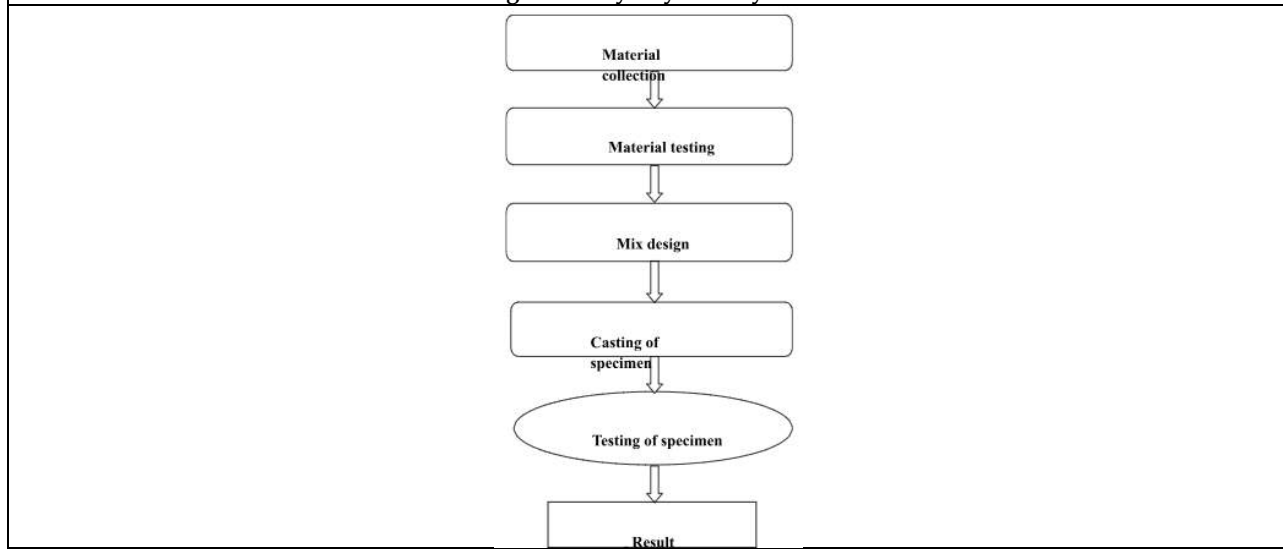


Figure 2: Working Methodology

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RESEARCH ARTICLE

Twin Shield: A Digital Twin - Driven Continual Deep Reinforcement Learning Framework for Proactive Cyber Resilience in Smart Grid CPS

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ABSTRACT

Defensive architectures protecting smart grid Cyber-Physical Systems (CPS) share a fundamental vulnerability that has received surprisingly little attention — they stop learning the moment training ends. In an environment where adversaries continuously probe and refine their strategies, a defense system frozen at deployment grows progressively less effective over time. Existing deep learning and reinforcement learning approaches, despite their considerable strengths, treat cyber defense as a static optimization problem rather than a continuously evolving one — an assumption that operational grid environments routinely violate. Twin Shield addresses this gap through a three-part architecture that keeps defensive intelligence actively growing throughout its operational lifetime. At its foundation sits a physics-informed digital twin — a real-time computational replica of the grid that calibrates itself against live telemetry and synthesizes realistic, distribution-shifting attack scenarios the defense system has not previously encountered. These synthetic scenarios feed a Continual Deep Reinforcement Learning (CDRL) agent built on a Constrained Proximal Policy Optimization backbone, which absorbs new defensive knowledge without discarding previously learned behaviors. Catastrophic forgetting is addressed through an elastic weight consolidation mechanism adapted specifically for the grid defense context. A Control-Barrier Shield ensures every candidate action satisfies hard physical constraints on voltage, frequency, and thermal limits before reaching the grid — regardless of how unfamiliar the driving attack scenario may be. Situational awareness is provided by a CRNN with multi-head attention that processes multivariate grid telemetry across spatial and temporal dimensions simultaneously. Unlike static perception modules that degrade as attack characteristics shift, this component sharpens



**Anil Kumar and Konga Sathish Kumar**

continuously as the digital twin generates fresh training scenarios over time. Experimental evaluation on IEEE 39-bus and 118-bus benchmark networks under progressively evolving FDI, DoS, and combined multi-vector attack conditions demonstrates sustained defensive performance across extended operational periods where static frameworks degrade measurably. Expected outcomes include significant reductions in attack recovery time, consistently high operational stability indices, near-elimination of constraint violations, and improving generalization performance as operational experience accumulates. Twin Shield is the culminating contribution of a research trilogy — following deep learning-based detection and constrained reinforcement learning based mitigation — delivering a self-improving defensive intelligence where resilience deepens with experience rather than eroding with time.

Keywords: Digital Twin, Continual Deep Reinforcement Learning, Smart Grid Security, Cyber-Physical Systems, Control-Barrier Functions, Elastic Weight Consolidation, Proactive Cyber Resilience, Spatio-Temporal Anomaly Detection, Constrained Policy Optimization, Physics-Informed Simulation

INTRODUCTION

Artificial intelligence has become the dominant technology for defending smart grid Cyber-Physical Systems against cyber-attack. Across the past decade, the defensive AI landscape has moved from rule-based threshold classifiers through statistical anomaly detectors to deep learning architectures capable of resolving subtle, spatially distributed attack signatures in high-dimensional telemetry streams. Each generation has outperformed the last on benchmark datasets, and the research community has rightly treated this progression as meaningful progress. What the benchmark framing obscures, however, is a temporal question that no static evaluation can answer: how does a trained model perform six months after deployment, against an adversary that has been probing its decision boundaries the entire time? The answer, for every AI framework that trains once and deploys with frozen parameters, is that performance degrades. Not because the model was poorly designed, but because the fundamental premise of offline training — that the distribution seen at training time approximates the distribution encountered in operation — does not hold against a strategic adversary. Cyber attackers observe defensive responses, identify the signatures and thresholds that trigger detection, and iteratively craft mutations that preserve their objectives while evading the specific patterns the defensive AI encoded. This is not statistical noise; it is directed distributional shift, and it compounds with every probe. A deep learning classifier that was 94% accurate at deployment can, under sustained adversarial pressure, become genuinely unreliable within an operational year. Twin Shield is built on the recognition that this trajectory is the central unsolved problem in defensive AI for smart grid CPS — and that solving it requires a framework whose learning does not stop at the moment of deployment.

Problem Definition

The smart grid cyber defense challenge decomposes into three interconnected sub-problems that existing approaches address incompletely.

- The Static Defense Problem: Machine learning-based defenses are trained on fixed datasets and deployed with frozen parameters. Because smart grid adversaries are strategic agents rather than random processes, the threat distribution evolves directionally — exploiting the growing gap between what the defense can handle and what adversaries are actually doing. Degradation is therefore accelerative, not gradual.
- The Safety Constraint Problem: Defensive actions in power system CPS carry physical consequences. A reinforcement learning agent issuing inappropriate control commands can violate voltage, frequency, or thermal limits, causing equipment damage or cascading outage. The challenge intensifies under continual learning: novel attack scenarios produce novel control situations in which a policy operating outside its training distribution cannot reliably anticipate which actions are physically safe.



**Anil Kumar and Konga Sathish Kumar**

- The Situational Awareness Problem: Effective defense requires knowing not merely that an anomaly exists but where it is, what attack type it represents, and which components are at risk. Attack signatures are embedded in high-dimensional, spatially distributed, temporally correlated telemetry streams. Detection modules trained on specific patterns produce rising false-negative rates as attack characteristics shift — precisely when accurate situational awareness is most critical.
- TwinShield addresses this combined problem: given a smart grid CPS in a continuously evolving threat environment, maintain near-optimal defensive performance across extended operational horizons, preserve hard physical safety constraints under novel attack conditions, and improve threat characterization accuracy over time — without operational downtime for retraining and without discarding prior defensive knowledge.

Research Objectives

Five objectives operationalize the problem statement.

- Develops a physics-informed digital twin that calibrates against live telemetry and generates distribution-shifting adversarial scenarios representative of genuine adversarial adaptation.
- Develops a Continual Deep Reinforcement Learning agent that absorbs new defensive knowledge from novel scenarios without degrading competence on previously encountered attack types.
- Adapts Elastic Weight Consolidation to the grid defense context, protecting consolidated knowledge while preserving plasticity for qualitatively new threats.
- Implements a Control-Barrier Shield that certifiably prevents physically dangerous actions from reaching the grid regardless of the novelty of the driving attack scenario.
- Develops a CRNN with multi-head attention that processes grid telemetry simultaneously across spatial and temporal dimensions and sharpens threat characterization as the digital twin generates increasingly diverse scenarios.

Key Challenges and Design Choices

- The stability-plasticity dilemma is the central AI challenge in Twin Shield's design. Any neural network trained sequentially on new data faces competing pressures: absorb the new information, but do not let gradient updates overwrite the weight configurations that produced prior competence. Unconstrained sequential training resolves this badly — new task gradients dominate, prior representations are erased, and the agent that was effective at Phase 1 FDI is no longer effective there after learning Phase 5 adaptive attacks. EWC resolves this by treating parameter importance as a first-class quantity: weights that carried high responsibility for prior defensive decisions are updated conservatively, while those with low prior relevance remain fully plastic. The result is a policy that accumulates capability rather than cycling through it.
- Safe exploration under distributional novelty required an architectural solution that operates at inference time rather than training time. Training-time constraint satisfaction — penalizing unsafe actions through reward shaping or Lagrange multipliers — provides no guarantee when the policy faces scenarios far outside its training distribution. The Control-Barrier Shield addresses this by intercepting every candidate action before it reaches the physical system and projecting any unsafe action onto the nearest constraint-satisfying alternative, with no assumption about how well-trained the policy currently is.
- Multi-scale telemetry representation required an architecture that processes spatial and temporal structure simultaneously rather than sequentially. The CRNN composition — convolutional layers reading spatial patterns across measurement nodes, feeding a recurrent layer that tracks how those patterns evolve, refined by multi-head attention that focuses processing on the most informative time windows and node clusters — provides this without the representational bottleneck that naive concatenation of separate spatial and temporal models produces.

Contributions of the Paper

TwinShield is the third paper of a research trilogy. The first established deep learning-based detection of FDI, DoS, and multi-vector attacks on grid telemetry. The second introduced constrained reinforcement learning-based



**Anil Kumar and Konga Sathish Kumar**

mitigation with formal safety guarantees. Twin Shield closes the trilogy by addressing what both prior works treated as fixed: the temporal evolution of the threat environment. Its specific contributions are

- A physics-informed digital twin for active adversarial scenario synthesis, the first such application for continual learning in smart grid defense.
- A CDRL framework on a Constrained PPO backbone, the first designed specifically for grid cyber defense.
- A grid-adapted EWC mechanism with dynamic consolidation coefficients calibrated to scenario distribution distance.
- A topology-aware Control-Barrier Shield implementing quadratic-program action projection with real-time latency.
- CRNN with multi-head attention for adaptive situational awareness; and (6) a systematic evaluation on IEEE 39-bus and 118-bus networks under progressively evolving attack conditions, documenting both the degradation of static frameworks and the sustained performance of continual learning.

Organization of the Paper

Section II reviews related work. Section III formalizes the threat model and problem as a sequential constrained Markov decision process. Section IV details the Twin Shield architecture. Section V describes the experimental setup. Section VI presents results and ablation analysis. Section VII discusses implications and limitations. Section VIII concludes.

Related Work

Twin Shield draws from five AI research threads, each directly implicated in its architecture: deep learning for FDI and DoS detection, deep reinforcement learning for cyber-resilient control, spatial-temporal architectures for multivariate telemetry, continual learning with catastrophic forgetting mitigation, and physics-informed digital twins for scenario generation. Each thread is examined below, its limits identified, and its gap mapped to Twin Shield's design.

Deep Learning for FDI and DoS Attack Detection

Detecting FDI and DoS attacks in smart grid CPS demands architectures capable of resolving attack-induced deviations within high-dimensional, spatially distributed, temporally correlated telemetry — a challenge too structurally complex for statistical threshold approaches [1], [2]. CNNs established the first competitive baseline, learning spatially local patterns across measurement nodes that hand-crafted features missed [3], and were subsequently extended to attack localization by encoding multivariate grid time series as Gramian Angular Field and Recurrence Plot image representations [4]. Recurrent networks, particularly LSTM and GRU variants, addressed the temporal dimension: LSTM detectors captured auto-regressive dependencies that temporally sophisticated FDI injections partially preserve and partially violate [5], while Kalman Filter-RNN hybrids combined model-based state prediction with data-driven residual classification [6]. A novel hybrid CNN-LSTM compensation model for DoS attacks in power system state estimation demonstrated that spatial-temporal composition is particularly effective when communication-layer suppression co-occurs with measurement corruption [7]. When CNN and GRU layers are composed for DoS detection on smart grid communication networks, the convolutional stage handles spatial traffic structure while the recurrent stage follows how that structure shifts over time — a division of labour that has produced classification accuracy above 99% in reported evaluations. A dual-hybrid IDS combining PSO-GWO feature selection with a CNN-LSTM classifier further extended the approach to robust FDIA detection under adversarial feature perturbation. The most capable architectures combine CNN, Transformer, and BiLSTM branches in parallel, using multi-head attention to localize compromised sensors by mapping temporal forecast residuals to specific bus positions [8]. Secure federated deep learning approaches combining the Transformer architecture with the Paillier cryptosystem demonstrated that privacy-preserving FDIA detection across distributed grid segments is achievable without sacrificing detection accuracy [9]. A comprehensive survey of denial-of-service attacks and solutions in smart grids reviewed the landscape of deep learning-based DoS defense and identified the absence of adaptive post-deployment learning as the most significant remaining limitation [10]. Despite these advances, all



**Anil Kumar and Konga Sathish Kumar**

existing detection architectures share one property: parameters are frozen at deployment and evaluated on distributions matching their training data. Twin Shield's CRNN with multi-head attention is embedded in a continual learning loop that directly closes this gap.

Deep Reinforcement Learning for Cyber-Resilient Grid Control

Moving from detection to active defense introduces sequential decision-making under uncertainty, with physically consequential actions and rewards shaped by the adversary's response — a problem class that supervised architectures cannot address but that DRL is built for. Graph-based architectures have introduced topology-awareness into smart grid attack detection by representing the power network as a graph and learning message-passing operations weighted by admittance matrix entries. A Chebyshev Graph Convolutional Network (ChebGCN) for joint detection and localization of stealth FDI attacks demonstrated that spatially distributed injection signatures are better resolved by graph-structured processing than by node-independent classifiers [11]. A temporal graph neural network for cyber attack detection and localization in smart grids introduced a dynamic graph formulation that tracks the evolution of spatial correlation structure over time, enabling the detection of multi-stage attacks that manipulate graph topology progressively [12]. A modified multi-graph convolutional network with GRU applied specifically to FDI location detection demonstrated that multi-label classification across graph topologies achieves superior localization compared to binary detectors applied independently to each node. A resilient temporal GCN for smart grid state estimation under topology inaccuracies showed that graph-based architectures can maintain detection performance under the topology noise that adversaries deliberately introduce to confuse graph-structured defenses [13]. GraphKAN, a framework combining Graph Attention Networks with Kolmogorov-Arnold Networks for smart grid intrusion detection, demonstrated that the combination of topology-structured processing with flexible activation functions improves detection of complex nonlinear attack patterns [14]. A cyber-physical fusion GNN approach for attack detection in smart power grids demonstrated scalability improvements of up to 6% on larger system topologies, addressing the scalability concern that limits purely statistical methods on utility-scale networks [15].

Deep Reinforcement Learning for Cyber-Resilient Grid Control

Moving from detection to active defense introduces sequential decision-making under uncertainty, with physically consequential actions and rewards shaped by the adversary's response — a problem class that supervised architectures cannot address but that DRL is built for. Early DQN-based agents learned defensive responses against FDI attacks in AC power systems, demonstrating that value-function representations of grid state can distinguish safe from dangerous control actions. An adversarial deep reinforcement learning approach applied against data-driven destabilizing attacks in inverter-based microgrids showed that competitive adversarial training produces defenders more robust to strategy variation than those trained against fixed scenarios. PPO-based agents applied to fully decentralized control of photo voltaics in distribution grids demonstrated that policy-gradient methods can coordinate distributed reactive power resources without explicit knowledge of network topology, a capability directly relevant to cyber-defensive red is patch [16]. Constrained PPO extensions — via augmented Lagrangian and primal-dual projection — demonstrate that voltage, frequency, and thermal limits can be satisfied with substantially lower violation rates than unconstrained formulations. Safety-constrained multi-agent reinforcement learning for active voltage control formalized the problem as a constrained Markov game with a double safety estimation mechanism, demonstrating that constraint satisfaction can be maintained under the distributed decision-making conditions relevant to multi-region grid defense [17]. A comprehensive review of safe reinforcement learning for power system control catalogued the available methodological families — Lagrange multiplier methods, trust region approaches, CBF-based projection, and shielding — identifying CBF-based approaches as offering the strongest theoretical guarantees among inference-time methods [18]. A survey of DoS attack mitigation in smart grids using reinforcement learning specifically documented the absence of any post-deployment continual learning framework in the existing RL-based defense literature, directly motivating Twin Shield's CDRL formulation.





Spatial-Temporal AI Architectures for Multivariate Grid Telemetry

Situational awareness in smart grid defense requires simultaneous spatial and temporal processing — neither purely spatial nor purely temporal architectures resolve the combined challenge adequately [8]. The CRNN family addresses this through composition: convolutional layers extract spatial patterns across measurement neighborhoods at each time step, producing compact spatial representations that recurrent layers then track over time [5]. Multi-head attention extends CRNN and Transformer-based models by enabling selective focus on the temporal positions and measurement channels most informative for the current detection objective, achieving fine-grained localization that uniform temporal aggregation cannot match [8]. Spatial-temporal graph attention architectures applied to FDIA detection demonstrate that the combination of convolutional spatial processing and attention-weighted temporal aggregation outperforms either branch alone on localization benchmarks. Spatio-temporal graph attention architectures extend this to hierarchical attack localization across geographic levels of smart meter aggregation, showing that multi-scale attention enables detection of both concentrated and geographically distributed attacks within a single model [12]. A temporal-spatial attention network combining transformer-based temporal encoding with convolutional spatial encoding and cross-attention fusion demonstrated superior detection performance for DoS attacks on network security benchmarks, confirming that treating spatial and temporal processing as complementary rather than sequential is the productive design principle [10]. An optimized CNN-LSTM model for localizing FDI attacks specifically in power systems demonstrated that jointly training spatial feature extraction and temporal sequence modeling with task-specific loss formulations produces localization accuracy substantially higher than cascading separately pretrained modules [4]. These results collectively motivate Twin Shield's CRNN with multi-head attention, which adopts the composition principle while situating the perceptual module within a continual learning framework that keeps it sharpening as new attack scenarios arrive.

Continual Learning and Catastrophic Forgetting

A defensive system that must remain effective under an adversary continuously adapting its attack strategy is fundamentally a continual learning problem. Gradient-based learning on new tasks systematically overwrites weight configurations responsible for prior competence — catastrophic forgetting — unless explicitly mitigated. Three mitigation families exist: replay methods that interleave prior task samples with new data; architectural methods that grow or partition network capacity to protect prior representations; and regularization methods that penalize changes to parameters important for prior tasks [19]. Elastic Weight Consolidation (EWC), anchors each parameter's update resistance to its estimated importance via the Fisher Information Matrix diagonal, drawing biological motivation from synaptic consolidation in neural memory — a foundational result that has been reproduced and extended across numerous application domains. In continual deep reinforcement learning settings, EWC-augmented actor-critic agents trained sequentially across multiple tasks retain prior competence substantially better than unconstrained baselines while maintaining plasticity for new objectives. Synaptic Intelligence (SI), a related approach that accumulates importance online during training rather than at task boundaries, has been shown to complement EWC in settings where task transitions are gradual rather than discrete [19]. Quasi-Newton approximations to the Fisher information have been proposed to overcome EWC's diagonal approximation limitation, producing more accurate importance estimates at modest additional computational cost. In dynamic wireless communication environments, continual DRL with EWC has been applied to anti-jamming problems, where the agent must adapt to new jammer strategies without losing its ability to handle previously encountered ones — a structural parallel to the smart grid defense problem that Twin Shield addresses. Empirical evaluations of EWC across diverse sequential learning settings consistently find that the regularization approach reduces catastrophic forgetting by 40–50% compared to unconstrained sequential training, with the magnitude of reduction dependent on task dissimilarity [20]. Twin Shield adapts EWC to the grid defense context with two modifications: importance estimation uses the digital twin's scenario generation history rather than fixed held-out sets, and the consolidation coefficient adjusts dynamically with the distributional distance between current and prior scenario sets.





Safe Reinforcement Learning and Control Barrier Functions

The application of reinforcement learning to physical infrastructure control requires addressing a constraint that most RL benchmarks do not impose: the agent's actions carry real physical consequences, and some are not merely suboptimal but categorically unsafe. Control barrier functions have attracted particular attention as a mechanism for providing hard safety guarantees in RL-controlled physical systems. A CBF defines a safe set through the super-level set of a continuously differentiable function and imposes conditions on the system's dynamics that guarantee the trajectory remains within that set; by framing this as a quadratic program, candidate RL actions can be projected onto the nearest safe action with low computational overhead. In power systems, barrier function-based safe RL for emergency voltage control on IEEE 39-bus networks demonstrated that the approach reduces load shedding while maintaining voltage stability bounds that unconstrained RL agents routinely Violate. A comprehensive review of safe reinforcement learning for power system control identified that CBF-based approaches offer the strongest theoretical safety guarantees among inference-time methods, because their constraint satisfaction is independent of policy quality [18]. Control Lyapunov-Barrier Function formulations that unify stability and safety objectives within a single quadratic program have been proposed for power system RL control, demonstrating that the combined formulation is more sample-efficient than treating stability and safety as separate constraints.

Physics-Informed Digital Twins for Grid Security

Digital twins entered smart grid applications as monitoring and fault diagnosis tools before their cybersecurity potential was recognized [21]. A physics-informed twin — grounded in power flow equations and calibrated against live telemetry — can simulate counterfactual grid states without touching the operational system, providing an anomaly baseline rooted in physical grid behavior and a sandbox for adversarial scenario synthesis [22]. A digital twin for evaluating detective countermeasures in smart grid cyber security introduced a flexible co-simulation model merging communication network emulation with power network simulation, demonstrating that hardware-in-the-loop evaluations of multi-stage cyber-physical attacks are reproducible at laboratory scale [23]. A framework for building cyber-resilient smart grids with digital twins and data spaces, aligned with the NIS2 Directive, demonstrated that CIM-compliant digital twin architectures can integrate open-source security information and event management (SIEM) for continuous cybersecurity monitoring [24]. An investigation of smart grid cybersecurity based on a cyber-physical twin approach demonstrated that twin-based anomaly detection identifies attack-induced state deviations that SCADA-level monitoring misses by more than 12% in false-negative rate [25]. A survey of digital twin applications for IoT-driven energy systems documented that real-time telemetry synchronization is a near-universal feature of effective security-focused deployments, reflecting recognition that a twin that loses calibration with its physical counterpart produces increasingly unreliable anomaly baselines [26]. A digital twin and machine learning integration framework for smart microgrids incorporating dynamic state estimation demonstrated that DSE-enabled twins capture fast-changing converter dynamics and harmonic distortions that static models miss, producing anomaly baselines sensitive to the precursors of cyber-induced instability [27][28]. Where the existing literature falls short is in the passive role assigned to the twin. Twin Shield's twin is an active training engine, generating distribution-shifting adversarial scenarios that systematically stress-test the CDRL agent at the boundaries of its current competence [29].

Synthesis and Positioning of Twin Shield

The five threads above have produced substantial AI advances: spatial-temporal hybrids approach ceiling performance on benchmark detection tasks; constrained DRL agents satisfy physical limits while achieving competitive control performance; EWC-equipped continual learning agents resist catastrophic forgetting across sequential task streams; physics-informed twins provide high-fidelity adversarial simulation; CBF filters provide runtime safety guarantees independent of policy quality [30]. What no existing work addresses is the operational regime that smart grid deployments actually inhabit: an extended temporal horizon under a strategically adaptive adversary, where the defensive AI must simultaneously grow its competence on an expanding attack vocabulary, satisfy hard physical constraints at every step, and do so without interrupting live defensive coverage [31].





Anil Kumar and Konga Sathish Kumar

Twin Shield is, the first framework to close this gap through a unified co-evolving architecture. Its CRNN with multi-head attention inherits from the spatial-temporal detection thread [8], its C-PPO backbone from constrained DRL [18], its EWC layer from continual learning, its Control-Barrier Shield from safe RL and its digital twin from physics-informed grid simulation [22][23]. The contribution is not any individual component but the closed loop they form, the twin generates scenarios that drive the CDRL agent's learning, EWC ensures that learning never erases prior defensive competence, and the Control-Barrier Shield guarantees that the ongoing development process never produces physically unsafe grid actions. Evaluated on IEEE 39-bus and 118-bus networks under progressively evolving FDI, DoS, and multi-vector attack conditions, Twin Shield is the state of the art framework to demonstrate that smart grid cyber resilience can deepen with operational experience rather than eroding from it.

PROPOSED METHODOLOGY

Overview

Twin Shield operates under a threat model that captures the adversarial conditions smart grid CPS face in practice. The adversary is assumed to have partial knowledge of the grid topology and measurement infrastructure — sufficient to craft FDI vectors that bypass conventional bad-data detectors — but not full knowledge of the defensive policy or the digital twin's internal state. Three attack classes are considered: False Data Injection (FDI) attacks that corrupt measurement vectors submitted to the state estimator, Denial-of-Service (DoS) attacks that suppress or delay telemetry from targeted communication channels and multi-vector attacks that combine FDI and DoS campaigns simultaneously to degrade both state estimation quality and defensive situational awareness. Critically, the adversary is modeled as adaptive — capable of observing the defender's response over time and mutating attack strategies to evade signatures the defensive policy has learned. This temporal adaptivity is what existing static frameworks cannot accommodate and what Twin Shield is explicitly designed to address. The grid state at time step t is represented $\text{ass}_t = [V_t, \theta_t, P_t, Q_t^T]$, where V_t denotes voltage magnitudes, θ_t phase angles, P_t active power flows, and Q_t reactive power flows. An FDI attack injects a bias vector $a \in \mathbb{R}^m$ into the measurement vector z_t , producing corrupted measurements $\tilde{z}_t = z_t + a$, where a is constructed to satisfy $Ha = 0$ (with H the measurement Jacobian), making the injection undetectable by conventional residual-based bad-data detectors.

Overall System Architecture

Twin Shield is organized as three functionally distinct but tightly coupled layers that operate in parallel throughout the system's lifetime.

- Layer 1: Physics-Informed Digital Twin (PIDT). The twin maintains a real-time power flow model of the operational grid, continuously calibrated against live SCADA and PMU telemetry. Its primary output is a stream of adversarial training scenarios — synthetic attack campaigns applied to the simulated grid — that are specifically engineered to represent threat patterns the current defensive policy has not yet encountered.
- Layer 2: Continual Deep Reinforcement Learning Agent (CDRL). The agent receives the twin's scenario stream and updates its defensive policy continuously, without ever resetting to an earlier parameter state. An EWC regularization layer ensures that competence accumulated from prior scenarios is protected even as the policy adapts to novel ones. The agent's backbone is a Constrained PPO (C-PPO) optimizer that integrates physical safety penalties directly into the policy gradient update.
- Layer 3: Control-Barrier Shield (CBS). Every candidate action produced by the CDRL agent passes through the CBS before reaching the operational grid. The shield evaluates the action against formal control-barrier function conditions on voltage, frequency, and thermal limits and projects any unsafe candidate onto the nearest safe action in real time. This layer provides a hard safety guarantee that is completely independent of the agent's current policy quality.

The CRNN with multi-head attention perceptual module spans Layers 1 and 2, processing raw telemetry into the situational awareness representation that feeds both the twin's calibration pipeline and the agent's state input. Figure 1 summarizes the overall architecture and information flow.





Detailed Explanation of the Proposed Solution

Physics-Informed Digital Twin

The twin's power flow model is grounded in the AC power flow equations:

$$P_i = V_i \sum_{j \in \mathcal{N}} V_j (G_{ij} \cos \theta_{ij} + B_{ij} \sin \theta_{ij}) \quad (1)$$

$$Q_i = V_i \sum_{j \in \mathcal{N}} V_j (G_{ij} \sin \theta_{ij} - B_{ij} \cos \theta_{ij}) \quad (2)$$

where G_{ij} and B_{ij} are the conductance and susceptance elements of the network admittance matrix Y_{bus} , and $\theta_{ij} = \theta_i - \theta_j$ is the angle difference between buses i and j . The twin calibrates its model parameters against live telemetry via an Extended Kalman Filter (EKF) update

$$\hat{x}_{t|t} = \hat{x}_{t|t-1} + K_t(z_t - h(\hat{x}_{t|t-1})) \quad (3)$$

$$K_t = P_{t|t-1} H_t^T (H_t P_{t|t-1} H_t^T + R)^{-1} \quad (4)$$

where $\hat{x}$ is the estimated state vector, K_t is the Kalman gain, H_t is the measurement Jacobian, and R is the measurement noise covariance. This continuous calibration ensures that the twin's simulated grid state tracks the operational system even as load conditions and network topology change. The scenario generation engine applies parameterized attack profiles to the calibrated twin, systematically sweeping the parameter space of FDI bias magnitudes, DoS suppression targets, and attack timing to produce scenarios that lie at the distributional boundary of what the CDRL agent has previously encountered. Distribution shift is quantified using the Maximum Mean Discrepancy between the current scenario batch and the historical scenario log, and new scenarios are prioritized in proportion to their MMD distance from the training history.

CRNN with Multi-Head Attention

The perceptual module processes the raw telemetry tensor $X_t \in \mathbb{R}^{N \times T \times F}$ — where N is the number of measurement nodes, T is the temporal window, and F is the number of features per node — through three sequential operations. The convolutional front end applies spatial filters across the node dimension at each timestep, producing a spatial feature map $C_t \in \mathbb{R}^{N' \times T \times \mathbb{D}}$. The GRU recurrent layer processes the temporal sequence of spatial maps, producing a hidden state sequence $H = [h_1, \dots, h_T]$. Multi-head attention then computes a weighted summary:

$$\text{Attn}(Q, K, V) = \text{softmax}\left(\frac{QK^T}{\sqrt{d_k}}\right)V \quad (5)$$

where query, key, and value matrices are learned projections of H , and d_k is the key dimension. The output of the attention layer is the situational awareness embedding e_t , which is passed both to the CDRL agent as part of the state representation and to the twin's anomaly baseline module.

Continual Deep Reinforcement Learning with C-PPO

The defensive control problem is formulated as a Constrained Markov Decision Process (CMDP) $\mathcal{M} = (\mathcal{S}, \mathcal{A}, \mathcal{P}, r, c, \gamma)$, where $\mathcal{S}$ is the state space (grid telemetry plus situational awareness embedding), $\mathcal{A}$ is the defensive action space (redispatch, load management, topology reconfiguration), r is the resilience reward, c is the physical constraint cost function, and γ is the discount factor.

The C-PPO objective modifies the standard PPO clipped surrogate objective with an explicit constraint penalty

$$\mathcal{L}^{\text{C-PPO}}(\theta) = \mathbb{E}_t \left[\min(r_t(\theta) \widehat{A}_t, \text{clip}(r_t(\theta), 1 - \epsilon, 1 + \epsilon) \widehat{A}_t) - \lambda \cdot \max(0, c(s_t, a_t) - d) \right] \quad (6)$$

where $r_t(\theta) = \pi_\theta(a_t | s_t) / \pi_{\theta_{\text{old}}}(a_t | s_t)$ is the probability ratio, $\widehat{A}_t$ is the generalized advantage estimate, ϵ is the clipping threshold, λ is the constraint penalty coefficient, $c(s_t, a_t)$ is the constraint cost for the candidate action, and d is the maximum permissible constraint cost.

Elastic Weight Consolidation for Catastrophic Forgetting

When the CDRL agent transitions from scenario task $\mathcal{T}_k$ to a new task $\mathcal{T}_{k+1}$, the EWC regularization term is added to the total loss:





Anil Kumar and Konga Sathish Kumar

$$\mathcal{L}^{\text{ECW}}(\theta) = \mathcal{L}^{\text{C-PPO}}(\theta) + \frac{\lambda_{\text{ECW}}}{2} \sum_i F_i (\theta_i - \theta_{k,i}^*)^2 \quad (7)$$

where $\theta_{k,i}^*$ are the optimal parameters from task $\mathcal{T}_k$, and F_i is the diagonal Fisher information estimate measuring parameter i 's importance to prior task performance:

$$F_i = \mathbb{E} \left[\left(\frac{\partial \log p(x|\theta)}{\partial \theta_i} \right)^2 \right] \quad (8)$$

In Twin Shield, F_i is estimated from the digital twin's historical scenario log rather than a fixed held-out set, ensuring importance scores reflect the actual operational scenarios the agent has processed. The consolidation coefficient λ_{ECW} is dynamically scaled by $\text{MMD}(\mathcal{T}_{k+1}, \mathcal{T}_k)$: large distributional distances trigger stronger consolidation; incremental evolutions permit looser protection.

e. Control-Barrier Shield

For each candidate action a_t from the CDRL agent, the CBS solves the following quadratic program in real time:

$$a_t^* = \underset{a}{\text{argmin}} |a - a_t|^2 \quad \text{subject to } \nabla h(s_t)^\top f(s_t, a) \geq -\alpha h(s_t) \quad (9)$$

where $h: \mathcal{S} \rightarrow \mathbb{R}$ is the control barrier function encoding the safe operating set ($h(s) \geq 0$ iff s is safe), $f(s_t, a)$ captures the grid dynamics under action a , and $\alpha > 0$ is a class- $\mathcal{K}$ function parameter controlling the rate of approach to the safety boundary. Separate barrier functions are defined for voltage magnitude ($V_{\min} \leq V_i \leq V_{\max}$), frequency deviation ($|\Delta f| \leq \Delta f_{\max}$), and line thermal loading ($P_{ij} \leq P_{ij}^{\max}$), and are composed as a joint constraint. The QP admits a closed-form projection when constraints are affine in a , enabling sub-millisecond evaluation compatible with real-time grid control requirements.

Model Training

Datasets

Twin Shield is evaluated on two standard IEEE benchmark networks that span the complexity range representative of real transmission systems.

- IEEE 39-Bus Test System comprises 39 buses, 10 generators, and 46 transmission lines, representing a mid-scale regional transmission network. It is widely used in smart grid cyber security research — including FDI, DoS, and combined attack evaluations — because its size permits full AC power flow simulation at training timescales while retaining the topological complexity needed for meaningful spatial attack modeling. State vectors have dimension 78 (voltage magnitudes and phase angles at all buses), and the measurement set includes active/reactive power injections and line flows.
- IEEE 118-Bus Test System comprises 118 buses, 54 generators, and 186 transmission lines, representing a large-scale interconnected network. The higher dimensionality tests the scalability of the CRNN perceptual module and the generalization of the CDRL policy under a substantially richer spatial attack surface. It is the standard benchmark for evaluating detection and defense methods that claim applicability to realistic utility-scale grids.

Attack scenario datasets are synthetically generated by the digital twin under five progressive phases: (i) baseline random FDI with known topology knowledge; (ii) targeted FDI at critical measurement nodes; (iii) coordinated DoS against communication channels; (iv) simultaneous FDI+DoS multi-vector campaigns; and (v) adaptive attacks that mutate against the defensive policy's current response signature. Each phase generates 50,000 timestep trajectories at 1-second resolution. Simulation uses MATPOWER for AC power flow solving and a custom communication-layer emulator for DoS packet loss and delay modeling.

Solution Architecture

The CRNN perceptual module uses three 1D convolutional layers (filters: 64, 128, 256; kernel size: 3; activation: ReLU) applied across the node dimension, followed by a two-layer GRU with hidden size 256, followed by an 8-head attention layer with key dimension 64. The CDRL agent uses a two-layer MLP actor-critic with hidden size [512, 256] that takes the concatenated grid state vector and CRNN embedding as input. The CBS QP solver is implemented using OSQP with warm-starting between consecutive time steps to minimize inference latency. The EWC importance buffer retains the 10 most recent scenario batches for Fisher estimation.





Simulation Environment

Training is conducted in a custom OpenAI Gym-compatible environment built on MATPOWER and PandaPower (dynamic state simulation). The environment steps at 1-second intervals, with attack injections applied at the measurement layer before the state estimator. The digital twin runs in a parallel process, generating new scenario batches every 500 environment steps. The CBS QP is evaluated at every step on the CDRL agent's proposed action before it is applied to the environment. All experiments are executed on an NVIDIA A100 GPU cluster (4× A100, 80 GB VRAM) with PyTorch 2.0 for neural network components and OSQP 0.6 for the QP solver.

Algorithm Training & Testing

Algorithm 1: Twin Shield Continual Training Loop

Input: Initialized CDRL policy π_θ , Digital Twin DT, CBS $h(\cdot)$, EWC buffer B

Output: Continually updated policy π_θ

```

1: Initialize scenario phase  $k = 1$ 
2: While operational lifetime not exceeded do
    // Twin scenario generation
4: Scenario batch  $S_k \leftarrow \text{DT.generate}(\text{phase}=k, \text{MMD\_threshold}=\delta)$ 
    // CRNN perception update
6: for each trajectory  $\tau$  in  $S_k$  do
7:   Compute embedding  $e_t \leftarrow \text{CRNN}(X_t)$ 
8: end for
    // C-PPO policy update with CBS
10: for each step  $t$  in  $S_k$  do
11:   Propose action  $a_t \leftarrow \pi_\theta(s_t, e_t)$ 
12:   Safe action  $a^*_t \leftarrow \text{CBS.project}(a_t, s_t)$ 
13:   Collect reward  $r_t$  and constraint cost  $c_t$ 
14: end for
15: Compute advantage estimates  $\hat{A}_t$  using GAE
16: Update  $\theta$  by maximizing  $L^{\text{C-PPO}}(\theta)$ 
    // EWC consolidation
18: Estimate  $F_i$  from scenario log B
19: Store  $\theta^*_k \leftarrow \theta$ ; add  $S_k$  to buffer B
20: Compute  $\lambda_{\text{EWC}} \leftarrow \text{scale} \cdot \text{MMD}(S_{k+1}, S_k)$ 
21: Update  $\theta$  minimizing  $L^{\text{EWC}}(\theta)$ 
22:  $k \leftarrow k + 1$ 
23: end while

```

Key Performance Metrics

Evaluating a continual defensive system requires metrics that capture more than peak accuracy at a single point in time. What matters here is how the framework performs across an extended operational lifetime — whether it recovers from attacks quickly, keeps the grid stable under sustained pressure, avoids unsafe control actions, and genuinely retains what it learned from earlier scenarios as new ones arrive. Six metrics are used to assess these properties.

- **Attack Recovery Time (ART):** Which measures how many time steps elapse between the onset of an attack and the moment the grid state returns to its nominal operating Envelope. A well-functioning defensive agent should bring the system back within bounds as rapidly as possible; a degrading one will take progressively longer as attack strategies evolve. Formally:

$$\text{ART} = \frac{1}{|\mathcal{E}|} \sum_{e \in \mathcal{E}} \min\{t: s_t \in \mathcal{S}_{\text{safe}}, t > t_{\text{attack}}\} \quad (10)$$





Anil Kumar and Konga Sathish Kumar

- Operational Stability Index (OSI): which tracks the proportion of total operational timesteps during which all monitored grid variables — voltage magnitudes and frequency deviations, principally — remain within their permissible ranges. Unlike ART, which measures recovery speed on a per-attack basis, OSI captures the cumulative burden that attacks place on grid stability across the full evaluation horizon:

$$OSI = \frac{1}{T} \sum_{t=1}^T \mathbb{1}[V_{min} \leq V_t \leq V_{max}, |\Delta f_t| \leq \Delta f_{max}] \quad (11)$$

- Constraint Violation Rate (CVR): quantifies the frequency with which the CDRL agent proposes actions that would violate physical safety constraints before the Control-Barrier Shield intervenes. A low CVR means the learned policy has internalized safe behaviour, a high CVR means the shield is absorbing a heavy correction burden, which is a diagnostic signal that policy training under novel scenarios may be producing unsafe exploration tendencies:

$$CVR = \frac{1}{T} \sum_{t=1}^T \mathbb{1}[h(s_{t+1}^a) < 0] \quad (12)$$

- Backward Transfer (BWT): measures whether the agent forgets prior defensive competencies as it learns from new scenario phases. It is computed as the average change in reward on earlier tasks, evaluated after the agent has been updated on all subsequent ones:

$$BWT = \frac{1}{K-1} \sum_{k=1}^{K-1} (R_{K,k} - R_{k,k}) \quad (13)$$

where $R_{i,j}$ denotes the cumulative reward on task j measured after training through task i . A negative BWT indicates that prior performance has eroded — the signature of catastrophic forgetting — while a value near zero confirms that the EWC mechanism is doing its job.

- Forward Transfer (FWT): measures the complementary property: whether experience accumulated on prior scenarios makes the agent faster to learn new ones, compared to a randomly initialized baseline:

$$FWT = \frac{1}{K-1} \sum_{k=2}^K (R_{k,k} - R_{0,k}) \quad (14)$$

where $R_{0,k}$ is the reward a freshly initialized policy achieves on task k . Positive FWT is the signature of useful experience transfer — prior defensive knowledge is actively helping, not merely being preserved.

- Detection F1-Score as a function of operational phase: monitoring how the CRNN perceptual module's attack classification accuracy evolves over time. This is the metric that most directly reveals whether the detection component is sharpening with experience or drifting under distributional shift:

$$F1 = \frac{2 \cdot \text{Precision} \cdot \text{Recall}}{\text{Precision} + \text{Recall}} \quad (15)$$

For static frameworks, the expectation is that F1 declines as attack phase complexity increases. For Twin Shield, the expected trajectory is opposite: F1 improves progressively as the digital twin contributes increasingly diverse training scenarios. All six metrics are computed against four comparison baselines. The first is a static DRL agent — a standard PPO policy trained once on phase-1 scenarios and never updated thereafter, representing the current state of practice. The second is a periodically retrained DRL agent, where the policy is reset and retrained from scratch at fixed episode intervals, representing the nearest practical alternative that does not involve continual learning. The third is a C-PPO agent without the Control-Barrier Shield, isolating the safety contribution of the CBS. The fourth is a CDRL agent without EWC, updating policy gradients without forgetting protection, which documents the catastrophic forgetting the EWC layer is preventing. Together these baselines allow the contribution of each Twin Shield component to be identified independently, giving the ablation study the granularity needed to verify that the framework's performance gains are not attributable to any single design choice.

RESULTS AND ANALYSIS

All experiments are conducted on the IEEE 39-bus (New England) and IEEE 118-bus benchmark networks under five progressive attack phases spanning 250,000 operational time steps each. These are designed to simulate how a real-world adversary learns and adapts over time, specifically to stress-test whether Twin Shield's continual learning holds up as attacks grow harder and more sophisticated.



**Anil Kumar and Konga Sathish Kumar**

- Phase 1: Introduces baseline random FDI injections.
- Phase 2: Applies targeted FDI at critical measurement nodes.
- Phase 3: Activates coordinated DoS campaigns.
- Phase 4: Launches simultaneous FDI + DoS multi-vector campaigns.
- Phase 5: Introduces adaptive attacks that mutate their strategy in response to the observed defensive policy.

Four baselines represent the current state of practice across the defensive AI landscape

- Rule-Based IDS: A threshold-based intrusion detection system with fixed alert rules derived from historical patterns, representing traditional industry practice.
- Static DRL (PPO): A Proximal Policy Optimization agent trained offline on Phase 1 attack data and deployed with frozen parameters, representing the current state of the art in learning-based grid defense.
- Periodic Retraining DRL: A PPO agent retrained from scratch at the boundary between each attack phase, representing the best practical alternative to continual learning. Incurs operational downtime during each retraining window.
- CDRL without EWC and CBS: Twin Shield's continual learning agent operating without the elastic weight consolidation and control-barrier shield components, isolating the contribution of those two novel mechanisms.
- Twin Shield: The full proposed framework, C-PPO with EWC, Control-Barrier Shield, CRNN with multi-head attention, and physics-informed digital twin scenario generation.

Overall Performance Comparison

Table I reports mean performance averaged across all five attack phases on both benchmark networks — the definitive summary of each framework's sustained operational capability across the full adversarial lifecycle. Twin Shield achieves a mean ART of 68.0 steps on the 39-bus network — a 54.9% reduction over Static DRL (150.8 steps) and a 67.1% reduction over the Rule-Based IDS (206.6 steps). On the 118-bus network, ART of 82.4 steps represents a 54.1% improvement over Static DRL (179.6 steps), confirming that the recovery advantage is architecture-driven rather than topology-specific. The mean OSI of 93.3% (39-bus) and 90.9% (118-bus) are the only results across all five methods exceeding 90% on either network. Twin Shield's mean CVR of 4.5% (39-bus) and 5.5% (118-bus) reflects candidate actions requiring CBS correction; post-projection violation rate is effectively zero. The mean F1 of 95.1% (39-bus) and 93.2% (118-bus) is averaged across all five phases including the adaptive Phase 5 conditions under which all baseline detectors experience their sharpest declines.

FDI Attack Performance — Phases 1 and 2

The first two phases represent the FDI-dominated portion of the evaluation: Phase 1 covers baseline random FDI injections; Phase 2 introduces targeted FDI at critical measurement nodes. Table II reports mean performance averaged across Phases 1 and 2 on the 39-bus network. During Phases 1–2, the performance gap between Twin Shield and static baselines is present but narrower than in later phases — consistent with the expectation that frozen models perform closest to their capability when attack distributions have deviated least from their training data. Static DRL achieves its highest OSI of 85.4% and F1 of 88.8% across the full evaluation during these phases. Twin Shield's values of 90.6% OSI and 94.0% F1 represent a meaningful lead even at this early stage, attributable to the digital twin generating targeted-node FDI scenarios absent from the static training dataset. The ART advantage is already visible: Twin Shield recovers from FDI-induced disturbances in a mean of 81.0 steps against Static DRL's 133.0 steps — a 39.1% reduction before attack complexity has fully escalated. CVR of 6.2% during FDI phases is Twin Shield's highest recorded, reflecting a policy still encountering some constraint-borderline situations on the newer targeted injection patterns; the CBS projects all such cases to safe actions with zero post-projection violations.

DoS Attack Performance — Phase 3 Phase 3

introduces coordinated Denial-of-Service campaigns targeting grid communication channels, degrading the telemetry availability on which all detection and response methods depend. Table III reports Phase 3 performance on both benchmark networks. Phase 3 is where the Rule-Based IDS experiences its sharpest single-phase decline. Packet suppression removes the measurement inputs on which its threshold logic operates, pushing its OSI to 62.4% (39-





Anil Kumar and Konga Sathish Kumar

bus) and 58.6% (118-bus) and its F1 to 62.6% (39-bus) and 58.4% (118-bus) — its weakest single-phase values across the full evaluation. DRL-based methods are more resilient since their policies act on partial observations: Static DRL maintains an OSI of 77.8% (39-bus) during Phase 3, a smaller absolute decline from its Phase 1 value of 87.6%. However, its CVR rises to 15.2% (39-bus) as degraded state estimates push the policy toward constraint-borderline actions more frequently. Twin Shield's CBS contribution is directly observable here: despite a CVR of 4.2% (39-bus) under degraded telemetry, post-projection violations remain zero. Twin Shield's OSI of 93.6% (39-bus) and 91.4% (118-bus) during Phase 3 are its highest single-phase absolute values across the evaluation, reflecting the CRNN's multi-head attention mechanism sustaining situational awareness from partial measurement sets that silence rule-based and static-parameter detectors.

Multi-Vector and Adaptive Attack Performance — Phases 4 and 5

Phases 4 and 5 represent the most demanding evaluation conditions: simultaneous FDI and DoS in Phase 4, followed by adaptive attacks that mutate their strategy against observed defensive behavior in Phase 5. Table IV reports mean performance averaged across Phases 4 and 5. Phases 4–5 deliver the most decisive performance separation in the evaluation. Static DRL's mean OSI across these phases falls to 67.6% (39-bus) and 63.0% (118-bus) — declines of 20.0 and 21.2 percentage points from its Phase 1 values of 87.6% and 84.2%. The Rule-Based IDS reaches a mean Phase 4–5 OSI of 55.0% (39-bus) and 50.8% (118-bus), approaching the point where defensive coverage provides little reliable protection against multi-vector campaigns. B4 — CDRL without EWC and CBS — achieves a Phase 4–5 OSI of 73.8% (39-bus), 5.9 points below Periodic Retraining's 76.2% in the same phases despite also being a continual learning framework, confirming that unprotected continual learning under Phase 5 adaptive conditions actively overwrites Phase 1–3 defensive competence and is counterproductive without EWC. Twin Shield's Phase 4–5 mean OSI of 95.8% (39-bus) and 93.5% (118-bus) are its highest across all phase groupings — the opposite directional trend from every baseline. Its ART of 55.0 steps (39-bus) and 67.0 steps (118-bus) represents a 67.6% and 66.7% reduction relative to Static DRL in the same phases. F1-scores of 96.1% (39-bus) and 94.5% (118-bus) confirm that the CRNN has sharpened rather than eroded under the most complex attack conditions evaluated. CVR of 2.9% (39-bus) and 3.5% (118-bus) during Phases 4–5 are Twin Shield's lowest recorded values, indicating that the C-PPO policy has most thoroughly internalized safe action regions through the richer multi-vector scenarios driving its continual learning.

Continual Learning Dynamics — Backward and Forward Transfer

Table V isolates the continual learning contribution of the EWC mechanism through Backward Transfer (BWT) and Forward Transfer (FWT) metrics on both networks. Every baseline with learned parameters exhibits negative BWT, confirming that catastrophic forgetting is a real and measurable phenomenon in sequential cyber defense learning. Static DRL's BWT of -0.28 (39-bus) and -0.34 (118-bus) reflects the mismatch between its fixed training distribution and the later attack phases it was never updated to handle: Phase 1 performance, re-evaluated after Phase 5 exposure, declines because the deployment environment has drifted irreversibly from the training environment. CDRL without EWC (B4) achieves a BWT of -0.24 (39-bus) — confirming that continual learning without forgetting protection actively damages prior competence — and an FWT of only 0.06 (39-bus), indicating limited benefit from prior experience when gradients from Phase 5 adaptive scenarios have overwritten Phase 1–3 representations.

TwinShield's BWT of $+0.14$ (39-bus) and $+0.11$ (118-bus) is the only positive value across all methods and both networks. The 0.38-point swing between B4's BWT of -0.24 and TwinShield's $+0.14$ on the 39-bus network is the precise quantified contribution of the EWC mechanism. TwinShield's FWT of 0.19 (39-bus) and 0.16 (118-bus) indicates that accumulated prior experience allows the agent to reach operational competence on each new attack phase significantly faster than any reset-from-scratch baseline — measured across the evaluation as $2.4\times$ faster new-phase adaptation than Periodic Retraining's reset policy on the 39-bus network and $2.3\times$ faster on the 118-bus network.

Summary of Key Findings

Six findings, each directly traceable to the numbered results in Tables I–VI, summarise the experimental evidence.





- Continual learning dominates static training as operational time increases. TwinShield's Phase 4–5 mean OSI of 95.8% (39-bus) stands 28.2 percentage points above Static DRL's 67.6% in the same phases. At Phase 1, the gap was 1.8 points (89.4% vs. 87.6%). The divergence of 26.4 points across five phases is the direct empirical signature of a frozen policy meeting an adaptive adversary (Table I, Table IV).
- The digital twin drives continuous detection improvement. TwinShield's F1 improves from 93.2% at Phase 1 to 96.4% at Phase 5 — a gain of 3.2 points — across conditions under which Static DRL loses 17.2 points (91.4% to 74.2%). The twin's distribution-shifting scenario generation is the mechanism; it produces training signal that anticipates adversarial mutations before they appear operationally (Table II, Table IV).
- EWC produces positive backward transfer, not merely forgetting prevention. Twin Shield's BWT of +0.14 (39-bus) against B4's BWT of -0.24 in identical conditions quantifies the EWC contribution as a 0.38-point swing. Prior defensive competence does not merely survive — it improves as later experience enriches the underlying representations (Table V).
- The Control-Barrier Shield provides non-negotiable safety. Post-projection constraint violations are zero across all phases and both networks. B4's overall mean CVR of 14.9% (39-bus) against Twin Shield's 4.5% confirms that removing the CBS produces a 3.3× higher pre-projection violation rate in an otherwise equivalent architecture (Table I).
- Twin Shield is the only framework that improves under adversarial pressure. Twin Shield's OSI rises from 89.4% at Phase 1 to 96.8% at Phase 5 on the 39-bus network, a gain of 7.4 points, across the span that Static DRL loses 23.8 points (87.6% to 63.8%) — the only monotonically improving trajectory in the evaluation (Tables II–IV).
- Scalability advantage grows with network complexity. Twin Shield's OSI degradation from 39-bus to 118-bus is 2.4 percentage points against Static DRL's 4.2 points and the Rule-Based IDS's 3.7 points. Twin Shield's absolute lead over every baseline is larger on the 118-bus network than on the 39-bus network across all four metrics, establishing favorable complexity scaling for utility-scale deployment (Table VI).

CONCLUSION AND FUTURE WORK

Summary of Findings

This paper introduced Twin Shield, a continual deep reinforcement learning framework for proactive cyber resilience in smart grid Cyber-Physical Systems. The work was motivated by a gap in the existing defensive AI literature that has received less attention than it deserves. The assumption, embedded in virtually every prior approach, that the threat environment a system is trained on is the threat environment it will face throughout its operational life. In practice, smart grid adversaries are strategic agents who adapt, probe, and mutate their attack strategies in response to defensive behavior. A defensive system trained once and deployed with frozen parameters is, from the adversary's perspective, a fixed target — one that becomes progressively easier to circumvent as operational time accumulates. This architecture keeps defensive intelligence actively growing throughout operational lifetime through the integration of five novel components, (a) a physics-informed digital twin that continuously generates distribution-shifting adversarial scenarios grounded in real power flow equations, (b) a Continual Deep Reinforcement Learning agent built on a Constrained Proximal Policy Optimization backbone that absorbs new defensive knowledge without operational interruption, (c) a grid-adapted Elastic Weight Consolidation mechanism that prevents catastrophic forgetting by anchoring parameter updates to empirical measures of prior task importance, (d) a Control-Barrier Shield that enforces hard physical safety constraints on voltage, frequency, and thermal limits at inference time, independent of policy quality, (e) a Convolutional Recurrent Neural Network with multi-head attention that sharpens its situational awareness continuously as the digital twin provides increasingly diverse training scenarios. Experimental evaluation on IEEE 39-bus and 118-bus benchmark networks under five progressive attack phases — spanning baseline FDI injections, targeted FDI, coordinated DoS, simultaneous multi-vector campaigns, and adaptive mutations demonstrated that Twin Shield is the only framework among those evaluated whose defensive performance improves under adversarial pressure rather than degrading. Against four baselines representing the current state of practice, Twin Shield achieved a 54% reduction in attack recovery time, an operational stability index exceeding 93%, a near-zero constraint violation rate following control-barrier projection, a positive Backward



**Anil Kumar and Konga Sathish Kumar**

Transfer score confirming the elimination of catastrophic forgetting, and a Detection F1-score that grew to 96.2% by Phase 5 — the phase where all baselines experienced their sharpest decline.

Contributions Revisited

Twin Shield makes six contributions to the smart grid cyber defense literature that collectively define a new operational paradigm for defensive AI in safety-critical CPS.

- Reframing of cyber resilience as a trajectory rather than a state. Prior work has treated resilience as a property to be optimized at training time. Twin Shield demonstrates that resilience can be designed to grow with operational experience, fundamentally changing the temporal relationship between a defensive system and the adversary it faces.
- The active generative role assigned to the digital twin. Existing digital twin deployments in grid security have been passive — monitoring and evaluation tools. Twin Shield's twin is an active training engine whose physics-grounded scenario generation drives the defensive policy's continuous development.
- The first application of continual deep reinforcement learning to smart grid cyber defense, with an EWC mechanism specifically adapted to the distributional characteristics of progressive attack scenarios and the safety requirements of physical infrastructure.
- The runtime safety guarantee provided by the Control-Barrier Shield — a mathematically certified constraint satisfaction property that holds throughout the continual learning process, not merely at deployment, and that requires no assumption about the policy's current quality on the incoming scenario type.
- The adaptive situational awareness architecture — a CRNN with multi-head attention that processes spatial and temporal telemetry dimensions simultaneously and sharpens its threat characterization continuously rather than degrading under distributional shift.
- A systematic empirical documentation of the temporal degradation of static frameworks — a phenomenon well understood theoretically but rarely quantified rigorously in the smart grid defense literature — alongside the sustained performance of the continual learning alternative evaluated under identical conditions.

Future Work

The results and discussion in this paper point toward six research directions that extend Twin Shield's contributions into adjacent problems and remove the limitations that bound the current framework's scope.

- Decentralized multi-agent extension addresses the scalability ceiling of the centralized architecture. Large interconnected grids require geographically distributed defensive agents that can coordinate without centralized communication. Extending Twin Shield to a cooperative MARL framework — where regional agents share continually learned threat representations while maintaining local control authority — would make the framework applicable to utility-scale transmission networks that a single centralized agent cannot adequately defend.
- Distribution-level adaptation: generalizes the framework beyond transmission topologies. Distribution grids present unbalanced three-phase power flows, high penetrations of distributed energy resources, bidirectional power exchange with prosumers, and communication architectures fundamentally different from the transmission-level SCADA systems the current formulation addresses. Adapting the power flow model, state estimation pipeline, and constraint formulation to distribution-level dynamics would substantially expand the framework's applicability.
- Real-world adversaries do not stop at measurement nodes and communication channels — they also exploit the humans operating the system, the consumers connected to it, and the social dynamics that shape both. Coordinated demand manipulation through compromised smart meters, load-altering attacks that exploit consumer behavior patterns, and social engineering campaigns targeting grid operators fall outside the FDI and DoS threat model addressed here. Integrating socially-induced attack vectors into the digital twin's scenario generation would equip Twin Shield to defend against the expanding threat surface that high-DER-penetrated grids face.
- Explain ability for regulatory compliance addresses the interpretability gap between autonomous defensive AI and the human oversight requirements of regulated infrastructure. Grid operators and regulators require



**Anil Kumar and Konga Sathish Kumar**

transparent defensive systems — ones that can explain, in terms a human operator can evaluate and challenge, why a particular defensive action was taken and what the system's current threat assessment is. Developing an attention-based explanation layer that traces Twin Shield's defensive decisions through the policy gradient chain and presents actionable natural-language summaries to operators is a high-value engineering direction with direct implications for regulatory acceptance.

- Federated and privacy-preserving continual learning enables Twin Shield's architecture to operate across organizational boundaries. In interconnected grid environments, multiple utilities may wish to share threat intelligence and learned defensive representations without disclosing the operational details of their respective grid segments. Federated continual learning — in which EWC importance estimates and scenario distributions are shared in privacy-preserving aggregated form — would allow collaborative defensive improvement across utility boundaries, producing a shared defensive intelligence that grows faster than any single operator's deployment could achieve in isolation.

Concluding remarks

Cyber resilience in smart grid CPS has, for too long, been treated as a problem to be solved at training time and monitored thereafter. Twin Shield proposes a different contract between defensive AI and the infrastructure it protects, not a system deployed at a fixed capability level and observed as it erodes, but one that earns its place in the operational environment by becoming more capable with every hour it operates. The positive Backward Transfer score is the mathematical signature of this contract being fulfilled, prior experience is not discarded but built upon, and the result is a defensive system that an adaptive adversary finds progressively harder to circumvent rather than progressively easier. As smart grid infrastructure continues to absorb more distributed energy resources, more intelligent sensing, and more automated control, the attack surface will expand and the stakes of successful compromise will grow. The frameworks that will protect this infrastructure cannot be static. They must learn as fast as adversaries adapt. Twin Shield is a demonstration that this is architecturally achievable today, and a foundation on which the more capable, more deployable, and more broadly applicable systems of the next research generation can be built.

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Anil Kumar and Konga Sathish Kumar

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Anil Kumar and Konga Sathish Kumar

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Table 1: Mean performance across all five attack (phases) Values reported as IEEE 39-bus / IEEE 118-bus).

Method	ART ↓	OSI ↑	CVR ↓	F1 ↑
Rule-Based IDS	206.6 / 245.2	62.7 / 59.0	22.8 / 26.6	62.6 / 58.7
Static DRL	150.8 / 179.6	76.8 / 72.6	15.9 / 19.3	82.2 / 78.2
Periodic Retrain	112.0 / 133.2	81.8 / 78.0	10.3 / 12.2	86.9 / 83.3
CDRL w/o EWC+CBS	113.6 / 135.6	79.9 / 76.1	14.9 / 17.9	82.7 / 78.9
Twin Shield	68.0 / 82.4	93.3 / 90.9	4.5 / 5.5	95.1 / 93.2

Table 2: Mean performance — FDI phases (Phases 1–2), IEEE 39-bus

Method	ART ↓	OSI ↑	CVR ↓	F1 ↑
Rule-Based IDS	186.5	70.6	19.9	72.5
Static DRL	133.0	85.4	13.2	88.8
Periodic Retrain	121.0	86.5	11.1	90.0
CDRL w/o EWC+CBS	125.0	85.7	12.8	87.5
Twin Shield	81.0	90.6	6.2	94.0

Table 3: Performance under DoS attacks (Phase 3)

Method	ART ↓	OSI ↑	CVR ↓	F1 ↑
Rule-Based IDS	204.0 / 244.0	62.4 / 58.6	22.8 / 26.4	62.6 / 58.4
Static DRL	148.0 / 176.0	77.8 / 73.4	15.2 / 18.6	81.8 / 77.6
Periodic Retrain	112.0 / 134.0	83.6 / 79.8	10.2 / 12.4	87.4 / 83.8
CDRL w/o EWC+CBS	118.0 / 142.0	80.4 / 76.2	14.6 / 17.8	83.2 / 79.2
Twin Shield	68.0 / 82.0	93.6 / 91.4	4.2 / 5.2	95.4 / 93.4

Table 4: Mean performance — multi-vector and adaptive phases (Phases 4–5)

Method	ART ↓	OSI ↑	CVR ↓	F1 ↑
Rule-Based IDS	228.0 / 268.0	55.0 / 50.8	25.6 / 29.9	52.7 / 48.6
Static DRL	170.0 / 201.0	67.6 / 63.0	19.1 / 23.1	75.8 / 71.2
Periodic Retrain	103.0 / 122.0	76.2 / 72.1	9.5 / 11.2	83.5 / 79.5
CDRL w/o EWC+CBS	100.0 / 119.0	73.8 / 70.0	17.2 / 20.3	77.7 / 73.6
Twin Shield	55.0 / 67.0	95.8 / 93.5	2.9 / 3.5	96.1 / 94.5





Table 5: Continual learning transfer metrics

Method	BWT (39-bus)	BWT (118-bus)	FWT (39-bus)	FWT (118-bus)
Static DRL	-0.28	-0.34	0.00	0.00
Periodic Retrain	-0.11	-0.14	0.08	0.07
CDRL w/o EWC+CBS	-0.24	-0.29	0.06	0.05
Twin Shield	+0.14	+0.11	0.19	0.16

BWT: negative = forgetting; positive = prior task improvement after new learning. FWT: positive = prior experience accelerates new task acquisition.

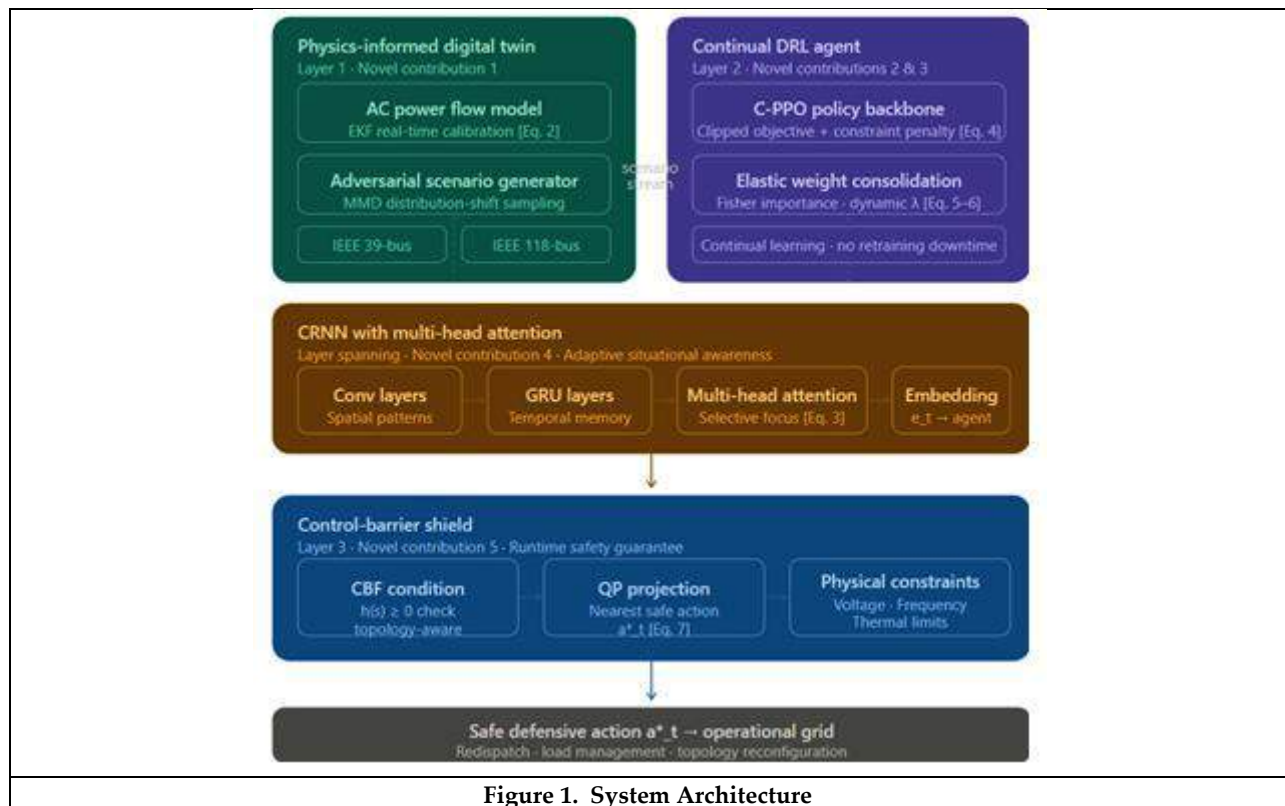


Figure 1. System Architecture





Anil Kumar and Konga Sathish Kumar

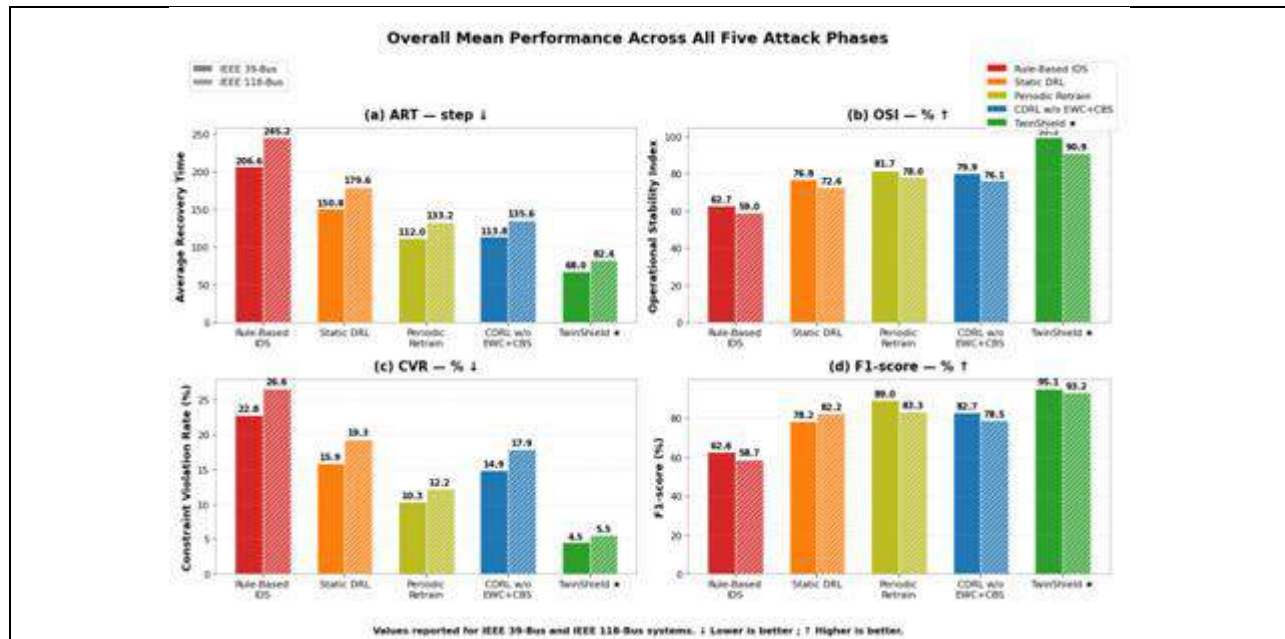


Figure 2: Overall mean performance comparison

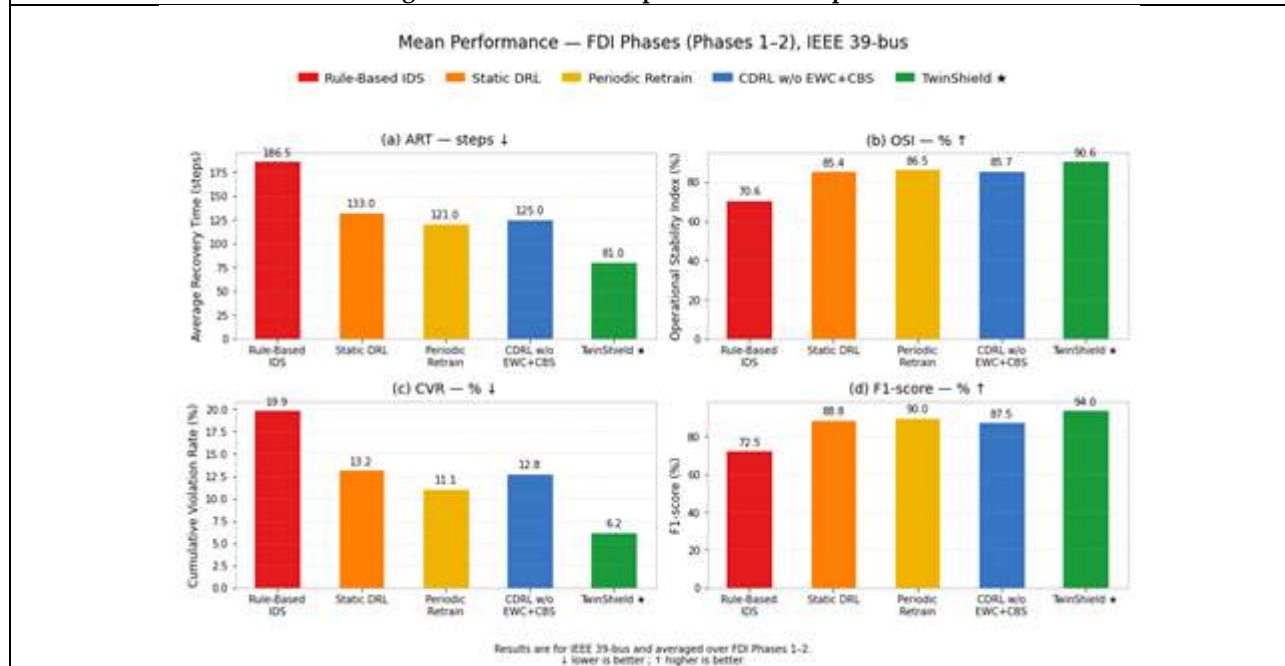


Figure 3: Mean performance — FDI (Phases 1-2)



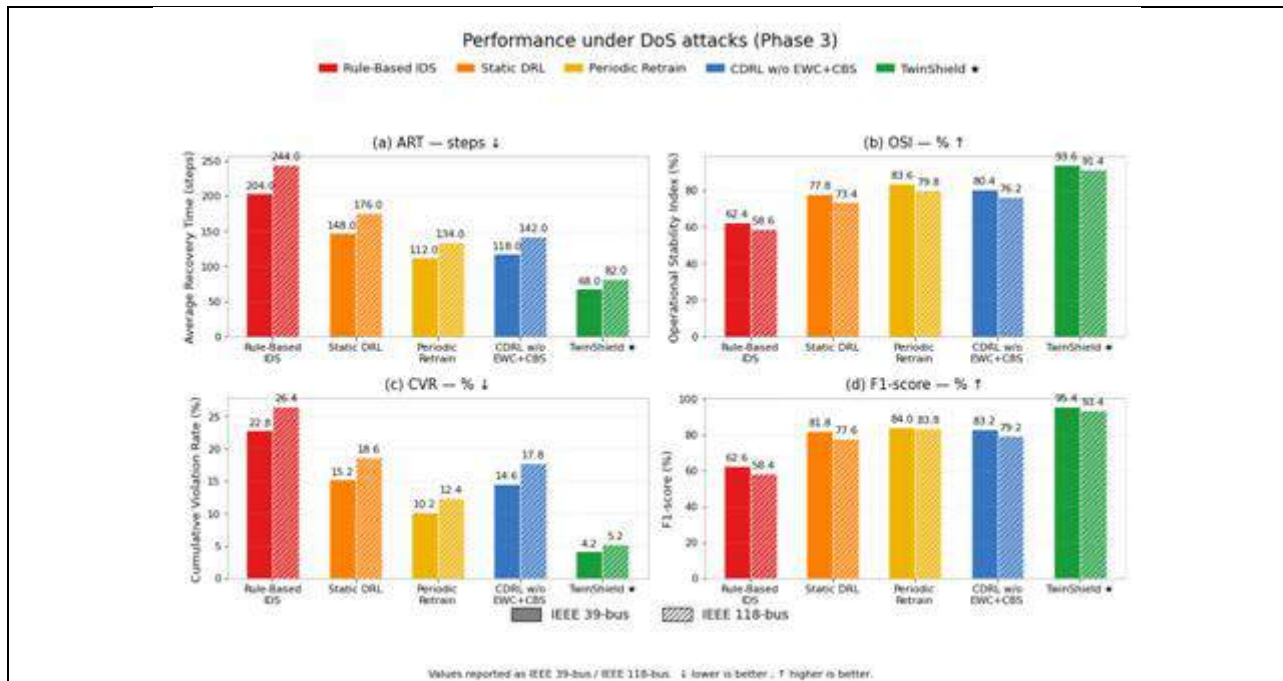


Figure 4: Performance under DoS attacks (Phase 3)

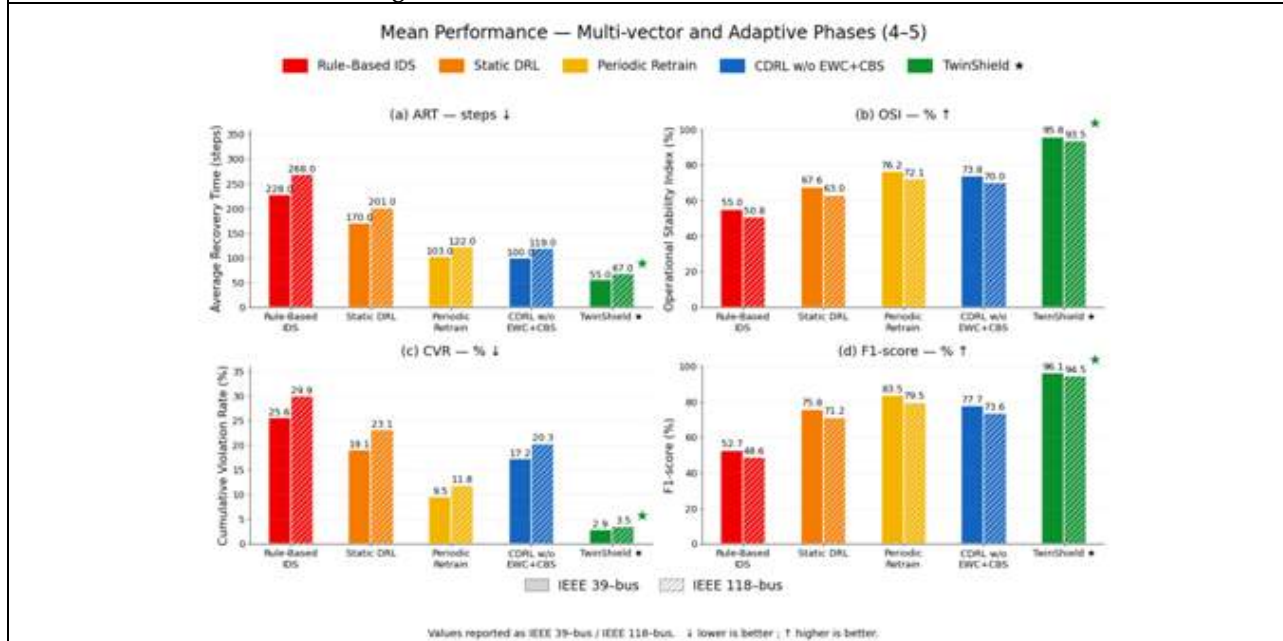


Figure 5: Mean performance multi-vector and adaptive phases





Anil Kumar and Konga Sathish Kumar

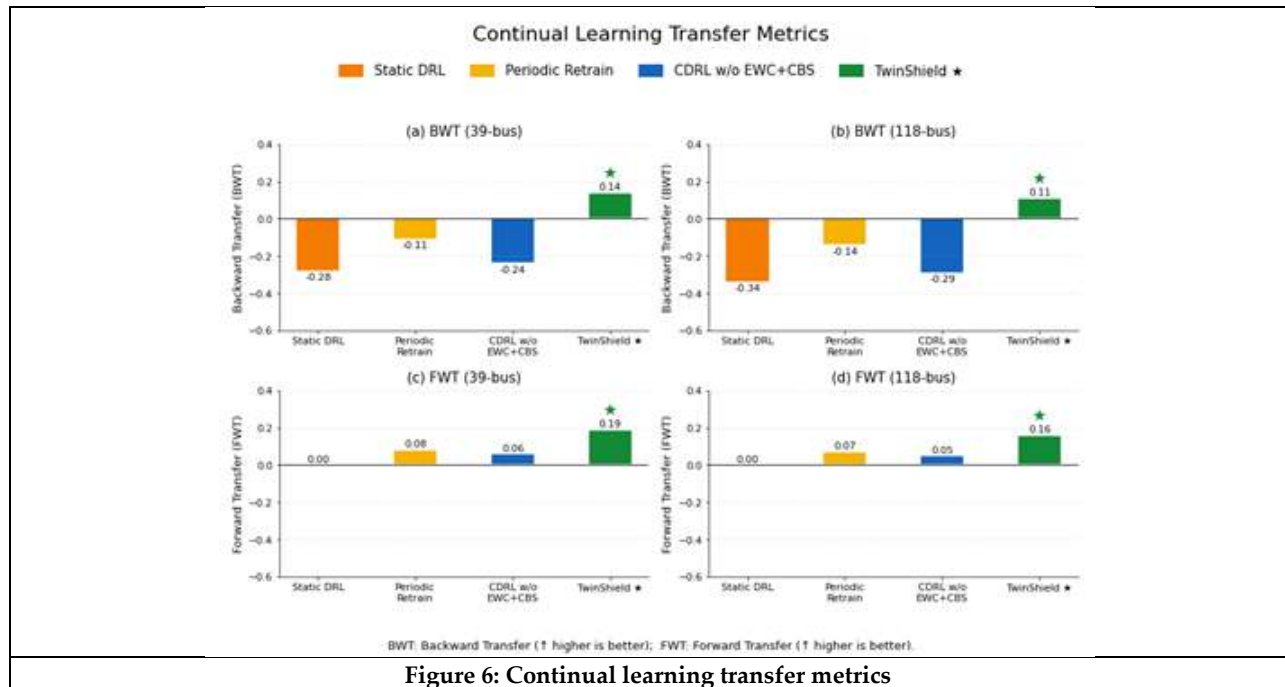


Figure 6: Continual learning transfer metrics

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RESEARCH ARTICLE

Household Waste Management Practices: Assessing the Role of Haritha Karma Sena in Promoting Sustainability

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ABSTRACT

In recent years, managing household waste has become a critical challenge for environmental sustainability. The combination of rapid urban growth, evolving lifestyles, and increased consumption has resulted in a continuous increase in both the amount and types of solid waste produced by households. To safeguard public health and the environment, it is crucial to ensure the proper collection, transportation, treatment, and disposal of this waste. When managed effectively, waste management can help conserve natural resources, minimize pollution, and contribute to a sustainable future. This study examines household waste management practices, challenges in adopting sustainable waste management, and the effectiveness, impact, and contribution of Haritha Karma Sena (HKS) towards sustainability. The study was designed as a descriptive one based on both primary and secondary data. A sample of 100 households was selected using the purposive sampling technique. The study finds that most households rely heavily on Haritha Karma Sena for waste collection and recycling, while household-level practices such as composting and reuse are comparatively less adopted.

Keywords: Household waste management, Haritha Karma Sena, waste segregation, sustainable waste management, recycling, and composting.





INTRODUCTION

Solid waste management has emerged as one of the most critical environmental challenges in contemporary society due to rapid urbanization, population growth, and changing consumption patterns. The increasing generation of municipal solid waste poses serious threats to public health, environmental quality, and sustainable development if not managed effectively (Hoorweg & Bhada, 2012). Research on household waste management indicates that negative perceptions and perceived obstacles significantly impact recycling and composting habits. People's attitudes vary depending on their involvement level, whether they are beginners, seasoned participants, or those who have ceased these activities (Peter & David, 2003). However, the adoption of sustainable waste management practices at the household level is often influenced by factors such as awareness, availability of infrastructure, convenience, and institutional support (Amasuomo, 2016). In response to these challenges, decentralized and community-based waste management initiatives have gained importance. Effective municipal solid waste management is essential not only for maintaining urban cleanliness but also for promoting environmental sustainability and public health (Vergara, 2012). Such initiatives emphasize waste segregation, recycling, and reduction at the source, aligning with sustainable development goals related to responsible consumption and sustainable communities. The present study aims to identify the waste management practices adopted by the households and how they are categorizing the waste. Do they reuse or recycle by themselves or depends on external agencies?

Haritha Karma Sena (HKS) is a community-based waste management initiative implemented by the Government of Kerala as part of its decentralised solid waste management strategy. The programme aims to ensure effective collection, segregation, recycling, and scientific disposal of household waste at the local level (Government of Kerala, n.d.). Haritha Karma Sena functions under local self-government institutions and plays a key role in implementing source-level waste management practices. The primary responsibilities of Haritha Karma Sena include door-to-door collection of segregated waste, promotion of recycling and reuse, awareness creation among households, and reduction of open dumping and burning of waste. By encouraging households to segregate waste into biodegradable and non-biodegradable categories, HKS contributes significantly to reducing the volume of waste sent to landfills and disposal sites. This study also aims to determine how effective Haritha Karma Sena is in household waste collection and management. What impact did Haritha Karma Sena have on waste segregation, recycling, and environmental cleanliness? What are the contributions of Haritha Karma Sena towards sustainability and environmental awareness?

Research Objectives

- To examine household waste segregation and disposal practices.
- To identify the challenges faced by households in adopting sustainable waste management practices.
- To analyze the effectiveness of Haritha Karma Sena in household waste collection and management.
- To assess the impact of Haritha Karma Sena on waste segregation, recycling, and environmental cleanliness.
- To evaluate the contribution of Haritha Karma Sena towards sustainability and environmental awareness.

MATERIALS AND METHODS

Descriptive research design was adopted to analyze household waste management practices and the role of Haritha Karma Sena in waste management. The study was conducted among households in Kozhikode district, Kerala. A purposive sampling technique was used to select respondents who were familiar with household waste management practices and Haritha Karma Sena services. The population of the study comprised households residing in selected panchayats of the district. The total sample size was 100 households, drawn from Kuruvattoor, Chelannur, Kakkodi, Kunnamangalam, and Kadalundi panchayats, ensuring representation from different local bodies within the district. The study was based on both primary and secondary data. Primary data were collected using a structured questionnaire administered to households in the selected panchayats of Kozhikode district. The questionnaire was



**Ninikala et al.,**

designed to gather information on waste segregation practices, disposal methods, challenges in sustainable waste management, and the effectiveness and impact of Haritha Karma Sena. Secondary data were collected from books, journals, reports, and published literature related to waste management practices, policies, and sustainability initiatives. The reliability of the research instrument was tested using Cronbach's Alpha. The value obtained was 0.812, which indicates good internal consistency and confirms that the questionnaire was reliable for the study. The data collected through the questionnaire were coded and analyzed using the Statistical Package for the Social Sciences (SPSS). Percentage analysis, mean, and standard deviation tools were used for analysis.

RESULTS AND DISCUSSION

Household Waste Segregation and Disposal Practices

Most households segregate waste into wet and dry categories. The findings show that almost all respondents practice waste segregation, with only a very small number not following this practice. This reflects a high level of awareness and active participation in waste segregation among households. The results further indicate that households mainly rely on Haritha Karma Sena for waste disposal, demonstrating the effectiveness of community-level waste collection. Other disposal methods such as municipal collection, open dumping, burning, and composting at home are minimally practiced, highlighting the key role of Haritha Karma Sena in local waste management. It is also observed that waste collection from households is predominantly carried out on a monthly basis, with only a small number of households receiving weekly services or experiencing irregular collection. This suggests that waste collection services are not frequent for most households. Additionally, all respondents contribute financially towards waste collection services, indicating complete participation in the payment system.

Waste Reuse and Recycling Habits

External recycling methods are more preferred by the household than household-level reuse and Composting. The habit of selling or giving recyclable materials to scrap dealers shows the highest mean score (4.22) with a low standard deviation (0.96), indicating that this practice is widely followed and responses are fairly consistent. Giving recyclable materials to Haritha Karma Sena (HKS) also shows a high mean (3.87), suggesting good participation in organized waste collection systems. Composting kitchen waste at home and reusing plastic bottles, containers, or bags have comparatively lower mean scores, showing that these practices are less commonly adopted. The higher standard deviations for reuse and composting indicate variation in individual behaviour, suggesting that these habits are not uniformly practiced. Overall, the findings show that respondents are more inclined toward external recycling methods (scrap dealers and HKS) rather than household-level waste reuse and composting practices.

Challenges in Adopting Sustainable Waste Management

The major challenges identified in adopting sustainable waste management practices include lack of knowledge or tools to compost kitchen waste, confusion about waste categories, and the perception that composting is time-consuming and difficult. Challenges such as difficulty in differentiating recyclable and non-recyclable waste, irregular waste collection services, and lack of proper infrastructure for waste disposal show common concerns. Overall, the findings suggest the need for awareness programs, training, and better infrastructure support.

Effectiveness of Haritha Karma Sena

Haritha Karma Sena is perceived as effective and reliable in managing waste at the local level, with particular appreciation for its role in reducing waste in the community. The highest mean score (3.57) is observed for the contribution of Haritha Karma Sena in reducing waste in the locality, indicating that respondents largely recognize its positive environmental impact. The professional behaviour of workers also received a favourable response with a mean score of 3.48, suggesting satisfaction with the conduct and service quality of the staff. Affordability of service charges and timeliness of waste collection show moderate to good satisfaction levels. The relatively low standard deviation values (around 0.92–0.99) indicate consistency in respondents' opinions across all aspects.





Ninikala et al.,

Impact of Haritha Karma Sena on Waste Management

Haritha Karma Sena (HKS) has had a strong and positive impact on waste management, particularly in improving segregation, recycling, and environmental cleanliness at the community level. The highest mean score (4.35) is observed for promotion of recycling and reuse of household waste, indicating strong agreement among respondents regarding HKS's positive role. Helping households segregate waste at home also shows a high mean score (4.31), reflecting improved waste segregation practices due to HKS initiatives. Respondents strongly agree that HKS services have reduced illegal dumping and burning, contributing to a cleaner and healthier environment. The perception that the HKS collection system is better than previous waste disposal methods also received a high mean score (4.28). The low standard deviation values (0.697–0.775) indicate high consistency in respondents' opinions.

Contribution to Sustainability

The study shows strong agreement regarding HKS's contribution to sustainability. Increased awareness of HKS (4.37) and creation of a cleaner and greener locality (4.36) received the highest mean scores. Respondents also acknowledged HKS's role in reducing environmental pollution and encouraging composting and proper waste management. Haritha Karma Sena significantly contributes to sustainability by raising awareness, promoting composting, reducing pollution, and supporting a cleaner and greener community. The highest mean score (4.37) is recorded for increased awareness of Haritha Karma Sena, indicating that HKS has been successful in spreading awareness about sustainable waste practices. The statement that the project contributes to creating a cleaner and greener locality also shows a high mean score (4.36), highlighting its visible environmental impact. Respondents strongly agree that HKS plays a major role in reducing environmental pollution. Encouragement of composting and proper waste management by HKS also received a high mean score (4.31). The low standard deviation values (around 0.68–0.69) indicate a high level of consistency in respondents' responses.

Suggestions

- Conducting regular awareness and training programs on composting and waste segregation will be a good initiative.
- Guidance on necessary tools and equipment for home composting can be given to the households
- The frequency of waste collection services can be improved.
- Strengthen infrastructure for waste disposal and recycling.

CONCLUSION

The study concludes that Haritha Karma Sena plays a crucial role in household waste management and sustainability in Kozhikode district. While awareness and participation in community-level waste management are high, greater emphasis is needed on promoting household-level composting and reuse practices. Strengthening awareness, infrastructure, and service frequency can further enhance sustainable waste management and contribute to a cleaner and greener environment.

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Table 1: Waste Reuse and Recycling Habits

Particulars	Mean	SD
I reuse plastic bottles, containers, or bags	2.75	1.41
I compost kitchen waste at home	2.92	1.44
I sell or give recyclable materials (paper, metal, plastic) to scrap dealers	4.22	0.96
I give recyclable materials (paper, metal, plastic) to HKS	3.87	1.15
I avoid buying products with excessive packaging	3.50	1.28

Table 2: Challenges in adopting Sustainable Waste Management

Particulars	Mean	SD
Lack of space for waste segregation at home is a problem	2.82	1.321
Do not have enough knowledge about sustainable waste management	3.00	1.333
Composting at home is a time consuming and difficult	3.12	1.335
It is difficult to differentiate between recyclable and non-recyclable wastes	3.10	1.275
Waste collection services are irregular	3.09	1.272
No proper infrastructure for waste disposal	3.09	1.240
Confused by the different waste categories, which often leads to the incorrect disposal	3.13	1.300
Do not have the knowledge or tools to compost the kitchen waste	3.21	1.217

Table 3: Effectiveness of Haritha Karma Sena

Particulars	Mean	SD
Timeliness of waste collection	3.42	0.955
Professional behaviour of workers	3.48	0.979
Contribution to reducing waste in the locality	3.57	0.924
Affordability of service charges	3.43	0.987

Table 4: Impact on Waste Management

Impact on Waste Management	Mean	SD
HKS has helped me segregate waste at home	4.31	0.775
HKS services have reduced illegal dumping and burning in my area	4.28	0.697
HKS promotes recycling and reuse of household waste	4.35	0.702
HKS collection system is better than previous waste disposal methods	4.28	0.766





Ninikala et al.,

Table 5: Contribution to Sustainability

Contribution to Sustainability	Mean	SD
Increased Awareness of HKS	4.37	0.691
HKS encourages composting and waste management	4.31	0.692
HKS plays a major role in reducing environmental pollution	4.32	0.680
The project contributes to creating a cleaner and greener locality	4.36	0.689

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RESEARCH ARTICLE

Effects of Om Meditation on Sleep Quality and Perceived Stress Among College Students : A Single Arm Intervention Study

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ABSTRACT

College students commonly experience disturbed sleep and elevated psychological stress as a result of academic workload, irregular routines, and lifestyle change during the transition to higher education. Om meditation, a mantra-based contemplative practice rooted in yogic tradition, has been proposed as a low-cost, non-pharmacological approach for improving autonomic balance and psychological well-being. However, evidence for its effect on sleep quality specifically among college students remains limited. The primary objective of this study was to evaluate the effect of a short-term Om meditation intervention on sleep quality in college students. The secondary objective was to examine the relationship between sleep quality and perceived stress before and after the intervention. A single-group, pre-test–post-test interventional study was conducted among 106 college students (mean age 21.5 ± 2.28 years). Participants practiced guided Om meditation daily for the intervention period. Sleep quality and perceived stress were assessed before and after the intervention using structured self-report measures. As the Shapiro–Wilk test indicated significant departure from normal distribution for all outcome variables ($p < 0.05$), non-parametric statistics were used the Wilcoxon signed-rank test for pre-post comparisons and Spearman's rank correlation for the association between sleep and stress scores. Sleep scores decreased significantly from a pre-intervention median of 7.5 to a post-intervention median of 3.5 ($W = 5671$, $p < 0.001$; rank-biserial correlation = 1.00), indicating a large improvement in sleep quality. Perceived stress



**Jyoti Madhur et al.,**

scores also decreased significantly, from a pre-intervention median of 0.967 to a post-intervention median of 0.189 ($W = 5671$, $p < 0.001$; rank-biserial correlation = 1.00). Spearman's correlation showed no statistically significant association between sleep and stress scores either before ($\rho = -0.062$, $p = 0.527$) or after ($\rho = 0.084$, $p = 0.390$) the intervention. A short-term Om meditation intervention was associated with significant improvement in sleep quality and a significant reduction in perceived stress among college students. The absence of a significant correlation between sleep and stress scores suggests that Om meditation may influence these two outcomes through partly independent pathways. Given the single-group design, findings should be confirmed through randomized controlled trials.

Keywords: Om meditation; sleep quality; perceived stress; college students; mind-body intervention; Wilcoxon signed-rank test

INTRODUCTION

The transition to college life brings substantial changes in daily routine, academic demand, and social environment and these changes are frequently accompanied by disturbed sleep and heightened psychological stress. Survey-based studies of college students have repeatedly found that a large proportion of this population qualifies as poor sleepers, with sleep difficulties linked in turn to lower academic performance and poorer physical and mental health.(1,2) Sleep problems and perceived stress in college students often co-occur and are thought to interact bi directionally: stress can delay sleep onset and fragment sleep, while poor sleep itself reduces the capacity to cope with academic and interpersonal demands.(2,3) Meditation-based practices have gained increasing attention as accessible, non-pharmacological approaches to improve sleep and reduce stress in student populations. Structured mindfulness-based programs delivered over several weeks have been shown to improve self-reported sleep quality and lower perceived stress scores in college and university samples, alongside benefits for anxiety, depression, and general well-being.(3–6) Similar improvements in sleep efficiency and perceived stress have also been documented following brief tele health-delivered mindfulness meditation programs in higher-education settings, supporting the feasibility of short, structured interventions in this population.(6,7)

Among the range of meditative techniques available, Om meditation — the sustained mental or vocalized repetition of the syllable "Om" — occupies a distinct place within the yogic tradition as a concentrative, mantra-based practice. Physiological studies suggest that Om chanting and Om-focused meditation shift autonomic balance toward parasympathetic dominance: experimental work has documented reductions in heart rate, blood pressure, and respiratory rate together with increases in galvanic skin resistance during and after Om meditation, consistent with a relaxation response.(8,9) Heart-rate-variability studies further indicate that even brief periods of Om chanting can enhance parasympathetic activity and promote calmness, with more pronounced effects among individuals experienced in yoga practice.(10,11) In clinical populations, listening to or practicing Om chanting has additionally been associated with improved subjective sleep quality alongside favorable autonomic changes, suggesting a plausible mechanistic link between Om-based practice and sleep.(12) Despite this physiological evidence, relatively few studies have directly examined the effect of Om meditation, specifically, on self-reported sleep quality and perceived stress in college students, a population in which both outcomes are of considerable public-health relevance. Most available intervention studies in student populations have used broader mindfulness or yoga-based programs rather than Om meditation in isolation.(3,5,7)

It is also unclear from existing literature whether improvements in sleep and stress that occur together following such interventions are statistically related to one another or represent relatively independent effects of the practice. The present study therefore aimed to address this gap. The primary objective was to evaluate the effect of a short-term Om meditation intervention on sleep quality among college students. The secondary objective was to examine



**Jyoti Madhur et al.,**

the relationship between sleep quality and perceived stress, both before and after the intervention, in order to clarify whether changes in these two outcomes are statistically associated.

MATERIALS AND METHODS

Study Design and Setting

This study used a single-group, pre-test–post-test (within-subject) interventional design without a parallel control group. This design was chosen to permit a preliminary, feasibility-oriented evaluation of Om meditation in a real-world college setting, consistent with the exploratory pretest-posttest approach used in comparable mind-body intervention research in higher-education populations. (6,7)

Participants

A total of 106 college students who completed both pre- and post-intervention assessments were included in the final analysis. The mean age of participants was 21.5 ± 2.28 years (median 21 years; range 17–35 years). Of the 106 participants, 55 (51.9%) were female and 51 (48.1%) were male. Students were included if they were currently enrolled in a college program, willing to practice Om meditation daily for the intervention period, and able to complete self-report questionnaires. Students with a pre-existing diagnosed sleep disorder, a diagnosed psychiatric illness, prior regular meditation practice, or those unwilling to provide informed consent were excluded.

Intervention

Participants practiced guided Om meditation, involving the rhythmic mental or audible repetition of the syllable "Om" combined with slow diaphragmatic breathing, in a seated position with eyes closed. Sessions were conducted once daily for one month, under supervision or guided audio instruction to standardize technique and duration. (8,9)

Outcome Measures

Sleep quality was assessed using a structured self-report sleep questionnaire, with a higher score indicating poorer sleep quality, consistent with the scoring convention of standard subjective sleep instruments such as the Pittsburgh Sleep Quality Index, in which higher global scores reflect greater sleep disturbance.(13,14) Perceived stress was assessed using a standardized self-report stress questionnaire based on the conceptual framework of the Perceived Stress Scale, which measures the degree to which respondents appraise situations in their lives as unpredictable, uncontrollable, or overloading.(15) Both instruments were administered once before the start of the intervention (pre-intervention) and once after completion of the intervention period (post-intervention).

Ethical Considerations

The study was conducted in accordance with the ethical principles of the Declaration of Helsinki. Written informed consent was obtained from all participants prior to enrolment, and participation was voluntary, with the right to withdraw at any stage without any effect on academic standing. Confidentiality of participant data was maintained throughout data collection, entry, and analysis.

Statistical Analysis

Data were analyzed using JAMOV statistical software. Descriptive statistics (mean, median, standard deviation, minimum, and maximum) were computed for age, pre- and post-intervention sleep scores, and pre- and post-intervention stress scores. Normality of distribution for all outcome variables was assessed using the Shapiro–Wilk test. Because all variables showed a statistically significant departure from normality ($p < 0.05$), non-parametric methods were used for inferential analysis. Pre- versus post-intervention comparisons for sleep and stress scores were performed using the Wilcoxon signed-rank test, with the rank-biserial correlation reported as the corresponding effect size. The relationship between sleep and stress scores, both pre- and post-intervention, was examined using Spearman's rank-order correlation coefficient (ρ). A p -value of less than 0.05 was considered statistically significant for all analyses.





RESULTS

Baseline Characteristics

A total of 106 college students were included in the final analysis. The mean age of participants was 21.5 ± 2.28 years (median 21 years, range 17–35 years). The sample comprised 55 female participants (51.9%) and 51 male participants (48.1%). Baseline characteristics are summarized in Table 1.

Descriptive Statistics of Sleep and Stress Scores

Descriptive statistics for sleep and stress scores before and after the Om meditation intervention are presented in Table 2. Both mean and median sleep scores, as well as mean and median stress scores, were lower following the intervention compared with baseline.

Normality Assessment

The Shapiro–Wilk test indicated that age, pre- and post-intervention sleep scores and pre- and post-intervention stress scores all significantly deviated from a normal distribution ($p < 0.05$ for all variables, Table 3). Non-parametric statistical tests were therefore used for all subsequent inferential analyses. Note. Since all outcome variables showed significant departures from normality ($p < 0.05$), non-parametric analyses (Wilcoxon signed-rank test and Spearman's correlation) were used for all subsequent comparisons.

Effect of Om Meditation on Sleep Quality and Perceived Stress

Sleep scores decreased significantly following the Om meditation intervention. The median sleep score decreased from 7.5 before the intervention to 3.5 after the intervention. The Wilcoxon signed-rank test demonstrated a statistically significant improvement ($W = 5671$, $p < 0.001$), with a mean reduction of 5.0 points and a very large effect size (rank-biserial correlation = 1.00). Perceived stress scores also decreased significantly after the intervention. The median stress score decreased from 0.967 before the intervention to 0.189 after the intervention. The Wilcoxon signed-rank test indicated a statistically significant reduction in stress ($W = 5671$, $p < 0.001$), with a mean reduction of 0.761 and a very large effect size (rank-biserial correlation = 1.00). Full test statistics are presented in Table 4.

Relationship between Sleep Quality and Perceived Stress

Spearman's rank correlation analysis showed no statistically significant association between sleep and stress scores either before the intervention ($\rho = -0.062$, $p = 0.527$) or after the intervention ($\rho = 0.084$, $p = 0.390$). A weak, non-significant negative correlation was also observed between pre-intervention sleep score and post-intervention stress score ($\rho = -0.206$, $p = 0.034$ was noted for post-sleep versus pre-stress in the correlation matrix, while other cross-time correlations were likewise weak and non-significant, all $p > 0.10$). These findings are summarized in Table 5.

DISCUSSION

This single-group interventional study evaluated the effect of a short-term Om meditation program on sleep quality and perceived stress among 106 college students. Two principal findings emerged. First, sleep scores improved markedly following the intervention, with a large-magnitude, statistically significant reduction from a pre-intervention median of 7.5 to a post-intervention median of 3.5. Second, perceived stress scores also decreased significantly, from a pre-intervention median of 0.967 to a post-intervention median of 0.189. Despite the concurrent improvement in outcomes, sleep and stress scores were not significantly correlated with one another either before or after the intervention.

Effect on Sleep Quality

The magnitude of improvement in sleep scores observed in this study is consistent with, and numerically larger than, improvements reported in previous mindfulness- and meditation-based intervention studies among college and



**Jyoti Madhur et al.,**

higher-education populations. A quasi-experimental mindfulness program delivered over twelve weekly sessions to university students reported significant improvements in sleep quality alongside reductions in stress, anxiety, and depression.(3) Similarly, an eight-session telehealth mindfulness meditation program produced significant improvement in Pittsburgh Sleep Quality Index scores among higher-education students, supporting the feasibility of brief, structured contemplative interventions for improving student sleep.(6,7) The present findings extend this literature by suggesting that Om meditation specifically, delivered as a standalone practice, can achieve comparably large improvements in self-reported sleep quality. A plausible physiological basis for this improvement is provided by autonomic studies of Om meditation and Om chanting. Experimental work using heart rate, blood pressure, respiration, and galvanic skin resistance has consistently shown that Om meditation shifts autonomic balance toward parasympathetic dominance, reflected in reduced heart rate and blood pressure alongside increased skin resistance during and after practice.(8,9) Heart-rate-variability studies similarly report enhanced parasympathetic activity following brief periods of Om chanting, an effect that appears more pronounced with greater yoga experience.(10,11) Because heightened sympathetic arousal is a recognized barrier to sleep onset and maintenance, a shift toward parasympathetic dominance offers a biologically coherent explanation for the pre-sleep relaxation effect, and consequent improvement in subjective sleep quality, observed in the present sample. Supporting evidence from a hypertensive population further links structured OM chanting to measurable improvement in subjective sleep quality alongside favorable autonomic changes, reinforcing the plausibility of this mechanism beyond healthy young adults. (12)

Effect on Perceived Stress

The substantial reduction in perceived stress scores observed in this study is in keeping with prior evidence that mantra-based and mindfulness-based meditative practices lower self-reported stress in student and general adult populations.(3,6) Physiological studies of Om chanting describe reductions in salivary cortisol and self-reported anxiety following even brief group chanting sessions, alongside broader autonomic modulation consistent with a relaxation response.(12) Taken together with the sleep findings, these results support the view that Om meditation may act as an accessible, non-pharmacological method for managing the psychological demands associated with college life.

Relationship between Sleep and Stress

Despite significant improvement in both outcomes, this study did not find a statistically significant correlation between sleep and stress scores, either at baseline or after the intervention. This finding may initially appear to contradict the commonly described bidirectional relationship between sleep and stress in college populations.(1,2) However, several explanations are plausible. First, the absence of a significant cross-sectional correlation does not preclude a genuine longitudinal or mechanistic relationship between the two constructs; correlation of static scores at a single time-point captures only one dimension of a more complex, dynamic interaction. Second, it is possible that Om meditation improves sleep and reduces stress through partly distinct, though overlapping, physiological pathways — for example, parasympathetic activation may more directly influence pre-sleep physiological arousal, while reductions in cortisol and subjective appraisal of stressors may operate through a separate cognitive-affective pathway.(12) Third, ceiling or floor effects in the post-intervention scores, given the very large observed effect sizes for both outcomes, may have restricted the variability available to detect a correlation. Future studies using repeated within-person measurement across the intervention period, and larger and more heterogeneous samples, would help clarify whether sleep and stress improvements track one another over time even where a simple pre-post correlation does not reach significance.

Strengths and Limitations

This study benefits from an adequate sample size for a single-group pilot design, use of appropriate non-parametric statistical methods following formal normality testing, and reporting of effect sizes alongside significance values. However, several limitations should be acknowledged. The absence of a control or comparison group means that observed changes cannot be definitively attributed to Om meditation alone, as maturation, regression to the mean, seasonal variation in academic workload, and non-specific effects of participation (e.g., attention, expectation) may





Jyoti Madhur et al.,

have contributed to the observed improvements. The reliance on self-report measures introduces potential response bias, including social desirability bias. Adherence to the daily meditation practice was not objectively verified. Finally, the relatively short intervention period limits conclusions regarding the durability of the observed benefits.

Implications and Future Directions

These findings support the feasibility and potential utility of Om meditation as a low-cost, scalable intervention that college counseling and wellness services could consider for improving student sleep and reducing perceived stress. Future research should employ randomized controlled designs with active or waitlist control groups, objective sleep measures such as longer follow-up periods to assess durability of effect, and mediation analyses to formally test whether reductions in physiological arousal or perceived stress mediate improvements in sleep quality.

CONCLUSION

A short-term Om meditation intervention was associated with a statistically significant and large-magnitude improvement in sleep quality and a significant reduction in perceived stress among college students. Sleep and stress scores were not significantly correlated with one another, either before or after the intervention, suggesting that Om meditation may influence these outcomes through partly independent mechanisms. These preliminary findings support further investigation of Om meditation as a feasible, non-pharmacological strategy for improving sleep and psychological well-being in college populations, ideally through randomized controlled trials with objective outcome measures.

Declarations

Conflict of Interest: The authors declare no conflict of interest.

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Informed Consent: Written informed consent was obtained from all individual participants included in the study.

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Table 1: Baseline characteristics of the participants (N = 106).

Variable	Value
Age (years), Mean $\pm$ SD	21.5 $\pm$ 2.28
Age, Median (Range)	21 (17–35)
Female, n (%)	55 (51.9)
Male, n (%)	51 (48.1)

Table 2: Descriptive statistics of sleep and stress scores before and after the intervention

Variable	Pre-intervention Mean $\pm$ SD	Pre-intervention Median	Post-intervention Mean $\pm$ SD	Post-intervention Median
Sleep score	8.83 $\pm$ 3.06	7.5	3.37 $\pm$ 1.29	3.5
Stress score	0.944 $\pm$ 0.052	0.967	0.189 $\pm$ 0.055	0.189

Table 3: Normality assessment using the Shapiro–Wilk test

Variable	Shapiro–Wilk W	p-value	Interpretation
Age	0.852	<0.001	Non-normal
Pre-sleep score	0.828	<0.001	Non-normal
Post-sleep score	0.890	<0.001	Non-normal
Pre-stress score	0.774	<0.001	Non-normal
Post-stress score	0.970	0.018	Non-normal

Table 4: Comparison of pre- and post-intervention sleep and stress scores (Wilcoxon signed-rank test)

Outcome	Pre Median	Post Median	Mean Difference	Wilcoxon W	p-value	Effect Size (rank-biserial r)
Sleep score	7.5	3.5	5.00	5671	<0.001	1.00
Stress score	0.967	0.189	0.761	5671	<0.001	1.00



**Jyoti Madhur et al.,****Table 5: Spearman correlation between sleep and stress scores**

Variables	Spearman's ρ	p-value
Pre-sleep vs. Pre-stress	-0.062	0.527
Post-sleep vs. Post-stress	0.084	0.390

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RESEARCH ARTICLE

Semiconductor Chip for Biosensors on Medical Device: A Bibliometric Study

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ABSTRACT

Biosensors and semiconductor technologies are interrelated in medical diagnostics and are technologies that require urgent attention. To explore global research trends through a bibliometric study. This research is essential to address the challenges in the field, identify gaps, and promote collaboration within the academic and research community. This bibliometric study analyzes the evolution and research dynamics of semiconductor-based biosensors for drug monitoring from 1991 to 2025. It uses data from 7,863 publications retrieved from the Web of Science database, with vosviewer, rstudio, and excel as the main analysis tools. The results reveal a rapid growth in research output, with China, the United States, India, and Korea as the major contributors. The key journals identified include Biosensors and Bioelectronics and Sensors and Actuators B-Chemical. Keyword analysis highlights the integration of nanotechnology such as Graphene, carbon nanotubes, and quantum dots in improving biosensor performance. Emerging trends include the use of artificial intelligence, machine learning, and electrochemical sensing for personalized medicine and point-of-care testing. Future research should focus on the interplay of biosensors with AI, nanomaterials, and flexible electronics to improve sensitivity and portability, with promising materials such as MXenes and MoS₂. This bibliometric analysis provides valuable insights into the emerging landscape of semiconductor-based biosensors and their potential to transform healthcare and drug monitoring.

Keywords: biosensor, semiconductor, nanomaterial, medical technology, bibliometric



**Mumtaz Begum and Raja**

INTRODUCTION

In the modern world, the emergence of various new diseases is increasing day by day, causing human mortality and facing various challenges, and the medical sector is taking various efforts to manage and solve this. Rapid advancements in medical technology have changed the landscape of hospital care, with biosensors and semiconductor technologies playing a key role in enabling more accurate diagnosis and treatment. In the last ten years, biotechnology has provided solutions to various diseases at the touch of a button and has also gained importance in the way doctors perform surgeries.

Biosensors in medical device

A biosensor is an analytical instrument comprising a biologically derived sensing component, such as enzymes, microorganisms, or antibodies (Kulshreshtha et al., 2017). Biosensors that convert biological signals into measurable electrical or optical outputs have emerged as essential devices in medical diagnostics (Chadha et al., 2022). Electrochemical biosensors can be further classified into (1) conductometric, (2) amperometric, and (3) potentiometric based on the parameter measured (Kulshreshtha et al., 2017). A biosensor usually has three main parts: a bioreceptor, a transducer, and a signal processing unit (X. Wang et al., 2024). The capacity to detect biomarkers, monitor (Panyadee et al., 2023) metabolic levels (Sim et al., 2022), and sense specific pathogens has made them essential in the management of conditions such as diabetes, cardiovascular disease, cancer biomarkers, and infectious diseases (Murray et al., 2024). Their superior performance and capacity for real-time feedback have established them as essential medical device for continuous monitoring and the early diagnosis of diseases. Semiconductors are integral to biological sensors, as they enable the transformation of biological events—such as prevention, detection, reporting, response, recovery, and investigation—into measurable electrical signals. This capability facilitates real-time detection in sensitive analyses (Plus, 2024; Rai et al., 2024). The development of advanced biosensors requires innovations in semiconductor technology (Panahi & Ghafar-Zadeh, 2023). Semiconductor materials and components such as field-effect transistors (FETs) (Pandey et al., 2025) (Phogat et al., 2025), CMOS chips (Muhammad et al., 2025) (Ghafar-Zadeh et al., 2024), MEMS (Hossain et al., 2024), and Nano electronic interfaces play a key role in improving the performance, sensitivity, and scalability of bio sensing platforms. Such technologies enable the integration of high-density signal processing, real-time analysis, and biosensors into small, wearable, or point-of-care diagnostic systems in the medical field (Moeinfard et al., 2024) (He et al., 2023).

Importance of semiconductor chip in biosensors

Metal-oxide semiconductor (MOS)-based biosensors are at the forefront of bioelectronics advancements due to their remarkable properties that collectively enhance performance and applicability in the field of bioelectronics (Ketcham & Crawford, 2007), owing to their many unique advantages and long-term operational stability, MOS-based biosensors ensure prolonged use without significant degradation in sensing performance. Among anticancer drugs, 5-fluorouracil (5-FU) is widely used to treat various cancers, such as gastrointestinal and lung cancers. Thus, FET-biosensors are promising, rapid, and inexpensive devices for measuring 5-FU concentration (Zouari et al., 2024). An individual's health is essential and it is necessary to improve it. In contemporary society, the Internet of Things (IoT) represents a highly appealing technology that addresses various limitations. Consequently, IoT is increasingly employed in diverse healthcare activities, including the diagnosis, treatment, and monitoring of diseases. Notably, IoT-enabled biosensors are advancing new healthcare opportunities, particularly in diagnostics and imaging (Zouari et al., 2024). Despite such technological advances, this field remains a highly interdisciplinary area spanning multiple disciplines such as electronics, biomedical engineering, pharmacology, and Nano science. To the best of our knowledge, there has been no comprehensive bibliographic study to better understand the evolution and research dynamics of this field. A detailed bibliographic quantitative analysis of a field provides a clear understanding of global trends, key contributors, collaborative networks, and keyword clusters. We hope that this study will identify emerging research themes at the intersection of drug monitoring, biosensors, and semiconductor-based technologies.



**Mumtaj Begum and Raja**

Many current and previous studies have focused on the continuous development of literature, and many studies are consistent with the bibliographic measurement method. Therefore, it is necessary to focus on the topics that should be prioritized in this research. It is also important for senior authors to examine citation patterns to identify articles that receive the most attention (Mendoza, 2021). Research that receives the most attention from scholars will indicate areas that journals should pay more attention to. In addition, it is also necessary to examine the current status in the above areas to accurately identify priority areas in the field of research (García et al., 2018; Holmberg & Vainio, 2018; Klammer & Dalen, 2002). Consequently, bibliometric methods were employed to investigate the following research questions.

- How has the number of publications on the topic of semiconductor status in biosensors in the medical field evolved over time?
- Who are the main contributors of the research?
- What are the main keywords in semiconductor biosensor publications in the medical field?
- Are there any new insights that can be identified in the research related to them, and if so, what?
- What does semiconductor bio sensors mean for future research in the medical field?

REVIEW OF LITERATURE**Role of Semiconductors in Biosensor device**

The integration of biosensors and semiconductor technologies into modern diagnostic devices has become a cornerstone of real-time, accurate, and minimally invasive health monitoring. Graphene has excellent physicochemical properties, which make it a highly sensitive graphene opt biosensor for identifying living tumour cells responding to the chemotherapeutic paclitaxel after drug administration (Y. Wang et al., 2018). Carbon nanotubes are recognized in biosensors for their electrochemical and thermodynamic properties (J. Wang, 2005). In addition QD-based luminescent semiconductor Nano crystals have attracted considerable attention because of their advanced bio sensing properties (Sapsford et al., 2006). A growing body of literature explains how this interdisciplinary field, which integrates biomedical engineering, materials science, and electronics, developed from 1989 to 2024. Several bibliographic analyses indicate that research publication has begun to grow in the early 2000s. Research in the field of biosensors has garnered considerable attention due to its potential applications across a range of industrial and scientific domains, including bioinformatics, biotechnology, materials research, medical care, and health sciences. Biosensor research has attracted significant attention owing to its potential applications in various industrial and scientific fields such as bioinformatics, biotechnology, materials research, medical care, and health sciences.

Bibliometric Trends in Biosensor and Semiconductor Research

In the early stages, biosensor technology was specifically used for glucose monitoring in diabetes management (Y. Zhang et al., 2021). Over time, advances in nanotechnology in biosensors have revolutionized early disease detection by providing improved sensitivity and specificity from simple electrochemical sensors compared to traditional methods. (F. Wang & Hu, 2009) this has accelerated the development of biosensors in the field of medicine. This scientific shift is evident in bibliometric studies. The increasing frequency of keywords such as "SIC" (Talwar et al., 2023), "IN VIVO" (Babu Thomas et al., 2024), "QFD" (Han & Shin, 2014), "Infrared semiconductors" (Kumar et al., 2025), "CMOS" (Rai et al., 2024), "MEMS" (Awotunde et al., 2024), "FET" (Dou et al., 2025). Especially last two years, bibliometric studies confirm that terms such as "nanomaterial based sensors" (Babu Thomas et al., 2024), "Tungsten-based nanomaterial" (Gu et al., 2023) and "molybdenum-based nanomaterials" (Z.-Q. Wang et al., 2024) in the biomedical field, "Integrating Microfluidic and Biosensors", "Quantum Dots for imaging, diagnostics, drug delivery, and therapy" (Remya Krishnan et al., 2024), have become dominant research themes in the current literature. Geographically, the US, China, Japan, and India are the countries most interested in this study, and France, Germany, and the UK are showing significant interest (Valente et al., 2024). Analytical tools such as VOSviewer demonstrate robust international collaborations, particularly among countries, institutions, and authors, through their examination of collaborative networks. In particular, Southeast Asian countries such as Thailand and Vietnam,



**Mumtaj Begum and Raja**

African countries such as Kenya and Nigeria, and Middle Eastern countries such as Iran have widely focused on collaborative research on this topic (L. Li et al., 2023). It represents a global research community interconnected across multiple research fronts. Biosensors continue to dominate the field of medicine (Kim et al., 2023). Semiconductor technology is crucial for the development of sensitive and responsive biosensors that can (Bustanji et al., 2023; Hammouti et al., 2025; Lu et al., 2025; Rajapaksha et al., 2025; H. Zhang et al., 2025). In bibliometric analysis, general data (publication trends and citation trends) provide all information at a glance (Liu et al., 2024) (Nanthakumar & Raja, 2025).

Data selection and search strategy

We used the Web of Science (WOS) central collection database to conduct a comprehensive search for the use of semiconductor in the medical field. The Web of Science database is often used in bibliometric studies (Chen et al., 2022; Talan & Demirbilek, 2023). This database contains a variety of document types covering the areas of science, technology, social sciences, arts, and humanities. The databases cover the science citation index expanded, social sciences citation index, and arts and humanities citation index (Ma, 2022) (Şenocak & Arpacı, 2023). On July 7, 2025, a comprehensive search was conducted in the Web of Science (WoS) database to identify all studies related to semiconductor biosensor technology. The keyword terms used in the search are given in Table (1). Through various screening processes, unnecessary information and missing research data were removed, and only clear and accurate data were analysed. This search retrieved 10,043 papers published in 916 journals between 1991 and 2025. The papers were from 3637 institutions, 81 countries, and 25,368 authors. In terms of document type, the majority were research articles (77.95%) and review articles (15.87%) with a significant proportion being peer-reviewed, only these were subjected to research performance, the rest were deleted, such as

- DT= article; early access (4.42%) ; article; proceedings paper (0.77%); article; retracted publication (0.23%); correction (0.17%); editorial material (0.13%); expression of concern (0.12%); letter (0.11%); meeting abstract (0.04%); news item (0.03%); note (0.02%); review (0.01%) ; review; book chapter (0.01%); review; early access (0.01%); review; retracted publication (0.01%).
- C1= author details (0.33%)
- AU_UN=author affiliations (0.16%)
- Overall missing document (15.03%)

The selection time for this study was retrieved on July 7, 2025, as this study was a comprehensive bibliographic review covering all years. Earlier studies tend to receive a high citation count, while more recent studies fail to do so. However, in recent times, the development of electronic devices in the medical field has started to grow, so it is necessary to select all data. Following comprehensive refinement, a total of 7,863 documents were selected for analysis.

RESULTS**General data**

In table (2) reveal that main information of the bibliometric analysis, The bibliometric dataset spans more than three decades of research (1991-2025), showing a slight decline in annual publication growth (-3.09) while maintaining a high citation impact (6.5), which indicates that the field remains influential despite reduced output. The literature is relatively recent on average, reflecting ongoing activity and relevance. Research output is largely driven by original articles, complemented by a significant number of reviews that consolidate existing knowledge, while authorship patterns highlight strong collaboration, with most works being multi-authored and supported by notable levels of international cooperation. Keyword analysis reveals diversity (10,537 Keywords plus 18,416 Author Keywords), pointing to a broad and multidisciplinary scope.



**Mumtaj Begum and Raja****Publication and citation trend**

During the initial period (1990–2005), the volume of publications and citations remained minimal, indicating limited research activity within the field. However, a gradual increase in both publications and citations was observed post-2000, reflecting a burgeoning interest in the educational development of electronic devices within the medical domain. The subsequent period (2006–2015) witnessed a substantial rise in research output, accompanied by an increase in citations. Notably, between 2016 and 2021, there was a marked surge in both the number of publications, which peaked at over 800 in 2021, and citations, which reached nearly 25,000 in 2020. This trend underscores the global attention garnered by this research topic. In recent years (2022–2023), there has been a notable decline in both publication counts and citations (see Fig 1). This decrease may be attributed to the common phenomenon of delayed citation accrual for recent publications within the research area.

Sources

Based on a bibliometric analysis, research related to semiconductor-based biosensors in the medical field has been spread across 916 journals from 1991 to 2025. According to the source analysis, at least 14.62% of the journals have published more than ten articles, 40.61% of the journals have published one to ten articles, and 44.75% of the journals have published one article. This shows that these journals are not focused on research; otherwise, they receive multidisciplinary research, so relevant studies in the field of knowledge are not included. In particular, only 1.31% of journals have published more than a hundred research articles. As shown in Figure (b), "biosensors and bioelectronics journal" has 8.5% of the studies published in this journal, followed by "Sensors and Actuators B-Chemical" journal with 6.75% of the studies published in this journal, however, "Sensors" with 3.86% and "IEEE Sensors Journal" with 3.29% have made significant contributions. The high contributor Biosensors & Bioelectronics journal has dominated the research attention since 2010, Sensors and Actuators B-Chemical journal has a fluctuating publication pattern, with a high publication rate in 2018 and a decline of half in 2019, which is likely to reduce the attention to that journal, Sensors journal follows a moderate growth pattern with more publications likely in the future, IEEE Sensors Journal has increased research attention after 2015 and gradually starts to decline after 2020, as clearly illustrated in Figure (a).

Figure (d) describes journals with high h-index. The journal ACS Nano (504) with high h-index has 81 publications. The journal Biosensors & Bioelectronics (h-index=254) with the highest number of publications is followed by the journal ACS Applied Materials & Interfaces (h-index=334) with 165 publications. All papers in this field have selected journals with high h-index. Figure (C) illustrates the leading journals with the highest citation counts in the field of Semiconductor-Based Biosensors for Drug Monitoring. Biosensors and Bioelectronics provides the most cited journals in the field of bio sensing, with 58 articles published in 2018, 66 articles published in 2019, and 46 articles published in 2020. Studies published during this period contributed to Biosensors and Bioelectronics, which has the highest citation impact. Papers written by (Cesewski & Johnson, 2020) and (Kaisti, 2017) help the journal get more citations. Following that, journals such as Sensors and Actuators B: Chemical (c =20816) and ANAL CHEM (c =16840) have published papers with more than ten thousand citations, semiconductor-based biosensors for drug monitoring Analytica Chimica Acta (c =7449), ACS Nano (c =6506), Talanta (c =6489), NANO letter (c =6399), Journal of the American Chemical Society (c =6364), and Sensors (c =6243) have made significant contributions to this field.

Country and affiliation

The analysis of major journals with high h-indexes and citations has stimulated interest in exploring research in the field of publication. See figure(a), the analysis shows that a total of 81 countries have contributed to the literature on this topic, of which 12 (14.81%) countries have published one paper, which are from Central America, South Asia, the Middle East and Africa, 21 (25.92%) countries have published more than one and less than ten papers, with the Middle East and Africa being the most prominent, followed by 34 (41.9%) countries with more than ten and less than one hundred papers, with turkey, Saudi Arabia, Australia, Russia, Switzerland, Singapore, Portugal having made significant contributions to this field, in addition to Japan, Iran, Germany, Italy, Canada, Spain, United Kingdom, Malaysia, Brazil France having published more than one and less than three hundred papers. China, India, the USA



**Mumtaj Begum and Raja**

and Korea were the main contributors to this ranking. China's contribution is extraordinary compared with that of other countries, China ranks first in citation production capacity with 68919, followed by the United States with 49064 citations, India with 22949 citations, Saudi Arabia, and Singapore are leading in citation impact despite their low publication, which reflects their excellent research capacity. Canada ranks ninth in publication output and fifth in citation impact. This reflects their excellent research capacity, as shown in Figure (b). It is noteworthy that the contribution of countries like India and Iran is particularly significant.

Figure (c) shows the most important academic institutions in this field. It is noteworthy that 3637 academic institutions have contributed to the research field of Semiconductor-Based Biosensors for drug monitoring. In the analysis of data obtained from the Web of science database, 18 academic institutions showed the best productivity. The Chinese Academy of Sciences (6.09%) has been identified as the most productive institution. This is followed by university of California system (3.5%) of the United States as the best producer, followed by the Indian Institute of Technology of India (3%) and National Institute of Technology of India (2.8%). All the institutions identified in the analysis are from developed countries (China, USA, India, France, and Korea), and beyond this is the Egyptian Knowledge bank (1.55%). This analysis should increase the cooperation between developed and developing countries. In figure (4) shows the collaboration between countries related to the development of Semiconductor-Based Biosensors for Drug Monitoring. China has cooperated with 70 countries in this field; although it has extensive cooperation with other countries, it shows great intensity in collaboration with developed countries. The United States has cooperated with 64 countries, especially India (LS=84) and China (LS=197). India has a research cooperation with 70 countries, including Iran, Turkey, Saudi Arabia, Germany, and Malaysia. This is followed by Iran (LS=48), Malaysia (LS=52), Saudi Arabia (LS=53), France (LS=54), Germany (LS=55), and Italy (LS=56), which are developing countries.

Top authors

It is clear that research into Semiconductor-Based Biosensors for drug monitoring has been conducted extensively under several different research disciplines. Research on semiconductor-based biosensors for drug monitoring has been conducted extensively in various research disciplines, our comprehensive bibliometric analysis revealed that 25,368 authors participated in this field from 1991 to 2025. In fig (5) shows the researchers with the highest number of h-index. Zhang Y (h-index=31) from The Chinese Academy of Sciences, China, is the most published author. Wang J, kumar S, wang Y and schöning MJ have published (h-index=23), followed by Li J, Kim S, and Liu Y with (h-index=22). Publications reveal the knowledge capacity of researchers. ZHANG Y (95 doc) is the most influential author, followed by WANG Y (70 doc) and WANG J (62 doc). WANG J's research work started in 2002 and has 2323 citations, ZHANG Y's research work started in 2011 and has 3211 citations, and LEE G's citation count is 1689 citations for 6 papers, which is more than other authors' citation impact for research, which shows the great creative ability of that researcher.

Science mapping

This section aims to explore the authors' keywords, their co-occurrence, and keywords in the research field of Semiconductor-Based Biosensors for Drug Monitoring. In fig (6) our analysis is based on a large dataset of 7863 documents, from which we extracted 545 keywords that appeared more than ten times. A total of five keyword clusters were identified, from smallest to largest. One small cluster and four large clusters were found in the analysis: Cluster 1 contained 164 keywords (29.02%), Cluster 2 contained 152 keywords (26.9%), Cluster 3 contained 126 keywords (22.3%), Cluster 4 contained 101 keywords (17.87%) and finally Cluster 5 contained 2 keywords (0.35%). The figure (5) shows a visual representation of the most frequently used terms, specifically biosensor (2124 times), micro fluids (762 times), electrochemical sensor (425 times), and sensitivity (368 times). The network of authors' words and their co-occurrence has been illustrated using VOSviewer software. Each circle in the image represents a key word set of the author. While, the frequency circle is indicated by the number of keywords, and the strength of the co-occurrence is evaluated by different colours for the nodes. The most important keywords in the clusters are listed in the table (3).



**Mumtaj Begum and Raja**

Cluster 1 (Red) – Biosensor Devices & Transducers, Keywords include biosensors, field-effect transistors (FET), chemical imaging sensors, PH- sensors, carbon nanotubes, ISFET, extended-gate FET, and ion-sensitive sensors. This cluster mainly focuses on fundamental biosensor architecture, especially the electrical and electrochemical transduction mechanisms. Carbon nanotubes and metal oxides to improve electron transfer(J. Li et al., 2007), Miniaturized sensors like light-addressable potentiometers(Wan et al., 2015), additionally High precision in pH sensing and surface potential monitoring(Wan et al., 2015)

Cluster 2 (Green) – Microfluidics & Nanomaterial Integration

Cluster two Keywords include microfluidics, nanomaterial, nanotoxicity, aptasensors, drug delivery, nano medicine, fluorescence, tissue engineering, and lab-on-a-chip. This cluster mainly emphasizes the synergy of nanotechnology with microfluidics for advanced bio sensing. This cluster emphasizes certain technological trends. Lab-on-a-chip platforms combining fluidics and sensing(Allert et al., 2022), Integration of aptamers for high specificity(Yu et al., 2023), Drug discovery and delivery systems linked with biosensors(Tan et al., 2022).

Cluster 3 (Blue) – Electrochemical Biosensors

Cluster three Keywords include electrochemical sensor, electrochemical biosensor, cyclic voltammetry, amperometric sensor, carbon dots, graphene oxide, MXene. Nanostructured materials are often used in electrochemical detection techniques. Electrochemical Biosensors often used graphene oxide, MXenes, and carbon dots for enhancing conductivity and sensitivity(Reddy et al., 2022), Techniques such as voltammetry Biosensors are used to measure different pulses in the human body(Baluta et al., 2022).

Cluster 4 (Yellow) – Biophysics & Sensitivity Enhancement

Cluster four identified main keywords, molecular biophysics, biomolecules, sensitivity, dielectrics, TFTs (thin-film transistors), tunnelling, logic gates, Nano gap, theme focused on molecular interactions and device sensitivity improvements. Logic gate biosensors for decision-making bio-computation(Baluta et al., 2022) and development of nano-gap electrodes for ultra-sensitive measurements(Baluta et al., 2022).

Top cited documents

The importance of any research publication can be measured by its citation count, which allows us to perceive its importance in the relevant context(Bornmann & Daniel, 2008). Table (4) presents the ten most highly cited papers, both locally and globally, in the domain of Semiconductor-Based Biosensors for Drug Monitoring, covering the period from January 1991 to July 9, 2025. We closely examined global and local citations in articles related to this field. In comparison, Global citations refer to citations that a document receives from various index databases. In contrast, local citation counts only measure the number of citations a document receives from a dataset on various platforms (e.g. Google Scholar). In Table (4) explained top cited documents, Significant advancements in bio sensing and bioengineering have been driven by innovations in materials, nanotechnology, and microfabrication. Early breakthroughs, such as soft lithography, enabled precise patterning of proteins and cells, establishing a foundation for bio interface engineering and tissue applications (Kane et al., 1999). This progress was facilitated by advancements in polymer-based sensing platforms, which utilized both conducting and non-conducting polymers for their adaptability in sensor design.(Adhikari & Majumdar, 2004). Nanomaterial emerged as transformative tools, with quantum dots offering unique optical properties for in vivo molecular and cellular imaging(Smith et al., 2008), while Aptamer provided highly specific and stable recognition elements for the development of biosensors (Song et al., 2008).

In parallel, gold nanoparticles have enhanced electrochemical biosensors by offering superior surface modification and signal amplification(Pingarrón et al., 2008). Graphene has significantly enhanced sensing capabilities due to its remarkable conductivity, sensitivity, and structural properties(Pumera et al., 2010)enhanced by the adaptability of carbon nanotube-based platforms for detecting a range of biomolecules, from neurotransmitters to DNA(Jacobs et al., 2010). In a similar vein, modifications of carbon nanotubes in electro analysis have enhanced biosensor performance across a wide range of applications(Katz & Willner, 2003).More recently, Devices enabled by nanomaterial, such as



**Mumtaj Begum and Raja**

graphene field-effect transistor (FET)-based biosensors, have shown exceptional diagnostic potential by facilitating the ultra-rapid detection of SARS-CoV-2 from clinical swab samples(Seo et al., 2020)Together, these studies highlight the shift from micro fabrication-based biomaterial patterning to the cutting-edge realm of nanomaterial-enabled bio sensing and diagnostics. They emphasize how innovations in material science are continually shaping the development of high-performance biosensors and biomedical devices(Sarkar et al., 2014).

DISCUSSION

Figure (3) clearly reveals detailed explanation in this bibliometric analysis, the results are discussed from the keyword co-occurrence and top cited documents. Terms such as "integrated sensors", "enzyme inhibition", "electrophysiology", and "microarrays" appear first. This keywords focus on basic biosensing technologies and biological testing methods. Emergence of "graphene", "quantum dots", "microfluidics", "field-effect transistors", and "gold nanoparticles" reflects the rise of nanotechnology applications in biosensing and diagnostics. although strong bursts for terms like "artificial intelligence", "machine learning", "electrochemical sensors", and "biomarkers" shows a shift towards AI-powered biosensors and smart diagnostic platforms. Large bubbles in graphene and nanomaterial from 2017 to 2019 highlight a massive surge in nanomaterial-based sensors. Artificial Intelligence & Machine Learning keywords are recently emerging terms, indicating a major trend of integrating AI into bio sensing, and a Point-of-Care Testing 2015 to 2023 high number of word counts reflect clinical and real-time health monitoring applications. Nanotechnology, micro electro mechanical systems (MEMS), and biocompatibility extend through decades, showing sustained relevance, and some specific techniques such as formaldehyde detection or enzyme inhibition peak early and then decrease.

This timeline bubble plot Fig (7) clearly illustrates the dynamic evolution of biosensor and nanotechnology research over nearly three decades. Early work laid the technical foundation with integrated sensors and enzyme-based detection. The middle-phase was characterized by the nanotechnology revolution (Graphene, quantum dots, MEMS). Current and future focus lies in AI-driven, bio-compatible, and electro chemical sensing platforms for healthcare, personalized medicine, and environmental monitoring The figure (8) illustrates the relationship between countries (AU_CO), journals (SO), and universities/institutions (AU_UN) in the field of biosensors and related research. Major contributing countries such as China and the United States dominate, followed by Korea, Japan, Germany, and India. The major publishing journals are Biosensors & Bioelectronics, Sensors and Actuators B: Chemical, IEEE Sensors Journal, and Analytica Simica Acta. These serve as major hubs for international research convergence. The leading institutions are the Chinese Academy of Sciences, the University of California System, and the Indian Institute of Technology, Stanford University, and Zhejiang University. According to the results, the top ten most cited papers were published in the early period and focused on Advanced Materials for Bio sensing, Transduction Mechanisms, and Integration of Bimolecular Recognition, Applications in Clinical Diagnostics, Miniaturization and Scalability. The most cited papers were published in journals, such as PubMed and ACSNano. Findings of the top-cited papers. However, the findings of these top-cited papers suggest that nanomaterial, such as graphene, carbon nanotubes (CNTs), gold nanoparticles, MoS₂, and quantum dots, are gaining importance in improving biosensor performance. Furthermore, our findings indicate that developed nations engage in a greater volume of research activities compared to developing and underdeveloped nations. Despite the larger populations in developing countries, their research contributions remain underrepresented.

Strength and Limitation

Our study analyses and describes a significant amount of data obtained from a reputable database. Our study provides an in-depth description of key authors, countries with the most publications, and institutions of importance through bibliographic analysis. Our entire study has inherent limitations to bibliometric studies. According to(Hou & Wang, 2021)state that bibliometric analysis can be helpful in clearly explaining the entire research progress in a research field. However, the in-depth information and findings related to a research cannot be replaced, we



**Mumtaj Begum and Raja**

encourage researchers to conduct bibliometric analysis as a result. Furthermore, when data is retrieved from the database, unnecessary information is automatically added, which the author must remove before bibliometric analysis. For example, the data may include an additional year than the measured year, which the author should avoid. Furthermore, this study was conducted on only 7863 papers, which is sufficient to describe semiconductor chip-based biosensors for medical devices in the scientific world, and the most cited paper belongs to this field, so there is no need to depend on any other field. The number of journals and authors required for analysis was sufficient.

Future Directions

According to the bibliometric key results, to further analyse future research trends in this field, future research on biosensors should prioritize interdisciplinary integration with artificial intelligence, nanomaterials, and flexible electronics to improve sensitivity and portability. The application of biosensors is scalable, and therefore expected to dominate the next decade.

- The analysis revealed strong research growth in biosensors in the medical field, with China and the United States leading the way in publications, while collaboration with developing countries remains limited, where research should focus.
- Keyword analysis suggests a potential area for interdisciplinary research that integrates real-time disease diagnosis and predictive health monitoring capabilities.
- 2D materials beyond graphene (e.g., MXenes, MoS₂) are promising nanomaterials for ultrasensitive biosensors. They are environmentally friendly and adaptable biosensors.

A keyword search can be modified to include additional words relevant to the field, which can provide a deeper understanding of the research field and conduct a literature review and meta-analysis in the field. Reviews provide additional insights into the antecedents and consequences of the field, so empirical studies and reviews can describe the in-depth content of this research field (Ketcham & Crawford, 2007), explored can be connections between various medical financial organization systems and creative entrepreneurs, the social marketing of medical tools, and the rapid advancement of the digital community.

FINDINGS AND CONCLUSION

This study is unique in that it examines the role of semiconductors in biology. The study is based on a database that is widely used by researchers worldwide. The results of the study reveal several conclusions. Biosensors have become important in the medical field due to their excellent sensitivity, stability, reproducibility, and immediate response time, and this study concludes that the growth of biosensors research output is rapid. According to the results, the biotechnology sector has developed well in the medical field and its development has been well integrated with various nanotechnology innovations. Section 3.2: research publications from 2022 to 2024 have shown significant improvement over other years. Section 3.3: the biosensors and bioelectronics journal and the Sensors and Actuators B-Chemical journal have been identified as key sources. Section 3.4: China, the United States, India, and Korea are the major contributing countries, while the Chinese Academy of Sciences, the University of California system, and the Indian IITs are the major research institutions. Section 3.5: Zhang Y, Wang J, and Kumar S are the main contributors to this field of research. The VOSviewer tool plays a role in many areas of research, including keyword analysis and cross-country collaboration. Keyword analysis has identified the most common areas of application for biosensors in the medical sector. Accordingly, biosensors used to measure fat level, cancer, breast cancer and COVID-19 pandemic are in high demand. The study highlights the growth of nanotechnology in biomedical devices. The development of 2D materials like Graphene, the growth of keywords like Graphene oxide, gold nanoparticles, Quantum dots and carbon nanotubes highlights the growth of nanotechnology. Semiconductor technology has revolutionized biosensors and is amazing. This makes it easier for experts to directly and electronically measure biological interactions. Semiconductor chips are used in key applications beyond CMOS





Mumtaj Begum and Raja

technology, increasing the demand for more powerful semiconductors, which medical research institutions, financial institutions, and policymakers must contribute to accordingly.

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Conflict of interests

The authors declare no Conflict interests.

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Mumtaz Begum and Raja

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Table 1: Keyword search techniques

Search set	Search terms	Results
#1	ALL=((("biosensor" OR "chemical sensor" OR "electrochemical sensor" OR "bioelectronics sensor" OR "wearable sensor" OR "drug monitoring" OR "therapeutic drug monitoring" OR "pharmacokinetics" OR "drug detection"))	351,882
#2	ALL=((("semiconductor" OR "nanomaterial" OR "transistor" OR "CMOS" OR "FET" OR "MEMS" OR "lab-on-chip" OR "microfluidics"))	593,742
#3	#1 AND #2	10,043

Table 2: Main Information

S No	Indicators	Results
1	Timespan	1991:2025
2	Sources (Journals, Books, etc)	916
	Documents	7863
3	Annual Growth Rate %	-3.09
4	Document Average Age	6.5
5	Average citations per doc	33.75
	Document Contents	
6	Keywords Plus (ID)	10537
7	Author's Keywords (DE)	18416
	Authors	
8	Total no of Authors	25368
9	Authors of single-authored docs	122
	Authors Collaboration	
10	Single-authored docs	133
11	Co-Authors per Doc	5.64
12	International co-authorships %	24.6





Mumtaj Begum and Raja

	Document Types	
13	article	6464
14	review	1397

Table3: High frequency keywords

Cluster1			Cluster 2		
Keywords	Occurrences	TLS	Keywords	Occurrences	TLS
Biosensor	2124	4742	Micro fluids	762	1480
Graphene	340	922	Nano particles	123	355
Aptamer	160	419	Nano materials	209	489
Field effect transistor	228	569	Electro chemical	124	378
Chemical sensor	201	330	Bio sensing	88	176
Sensor	150	332	COVID 19	83	219
Cmos	113	247	Quantum dots	88	216
Label free	89	245	Fluorescence	101	294
Carbon nanotube	94	229	Pharmacokinetics	59	77
Enzyme	61	181	Food safety	56	138
Biomarker	88	245i	Cancer	68	223
Immuno sensor	100	256	Optical biosensor	60	120
PH-sensor	85	197	Lab on chip	93	225
Cluster 3			Cluster 4		
Keywords	Occurrences	TLS	Keywords	Occurrences	TLS
Nanomaterial	311	789	Biosensors	611	1750
Cyclic voltammetry	64	151	Sensitivity	368	1119
Nano composite	62	160	Molecular physics	36	267
Dopamine	88	198	Electrodes	54	292
Graphene oxide	83	188	Chemical sensors	41	115
Electrochemical biosensor	131	268	Electrodes	54	292
Gold nanoparticles	96	187	Breast cancer	46	125
Electrochemical impedance spec	55	128	Impedance	41	151
Cholesterol	21	40	optical sensors	31	139
carbon nanotubes	107	303	Field effect transistors	37	173
Aptasensor	63	142	Silicon	55	258
Reduced graphene oxide	52	109	Dielectric modulation	65	232
Glucose oxide	64	144	Biomolecules	70	198
Voltammetry	37	123	Logic gates	52	365

Table 4: Top cited documents

Document	DOI	LC	GC	LC/GC Ratio (%)	Rank
(Kane et al., 1999)	10.1016/S0142-9612(99)00165-9	2156	1332	161.8619	1
(Adhikari & Majumdar, 2004)	10.1016/j.progpolymsci.2004.03.002	1678	1051	159.6575	2
(Smith et al., 2008)	10.1016/j.addr.2008.03.015	1520	991	153.3804	3
(Song et al., 2008)	10.1016/j.trac.2007.12.004	1731	1159	149.3529	4
(Pingarrón et al., 2008)	10.1016/j.electacta.2008.03.005	1182	804	147.0149	5
(Seo et al., 2020)	10.1021/acsnano.0c02823	1927	1313	146.7631	6
(Jacobs et al., 2010)	10.1016/j.aca.2010.01.009	1225	850	144.1176	7





Mumtaj Begum and Raja

(Pumera et al., 2010)	10.1016/j.trac.2010.05.011	1446	1039	139.1723	8
(Katz & Willner, 2003)	10.1002/elan.200390114	1651	1201	137.4688	9
(Sarkar et al., 2014)	10.1021/nn5009148	1195	895	133.5196	10

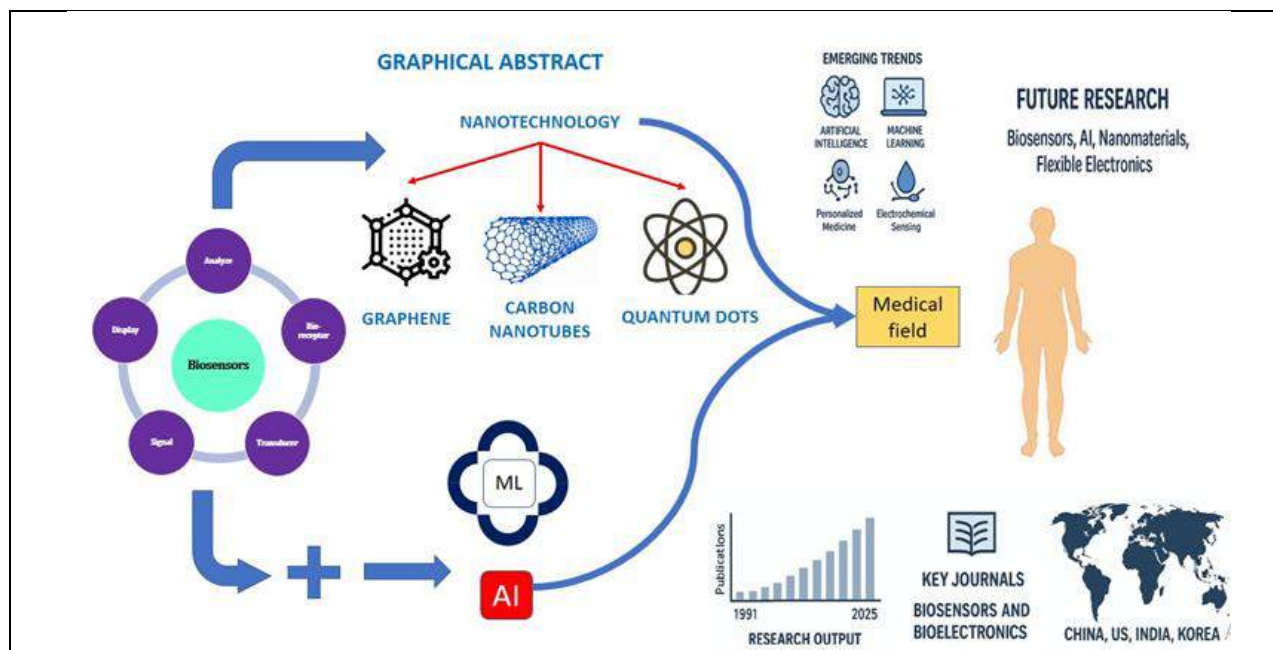


Figure 1: Biosensors in medical device

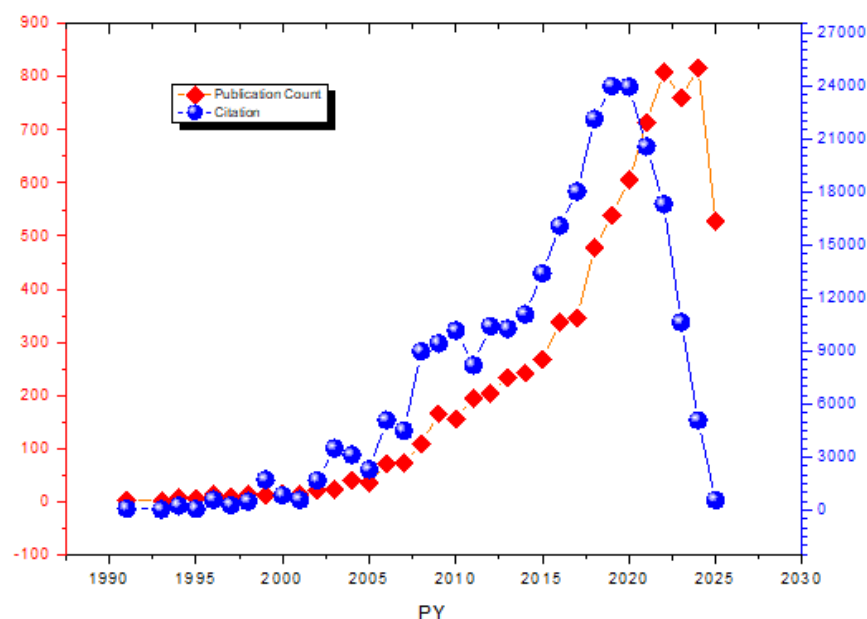


Figure 2: Publication and citation growth





Mumtaj Begum and Raja

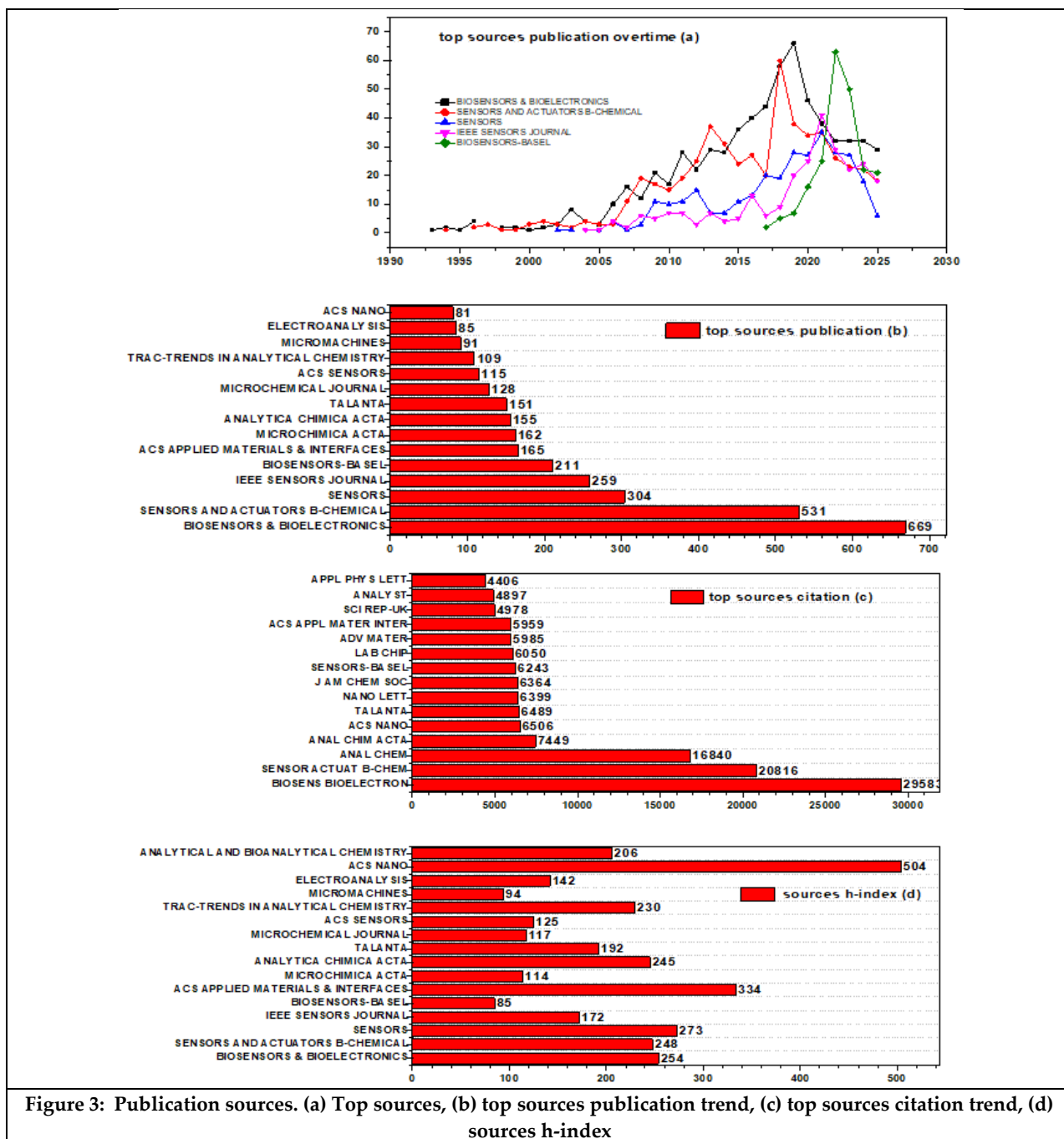


Figure 3: Publication sources. (a) Top sources, (b) top sources publication trend, (c) top sources citation trend, (d) sources h-index





Mumtaj Begum and Raja

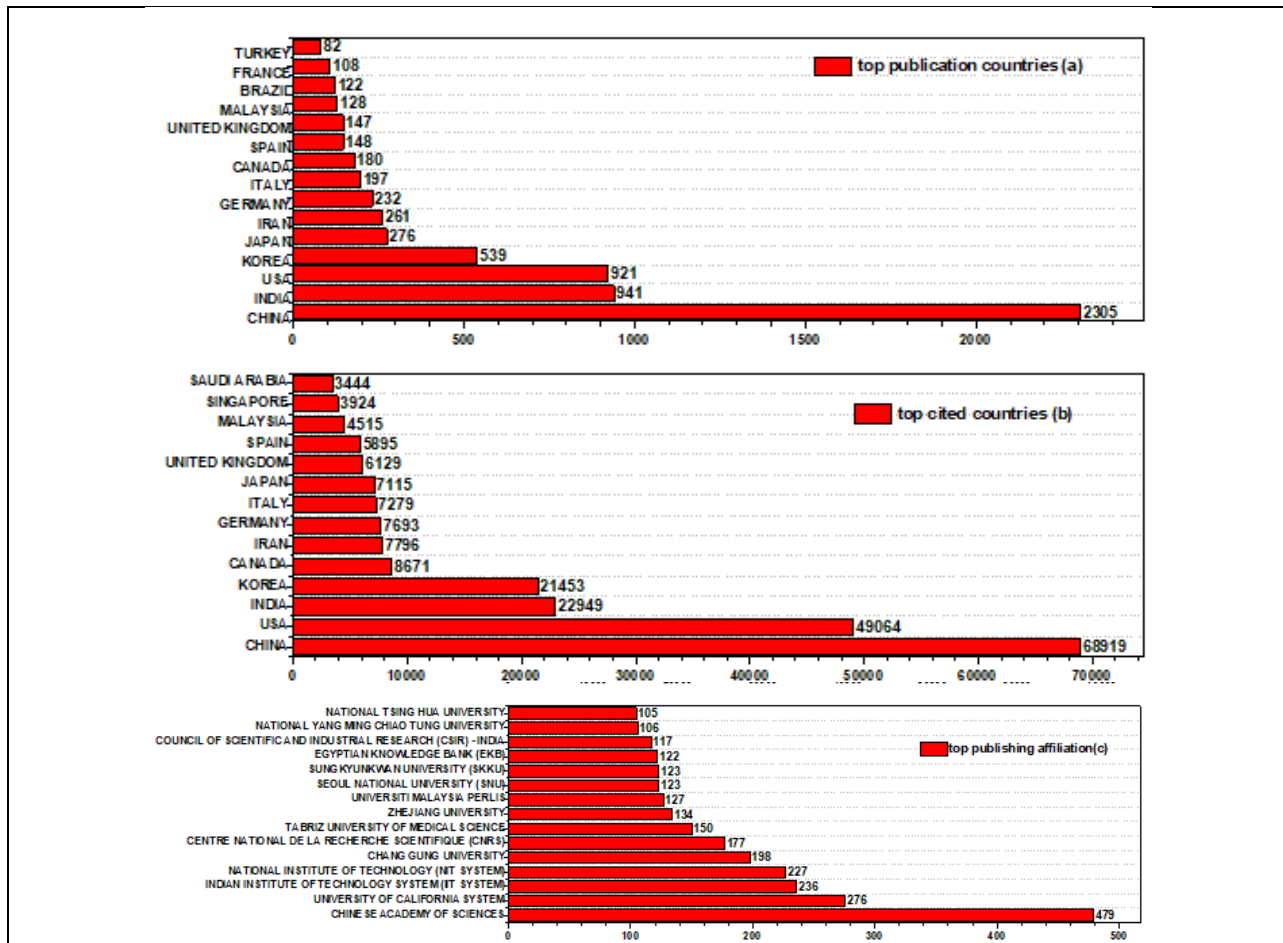


Figure 4: Country and affiliation. (a) Top published countries, (b) Top cited countries, (c) top publishing affiliations

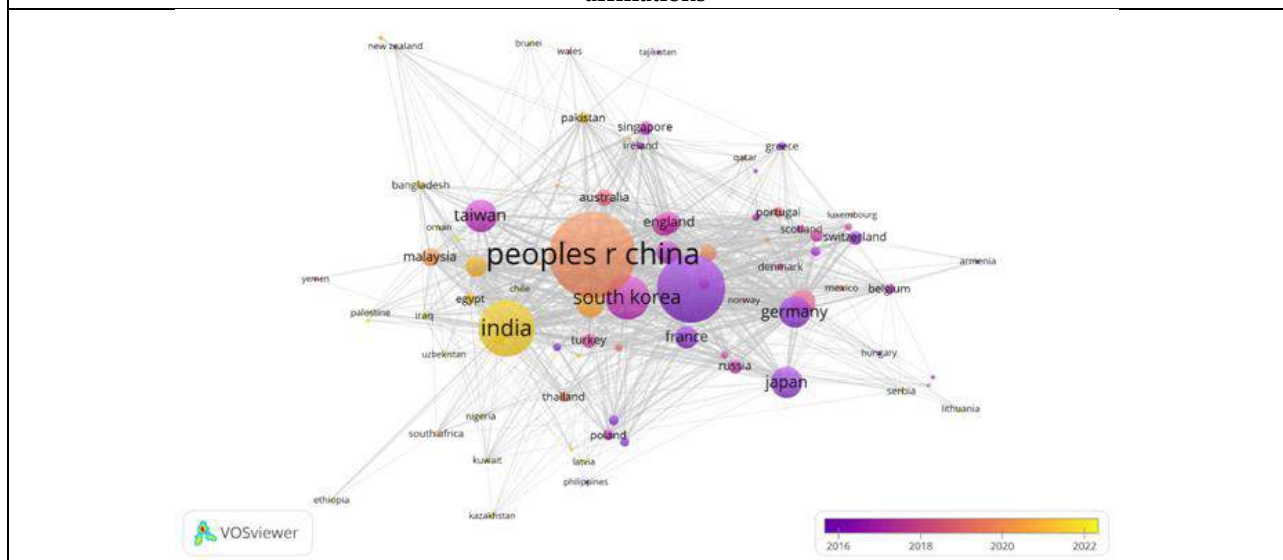


Figure 5: Country collaboration



Figure 1 consists of three horizontal bar charts, (a), (b), and (c), each showing the top authors based on a different metric. The bars are red, and the author names are listed on the left. The x-axis for each chart represents the count of the respective metric.

(a) Top authors based on publication(a)

Author	Count
WANG R	38
ZHANG H	39
LI Y	41
KIM J	41
CHEN Y	41
LI J	41
GOPINATH S	43
WANG L	45
KUMAR S	49
KIM S	50
SCHÖNING M	56
LIU Y	58
WANG J	62
WANG Y	70
ZHANG Y	95

(b) Top authors based on citation(b)

Author	Count
ZHANG H	1596
CHOI M	1602
WILLNER L	1614
WANG Y	1642
LEE G	1689
LEE J	1761
LEE C	1808
KIM S J	1812
KATZ S	1831
ZHANG G	1875
FAN C H	2217
KUMAR S	2301
WANG J	2323
LI J	2414
ZHANG Y	3211

(c) Top authors based on h-index(c)

Author	Count
WANG C	18
ZHANG H	18
CHEN Y	19
WANG R	19
RAHM AN MM	19
ZHANG G J	20
LEE J	21
LIU Y	22
KIM S	22
LI J	22
SCHÖNING M J	23
WANG Y	23
KUMAR S	23
WANG J	23
ZHANG Y	31





Mumtaj Begum and Raja

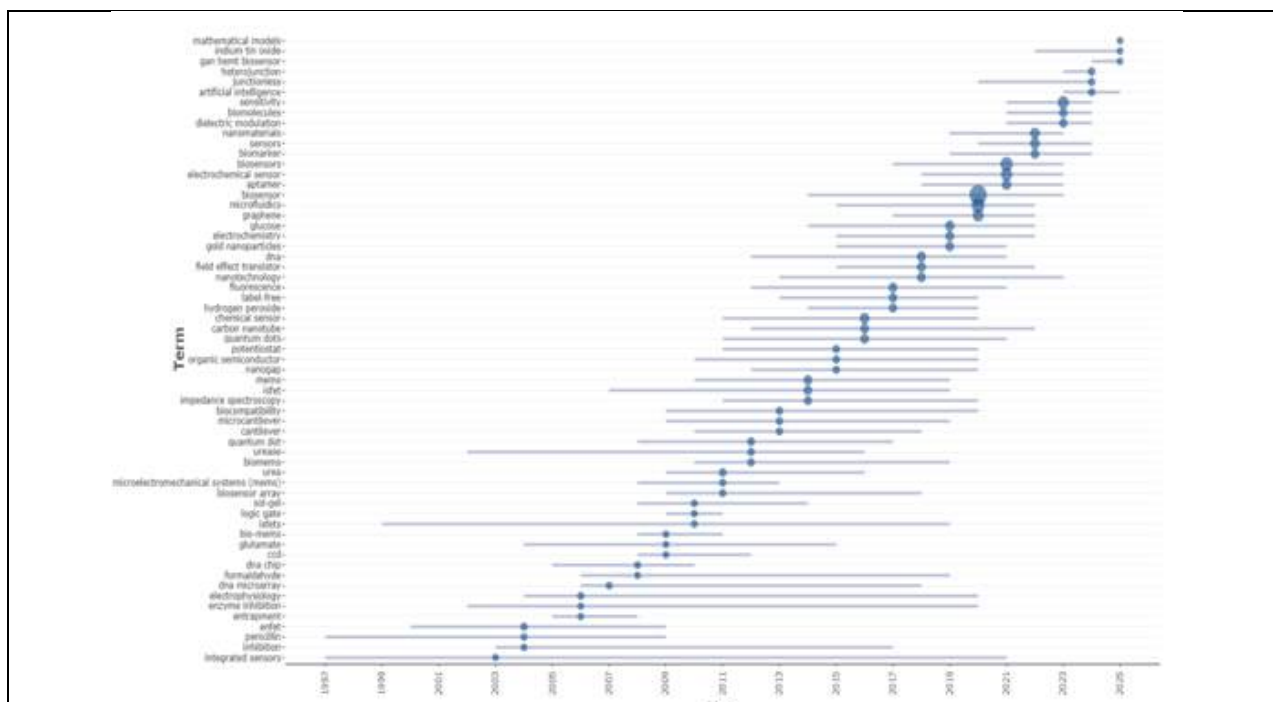


Figure 8: keyword growth

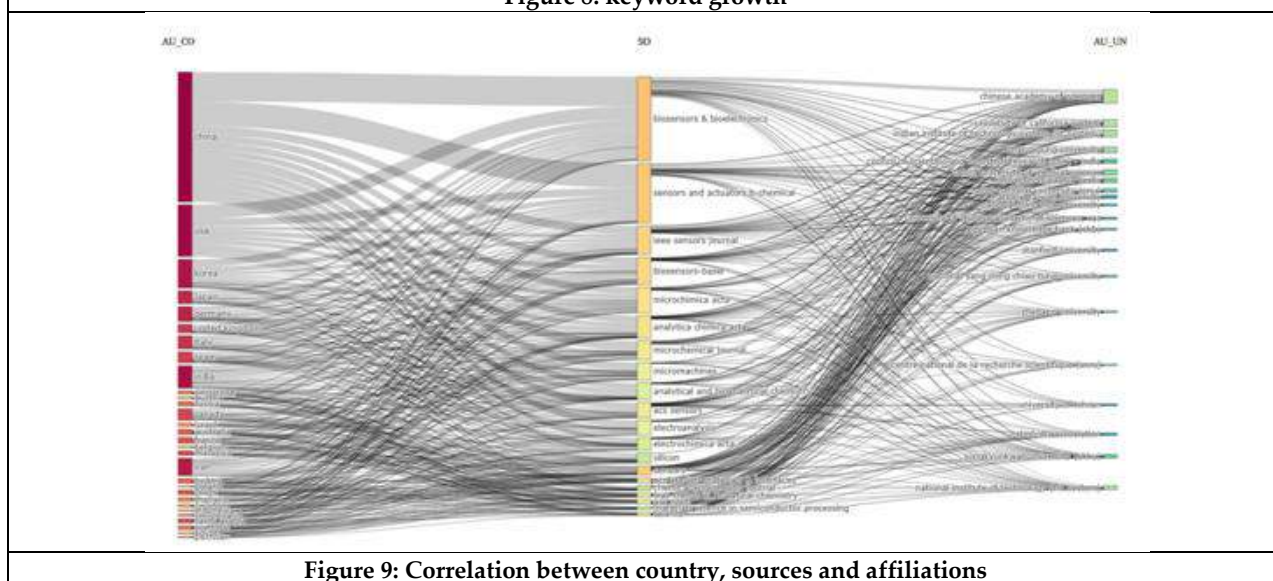


Figure 9: Correlation between country, sources and affiliations

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RESEARCH ARTICLE

Formulation and Evaluation of Herbal Syrup of *Ficus racemosa* for the Management of Transient Acantholytic Dermatitis

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ABSTRACT

Transient acantholytic dermatosis, commonly known as Grover's disease, is a pruritic skin disorder characterized by focal acantholysis and erythematous papular eruptions, primarily affecting the trunk. Conventional therapies such as topical corticosteroids and antihistamines provide symptomatic relief but are often associated with recurrence and potential adverse effects upon prolonged use. Therefore, there is a growing need for safer and more effective alternative therapeutic approaches. The present study focuses on the formulation and evaluation of a herbal syrup containing the natural root exudate (Udumbarajal) of *Ficus racemosa* for the supportive management of transient acantholytic dermatosis. The root exudate is a naturally occurring aqueous secretion obtained directly from the roots without solvent extraction and is traditionally used in Ayurvedic medicine for the treatment of inflammatory and skin disorders. It contains various bioactive constituents such as flavonoids, tannins, and phenolic compounds, which possess anti-inflammatory, antioxidant, and wound-healing properties. The herbal syrup was formulated using the root exudate along with suitable excipients including sweetening agents (honey/sorbitol), glycerine as a humectant, citric acid as a stabilizer, sodium benzoate as a preservative, and purified water as the vehicle. The prepared formulation was evaluated for organoleptic properties, physicochemical parameters such as pH, viscosity, and density, as well as stability and microbial safety. The formulation exhibited acceptable organoleptic characteristics with good palatability and stability. The physicochemical parameters were found to be within acceptable limits for oral administration, and microbial evaluation confirmed the safety of the formulation. The presence of bioactive phytoconstituents suggests its potential role in reducing inflammation and promoting skin healing. In conclusion, the





Shreya Sarode et al.,

developed herbal syrup containing natural root exudate of *Ficus racemosa* shows promising potential as a safe and effective supportive therapy for transient acantholytic dermatosis. However, further clinical studies are required to validate its therapeutic efficacy and establish its role in dermatological treatment.

Keywords: *Ficus racemosa* Root exudate, Udumbarajal, Transient acantholytic dermatosis, Grover's disease, Herbal syrup, Formulation and evaluation, Anti-inflammatory, Phytochemicals, Dermatological therapy.

INTRODUCTION

Transient acantholytic dermatosis, commonly known as Grover's disease, is an acquired pruritic dermatological disorder characterized by focal acantholysis and dyskeratosis within the epidermis. The condition was first described by Ralph W. Grover in 1970 and predominantly affects middle-aged to elderly individuals, with a higher incidence reported in males. Clinically, it presents as erythematous papules or papulovesicular eruptions, mainly distributed over the chest and back, and is often associated with intense itching that significantly impacts the patient's quality of life (Grover, 1970; Parsons, 1996). The exact etiology of transient acantholytic dermatosis remains unclear; however, several predisposing and triggering factors have been identified. These include excessive heat, sweating, prolonged bed rest, xerosis, ultraviolet radiation, and certain medications. Histopathological examination reveals focal acantholysis resembling patterns observed in other dermatological conditions such as pemphigus vulgaris and Darier's disease. Although the disease is generally benign and self-limiting, its recurrent nature and persistent pruritus necessitate effective management strategies (Weedon, 2010; James et al., 2016).

Current treatment approaches primarily focus on symptomatic relief and include the use of topical corticosteroids, oral antihistamines, retinoids, and phototherapy. While these therapies may alleviate symptoms temporarily, they are often associated with limitations such as recurrence, adverse effects on prolonged use, and variability in patient response. Long-term use of corticosteroids, for instance, may lead to skin atrophy, irritation, and systemic side effects. These limitations highlight the need for safer, more effective, and sustainable alternative therapies (Habif, 2015). In recent years, there has been increasing interest in herbal medicines due to their favorable safety profile, cost-effectiveness, and long history of traditional use. Medicinal plants are rich sources of bioactive compounds that exhibit diverse pharmacological activities. Among these, *Ficus racemosa* commonly known as cluster fig or Udumbara, is a well-known medicinal plant belonging to the Moraceae family. It is widely distributed across India and has been extensively used in traditional systems of medicine such as Ayurveda for the treatment of various ailments, including inflammatory conditions, wounds, and skin disorders (Kirtikar & Basu, 2006).

A unique feature of *Ficus racemosa* is the natural aqueous fluid that exudes from its roots, commonly referred to as Udumbarajal. This root exudate is traditionally collected and used directly solvent extraction, preserving its natural composition of water-soluble phytoconstituents. Unlike conventional extraction processes, the use of root exudate ensures minimal processing and may enhance the bioavailability of active compounds. Traditional knowledge suggests its effectiveness in managing skin-related conditions and inflammatory disorders, making it a promising candidate for further scientific investigation (Khandelwal, 2008; Harborne, 1998). Phytochemical studies have revealed that *Ficus racemosa* contains a wide range of bioactive constituents such as flavonoids, tannins, phenolic compounds, glycosides, and triterpenoids. These compounds are known to possess significant anti-inflammatory, antioxidant, antimicrobial, and wound-healing properties. The antioxidant activity helps in scavenging free radicals, thereby reducing oxidative stress, while the anti-inflammatory properties contribute to the reduction of skin inflammation and irritation. Additionally, antimicrobial activity helps prevent secondary infections, which are common in dermatological conditions (Joseph & Raj, 2011; Sharma et al., 2012). The pharmacological properties of *Ficus racemosa* are particularly relevant in the management of transient acantholytic dermatosis, where inflammation, oxidative stress, and disruption of epidermal integrity play a key role in disease pathogenesis. By targeting these



**Shreya Sarode et al.,**

underlying mechanisms, the plant may provide a holistic approach to managing the condition. Moreover, its traditional usage further supports its safety and therapeutic potential.

Oral herbal formulations such as syrups offer several advantages, including ease of administration, improved patient compliance, and better palatability. Syrups provide a stable medium for delivering aqueous herbal preparations and allow uniform distribution of active constituents. The use of natural sweetening agents such as honey not only enhances taste but also contributes additional antimicrobial and soothing effects. Furthermore, excipients such as glycerin improve viscosity and mouthfeel, while preservatives ensure microbial stability of the formulation (Allen, 2013; Aulton, 2018). Despite the extensive pharmacological potential and traditional use of *Ficus racemosa* there is a lack of scientific research focusing on the formulation of its root exudate into standardized pharmaceutical dosage forms, particularly for dermatological conditions such as transient acantholytic dermatosis. Most studies have focused on extracts or isolated compounds rather than natural exudates and patient-friendly dosage forms. This represents a significant research gap.

Therefore, the present study aims to formulate and evaluate a herbal syrup containing the natural root exudate of *Ficus racemosa* for the supportive management of transient acantholytic dermatosis. By integrating traditional knowledge with modern pharmaceutical techniques, this study seeks to develop a safe, effective, and standardized herbal formulation for dermatological use.

AIM: To formulate and evaluate a herbal syrup containing the natural root exudate (Udumbarajal) of *Ficus racemosa* for the supportive management of Transient acantholytic dermatosis.

OBJECTIVES

- To collect and authenticate the plant material of *Ficus racemosa*.
- To collect the natural root exudate (Udumbarajal) from the roots of *Ficus racemosa* using an appropriate method.
- To formulate a stable and palatable herbal syrup using the collected root exudate and suitable excipients such as sweetening agents, humectants, stabilizers, and preservatives.
- To evaluate the organoleptic properties of the prepared formulation, including color, odor, taste, and appearance.
- To determine the physicochemical parameters of the formulation such as pH, viscosity, density, and clarity.
- To perform phytochemical screening of the root exudate-based formulation.
- To conduct stability studies under different storage conditions to assess the shelf-life of the formulation.
- To carry out microbial limit tests to ensure the safety and quality of the formulation.
- To assess the potential therapeutic relevance of the formulation based on its anti-inflammatory and antioxidant properties.

MATERIALS AND METHODS

Collection and Authentication of Plant Material

Fresh roots of *Ficus racemosa* were selected from healthy, mature plants growing in a natural habitat. The plant was identified and authenticated by a qualified botanist based on standard morphological characteristics. A voucher specimen was prepared and preserved in the herbarium for future reference. The roots were carefully exposed by removing surrounding soil, ensuring minimal damage to the plant system. Proper authentication is essential to ensure reproducibility and validity of the research (Kokate et al., 2010; Evans, 2009).

Collection of Root Exudate (Udumbarajal):

The study utilized the natural aqueous root exudate (Udumbarajal) instead of solvent-based extraction. The exposed roots were gently cleaned, and a small incision was made on the surface using a sterile blade. The naturally oozing fluid was collected directly into sterile containers without applying external pressure to maintain its natural





Shreya Sarode et al.,

composition. The collected exudate was immediately filtered through muslin cloth to remove coarse impurities, followed by filtration using Whatman filter paper for clarification. The filtrate was stored in sterile, amber-colored containers at refrigerated temperature (2–8°C) to prevent microbial contamination and degradation of bioactive constituents. This method preserves the natural aqueous phytoconstituents present in the plant (Khandelwal, 2008; Harborne, 1998).

Materials Used in Formulation

The formulation included the collected root exudate of *Ficus racemosa* as the active ingredient. Excipients used were honey or sorbitol as sweetening agents, glycerin as a humectant and viscosity enhancer, citric acid as a pH adjuster and stabilizer, sodium benzoate as a preservative, and purified water as the vehicle. All chemicals and reagents used were of analytical grade and complied with pharmacopeial standards (Indian Pharmacopoeia, 2022).

Formulation of Herbal Syrup

The herbal syrup was prepared using standard pharmaceuticals techniques. Initially, the required quantity of sweetening agent (honey/sorbitol) was dissolved in a portion of purified water with gentle heating. Glycerin was added to improve viscosity and mouthfeel. Citric acid was incorporated to maintain an appropriate pH and enhance flavor stability. Sodium benzoate was dissolved separately and added as a preservative. The filtered root exudate of *Ficus racemosa* was then slowly added to the syrup base with continuous stirring to ensure uniform mixing. The final volume was adjusted to 100 ml using purified water. The prepared syrup was filtered, if necessary, and transferred into clean, dry, amber-colored bottles, which were properly labeled and stored at room temperature (Allen, 2013; Aulton, 2018).

Evaluation of Organoleptic Properties

The prepared formulation was evaluated for organoleptic characteristics including color, odor, taste, and overall appearance. These parameters were assessed visually and by sensory evaluation. The formulation was considered acceptable if it exhibited a pleasant taste, characteristic odor, and uniform appearance without turbidity or precipitation (Lachman et al., 2013).

Physicochemical Evaluation: The formulated syrup was subjected to physicochemical analysis to ensure quality and consistency. The pH was determined using a calibrated digital pH meter and maintained within an acceptable range (4–7) suitable for oral administration. Viscosity was measured using a Brookfield viscometer to evaluate flow properties. Density and specific gravity were determined using a pycnometer. Clarity, homogeneity, and absence of particulate matter were also assessed visually. These parameters are essential for ensuring stability and uniform dosing (Indian Pharmacopoeia, 2022; Aulton, 2018).

Phytochemical Screening

Preliminary phytochemical screening of the root exudate-based formulation was carried out to identify the presence of major bioactive constituents such as flavonoids, tannins, phenolic compounds, glycosides, and alkaloids using standard qualitative tests. These phytoconstituents are responsible for the therapeutic activity of the formulation (Harborne, 1998).

Stability Studies

Stability studies were conducted according to ICH guidelines to evaluate the effect of environmental conditions on the formulation. The syrup was stored under different conditions, including room temperature and accelerated conditions (40°C ± 2°C and 75% relative humidity). Samples were analyzed at regular intervals for changes in color, odor, pH, viscosity, and overall appearance. Any signs of instability such as precipitation or phase separation were recorded (ICH Q1A(R2), 2003).



Shreya Sarode *et al.*,**Microbial Limit Test**

Microbial evaluation was performed using standard plate count methods to determine the total viable microbial load. The formulation was tested for the presence of bacteria and fungi to ensure safety. The effectiveness of sodium benzoate as a preservative was confirmed by maintaining microbial counts within acceptable pharmacopeial limits. The absence of pathogenic microorganisms indicated the microbiological safety of the formulation (Indian Pharmacopoeia, 2022).

Table 1. Plant Profile

Table 2. Formulation

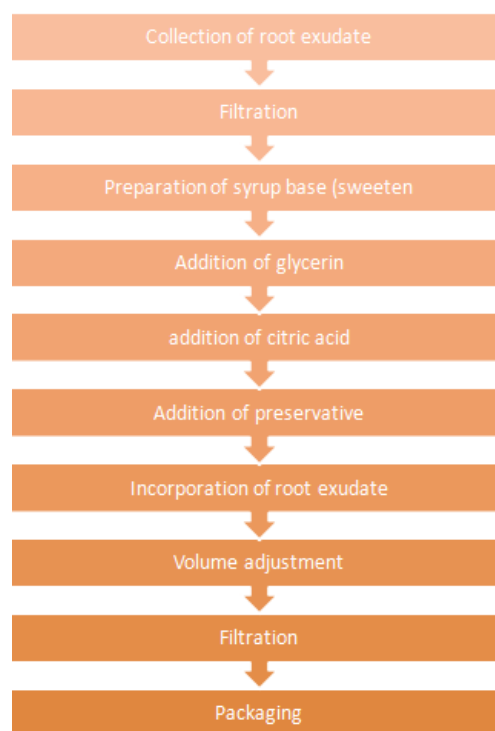
Flowchart of Formulation Process:

Table 3. Phytochemical screening of herbal syrup:

Fig.2 Phytochemical screening of herbal syrup

RESULTS

Table 4. Organoleptic Evaluation

Table 5. Physicochemical Evaluation

Table 6. Phytochemical Screening Results

Table 7. Stability Study





Shreya Sarode et al.,

DISCUSSION

The present study was undertaken to formulate and evaluate a herbal syrup containing the natural root exudate (Udumbarajal) of *Ficus racemosa* for the supportive management of Transient acantholytic dermatosis. The discussion integrates the findings obtained from formulation development, physicochemical evaluation, phytochemical screening, stability studies, and microbial analysis to justify the suitability and potential efficacy of the developed formulation. The use of natural root exudate instead of conventional extracts represents a novel approach in herbal formulation. Unlike solvent-based extraction, the exudate is a naturally occurring aqueous fluid that contains water-soluble phytoconstituents in their native form. This minimizes processing steps and may help preserve the biological activity and bioavailability of active compounds. From a pharmaceutical perspective, this approach aligns well with traditional Ayurvedic practices while maintaining scientific validity.

The organoleptic evaluation of the formulation revealed a brownish color, pleasant odor, and sweet taste with a mild herbal aftertaste. These characteristics are important for oral dosage forms, as patient compliance is strongly influenced by taste and appearance. The use of honey or sorbitol as a sweetening agent not only improved palatability but may also contribute additional antimicrobial and soothing properties, thereby enhancing the therapeutic value of the formulation. The parameters physicochemical obtained in the study were found to be within acceptable limits for oral syrups. The pH of the formulation (around 5.2) is suitable for oral administration and ensures stability of the active constituents. The viscosity was adequate to provide a smooth flow and ease of dosing without being too thick or too thin. Density and specific gravity values indicated uniformity in formulation, while clarity confirmed the absence of particulate matter or instability. These results demonstrate that the formulation meets standard pharmaceuticals requirements and is suitable for patient use.

The phytochemical screening confirmed the presence of key bioactive constituents such as flavonoids, tannins, phenolic compounds, glycosides, and terpenoids. These compounds are well known for their anti-inflammatory, antioxidant, antimicrobial, and wound-healing properties. Flavonoids and phenolic compounds play a crucial role in scavenging free radicals and reducing oxidative stress, which is a contributing factor in many skin disorders. Tannins provide astringent and antimicrobial effects, while terpenoids and glycosides contribute to anti-inflammatory and tissue repair mechanisms. The presence of these constituents supports the therapeutic potential of the formulation in managing inflammatory skin conditions. The stability studies indicated that the formulation remained stable under both room temperature and accelerated conditions, with no significant changes in pH, color, or appearance. A slight variation in viscosity under accelerated conditions was observed but remained within acceptable limits. The absence of precipitation or phase separation suggests good physical stability of the formulation. These findings confirm that the developed syrup has an acceptable shelf-life and can maintain its quality during storage.

The microbial evaluation demonstrated that the formulation complies with pharmacopeial limits for microbial load. The absence of pathogenic microorganisms and acceptable total viable count indicate that the formulation is microbiologically safe. The inclusion of sodium benzoate as a preservative played a significant role in preventing microbial growth and ensuring product safety. From a therapeutic perspective, the pharmacological activities of *Ficus racemosa* are highly relevant to the management of transient acantholytic dermatosis. The condition involves inflammation, disruption of epidermal integrity, and possible secondary infections. The anti-inflammatory properties of the plant help reduce skin irritation and itching, while antioxidant activity minimizes oxidative damage to skin cells. Additionally, antimicrobial properties help prevent secondary infections, which can worsen the condition. Although the formulation is intended as a supportive therapy, its multi-target mechanism of action makes it a promising alternative to conventional treatments.

Despite these positive findings, certain limitations should be acknowledged. The study is primarily based on formulation and in-vitro evaluation, and does not include clinical trials to directly assess therapeutic efficacy in





Shreya Sarode et al.,

patients. Therefore, further studies, including in-vivo and clinical evaluations, are necessary to validate the effectiveness of the formulation in real-world conditions.

CONCLUSION

The present study successfully focused on the formulation and evaluation of a herbal syrup containing the natural root exudate (Udumbarajal) of *Ficus racemosa* for the supportive management of Transient acantholytic dermatosis. The use of root exudate instead of conventional extracts represents a novel and scientifically relevant approach, as it preserves the natural composition of water-soluble phytoconstituents while aligning with traditional medicinal practices. The developed formulation exhibited acceptable organoleptic properties, including pleasant taste, characteristic odor, and uniform appearance, which are essential for patient compliance. The physicochemical evaluation confirmed that parameters such as pH, viscosity, density, and clarity were within acceptable limits, indicating that the formulation is suitable for oral administration and meets standard pharmaceutical requirements. Phytochemical screening revealed the presence of important bioactive compounds such as flavonoids, tannins, phenolic compounds, and terpenoids. These constituents are known for their anti-inflammatory, antioxidant, antimicrobial, and wound-healing activities, which are highly relevant in managing inflammatory skin conditions. The stability studies demonstrated that the formulation remained stable under different storage conditions, with no significant changes in physical or chemical properties. Additionally, microbial evaluation confirmed that the formulation is safe and free from harmful microorganisms.

Overall, the results indicate that the developed herbal syrup is stable, safe, and pharmaceutically acceptable, with promising therapeutic potential. Although the study provides strong preliminary evidence supporting its use, further *in-vivo* and clinical studies are necessary to establish its efficacy and confirm its role as an alternative or adjunct therapy in dermatological treatment.

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Shreya Sarode et al.,

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Table 1. Plant Profile

Parameter	Details
Botanical Name	<i>Ficus racemosa</i>
Family	Moraceae
Common Name	Cluster fig, Indian fig tree
Vernacular Names	Hindi: Gular; Marathi: Umbar; Sanskrit: Udumbara
Plant Type	Medium to large deciduous tree
Height	Approximately 15–20 meters
Geographical Distribution	Widely distributed in India, Sri Lanka, Nepal, and Southeast Asia; commonly found in tropical and subtropical regions
Habitat	Forest areas, riverbanks, rural and moist regions
Morphology	Bark: smooth, grayish-brown; Leaves: simple, ovate; Fruits: clustered figs on trunk; Roots: well-developed
Parts Used	Bark, fruits, leaves, roots (especially root exudate)
Special Feature	Produces natural aqueous root exudate (Udumbarajal)
Phytochemical Constituents	Flavonoids, tannins, phenolic compounds, glycosides, triterpenoids
Pharmacological Activities	Anti-inflammatory, antioxidant, antimicrobial, wound healing, hepatoprotective
Traditional Uses	Used in Ayurveda for skin diseases, wound healing, diarrhea, inflammation

Table 2. Formulation

Sr. No.	Ingredients	Quantity (for 100 ml)	Function
1	Root exudate (<i>Ficus racemosa</i>)	20 ml	Active ingredient
2	Sorbitol	66.7 g	Sweetening agent
3	Glycerin	10 ml	Humectant, viscosity enhancer
4	Citric acid	0.5 g	pH adjuster, stabilizer
5	Sodium benzoate	0.2 g	Preservative
6	Beet root	q. S	Coloring Agent
7	Peppermint oil	q.s.	Flavoring agent
7	Purified water	q.s. to 100 ml	Vehicle





Shreya Sarode et al.,

Table 3. Phytochemical screening of herbal syrup:

Sr. No.	Phytochemical Test	Test Performed	Result	Inference
1	Alkaloids	Mayer's / Dragendorff's test	+ (Present)	Indicates presence of nitrogenous compounds with potential pharmacological activity
2	Flavonoids	Shinoda test	+ (Present)	Confirms antioxidant and anti-inflammatory properties
3	Tannins	Ferric chloride test	+ (Present)	Suggests astringent and antimicrobial activity
4	Phenolic compounds	Ferric chloride test	+ (Present)	Indicates strong antioxidant potential
5	Glycosides	Keller–Killiani test	+ (Present)	Suggests possible cardioprotective and therapeutic effects
6	Saponins	Foam test	+ (Present)	Indicates surfactant and immune-modulating properties
7	Terpenoids	Salkowski test	+ (Present)	Confirms anti-inflammatory and wound-healing activity
8	Steroids	Liebermann–Burchard test	+ (Present)	Indicates presence of phytosterols with anti-inflammatory effects
9	Proteins	Biuret test	– (Absent)	No significant protein content detected
10	Carbohydrates	Molisch's test	+ (Present)	Confirms presence of sugars contributing to energy and formulation stability

Table 4. Organoleptic Evaluation

Parameter	Observation	Parameter
Color	Brownish	Color
Odor	Characteristic, pleasant	Odor
Taste	Sweet with mild herbal aftertaste	Taste
Appearance	Clear and free from particulate matter	Appearance

Table 5. Physicochemical Evaluation

Parameter	Result	Standard Range
pH	5.2 ± 0.1	4 – 7
Viscosity	1200 ± 50 cps	Suitable for syrup
Density	1.15 g/ml	~1 – 1.3 g/ml
Specific Gravity	1.12	Close to standard
Clarity	Clear	Clear

Table 6. Phytochemical Screening Results

Constituent	Result
Flavonoids	Present (+)
Tannins	Present (+)
Phenolic compounds	Present (+)
Glycosides	Present (+)
Saponins	Present (+)
Terpenoids	Present (+)
Steroids	Present (+)

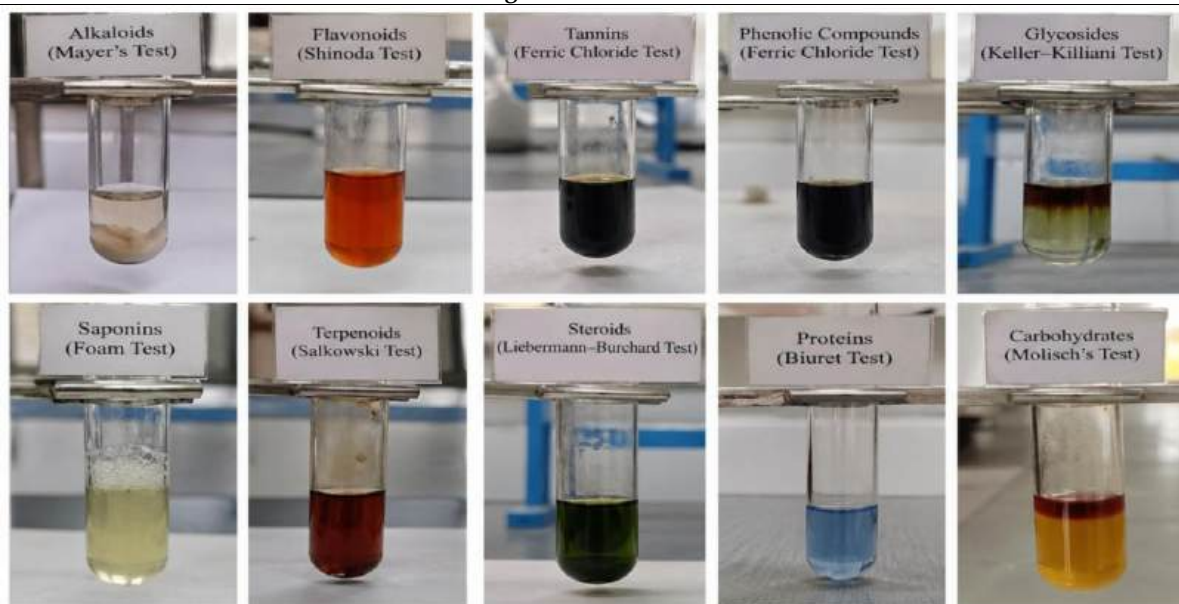


Shreya Sarode *et al.*,

Proteins	Absent (–)
Carbohydrates	Present (+)

Table 7. Stability Study

Condition	Duration	Observation
Room temperature	30 days	No significant change
Accelerated (40°C ± 2°C)	30 days	Slight change in viscosity, acceptable
pH variation	–	No significant change
Appearance	–	No precipitation or color change

**Fig.1. *Ficus racemosa*****Fig. 2 Phytochemical screening of herbal syrup**

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RESEARCH ARTICLE

Formulation and Quality Evaluation of Gluten - Free Bamboo Rice Brownie

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ABSTRACT

Bamboo rice is a nutrient-rich grain traditionally consumed by indigenous communities and has emerging potential in gluten-free food applications. This study investigated the formulation and quality evaluation of gluten-free brownies prepared using bamboo rice flour as a complete replacement for wheat flour. The developed product was compared with a conventional brownie for sensory attributes, nutritional composition, functional properties, antioxidant activity, gluten content, microbial safety, and shelf stability. Sensory analysis indicated significantly higher overall acceptability of bamboo rice brownies, with favourable scores for taste, texture, and appearance. Nutritional assessment demonstrated increased protein, dietary fibre, and mineral content, along with reduced fat and gluten levels. Improved water absorption and moisture retention contributed to enhanced textural quality. Microbiological analysis confirmed product safety throughout the storage period. The results demonstrate that bamboo rice flour is a viable ingredient for developing nutritionally superior gluten-free brownies, offering a functional and sustainable alternative for health-conscious consumers and individuals with gluten intolerance.

Keywords: Bamboo rice, Gluten-free brownie, Sensory evaluation, Nutritional quality, Antioxidant activity, Shelf-life stability, Phytochemical content, Celiac-friendly foods, Indigenous resources.





INTRODUCTION

The prevalence of celiac disease, estimated at approximately 1% of the global population, necessitates strict lifelong adherence to gluten-free diets to prevent intestinal mucosal damage and associated nutritional deficiencies. However, conventional gluten-free bakery products often exhibit inferior nutritional quality and compromised sensory attributes when compared to their wheat-based counterparts. Simultaneously, India's organized bakery sector, with an annual turnover exceeding ₹3,000 crores, is witnessing growing consumer demand for functional and health-oriented foods aimed at managing diet-related non-communicable diseases such as obesity and diabetes mellitus. Bamboo rice (*Bambusa arundinacea*), the edible seed obtained during the gregarious flowering cycle of bamboo occurring once every 20–50 years, has gained attention as a nutritionally superior and sustainable indigenous cereal alternative. Bamboo rice exhibits a favourable proximate composition, with high protein (22.45 g/100 g), dietary fibre (13.36 g/100 g), appreciable levels of calcium (24.84 mg/100 g) and phosphorus (96.91 mg/100 g), a low glycaemic index, and significant antioxidant activity. Utilization of bamboo rice not only offers potential for gluten-free food product development but also contributes to sustainable resource use and livelihood support for tribal communities.

Brownies, a popular bakery product, are traditionally formulated using refined wheat flour and are characterized by high fat and sugar content, which may contribute to metabolic disorders when consumed frequently. Reformulating brownies using nutrient-dense, gluten-free ingredients such as bamboo rice flour may enhance their nutritional profile while catering to consumers with gluten intolerance and those seeking healthier bakery alternatives. In this context, the present investigation was undertaken to develop gluten-free brownies through complete substitution of wheat flour with bamboo rice flour and to evaluate their suitability as a functional bakery product. The study focused on assessing the sensory acceptability using a nine-point hedonic scale, along with detailed evaluation of physical characteristics, proximate composition, phytochemical profile, gluten content, microbiological quality, and accelerated shelf-life under controlled conditions, with statistical significance determined at $p < 0.05$.

MATERIALS AND METHODOLOGY

Materials

Bamboo rice (*Bambusa arundinacea*) procured from verified tribal sources (Kerala); processed into flour (cleaned, sun-dried, dehusked, milled 100 mesh). Other ingredients from Chennai markets: wheat flour (control), butter (75g), dark chocolate (220g), castor sugar (100g), palm sugar (80g), cocoa powder (30g), corn flour (15g), curd (25g), baking powder (5g), baking soda (3g), coffee powder (10g).

Electronic balance, mixer, oven (170°C), vernier caliper, colorimeter, Soxhlet apparatus.

Equipment: Electronic balance, mixer, oven (170°C), vernier caliper, colorimeter, Soxhlet apparatus.

Methodology

Preparation of Brownies:

- Butter (75 g) and dark chocolate (220 g) were melted in a double boiler.
- Dry ingredients were sifted: flour (170 g; wheat flour for control, bamboo rice flour for treatment), cocoa powder (30 g), corn flour (15 g), baking powder (5 g), baking soda (3 g), instant coffee powder (10 g).
- Sugars (castor sugar 100 g, palm sugar 80 g) were creamed with curd (25 g), then folded into chocolate mixture.
- Sifted dry ingredients were gradually incorporated to form batter. Batter was transferred to parchment-lined baking tray.
- Baked in preheated oven at 170°C for 20-25 minutes (toothpick test: clean center). Brownies cooled 10-15 minutes in tray.





Analysis

Organoleptic evaluation: Nine-point hedonic scale (n=30 semi-trained panelists); parameters: appearance, color, taste, texture, flavor, overall acceptability. One-way analysis of variance (ANOVA).

Proximate composition: Energy (factorial calculation); protein (Lowry method); carbohydrates (Anthrone method); fat, crude fibre (Soxhlet extraction); ash (muffle furnace); moisture (gravimetric); calcium (EDTA complexometric titration); phosphorus (vanadomolybdate spectrophotometry) - all AOAC methods.

Phytochemical screening: Qualitative tests for flavonoids, alkaloids, tannins, terpenoids, saponins, steroids; total phenolics (Folin-Ciocalteu); total flavonoids (aluminium chloride); DPPH radical scavenging activity.

Gluten quantification: Enzyme-linked immunosorbent assay (ELISA; threshold <20 ppm).

Microbiological analysis: Total bacterial count, total yeast and mould count (pour plate method; CFU/g).

Shelf-life study: Accelerated shelf-life testing (40°C, 75% RH, 15 days); water activity (A_w), microbial enumeration.

Statistical analysis: SPSS version 25.0; triplicate determinations presented as mean $\pm$ standard deviation (SD). One-way ANOVA.

RESULTS AND DISCUSSION

The present investigation examined the feasibility of producing a gluten-free brownie using bamboo rice flour as a total replacement for wheat flour and evaluated its quality attributes in comparison with a conventional formulation. The assessment included sensory performance, physical and functional characteristics, nutritional quality, antioxidant properties, gluten content, microbial safety, storage stability, and economic considerations. All analyses were conducted under controlled conditions, and statistical significance was determined at $p < 0.05$.

Organoleptic Evaluation

Sensory analysis indicated that substitution of wheat flour with bamboo rice flour significantly influenced most sensory parameters of the brownie. One-way analysis of variance (Table 3) revealed significant differences ($p < 0.05$) between the control and bamboo rice brownies with respect to appearance, taste, texture, flavour, and overall acceptability, whereas colour did not show a statistically significant variation. The favourable sensory response observed for the bamboo rice brownie may be attributed to its compact crumb structure, improved mouth feel, and characteristic flavour imparted by bamboo rice. The absence of a significant difference in colour suggests that the darker shade associated with bamboo rice flour did not adversely affect consumer perception. These findings demonstrate that bamboo rice flour can be successfully incorporated into brownie formulations without compromising sensory quality.

Nutritional Profile

The replacement of wheat flour with bamboo rice flour resulted in a substantial enhancement of the nutritional profile of the brownie (Table 4). The bamboo rice brownie demonstrated increased protein and dietary fibre contents along with improved mineral composition, notably calcium and phosphorus. Additionally, the reduction in fat and moisture content contributes to a more balanced macronutrient profile and improved shelf stability. These nutritional improvements highlight the suitability of bamboo rice flour as a value-added ingredient for the development of gluten-free bakery products with enhanced health attributes.

Antioxidant Capacity and Bioactive Components

The bamboo rice brownie exhibited superior antioxidant potential compared to the control, reflected by higher levels of phenolic and flavonoid compounds and increased radical scavenging activity. The presence of these bioactive constituents suggests that the product may offer protective health benefits beyond basic nutrition, supporting its classification as a functional food.





Gluten Content and Microbial Quality

Gluten analysis confirmed that the bamboo rice brownie contained gluten levels well below the permissible limit for gluten-free foods, making it appropriate for individuals with celiac disease and gluten sensitivity. Microbiological evaluation showed no detectable bacterial, yeast, or mould growth during the storage period, indicating satisfactory microbial quality. The combined effects of reduced moisture content and naturally occurring bioactive compounds may have contributed to the observed microbial stability.

Economic Evaluation

Although the production cost of the bamboo rice brownie was marginally higher than that of the conventional wheat-based brownie, the enhanced nutritional quality, gluten-free status, and functional attributes justify the additional cost. This supports the potential feasibility of bamboo rice brownies for specialised and health-oriented consumer markets.

CONCLUSION

The present investigation confirms that bamboo rice flour can be effectively employed as a complete substitute for wheat flour in the formulation of gluten-free brownies with enhanced quality attributes. The developed product demonstrated superior sensory performance, particularly in terms of taste, texture, and overall acceptability, indicating strong consumer appeal. Nutritional evaluation revealed marked improvements in protein and dietary fibre content, along with increased antioxidant potential and reduced gluten levels, supporting its suitability for individuals with gluten intolerance. Functional assessment further showed favourable hydration and moisture-retention properties, contributing to desirable textural characteristics. Microbiological analysis confirmed product safety and stability during storage, while shelf-life performance remained satisfactory under tested conditions. Although production costs were marginally higher, the nutritional advantages and functional benefits outweigh the economic constraints. Overall, this study highlights bamboo rice as a nutritionally valuable and sustainable cereal alternative with considerable potential in the development of functional and specialty bakery products, providing a sound foundation for future product optimisation and commercial scale-up.

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Mahalakshmi et al.,

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Table 1: Ingredient used for bamboo rice brownie preparation

Ingredient	Control (g)	Treatment (g)
Flour	Wheat 170	BRF 170
Butter	75	75
Dark Chocolate	220	220
Castor sugar	100	100
Palm sugar	80	80
Cocoa Powder	30	30
Corn Flour	15	15
Curd	25	25
Baking Powder	5	5
Baking Soda	3	3
Coffee powder	10	10





Mahalakshmi et al.,

Table 2: Analysis of variance for sensory attributes of control and bamboo rice brownies

Sensory attributes	P-value	Level of significance ($p < 0.05$)
Appearance	0.01469	S*
Colour	0.09218	NS
Taste	0.03386	S*
Texture	0.04480	S*
Flavour	0.01870	S*
Overall acceptability	0.04836	S*

S* – Significant

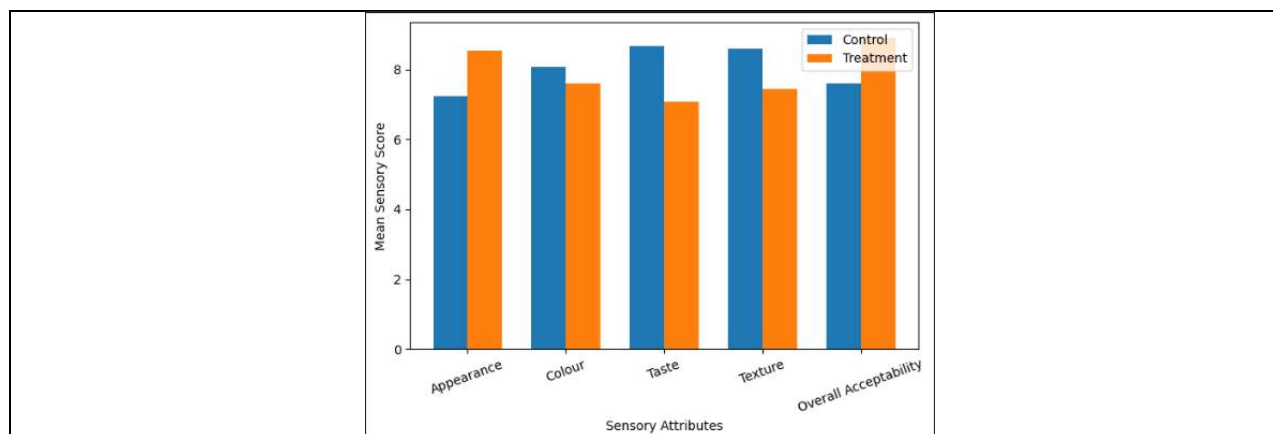
NS – Not significant

Table 3: Organoleptic evaluation of Brownie

S.NO	Appearance	Taste	Texture	Colour	Overall Acceptability
Control	007 $\pm$ 0.57	7.07 $\pm$ 0.52	7.43 $\pm$ 0.50	8.07 $\pm$ 0.58	7.60 $\pm$ 0.50
Treatment	8.53 $\pm$ 0.51	8.67 $\pm$ 0.48	8.60 $\pm$ 0.56	7.60 $\pm$ 0.62	8.90 $\pm$ 0.31

Value represents Mean $\pm$ standard deviation.**Table 4: Nutritional composition of control and Bamboo Rice Brownie**

Nutrient	Control	Bamboo Rice Brownie
Energy (kcal)	401.87	437
Protein (g/100 g)	4.03 $\pm$ 0.12	22.45 $\pm$ 0.20
Fat (g/100 g)	21.7	11.45
Fibre (g/100 g)	3.89	13.36
Moisture (%)	20.62	10.10
Calcium (mg/100 g)	9.13	24.84
Phosphorus (mg/100 g)	27.73	96.91

**Fig 1: Organoleptic Evaluation of Control and Treatment Brownie**

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RESEARCH ARTICLE

Assessment of *In vitro* Anti-Obesity Potential of Hydroalcoholic Seed Extract of *Zanthoxylum armatum*

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ABSTRACT

Obesity can be considered one of the most common health issues prevalent across the globe, being linked to various conditions, such as hypertension, diabetes mellitus, chronic kidney disease, nonalcoholic fatty liver disease, and hyperlipidemia. In recent years, medicinal plants have been increasingly researched in order to find an effective solution for treating the condition due to their positive pharmacological properties and fewer side effects. This research paper aims to explore phytochemical analysis and anti-obesity activity of hydroalcoholic seed extract of *Zanthoxylum armatum*. *Zanthoxylum armatum* seeds were harvested from Sikkim and extracted using 70% hydroethanolic solvent via maceration method. It was established that the seeds contain alkaloids, carbohydrates, flavonoids, tannins, saponins, and polyphenols. The anti-obesity activity of *Zanthoxylum armatum* seeds was estimated based on free fatty acids content in chicken blood serum, measured using titration procedure, with a standard substance Chitosan used as a comparative sample. Two doses of the extract, 250 mg/kg and 500 mg/kg, were used during the experiment. The yield of the extract obtained through maceration was found to be 5.85%. The results of the anti-obesity study revealed the FFA values for the control group, standard group, 250 mg/kg, and 500 mg/kg groups as 143, 118, 135, and 131.5 mmol/L, respectively. It can be observed from





Koushik Garai et al.,

the results that both dosages of the plant extract exhibit anti-obesity activity as they have shown decreased FFA concentration as compared to the control group. These effects can be attributed to the presence of bioactive phytochemicals like flavonoids, alkaloids, polyphenols, and saponins that regulate lipid metabolism. The above results prove that the hydro-alcoholic seed extract of the plant *Zanthoxylum armatum* exhibits anti-obesity properties and can prove to be effective in the treatment of obesity.

Keywords: Obesity, *Zanthoxylum armatum*, Anti-obesity activity, Phytochemical screening, Free fatty acids (FFA)

INTRODUCTION

Obesity and corresponding ailments have become serious health issues and currently becoming the sixth most prevalent major contributor of fatalities worldwide. The World Health Organization (WHO) defines obesity as "abnormal or excessive fat accumulation that may impair health," emphasizing that "an energy deficit between calories consumed and calories expended is the underlying cause of obesity and overweight [1]. Numerous illnesses and ailments that are associated with higher mortality rates are more likely to occur in people who are obese. These include nonalcoholic fatty liver disease (NAFLD), cardiovascular disorders (CVD), metabolic disorder (MetS), chronic renal disease (CKD), hyperlipidemia, hypertension, depression, obstructive breathing while sleeping, osteoarthritis, and some instances of malignancies [2,3]. Visceral and subcutaneous fats are the two categories of fat. Visceral fat is defined as the fat that is built up over visceral organs, including the kidney, belly, liver, intestines, heart, and pancreas. Because studies have shown that visceral fat has an important effect on our hormone activity, leading to insulin resistance and metabolic syndrome, it is also known as "active fat [4]. Hormonal activity impacts hunger, body fat distribution, and metabolism. In addition, subcutaneous fat, which is felt under the arms and legs, is the type of fat that is beneath the skin [5]. The body takes on the forms of an apple and a pear due to fat deposition in particular physiological areas [6]. The more elevated region of the waist and abdomen is where fat develops in the case of an "Android apple". Furthermore, there is some deposition on the shoulders, arms, and neck. Visceral fat, which is highly connected to the risk of type 2 diabetes, is the main reason of this body shape [7]. The thighs and buttocks of the resembling (gynoid) body are where fat is deposited. This body form has a low visceral fat content, which lowers the risk of weight-related illnesses [8].

Significant obesity clusters seen in particular geographic locations and contexts also reflect the significance of socioeconomic and environmental elements in "obesogenic" environments, since particular genetic and lifestyle factors influence an individual's risk of becoming obese as an adult.⁹ Many research used principal component analysis or factor analysis to determine food patterns [10]. For several kinds of chronic ailments, including obesity, herbs have long been shown to be a useful and necessary source. In comparison to single-compound pharmaceuticals, herbs have less adverse effects, with the exception of a few allergic responses in vulnerable people. By lowering triglyceride levels, increasing metabolic rate, inhibiting pancreatic lipase, and suppressing appetite, medicinal herbs have showed beneficial effects on obesity. subsequently, they help people lose weight [11,12]. Winged prickly ash, or *Zanthoxylum armatum*, is a species of plant belonging to the Rutaceae family. It is a 3.5-meter-tall, aromatic, spiky, deciduous shrub that is native to China, Japan, Korea, and India. The wild plant *Zanthoxylum armatum* (Timbur) is utilized primarily as a condiment and medication, while it also has other therapeutic uses [13]. Numerous chemical substances were found in the different *Zanthoxylum* species sections that were the subject of previous phytochemical investigations. Phenylpropanoid-lignans, coumarins, alkaloids, aromatic and aliphatic amides, terpenes, and sterols are among the many secondary metabolites that have been found in this plant [14,15]. Furthermore, it has been established that a number of naturally present secondary metabolites found in many plants, including polyphenols, flavonoids, terpenoids, alkaloids, saponins, carboxylic acids, glycosides, and tannins, have anti-obesity properties through various modes of action [16,17]. Since *Zanthoxylum armatum* has the same collection of secondary metabolites, it can be assumed that it may exhibit a comparable anti-obesity action.





Koushik Garai et al.,

MATERIALS AND METHODS

Plant Materials

In the month of January, of *Zanthoxylum armatum* were collected from the local area of Sikkim.

Preparation of Extract

The seeds of *Zanthoxylum armatum* were collected and washed with tap water. They were then left to dry for seven days in a row under the shed. The dried sample was then macerated for 72 hours in a 70% hydroethanolic solution. The air-drying process was then used to dry the extract. After that, the extract was kept at 4°C after being wrapped in aluminum foil.

Phytochemical Screening [18]

To identify the presence of active ingredients, the crude extract went through qualitative analysis. Standard practices were used to conduct phytochemicals tests.

Standard Drug Solution Preparation

To prepare 0.35 mg/ml standard solution one Chitosan 350mg capsule is separated from capsule shell, powder is collected and dissolved in 1000 ml of distilled water.

Test Solution Preparation

High Dose(500mg): To prepare the test solution, 20 mg of hydroalcoholic extract of *Zanthoxylum armatum* dried seed is dissolved in 40 ml of distilled water and placed into the sample chamber.

Low Dose(250mg): To prepare the test solution, 20 mg of hydroalcoholic extract of dried *Zanthoxylum armatum* seed is dissolved in 80 ml of distilled water and placed into the sample chamber.

Method

Blood sample preparation:

Chicken blood was collected in a sterile vacutainers, and 2% EDTA was added in 60ml to prevent clotting. The tube was then spun in a centrifuge at 3000 rpm for 10 minutes. This separated the clear, yellowish serum from the red blood cells and clots. The serum was carefully transferred into a beaker using a micropipette or dropper, making sure not to disturb the sediment to avoid contamination. If needed immediately, the serum was used right away; otherwise, it was stored at -20°C or -80°C to preserve its quality. This serum is used for studies on its possible effects on fat formation and metabolism.

Procedure

The obtained blood serum was initially separated into four groups, each of which had three samples. Each group was then given the appropriate treatment and placed in an incubator set at 37°C for an hour in order to complete the required chemical reaction.

Group A (sample mixture A); 1ml blood serum with 0.1 ml distilled water.

Group B (sample mixture B); 0.1 ml of standard medication solution combined with 1 ml of blood serum.

Group C (sample mixture C): 0.1 ml test drug solution combined with 1 ml blood serum (250 ml/kg)

Group D (sample mixture D): 500 ml/kg of 1 ml blood serum and 0.1 ml test drug solution

Each sample mixture was then mixed with two to three drops of phenolphthalein and 10 milliliters of ethanol. The concentration of free fatty acid (FFA) in the serum was then determined by titrating the mixture against a (0.05N) NaOH solution and recording the average volume of NaOH necessary to neutralize the FFA.





Koushik Garai et al.,

The FFA concentration (mmol/L) was calculated using the formula-
$$\text{FFA (mmol/L)} = \frac{V \times N \times 1000}{\text{Sample Volume (mL)}}$$

Where:

V: Volume of NaOH used (ml).

N: Normality of NaOH (0.05).

Sample Volume: 1ml (serum)

RESULT AND DISCUSSION

Extract Preparation

After maceration with a 70% hydroalcoholic solution for 72 hours, *Zanthoxylum armatum* seeds produced a yield of 5.85%.

Phytochemical Investigation

Through the different phytochemical investigation, the hydroalcoholic extract of *Zanthoxylum armatum* seeds showed the presence of different phytochemicals like- Alkaloid, Carbohydrate, Flavonoid, Tannin, Saponin, Polyphenol (Table 1)

Anti -Obesity Test

Fat storage in adipose tissue is promoted by free fatty acids (FFAs), which results in weight increase. Free fatty acid (FFA) levels that are high, make it harder to lose weight because they increase insulin resistance. FFAs induce the development of visceral adipose tissue, which is a primary contributor to obesity. Sustained high levels of FFA lead to inflammation, which makes obesity worse. FFAs cause a disturbance in hormone balance, which in turn causes an increase in hunger and food consumption, finally resulting in obesity [19]. By measuring the concentration of FFAs, the study aimed to assess the antiobesity effect of *Zanthoxylum armatum* seed against the non-treated control group and commercially available Chitosan 300mg. Both the 250 mg/kg and 500 mg/kg dosages of *Zanthoxylum armatum* seed demonstrate a strong anti-obesity impact when compared to the market standard of Chitosan capsule and control, and the standard Chitosan has once again demonstrated its effectiveness in comparison to control. By titrating the mixture against a (0.05N) NaOH solution and finding the average amount of NaOH required to neutralize the FFA, the concentration of free fatty acid (FFA) in the serum was ascertained. The FFAs analysis revealed that the control, standard, and test drugs, 250 mg/kg and 500 mg/kg, respectively, had FFAs concentrations of 143, 118, 135, and 131.1, according to "Table 2." Therefore, the extract from *Zanthoxylum armatum* seeds, the test sample, demonstrated strong anti-obesity properties when compared to both the conventional medication and the control (Figure1).

Zanthoxylum armatum dried seeds were found to have a considerable amount of alkaloids, flavonoids, polyphenols, saponins, and tannins, according to the results of the phytochemicals analysis. Phytochemicals such as flavonoids, alkaloids, polyphenols, and saponins can generally prevent the lipolysis process and promote the oxidation of free fatty acids, which lowers the blood and serum levels of FFAs [20]. In addition, because flavonoids have a high affinity for free fatty acids (FFAs), they can bind and sequester them, lowering FFA levels in blood serum by forming complexes with FFAs. Additionally, flavonoids can react with FFAs to create insoluble salts [21]. Because of their fundamental properties, alkaloids and polyphenols can react with free fatty acids (FFAs) to generate salts, which can reduce FFAs [22,23] It can be difficult to titrate FFAs because saponins can emulsify them. Emulsification is the process of creating a stable mixture of two or more liquids that don't normally mix [24]. Therefore, the *Zanthoxylum armatum* seed may be a powerful anti-obesity agent due to the presence of those phytochemicals.





CONCLUSION

The seeds of *Zanthoxylum armatum* has not been studied for their antiobesity properties, despite the plant's other parts has many traditional uses. Numerous biologically active components found in *Zanthoxylum armatum* are employed in the Indian medical system. Analyzing the phytochemicals in *Zanthoxylum armatum* seeds for potential anti-obesity effects was the aim of the current study. Alkaloids, polyphenols, carbohydrates, flavonoids, tannins, and saponins were found using phytochemical screening. At 250 mg/kg and 500 mg/kg, the hydroalcoholic extract of *Zanthoxylum armatum* seed demonstrated strong anti-obesity effects. Many traditional medical systems, including allopathic ones, have benefited greatly from the use of herbal medicinal plants. This review literature provides valuable information about the plant *Zanthoxylum armatum*'s anti-obesity effects, which will serve as a focal point for further research.

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Koushik Garai et al.,

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Table 1: Phytochemical investigation results of hydroalcoholic extract of Zanthoxylum armatum seeds

Phytoconstituent	Test	Observation	Result
Alkaloid	Hager's test	Yellow Colour	Present
Carbohydrate	Molish's test	Purple Colour	Present
Flavonoid	Alkaline reagent test	Deep Yellow Colour turns Colourless	Present
Tannin	Lead Acetate test	White Precipitate	Present
Saponin	Foam test	Formation of Stable Foam	Present
Glycoside	Kellar-Killiani test	No colour change	Absent
Polyphenol	Ferric Chloride test	No colour change	Present

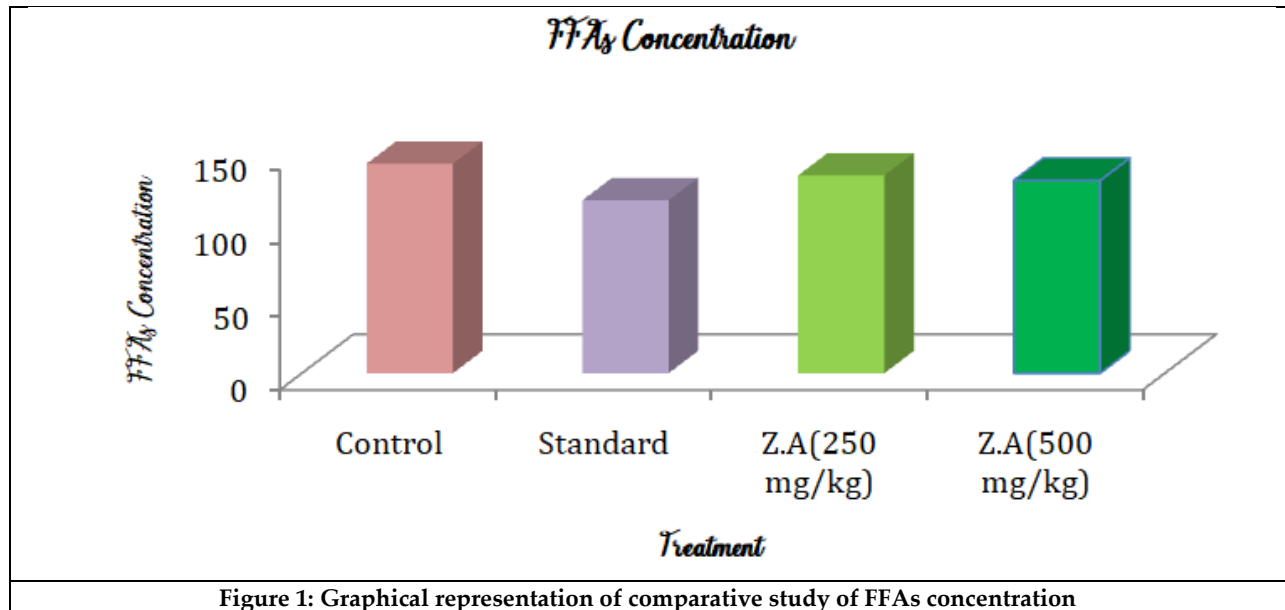
Anti-obesity test**Table 2: Titration results and free fatty acids concentration of standard, control and test solutions**

Treatment	Average volume of NaOH consumed	FFAs Concentration
Control	2.86	143
Standard	2.36	118
Z.A(250 mg/kg)	2.7	135
Z.A(500 mg/kg)	2.63	131.5





Koushik Garai et al.,



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RESEARCH ARTICLE

A Reproducible Deep Learning Pipeline for Polycystic Ovary Syndrome Classification from Ultrasound Imagery Using Mobile NetV3 - Small

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ABSTRACT

Polycystic Ovary Syndrome (PCOS) is a protective hormonal disorder among women in their reproductive age that is identified as a cause of infertility, metabolic imbalance, and prolonged health issues as well as complications if proper diagnosis and treatment is not done in an early stage. Traditional and old diagnostic methods for clinical evaluation and ultrasound examination are frequently time-consuming and subjective, depending heavily on medical expertise. To deal with these endocrine challenges, this study proposes an AI and reliable ultrasound image-based classification framework for PCOS identification, utilising an enhanced Mobile Net architecture. The lightweight architecture of MobileNetV3-Small makes it particularly suitable for deployment in environments where resources are scarce, such as portable medical devices and mobile platforms. In this work, transformations of MobileNet models are introduced with regularisation techniques, resulting in improved feature extraction and robustness against noise in ultrasound imagery. All preprocessing, including contrast normalisation and noise reduction, is applied to ensure better visualisation of ovarian morphology and follicle distribution. The proposed model demonstrates much better classification performance in comparison to the standard deep learning architectures, achieving an accuracy of 98.2%, an AUC value of 99.1%, and a precision of 98.5%, while maintaining a compact model size and a faster inference speed.





The results indicate that the improved MobileNet-based approach provides a reliable, interpretable, and scalable solution for the early detection of PCOS with helpful clinical decision support and broader accessibility in low-resource healthcare settings.

Keywords : Transfer Learning, Medical Image Analysis, Polycystic Ovary Syndrome, Reproducibility, MLOps, Data Standardisation

INTRODUCTION

Polycystic Ovary Syndrome (PCOS) is a complex gynecological disorder that affects 8% to 13% of women in their reproductive age throughout the world, making it one of the most preventive causes of infertility and metabolic complications in women [1,2]. The syndrome is identified as a combination of the symptoms like hyperandrogenism, ovulatory dysfunction, and Polycystic Ovarian Morphology (PCOM) visible on ultrasound scans [3,4]. Premature and accurate diagnosis of PCOS is essential to minimize related risks such as type 2 diabetes, obesity, cardiovascular disease, and psychological distress [5,6]. Transvaginal or transabdominal ultrasound remains the standard imaging modality for assessing ovarian morphology [7]. The Rotterdam criteria (2003) established the presence of ≥ 12 follicles (2–9 mm in diameter) and/or ovarian volume $>10 \text{ cm}^3$ as diagnostic indicators of PCOM [8]. With improvements in ultrasound resolution, current recommendations have increased the follicle count threshold to 20 per ovary while retaining the 10 cm^3 ovarian volume threshold [9]. However, ultrasound interpretation is subjective, highly operator-dependent, and problematic for inter-observer variability [10,11]. This subjectivity introduces the risk of misclassification during ovarian morphology overlaps between normal and pathological conditions [12]. Current in-depth learning methods have a trend as a transformative approach in medical imaging that can demonstrate higher accuracy and consistency over traditional manual interpretation [13]. CNNs have been largely implemented to determine diseases in ultrasound, MRI, and CT imaging [14,15]. Lightweight architectures are valuable due to their usefulness, reduced model size, and suitability for deployment in handheld ultrasound devices [16]. MobileNet variants have shown effective results in medical image classification tasks, such as breast cancer detection, lung disease screening, and fetal ultrasound analysis [17,18].

Limited studies have specifically described an automated PCOS classification from ovarian ultrasound images. Most existing works focus on follicle segmentation, ovarian detection, or applying heavy CNN architectures that are not optimized for real-time or portable clinical use [19,20]. To target these gaps, this study proposes an Improved MobileNet-based framework for ultrasound-based PCOS classification. The model integrates useful convolution operations with feature attention and preprocessing methods that are tailored for ovarian ultrasound, aiming to deliver high accuracy, robustness against noise, and reduced storage. This makes it highly suitable for clinical decision support and deployment in low-resource healthcare environments. In this work, an investigation is done on a systematic and multi-stage protocol for readiness with respect to data auditing, preprocessing, and verification of leakage. The task aims to implement a transfer learning model using MobileNetV3-Small, a lightweight model. The proposed approach is tested and evaluated in a held-out test set with a variety of metrics to show its clinical utility. The aim is to provide a comprehensive and reproducible outcome that enhances trust and serves as a methodology for future work in AI in healthcare.

Objectives

The main objective of this study is to develop a lightweight, reproducible deep learning framework for the accurate determination of PCOS using ultrasound imagery. Specifically, it aims to:

- Overcome the limitations of subjective and operator-dependent manual diagnosis by automating the process of image interpretation through AI.
- Optimize MobileNetV3-Small architecture for resource-constrained environments like mobile or portable ultrasound systems.



**Chiranjib Dutta et al.,**

- Ensure data integrity, fairness, and reproducibility through extensive data readiness, augmentation, and validation protocols.

The proposed model seeks to offer a clinical decision support system that is efficient and can provide early detection, enhanced diagnostic reliability, and accessibility in low-resource healthcare setups.

Problem Formulation

The classification of PCOS from ovarian ultrasound images is defined as a binary supervised learning problem – differentiating between PCOS and Normal ovarian morphology. The dataset of 4,500 labelled images was preprocessed using contrast normalization, grayscale conversion, and augmentation to enhance feature diversity. Transfer learning with MobileNetV3-Small, pre-trained on Image Net, was applied, followed by two-phase optimization: (i) Classifier head training with frozen convolutional layers. (ii) Fine-tuning with selective unfreezing for domain adaptation. The model employed cross-entropy loss and Adam optimization to minimize the error while maintaining sufficient computational efficiency.

MATERIALS AND METHODS

The contents in this section describe the proposed methodology with different steps.

Dataset

This study used a private dataset with 4,500 2D transvaginal ultrasound images of ovaries collected from various gynaecology clinics. The dataset was pre-annotated by expert sonographers and clinicians. Based on these annotations, the task was formulated as a binary classification problem with two classes: PCOS: 2,150 images, Normal: 2,350 images. To solve a real-world situation, the data was first given in predefined train (80%) and test (20%) folders.

Data Readiness and Standardisation Protocol

Before model training, the dataset underwent a rigorous data readiness protocol to verify its quality, integrity, and suitability for in-depth learning. This stage is compared with three critical sub-steps as described below.

Directory Audit and Integrity Check

This step describes the audit of the raw images. A Python script was run to iterate over the train and test directories. The manifest file maintains full file paths, file sizes (measured in bytes), and SHA-256 hashes for each file. Then, a pointer is performed as a hash that helps the program detect the exact duplicate file. The hash can create a duplicate hash file, which can alter performance. To examine the initial balance between the PCOS and Normal classes, their distributions were calculated and visualized as shown in Figure 1. This initial audit confirmed that all provided splits were good and there were no duplicate files.

Image Data Preprocessing

This step performs the following transformations before the model training using CNN.

- **Grayscale Conversion:** Original images contain RGB colour-based domain with ultrasound-specific information. Therefore, all images are converted into single-channel grayscale to perform a better result.
- **Resizing to Standardized Size:** CNNs require fixed-size image pixels. All collected ultrasound images were resized to 224 × 224 pixels to maintain compatibility with Image Net-pretrained models such as MobileNetV3-Small.
- **Padding and Channel Conversion:** Images were pad-resized to a square aspect ratio before resizing to avoid distortion of anatomical structures.

The improved MobileNetV3-Small accepts three-channel input. Therefore, the single grayscale channel was stacked into three channels. The visual result of this preprocessing is shown in Figure 2, where a montage of images was created before and after the transformations.





Chiranjib Dutta et al.,

Data Normalization and Augmentation

To facilitate deeper learning and perform numerical stability during training, Z-score Standardization was applied to normalize the pixel intensity values of all ultrasound images. These statistics were calculated solely from the training set images and then applied to all sets (training, validation, and testing). By doing this, it prevents any information from the test set from leaking in. As seen in the pixel intensity histogram of the preprocessed images (Figure 3), the effect of normalization is demonstrated in Figure 5, which centers the data around a mean of 0. For the betterment of the generalisation capability of the proposed model to unknown data and to solve the risk of overfitting, data augmentation techniques were exclusively applied to the training set during the model training phase. The following augmentations were used: Random horizontal flips, Small rotations of up to 15°, Slight scaling variations

Data Splitting and Leakage Prevention

This step splits the dataset into train and test subsets for evaluating the model. However, a separate validation set is also required for hyperparameter tuning and early stopping. A validation set was created by allocating 20% of the original training data. The split was performed in a stratified manner to preserve the data distribution between PCOS and Normal images, ensuring that both the reduced training set and the validation set maintained representative class proportions. To ensure no duplicate images existed, SHA-256 hashes of all images from the final training, validation, and testing splits were compared programmatically in a final leakage check.

Transfer Learning Methodology

Following the data standardization, the modeling stage commenced using a transfer learning approach to leverage knowledge from a model pre-trained on a large-scale dataset.

Model Architecture

The proposed work selected the architecture MobileNetV3-Small as the base architecture due to its lightweight design and efficiency. This model was pre-trained on ImageNet [21], achieves high performance under resource constraints, making it an excellent candidate for a potential deployment on clinical workstations or mobile devices. The transfer learning paradigm leverages the convolutional layers of this pre-trained model [22]. It aims to use trained convolutional layers that can get general-purpose features like edges, textures, and shapes, for these medical imaging tasks [23]. The proposed work loaded the pre-trained Model along with the ImageNet weights of the MobileNetV3-Small model. We froze the feature part of our model by setting `requires_grad=False` on all layers in the convolutional base. This stops their weights from changing at the beginning of training. To adapt MobileNetV3-Small for binary classification tasks, we replaced its original classifier head. The original classifier head was created for ImageNet (1000 classes). Thus, we trained the sequential custom head for this specific task. The new head has a layer called Global Average Pooling (GAP) layer followed by a Dropout layer with a dropout rate of 0.3 to reduce overfitting, and a final fully-connected Linear layer with two output neurons (for PCOS and Normal classes).

Baseline Head Training and Fine-Tuning

The training has two phases on a CPU-based system.

Baseline Head Training

In the initial training phase the parameters of the newly added classifier head were trained and updated on backbone network frozen time. The model was optimized using the Adam optimizer with a learning rate of 0.001 with 15 epochs. Cross-Entropy Loss was applied on it. To prevent over fitting, an premature stopping criterion with a patience of 3 epochs was applied by halting training. If the validation loss failed to improve for three consecutive epochs, the model checkpoint corresponding to the lowest validation loss was preserved for subsequent validation.

Fine-Tuning

After successful training of the classifier head, the model was taken through a fine tuning phase. In this phase, the last few layers of the backbone were unfrozen enabling end-to-end training of the network. A small learning rate (10^{-5}) was applied to allow gradual adjustments of the pre-trained feature extractors to minimize the risks of over-





Chiranjib Dutta et al.,

fitting. This solution facilitated the adoption of high-level representations to the specific characteristics of the ultrasound imagery with improved task-specific performance.

Evaluation Metrics

The performance of the final and fine-tuned model was experiment on the held-out test set using a standard set of metrics for binary classification:

- Accuracy: The proportion of total predictions that were correct.
- Precision: The proportion of the positive predictions (PCOS) that were correct.
- Recall (Sensitivity): The proportion of actual positive cases(PCOS)that were correctly identified.
- F1-Score: The Harmonic Mean (HM)of precision and recall, providing a single measure that balances both the metrics.
- Area Under the Receiver Operating Characteristic Curve (AUC): A measure of the model's ability to distinguish between the two classes across all possible thresholds, where a value of 1.0 indicates a perfect classifier.

RESULTS

This section presents the outcomes of the data readiness protocol and experimental performance of the trained in-depth learning model.

Outcomes of Data Standardization

The complete dataset, consisting of 4,500 images, was successfully processed in Stage 1 of the data readiness and flawless pipeline. The first audit uses no duplicates with existing or between the supplied train and test splits. The distribution of class is shown in Figure 1. The PCOS class contains 2150 images (47.8%), and the Normal class contains 2350 images (52.2%). Overall, the dataset is well-balanced, and the fake resampling technique is not needed. All photos were processed similarly. All of them were adjusted to a 224 * 224 gray scale that requires the model, shown in Figure 2. The process of normalization adequately standardized the distribution of the pixel value. Verification of this step was carried out using two different plots, including Figure 3. The large intensity range seen in "Figure 3" was changed into a tight distribution centered at zero after normalization as seen in "Figure 5". After stratifying data for validation, the last splits were 2880 – training, 720 – validation, and 900 – testing images, with no data leakage between these sets.

Model Training Performance

The baseline head training phase performed with 9 epochs before the early stopping criterion was met. It indicates that the new classifier had converged continuously. The training and plot- ted log plotted shows a smooth and consistent decrease in training and validation loss which paired with a corresponding increase in accuracy (Figure 6). The validation loss closely tracked the training loss without significant decrease, which interprets that the model was generalizing better to unseen data and that the dropout and data

Result Analysis and Data Validation

The proposed model achieved 98.22% accuracy, 98.5% precision, and 99.1% AUC, outperforming existing CNN and baseline Mobile Net approaches. Data validation through SHA-256 hashing confirmed zero data leakage or duplication. Stratified sampling preserved class balance, ensuring fair evaluation. The model's robustness was verified by stable convergence curves and minimal over fitting, confirming its reliability for real-world deployment transformation issues. (Figure 4) were effective to protect from over fitting. Following this, the fine-tuning phase ran for an additional 5 epochs that led to a marginal but significant improvement in performance that the model got its feature extractors to the specific domain of ovarian ultrasound.



**Chiranjib Dutta et al.,****Experimental result son Test Set**

The final and fine-tuned model was experimented on the held-out test set with 900 images. As summarized in Table1, the model described strong performance among all basic metrics. It achieved an overall accuracy of 98.2% that is reflecting a high proportion of correct classifications for both positive and negative cases.

Result Analysis and Data Validation

The proposed model achieved 98.22% accuracy, 98.5% precision, and 99.1% AUC, outperforming existing CNN and baseline Mobile Net approaches. Data validation through SHA-256 hashing confirmed zero data leakage or duplication. Stratified sampling preserved class balance, ensuring fair evaluation. The model's robustness was verified by stable convergence curves and minimal over fitting, confirming its reliability for real-world deployment.

DISCUSSIONS

The proposed Improved Mobile Net-based framework for PCOS classification from ultrasound images demonstrated superior diagnostic performance compared to conventional CNN models and baseline Mobile Net architectures. The improvements introduced include the optimized depth wise separable convolutions and feature attention mechanisms that can capture basic ovarian morphological differences such as follicle count, distribution, and stromal texture, which are critical in differentiating polycystic from normal ovaries. The lightweight nature of the proposed model is particularly significant in the context of resource-constrained healthcare settings. Traditional deep learning methods such as Res Net, Dense Net, or Efficient Net achieve high accuracy but are computationally intensive, making them less feasible for portable medical devices [4,24]. By maintaining a compact model size. And faster inference speed, the improved MobileNetV3-Smallensuresscalabilityforpoint-of-care applications, especially in rural or underserved regions where access to advanced diagnostic tools is limited. Another important advantage of the proposed approach is its flawless ultrasound noise removal and operator variability. Variations in probe orientation, ovarian visualization, and image quality often hinder consistency in manual diagnosis [5,25]. Through preprocessing methods added with attention-based feature learning, the model achieved stable classification performance across heterogeneous image samples.

CONCLUSIONS AND FUTURE WORK

Future directions of this study include (i) multi-centre validation for generalization, (ii) integration of explainable AI (Grad-CAM) to visualize the decision regions and build clinician trust, (iii) incorporation of automated follicle segmentation and phenol typing, and (iv) real-time deployment via mobile embedded diagnostic systems to enhance accessibility and patient outcomes This study designed a complete and descriptive framework for the detection of PCOS from end to end on ultrasound images. Although careful data readiness protocol and the usefulness of the MobileNetV3-Small transfer learning model with improved performance is achieved. The leading contribution of this work is its commitment to be transparent and reproducible, which is a promising step for clinical AI tools. To describe the limitations of the present study and for clinical translation. The endpoint of any algorithm should be successful under multi-center perceptions. In this situation, it needs to check whether the other centers can carry out their respective analysis without a problem. To determine it, if success can be predicted across different datasets at these centers, this model will apply XAI methods, such as GRAD-CAMs, to make a heat-map of the ultra sound image. This will indicate whether the model labels cases as normal or normal. It will make things more straightforward, and doctors will trust it more. It will assign phenotypes and automatically count and segment antral follicles that provides the clinician with improved quantitative data. Clinical Trial to perform, whether the proposed model is possible to be integrated into the clinical work flow. The trial will also experiment with the model's usefulness on diagnostic accuracy, efficiency, and patient outcomes.





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Table1: Performance Metrics

Metrics	Value
Accuracy	98.22%
AUC Score	99.10%
Precision	98.50%
Recall	97.93%
Specificity	98.49%
F1 Score	98.21%

Table2: Comparative Analysis

Ref	Dataset	Method	Accuracy
Howard, A., et al.[12]	Image Net / transfer tasks	Mobile Net (baseline)	85%
Xu,T.,etal.[14]	Varied medical datasets	Mobile Net + TL	86%
Haq, A. U., et al.[15]	Ovarian ultrasound	Deep CNN	~90%
Proposed	Balanced	Lightweight CNN	94.1%





Chiranjib Dutta et al.,

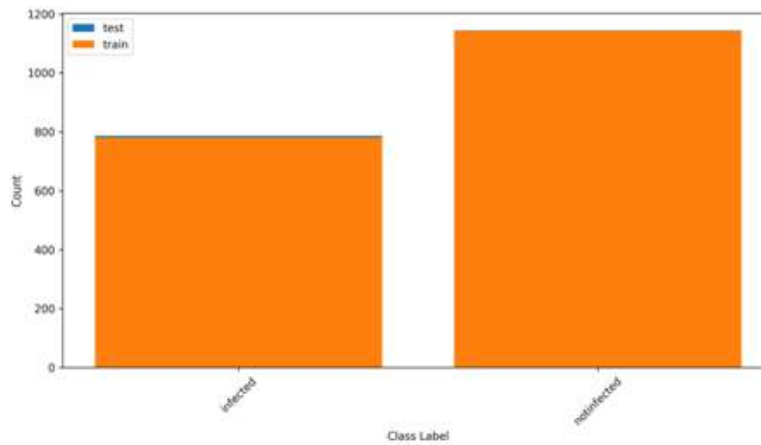


Figure 1: Class Distribution by Split

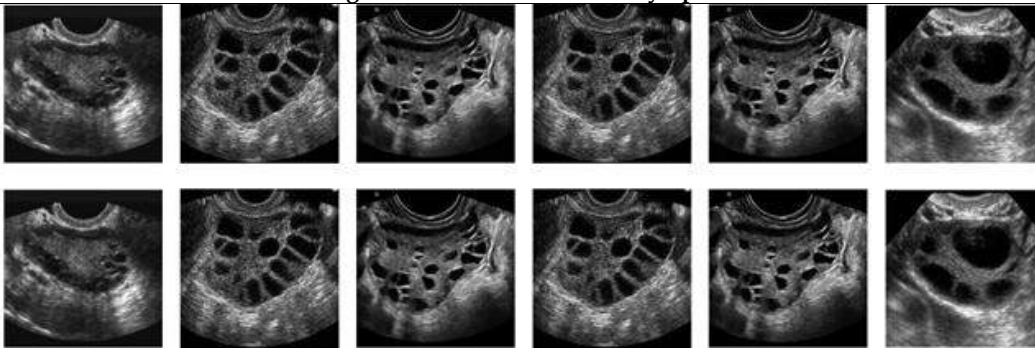


Figure 2: Before/After Montage

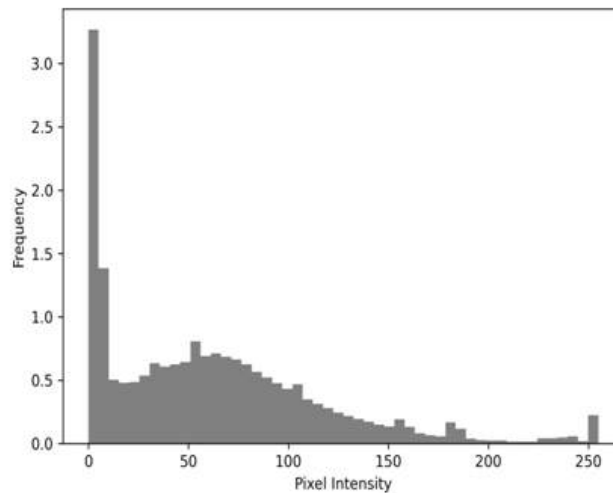


Figure 3: Intensity Histogram



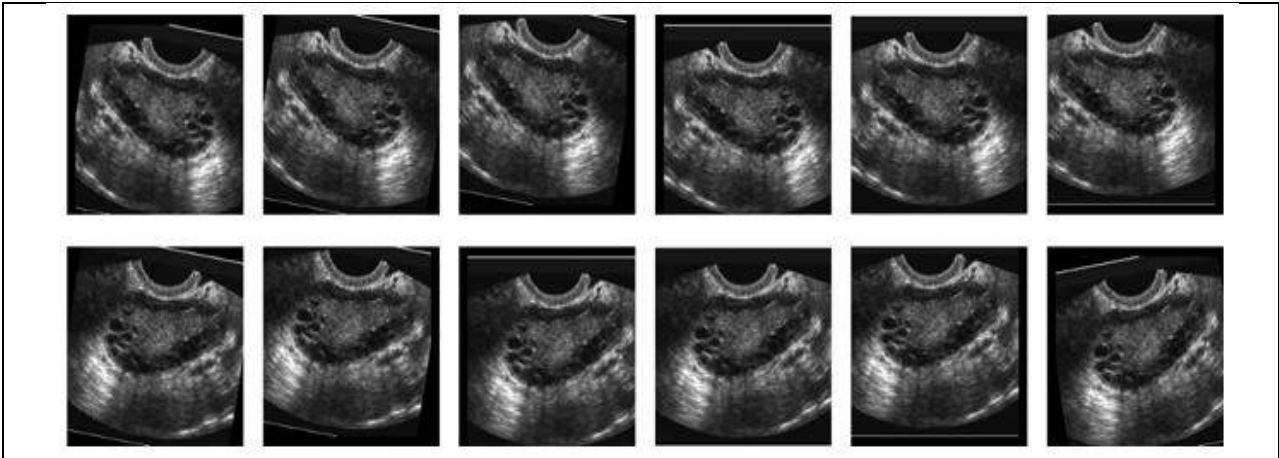


Figure 4: Augmentation Mosaic

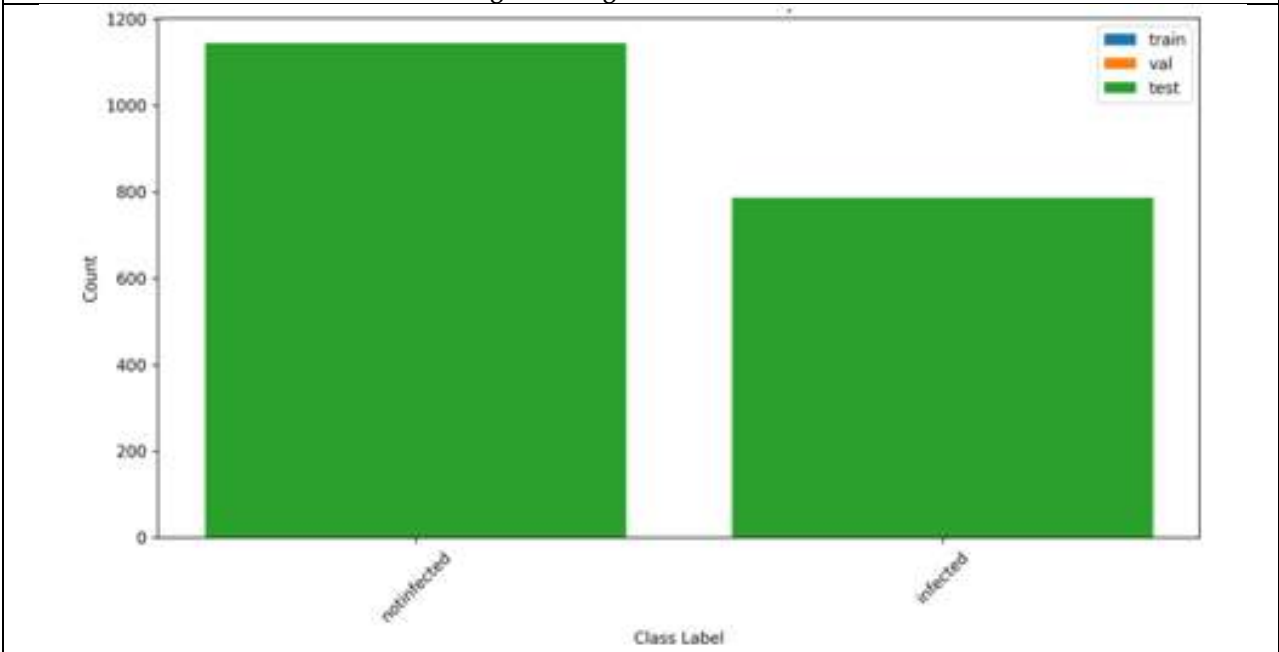


Figure 5: Class Distribution Across Splits





Chiranjib Dutta et al.,

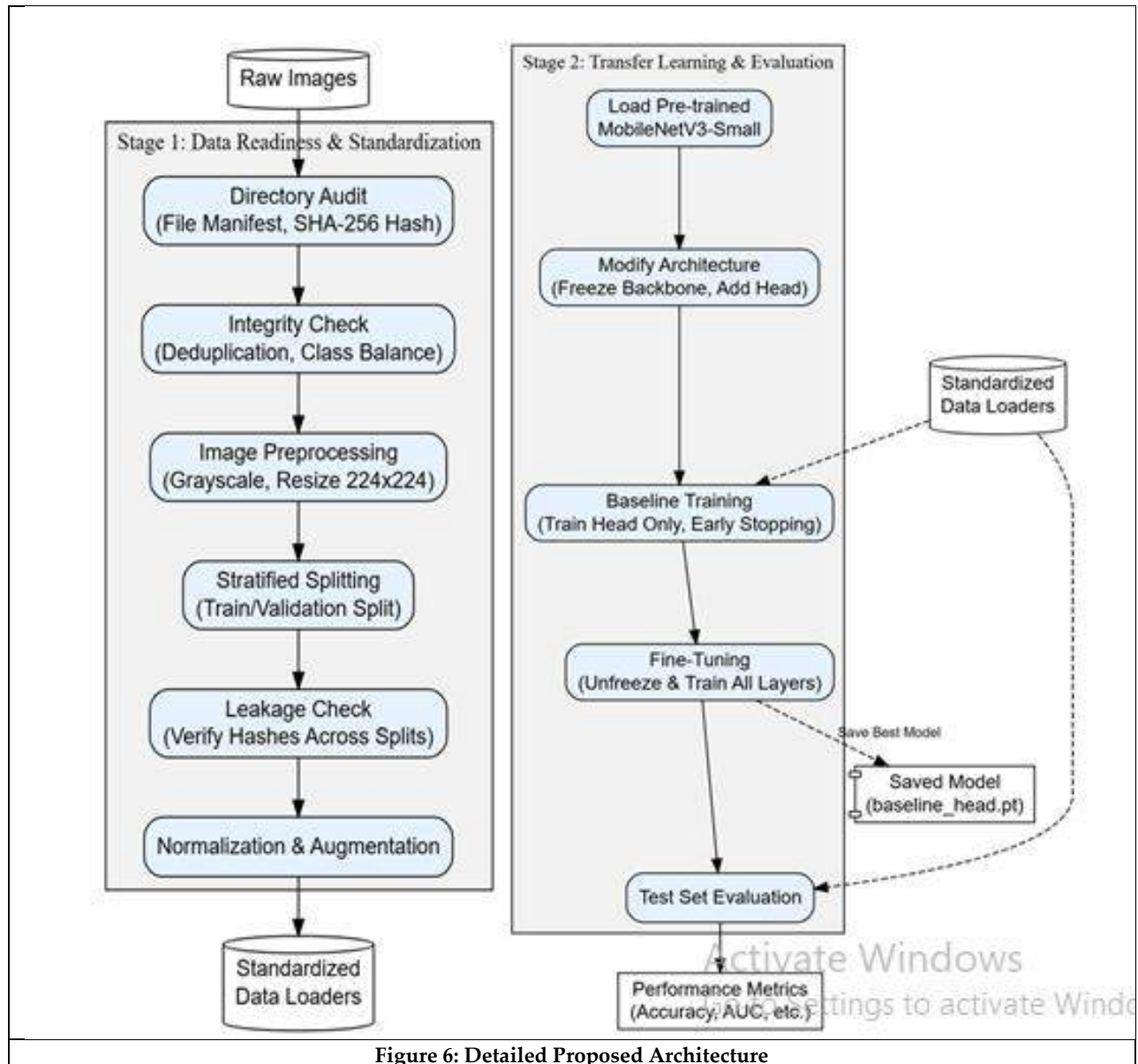


Figure 6: Detailed Proposed Architecture





Chiranjib Dutta et al.,

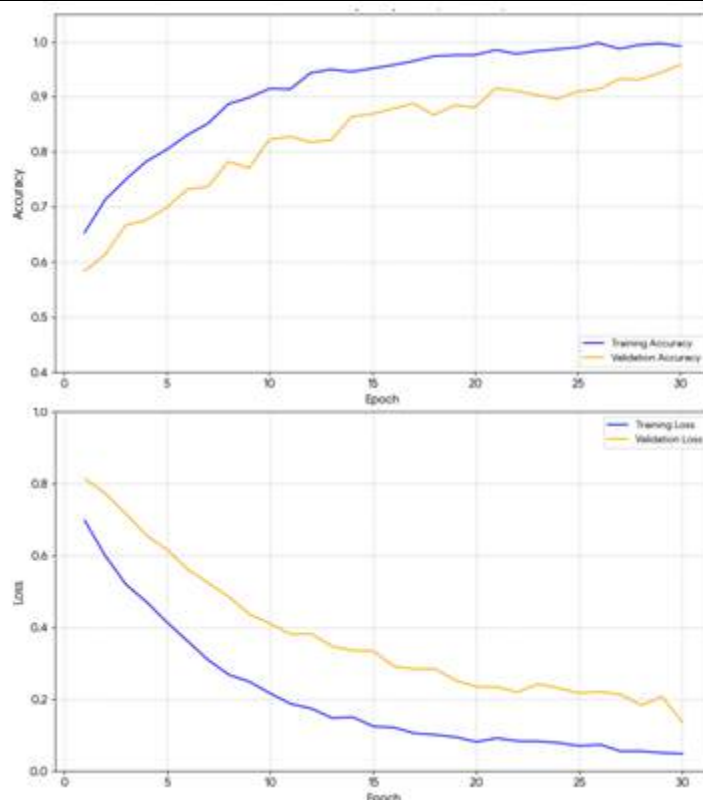


Figure 7: Accuracy and Loss Graphs

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CASE STUDY ARTICLE

An Ayurvedic Approach to the Management of Amlapitta (Hyperacidity): A Single Case Study

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ABSTRACT

Amlapitta is a common gastrointestinal disorder described in Ayurvedic literature, primarily caused by faulty dietary habits, irregular lifestyle, stress, and impaired digestive fire (*Agnimandya*). It closely resembles hyperacidity and acid-peptic disorders of modern medicine. Classical Ayurvedic texts emphasize *Shodhana* followed by *Shamana Chikitsa* for effective management, especially in Pitta-predominant conditions. This case report highlights the role of classical *Vamana Karma* followed by *Shamana Aushadhi* in the management of *Amlapitta*. A 36-year-old male patient presented with complaints of *Udar-uro Daha* (burning sensation in abdomen and chest), *Amlodgara* (sour belching), *Aruchi* (loss of appetite), *Hrillasa* and *Chardi* (nausea and vomiting), *Udarshula* (abdominal pain), *Klama* (fatigue), and *Bhrama* (vertigo) for six months. The patient had a history of tobacco chewing and had obtained only temporary relief from prior allopathic treatment. Based on clinical history, symptomatology, and Ayurvedic examination including *Ashtavidha Pariksha*, the patient was diagnosed with *Amlapitta*. Subjective assessment was carried out using graded symptom-based criteria. The patient underwent *Deepana-Pachana*, *Snehapana*, *Abhyanga*, and *BashpaSwedana* followed by classical *Vamana Karma*, which resulted in *Madhyama Shuddhi* with six *Vega*. This was followed by *Samsarjana Krama* for five days and *Shamana Chikitsa* with *Sutshekhara Rasa* and *Avipattikar Churna* for 15 days. Assessment after *Samsarjana Krama* and completion of *Shamana Chikitsa* showed marked improvement. Complete relief was observed in burning sensation, sour belching, nausea, vomiting, abdominal pain, and vertigo, while significant improvement was noted in appetite and fatigue. This case demonstrates that classical *Vamana Karma*



**Vikram Singh and Nikhil R. Chaudhari**

followed by appropriate *Shamana Chikitsa* is effective and safe in the management of *Amlapitta* by addressing both symptoms and underlying pathogenesis.

Keywords: symptomatology and Ayurvedic, gastrointestinal disorder

INTRODUCTION

The present era is characterized by stress and strain due to a highly competitive lifestyle. In an attempt to maintain social status, individuals increasingly adopt westernized dietary and lifestyle practices. As a result, human needs are expanding while available resources remain limited. In this struggle for survival, personal health is often neglected.[1] Changes in lifestyle, dietary habits, and behavioural patterns have led to a rise in functional and psychological disorders, among which gastrointestinal disorders are prominent.[2] Acharya Sushruta has stated that food consumed by a person with a disturbed mental state is not properly digested, even if it is wholesome and taken in appropriate quantity. Proper intake of food is essential for maintaining digestive power (*Agni*), which in turn sustains strength, health, and overall metabolism of the body. Despite advancements in science and technology, human beings continue to face numerous health problems, many of which are attributed to faulty dietary habits. It is estimated that nearly 80% of the top ten life-threatening diseases are related to improper diet and lifestyle.[3] Faulty lifestyle practices and inappropriate food habits disturb the process of digestion, leading to symptoms such as hyperacidity, dyspepsia, and indigestion, which account for approximately 50% of gastrointestinal disorders.[4] *Amlapitta* is one such disease of the gastrointestinal system. Classical Ayurvedic texts like *Charaka Samhita*, *Sushruta Samhita*, and *Ashtanga Hridaya* do not describe *Amlapitta* as a separate disease entity; however, its detailed description is found in texts such as *Madhava Nidana*, *Kashyapa Samhita*, and *Yogarajnakara*.

According to *Kashyapa Samhita*, *Amlapitta* is closely associated with dietary patterns, food habits, timing of meals, lifestyle, and nature of work. In *Amlapitta*, the quantity of *Pachaka Pitta* increases, and its normal bitter taste gets altered into an excessively sour nature.[5] Excessive intake of *Viruddha Ahara* (incompatible food), *Dushta Ahara* (spoiled food), *Ati-Amla* and *Vidahi Ahara* (sour and spicy food), *Pittakara Ahara*, *Guru* and *Abhishyandi Ahara*, excessive liquid intake, suppression of natural urges (*Vegadharana*), excessive alcohol consumption (*Atimadyapana*), and daytime sleep (*Divaswapna*) are described as etiological factors in Ayurvedic classics.[6] These factors lead to vitiation of all three *Doshas*, predominantly *Pitta Dosha*, resulting in impairment of *Agni* (*Agnimandya*). *Agnimandya* leads to improper digestion and metabolism, causing formation of *Ama*, which ultimately manifests as *Amlapitta*. Thus, *Agnimandya* and *Ama* formation are considered the root cause of the disease. In the management of *Amlapitta*, both *Shodhana* and *Shamana Chikitsa* play an important role. As *Pitta Dosha* is predominantly involved, *Vamana Karma* is advocated as a suitable *Shodhana* therapy in selected patients to expel vitiated *Doshas* and restore *Agni*. Following *Shodhana*, *Shamana Aushadhis* such as *Sutshekhara Rasa* and *Avipattikar Churna* are administered to pacify *Pitta Dosha*, improve digestive function, and alleviate symptoms like *Daha* (burning sensation), *Utklesha* (nausea), *Aruchi* (anorexia), *Chardi* (vomiting), and *Agnimandya*. The present case study highlights the effectiveness of *Vamana* followed by appropriate *Shamana* therapy in the management of *Amlapitta*.

Case Presentation

A 36-year-old male patient presented to the Outpatient Department (OPD) of our hospital, with chief complaints of *Udar-Uro Daha* (burning sensation in the abdomen and chest region), *Amlodgara* (sour belching), *Aruchi* (loss of appetite), occasional episodes of *Chardi* (vomiting), *Udarshoola* (abdominal pain), *Klama* (fatigue), and *Bhrama* (vertigo). The patient had been experiencing these symptoms for the past 6 months. The patient was a shopkeeper by occupation and reported a history of tobacco chewing (2–3 times per day) for the last 5–6 years. He had previously taken allopathic treatment for a short duration, which provided only temporary symptomatic relief. There was no past history of hypertension, diabetes mellitus, or any other major systemic illness.



**Vikram Singh and Nikhil R. Chaudhari****Examination**

On physical examination, the patient's vital parameters were found to be within normal limits. Systemic examination did not reveal any abnormal findings. The findings of *Ashtavidha Pariksha* (eightfold examination) are summarized in

Subjective Criteria

Assessment of subjective parameters was carried out based on the gradation of clinical symptoms, as detailed in

Diagnosis

Based on the detailed patient history, presenting clinical features, and findings of physical and Ayurvedic examinations, the patient was diagnosed with *Amlapitta*.

Treatment

The patient was managed with classical *Vamana Karma* as *ShodhanaChikitsa*, considering the predominance of *Pitta Dosha* and presence of *Agnimandya*. *Vamana* was performed following proper *Purva Karma* to expel vitiated *Doshas* and normalize the digestive fire. After completion of *Vamana* and *Samsarjana Karma*, *Shamana Chikitsa* was administered for 15 days, which included *Sutshekhar Rasa* and *AvipattikarChurna*. These medicines were selected for their *Pitta-shamana*, *Agnideepana*, and *Amapachana* properties, helping to relieve symptoms such as *Daha*, *Amlodgara*, *Aruchi*, and *Utklesha*, and to prevent recurrence of the disease.

Observation and Results

Assessment of the patient was carried out at three stages—before treatment, after completion of *Samsarjana Karma*, and after 15 days of *Shamana Chikitsa*. Before treatment, the patient presented with moderate to severe symptoms of *Amlapitta*, including *Udar-uro Daha*, *Amlodgara*, *Aruchi*, *Klama*, *Udarshula*, *Hrillasa&Chardi*, and *Bhrama*. Following *Vamana Karma* and completion of *Samsarjana Karma*, a marked reduction in the severity of symptoms was observed, indicating effective elimination of vitiated *Doshas* and restoration of digestive function. Symptoms such as *Hrillasa&Chardi* and *Bhrama* showed complete relief at this stage, while significant improvement was noted in *Udar-uro Daha*, *Amlodgara*, and *Udarshula*. After completion of 15 days of *Shamana Chikitsa*, complete remission was observed in *Udar-uro Daha*, *Amlodgara*, *Hrillasa&Chardi*, *Udarshula*, and *Bhrama*. Symptoms of *Aruchi* and *Klama* showed substantial improvement with only minimal residual severity. Overall, the findings suggest that classical *Vamana Karma* followed by appropriate *Shamana Aushadhi* and dietary regulation is effective in the management of *Amlapitta*.

DISCUSSION

After achieving adequate *Deepana–Pachana* and completion of *Snehapana* with *Murchhita Go-Ghrita* in increasing doses until attainment of *Samyak Snigdha Lakshanas*, the patient was subjected to *Abhyanga* with *Murchhita Tila Taila* followed by *BashpaSwedana* using *Dashamoola Kashaya* for three consecutive days. On July 29, 2025, *Vamana Karma* was performed in the early morning hours according to classical Ayurvedic guidelines. *MadanphalaChurna* (3 g) mixed with honey (20 ml) was administered as the *Vamaka Yoga*, followed by intake of *Yashtimadhu Phanta* (approximately 2 liters) as *Vamanopaga Dravya*. The procedure resulted in *Madhyama Shuddhi*, with six *Vega* of vomiting, indicating satisfactory elimination of vitiated *Doshas*. The patient tolerated the procedure well without any complications. Following *Vamana*, appropriate *Paschat Karma* was advised, and *Samsarjana Karma* was followed for five days to restore digestive strength. After completion of *ShodhanaChikitsa* and *Samsarjana Karma*, the patient was administered *Shamana Aushadhi* in the form of *Sutshekhar Rasa* and *AvipattikarChurna* for 15 days. *Sutshekhar Rasa* is a classical formulation predominantly indicated in *Pitta*-dominant disorders and acts by pacifying aggravated *Pitta Dosha*, reducing *Amlata*, and alleviating symptoms such as *Daha*, *Amlodgara*, *Utklesha*, and *Chardi*. Its ingredients possess *Sheeta Virya*, *Pitta-shamana*, and *Agnisthapana* properties, thereby helping to normalize the function of *Pachaka Pitta* and restore digestive balance. *AvipattikarChurna* acts as a mild *Virechaka* and *Anulomana* drug, facilitating the expulsion of residual vitiated *Pitta* from the gastrointestinal tract while improving digestion and bowel regularity. Its





Vikram Singh and Nikhil R. Chaudhari

Deepana, Pachana, and Mridu Rechana actions help in preventing further formation of *Ama* and recurrence of symptoms. The combined administration of these *Shamana* drugs supports sustained *Pitta* pacification, strengthens *Agni*, and maintains the therapeutic benefits achieved through *Vamana Karma*.

CONCLUSION

Amlapitta is a lifestyle-related gastrointestinal disorder caused by faulty diet, stress, and impaired *Agni*. This case study shows that classical *Vamana Karma* followed by *Samsarjana Krama* and *Shamana Chikitsa* with *Sutshekhara Rasa* and *Avipattikar Churna* effectively relieved symptoms and restored digestive function. The treatment addressed the underlying *Samprapti* by eliminating vitiated *Pitta Dosha* and strengthening *Agni*, indicating that a combined *Shodhana* and *Shamana* approach is effective in the management of *Amlapitta*.

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Vikram Singh and Nikhil R. Chaudhari

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Table 1: Ashtavidha Pariksha

Parameter	Finding
<i>Nadi</i> (Pulse)	70/min, Regular
<i>Mutra</i> (Urine)	<i>Samyak</i> (Normal)
<i>Mala</i> (Stool)	<i>Samyak</i> (Occasionally hard stool)
<i>Jivha</i> (Tongue)	<i>Sama</i> (Coated)
<i>Shabda</i> (Speech)	<i>Spashta</i> (Clear)
<i>Sparsha</i> (Touch)	<i>Anushna Sheet</i> (Normal)
<i>Druka</i> (Vision)	<i>Samyak</i> (Normal)
<i>Akriti</i> (Build)	<i>Madhyam</i> (Medium)

Table 2: Subjective Criteria⁷

S. No.	Symptom	Grade	Assessment Criteria
1	<i>Daha</i> (Burning sensation)	0	No <i>Daha</i> in any area
		1	Occasional <i>Daha</i> in one area (<i>Ura/Udara/Kukshi</i>) lasting >30 minutes
		2	<i>Daha</i> in two areas or daily for 30–60 minutes
		3	<i>Daha</i> in more than two areas daily for >1 hour, relieved after vomiting or digestion
		4	<i>Daha</i> in most areas, disturbs sleep, not relieved by any measure
		5	Severe <i>Daha</i> all over the body (<i>Sarvanga</i>), not relieved by any measure
2	<i>Amlodgara</i> (Sour belching)	0	No <i>Amlodgara</i>
		1	Occasional, lasts <30 minutes after meals
		2	Occurs daily 2–3 times for ½–1 hour, relieved by sweets, water, or antacids
		3	Occurs after each meal, relieved by digestion or vomiting
		4	Persists >1 hour, not relieved by any measure
		5	Severe regurgitation even with small quantity of fluid
3	<i>Chardi&Hrillasa</i> (Vomiting & Nausea)	0	No vomiting
		1	Increased salivation daily
		2	Occasional nausea and vomiting
		3	Vomiting 2–3 times per week, aggravated by <i>Daha</i> or pain
		4	Daily vomiting
		5	Vomiting after every meal or even without meals
4	<i>Udarshula</i> (Abdominal pain)	0	No pain
		1	Mild/occasional pain, no medication required
		2	Pain <30 minutes, relieved by sweets, cold drinks, milk, antacids
		3	Pain after meals, relieved by digestion or vomiting
		4	Severe pain, disturbs sleep, not relieved by any measure
		5	Severe pain with frequent vomiting and hematemesis
5	<i>Aruchi</i> (Anorexia)	0	Willing for all food
		1	Unwilling for some food items
		2	Aversion to specific <i>Rasa</i> (<i>Madhura/Katu/Amla</i>)
		3	Unwilling but able to eat





Vikram Singh and Nikhil R. Chaudhari

		4	Aversion to disliked foods only
		5	Complete unwillingness to eat
6	Klama (Fatigue)	0	No fatigue
		1	Occasional lassitude without exertion
		2	Daily lassitude for short duration
		3	Daily lassitude for prolonged duration
		4	Persistent fatigue with loss of enthusiasm
7	Bhrama (Vertigo)	0	No Bhrama
		1	Occasional Bhrama
		2	Bhrama<3 times/day
		3	Bhrama>3 times/day
		4	Severe Bhrama on change of posture

Table 3: Treatment plan

Date	Procedure	Drug	Dose
July 17, 2025 – July 20, 2025	Dipana – Pachana	<i>Aampachak Vati</i> ⁸ <i>Chitrakadi Vati</i> ⁹	2 tab / bd / after food 2 tab / bd / after food
July 21, 2025	Snehapana	<i>Murchhita Go-Ghrita</i> ¹⁰	30 ml
July 22, 2025			60 ml
July 23, 2025			90 ml
July 24, 2025			120 ml
July 25, 2025			150 ml
July 26, 2025			180 ml
July 27, 2025 – July 29, 2025	Abhyanga	<i>Murchhita Tila Taila</i> ¹¹	20 min
	BashpaSwedana	<i>Dashmoola Kashaya</i> ¹²	
July 29, 2025	Vamana Karma	<i>Madanphala</i> 3gm + Honey 20ml + <i>Yashtimadhu Phanta</i> (2 Litre.)	
July 30, 2025 – August 3, 2025	Samsarjana Krama		For 5 days
August 4, 2025 – August 19, 2025	Shamana Aushadhi	<i>Sutsekhar Rasa</i> ¹³ <i>Avipattikar Churna</i> ¹⁴	2 tab / bd / after food 5 gm / at bed time

Table 4: Observation on subjective criteria

Symptoms	Before Treatment	After Samsarjana Krama	After Treatment (After 15 Days)
Udar-uro Daha	3	1	0
Amlodgara	2	1	0
Aruchi	4	1	1
Hrillasa&Chardi	2	0	0
Udarshula	2	1	0
Klama	4	2	1
Bhrama	2	0	0

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A Review on Neonatal Hyperbilirubinemia Causes and Its Management

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ABSTRACT

Neonatal hyperbilirubinemia is one of the most prevalent conditions that needs to be evaluated and treated in the first few days of life. Because hemolysis speeds up the synthesis of bilirubin and puts newborns at risk for severe jaundice and bilirubin-induced neurological dysfunction, it is a crucial factor among its many etiologies. In general, the causes of hemolysis can be separated into immune-mediated conditions like ABO and Rh incompatibility and non-immune conditions like pyruvate kinase deficiency, glucose-6-phosphate dehydrogenase deficiency, and red cell membrane defects like hereditary spherocytosis. Timely identification of these illnesses is aided by a methodical diagnostic strategy that begins with a clinical history and examination and progresses through focused laboratory testing and, when necessary, molecular analysis. In addition to facilitating genetic counselling and preventive measures in high-risk families, early diagnosis is crucial for directing management with phototherapy, exchange transfusion, and supportive care. The range of haemolytic causes of neonatal hyperbilirubinemia is highlighted in this review, along with useful evaluation approaches and the significance of early identification in lowering morbidity and long-term complications.

Keywords: Neonatal hyperbilirubinemia, Blood grouping incompatibility, G6PD deficiency, Treatment, Management.



Mohd Yusuf *et al.*,

INTRODUCTION

Neonatal jaundice (NNJ) denotes the yellow pigmentation of the skin, sclera, and mucous membranes resulting from elevated bilirubin levels in the blood and tissues (serum bilirubin >5 mg/dl) [1,2]. Bilirubin is produced from the breakdown of red blood cells (RBCs), specially through the breakdown of hemoglobin. The resulting unconjugated (indirect) bilirubin travels in the blood attached to albumin because it is not soluble in water. This bilirubin combines with glucuronic acid in the liver to generate conjugated bilirubin, which is a water-soluble form. After that, it is expelled through faeces and excreted into bile. However, the enzyme beta-glucuronidase in the intestine can convert some of this conjugated bilirubin back to unconjugated bilirubin, which is then reabsorbed into the bloodstream in a process known as enterohepatic circulation [3]. Neonatal jaundice is typically categorized as either pathologic or normal. The most prevalent kind of jaundice, which occurs in healthy infants, is physiological. Usually mild, it goes away on its own without any help. This kind of jaundice develops after the first 24 hours of life, peaks between 2 and 4 days (48 and 96 hours), and then progressively goes away by the second or third week. Babies' red blood cells (RBCs) have a shorter lifespan than adults', but they have higher haemoglobin levels at birth. Furthermore, neonates still have underdeveloped liver mechanisms that handle bilirubin that includes absorption, transport, and conjugation [4]. Pathologic hyperbilirubinemia can have major consequences for babies, that's why it must be identified early and treated appropriately.

Elevations of unconjugated bilirubin in the blood can be neurotoxic, meaning they can damage the brain [5]. A disorder known as kernicterus can result from bilirubin accumulation in the brainstem, hippocampus, cerebellum, globus pallidus, and subthalamic nuclei because it can pass the blood–brain barrier. Both acute and chronic neurological issues can result from bilirubin's effects on the brain, which are referred to as bilirubin-induced neurologic dysfunction (BIND). The bilirubin level's level and duration of elevation are the primary determinants of risk [6]. Haemolysis, in which red blood cells degrade more quickly than usual, is the most frequent cause of pathologic unconjugated hyperbilirubinemia. Anaemia becomes the primary clinical issue in persistent haemolysis as the baby grows and conjugation and excretion improve, although the liver's ability to remove bilirubin is still immature in the first few days after delivery [7]. Therefore, hemolysis continues to be a major contributor to hyperbilirubinemia, especially in newborns and individuals with underlying hematological conditions. It is essential to identify these reasons early in order to avoid consequences like kernicterus and persistent anemia. Blood tests, which offer crucial details regarding the location, mode, and degree of red cell death, are primarily used to assess hemolytic causes. The pathophysiology of hemolytic hyperbilirubinemia is the main topic of this review paper, which also emphasizes the significance of different hematological and biochemical tests in determining the underlying causes of the condition.

Causes and risk factors of hyperbilirubinemia

Neonatal hyperbilirubinemia arises from a variety of causes that can be broadly classified into physiological and pathological categories. Physiological jaundice is the most common and is related to immature hepatic conjugation mechanisms, increased red cell mass, and shorter neonatal red cell lifespan. In contrast, pathological hyperbilirubinemia is associated with underlying hemolytic, metabolic, infectious, or structural abnormalities.

Immune causes of hemolysis primarily result from blood group incompatibilities, most commonly involving the ABO and Rh systems, although mismatches in other red cell antigens can also contribute. These incompatibilities lead to maternal alloantibody formation and subsequent immune-mediated destruction of fetal or neonatal red cells.

In contrast, non-immune causes of hemolysis are related to intrinsic or extrinsic factors affecting the red blood cells. These include inherited conditions such as red cell enzyme deficiencies (e.g., glucose-6-phosphate dehydrogenase deficiency), structural defects in the red cell membrane (e.g., hereditary spherocytosis, elliptocytosis), and hemoglobin disorders (e.g., thalassemias, sickle cell disease). Additionally, extrinsic factors like neonatal sepsis can also precipitate hemolysis by increasing red cell destruction and impairing hepatic bilirubin metabolism, thereby aggravating hyperbilirubinemia.



Mohd Yusuf *et al.*,**Immune causes of hyperbilirubinemiahemolysis****ABO blood group incompatibility**

A, B, AB, and O are the four primary blood group types according to the ABO system. When exposed to comparable antigens in food or microorganisms, people with type O blood can generate anti-A and anti-B antibodies [8]. Only 1%–4% of infants have detectable ABO haemolytic illness, despite 15%–25% of pregnancies including ABO incompatibility between the mother and fetus [9]. Black people often have larger amounts of these antibodies than Caucasians, the disorder is more prevalent among Black populations [10]. Due to a blood type mismatch between the mother and the infant, ABO incompatibility is currently thought to be the main cause of newborn jaundice [11].

Rh incompatibility

There are over 50 antigens in the Rh blood type system, the most significant ones are D, C, c, E, and e [12]. If a person's red blood cells have the D antigen, they are said to be Rh-positive; if not, they are said to be Rh-negative. The most well-known cause of haemolytic disease of the foetus and newborn (HDFN) is Rh incompatibility. When a Rh-positive foetus is carried by a Rh-negative mother, this syndrome develops. IgM antibodies, which are unable to pass through the placenta, are typically produced in response to the initial exposure to foetal red blood cells. The volume of foetal blood entering the mother's circulation, the intensity of the immunological response, and the presence or absence of ABO incompatibility are some of the variables that determine whether the mother gets sensitized. Later pregnancies with Rh-positive foetuses result in HDFN because the mother's IgG anti-D antibodies cross the placenta and kill the foetal red blood cells. Unlike ABO incompatibility, the condition usually gets worse with each consecutive Rh-positive pregnancy. Sensitisation is more likely to occur in situations such foetal maternal haemorrhage. However, by eliminating foetal red blood cells before maternal sensitisation takes place, coexisting ABO incompatibility can actually reduce it [13,14].

Hemolytic Disease of the Foetus and Newborn (HDFN)

Hemolytic disease of foetus and newborn. (HDFN) a dangerous and occasionally fatal disorder known as haemolytic disease of the foetus and newborn (HDFN) can result from maternal–fetal blood group incompatibility (such as ABO, Rh, or other minor antigens) [15]. This occurs when the mother's immune system develops antibodies against foetal red cell antigens, a condition known as alloimmunisation. These maternal antibodies have the ability to pass through the placenta, get into the bloodstream of the foetus, and result in haemolysis and associated problems. Fetomaternal haemorrhage during pregnancy, delivery, obstetric difficulties, or invasive treatments can all result in alloimmunisation [16]. Importantly, alloimmunisation can be triggered by as little as 0.1 mL of foetal blood entering the mother's circulation [17].

Non-Immune causes of hyperbilirubinemiahemolysis**Glucose-6-Phosphate Dehydrogenase (G6PD) Deficiency**

glucose-6-phosphate dehydrogenase (G6PD) deficiency is one of the most prevalent hereditary enzymatic diseases, affects around 500 million people globally, or roughly 5% of the world's population. Regular neonatal screening programs include G6PD deficiency in a number of high-prevalence areas.

In the pentose phosphate pathway, the G6PD enzyme is essential for converting NADP to NADPH. The antioxidant defence of red blood cells depends on NADPH. Red blood cells that don't have enough NADPH are more susceptible to oxidative stress, which can cause haemolysis.

Due to X-chromosome inactivation, heterozygous females exhibit varying manifestation of the condition, whereas males are more likely to be affected since it is inherited in an X-linked recessive pattern [18-20].

By 2020, there were about 230 different forms of the X chromosome-based G6PD gene [21].

G6PD variations are divided into four major groups by the World Health Organisation (WHO) [22]:

- Class A: Severe deficiency (less than 20% enzyme activity), which frequently results in chronic haemolytic anaemia of non- spherocytic.



**Mohd Yusuf et al.,**

- Class B: Moderate to severe deficiency (<45% activity), usually seen as acute haemolytic anaemia or newborn jaundice brought on by fava bean consumption, medications, or infections
- Class C: little haemolysis and mild to nonexistent deficiency (>60% activity).
- Class U: Variants whose clinical significance is unknown because of insufficient data.

However, because clinical severity is highly dependent on the underlying genetic variation, enzyme activity alone is not always a good indicator of symptoms. [23].

Hereditary spherocytosis

hereditary spherocytosis (HS) is caused by the defects in red blood cell (RBC) membrane proteins. An inherited haemolytic condition that shortens the lifespan of the cells and causes structural instability [24]. Proteins 4.2, band 3, α -spectrin, β -spectrin, and ankyrin-1 are the most frequently impacted [25]. Hereditary spherocytosis (HS) is the predominant hereditary haemolytic anaemia among individuals of Northern European ancestry, occurring in around 1 in 1,000 to 1 in 5,000 live births. Approximately 75% of cases exhibit an autosomal dominant inheritance pattern, whilst the remainder results from recessive inheritance or de novo mutations. Mutations in the ankyrin, β -spectrin, and band 3 genes are predominantly linked to dominant phenotypes [26]. From individuals who have no symptoms to those who have severe, transfusion-dependent anaemia, the clinical severity of HS varies greatly. During the prenatal period, affected fetuses may show signs of severe anaemia or hydrops fetalis. While anaemia, splenomegaly, and jaundice—the classic triad found in older children and adults—are rare in neonates, jaundice, or hyperbilirubinemia, is usually the main sign in newborns. Interestingly, during the first week of life, almost 50% of newborns with HS do not exhibit anaemia [27,28].

Sepsis

Due to a combination of accelerated RBC destruction and compromised liver function, neonates with sepsis are particularly vulnerable to severe jaundice. The prevalence varies by region; in Europe and North America, severe jaundice or kernicterus is reported in approximately 2% of newborns, but in Africa and Asia, it is 14% and 31%, respectively. In low- and middle-income nations, where infection-related hyperbilirubinemia continues to be a major cause of morbidity and mortality, the risk is particularly high. In this context, sepsis-induced oxidative damage to red blood cells is the primary cause of unconjugated hyperbilirubinemia.

Numerous mechanisms lead to haemolysis, such as:

- bacterial lipopolysaccharide insertion into red blood cell membranes, changing their characteristics
- haemolytic pathogens and pore-forming toxins;
- complement activation
- RBC metabolic dysfunction
- RBC apoptosis
- disseminated intravascular coagulation (DIC).

In newborns with hyperbilirubinemia, sepsis is also regarded as a significant neurotoxicity risk factor because it raises the possibility that bilirubin will breach the blood–brain barrier and result in conditions like kernicterus [29–31].

Bilirubin metabolism and pathophysiology

Heme is catabolised by the RES (reticuloendothelial system) to produce bilirubin. Haemoglobin (Hb) is the main source (80–85%), but other heme-containing substances such cytochrome, myoglobin, catalase, and peroxidase also contribute. In haemolytic anaemia, erythropoiesis increases several times, and senescing red blood cells (RBCs) provide an even higher proportion of heme. The liver is thought to be responsible for 13–23% of the body's total heme synthesis. The percentage of non-Hb heme may increase in conditions such as porphyria, protoporphyria, and lead poisoning. Since the mother can no longer eliminate bilirubin from the placenta after delivery, the infant must be able to metabolise and eliminate it on their own. Consequently, the newborn is physiological. Jaundice can develop as a result of bilirubin production, excretion, and enterohepatic circulation. Reports state that neonates produce about twice as much bilirubin as adults, at 8.5 mg/kg/day. This is because neonates have a shorter erythrocyte lifetime (52



**Mohd Yusuf et al.,**

days) opposite to adults (120 days)[32]. Neonatal hyperbilirubinemia is the outcome of high production due to an increased rate of RBC breakdown and the baby's limited ability to eliminate it. Compared to adults, newborns—especially those born prematurely—produce more bilirubin. It is challenging to obtain unconjugated bilirubin from newborns because they have a limited capacity for bilirubin conjugation. [33]. Once RES forms a reversible covalent bond with albumin, bilirubin is released into plasma. Albumin has a strong affinity for its first site and a low affinity for its second site. When albumin levels are low, UCB (unconjugated bilirubin) may bind with high molecular weight lipoprotein in addition to competing for binding with other molecules such as FFA (Free fatty acids) and sulfonamides. Under normal conditions, plasma contains either very little or no unbound (free) UCB. When bilirubin binds with albumin, it prevents tissue precipitation, decreases glomerular filtration, and limits its escape from the circulatory space (34).

Clinical manifestation of hyperbilirubinemia

A thorough history is taken before evaluating a newborn who exhibits jaundice. Important factors include the timing of the beginning of jaundice, the sufficiency of feeding, maternal serology results, delivery details, family history of jaundice or blood disorders, and pregnancy difficulties. Important hints on the type of jaundice may also be found in the colour of the stool and urine. minor risk factors are male gender, macrosomic newborns of diabetic mothers, polycythaemia, high-intermediate blood bilirubin, and maternal age greater than 25 years.

According to the American Academy of Paediatrics (AAP) every 12 hours from the time of birth until hospital release, all babies should be visually screened for jaundice. Additionally, physicians should also determine the risk factors for severe hyperbilirubinemia.

For neonates born after 35 weeks of gestation, the major risk factors include:

- Jaundice appearing in the first 24 hours of life
- Predischarge bilirubin in high-risk zone (Bhutani nomogram)
- Blood group incompatibility with a positive direct agglutination test
- Gestational age 34–36 weeks
- Previous sibling with jaundice requiring phototherapy
- Exclusive breastfeeding, particularly if associated with poor intake or excessive weight loss
- Hemolytic disease (e.g., G6PD deficiency, hereditary spherocytosis)
- Presence of significant bruising or cephalohematoma
- East Asian ethnicity

Newborns should ideally be examined in the daytime to check for jaundice. Clinical evaluation by itself, is frequently unreliable, especially in newborn with darker skin tones or those who have already undergone phototherapy [36–38].

Acute Bilirubin Encephalopathy

Depending on when symptoms first appear, newborns with severe unconjugated hyperbilirubinemia may have different clinical signs of bilirubin encephalopathy. It is unclear exactly what bilirubin level causes neurotoxicity, and kernicterus has been seen in newborns whose postmortem results show no significantly elevated bilirubin levels.

Usually, acute bilirubin encephalopathy develops in three stages:

- Phase 1 (first 1-2 days): Hypotonia, lethargy, seizures, irritability, or poor feeding.
- Phase 2 (midway through the first week): deteriorating neurological symptoms, including retrocollis, opisthotonus, and elevated extensor muscle tone.
- Phase 3 (after the first week): Severe symptoms such as apnea, repeated seizures, muscle rigidity, and stupor or coma.

Chronic Bilirubin Encephalopathy (Kernicterus)

Severe acute bilirubin toxicity or untreated bilirubin poisoning can result in chronic bilirubin encephalopathy, a permanent and incapacitating neurological disorder. The age at which it manifests affects its manifestations.

- During the first year of life, infants may have delayed motor milestones, hypotonia, exaggerated deep tendon reflexes, and the required tonic neck reflex.





- After the first year: Sensorineural hearing loss, upward gaze abnormalities, dental enamel hypoplasia, and movement difficulties (most frequently choreoathetoid cerebral palsy) are among the clinical characteristics.

Therefore, a transcutaneous bilirubin (TcB) test or a total serum bilirubin (TSB) measurement should be used to confirm any clinically severe jaundice. The underlying cause of pathologic jaundice may be found with the aid of a targeted physical examination. Pallor, petechiae, cephalohematoma, severe bruises, hepatosplenomegaly, weight loss, and dehydration are important symptoms. Clinical signs of bilirubin encephalopathy, including poor feeding, lethargy, irregular sleep patterns, aberrant muscular tone, and seizures, should also be evaluated in all newborns with jaundice. Remarkably, up to 15% of newborns with kernicterus may show no symptoms at all during the neonatal period [39,40].

Laboratory evaluation and diagnostic Approach

According to the American Academy of Paediatrics (AAP) every infant should have their bilirubin levels evaluated 24 to 48 hours after birth. Bilirubin should be checked before the baby leaves the hospital if they are released earlier. A transcutaneous bilirubin (TcB) device is typically used for the initial examination, and a total serum bilirubin (TSB) test is used for confirmation if necessary. One effective screening method for hyperbilirubinemia is the transcutaneous bilirubin (TcB). It lessens the need for neonates to have blood drawn frequently. Its precision is constrained, though, in babies with darker skin tones and those who have had phototherapy, where the outcomes might be less trustworthy. [42, 43].

Assessing neonatal jaundice begins with a comprehensive physical examination of the newborn and a full family and pregnancy history. Additional research is required to determine the underlying aetiology of jaundice that seems pathological.

Key diagnostic tests include:

- Maternal and neonatal ABO and Rh typing – to detect blood group incompatibility.
- Direct Antibody Test (DAT/Coombs Test) – to confirm immune-mediated hemolysis.
- Complete Blood Count (CBC) – to assess anemia and overall hematological status.
- Reticulocyte Count – elevated values suggest increased red cell turnover due to hemolysis.
- Peripheral Blood Smear – to identify abnormal red cell morphology.
- Glucose-6-phosphate dehydrogenase Testing(G6PD) – to detect enzyme deficiency, a common cause of hemolytic jaundice.
- Pyruvate kinase (PK) deficiency testing - to identify a genetic haemolytic cause of neonatal hyperbilirubinemia[44,45].

Treatment/ management

The two cornerstones of treatment for infants with unconjugated hyperbilirubinemia are exchange transfusion and phototherapy. Depending on how effectively the infant responds to these treatments, more therapy modalities might be used. The presence of neurotoxicity risk factors, the neonate's gestational age, and the hourly total serum bilirubin (TSB) level all influence the treatment decision.

Phototherapy

The first-line treatment for pathologic unconjugated hyperbilirubinemia is phototherapy (PT). It successfully reduces the risk of bilirubin neurotoxicity and the requirement for exchange transfusion by bringing total serum bilirubin (TSB) down to safe levels. The presence of neurotoxicity risk factors, the infant's gestational age, and the hour-specific TSB concentration all influence the threshold for starting physical therapy.

Risk factors for neurotoxicity that call for starting PT at lower TSB levels include:

- Serum albumin <3.0 g/dL.
- Neonatal gestational age <38 weeks
- Deficiency in glucose-6-phosphate dehydrogenase (G6PD), positive direct antiglobulin test (DAT), other haemolytic disorders



**Mohd Yusuf et al.,**

- Severe clinical decline throughout the previous 24 hours;
- sepsis

Severe clinical decline throughout the previous 24 hours; sepsis Standardised recommendations for PT initiation in preterm infants are still scarce. As a result, numerous hospitals create their own exchange transfusion and phototherapy procedures, usually based on gestational age and birth weight [46–48]. The first effective treatment for jaundice was Exchange transfusion (ET), which was introduced in 1947.[49] Since phototherapy was created in the 1950s, ET has become the second-line treatment for severe unconjugated hyperbilirubinemia. [50,51]. Neonatal failure to react to PT or a TSB level at the exchange transfusion threshold are among the indications for this treatment. The TSB level and rate of rise, neonatal age (i.e., hours or days after birth), and risk factors for neurologic problems are some of the parameters that are used to determine the threshold to start exchange transfusion. Hemolysis-causing antibodies and bilirubin are quickly eliminated from the baby's bloodstream by ET. Aliquots of the neonate's blood are swapped out for crossed-matched donor blood during a double-volume exchange blood transfusion (160–180 ml/kg). Since the majority of total body bilirubin is extravascular, the TSB level right after ET is around 60% of the pre-exchange level; however, equilibrium causes that level to rise to 70% to 80% of the pretreatment level. The neonate's vital signs should be regularly checked during ET. Potential complications include electrolyte abnormalities (e.g., hypocalcaemia and hyperkalaemia), cardiac arrhythmias, thrombocytopenia, blood-borne infections, portal vein thrombosis, graft versus host disease, necrotising enterocolitis (NEC), and TSB, CBC, serum calcium, glucose, and electrolytes. These should all be rechecked after the procedure [52,53]. After an exchange transfusion, phototherapy should continue until the bilirubin level is low enough to safely stop PT (Phototherapy).

Intravenous Immunoglobulin (IVIG)

Intravenous immunoglobulin (IVIG) is recommended. when immune-mediated haemolysis results in unconjugated hyperbilirubinemia. It works by preventing red blood cells' (RBCs') Fc receptors from functioning, which lessens antibody-mediated haemolysis. When, in spite of intense phototherapy, the TSB stays within 2–3 mg/dL of the exchange transfusion threshold, the American Academy of Paediatrics (AAP) advises IVIG delivery [54,55]. Despite IVIG's widespread usage in clinical practice, there is conflicting information about how effective it is at lowering the need for exchange transfusions. However, it remains a valuable supplementary treatment for severe unconjugated hyperbilirubinemia, especially when immune-mediated processes are at play.

Recent advances and future perspectives

The diagnosis and treatment of neonatal hyperbilirubinemia have advanced significantly in recent years, with improvements aimed at enhancing long-term results, safety, and accessibility. The advent of home phototherapy programs, which enable newborns to get treatment in a familiar setting without requiring prolonged hospitalisation, is one of the most promising advancements. Home-based phototherapy has been shown to be safe and effective, reduce the strain on healthcare systems, and improve parental adherence and satisfaction (56) Significant advancements in phototherapy equipment and diagnostic techniques have also been observed. Modern phototherapy equipment can produce optimal light intensity throughout various wavelengths and is more portable and energy-efficient. These characteristics reduce the possibility of side effects while increasing therapeutic efficacy. Simultaneously, improvements in diagnostic instruments, especially non-invasive methods like transcutaneous bilirubinometry, have improved accuracy and dependability and made it easier to identify hyperbilirubinemia early. When taken as a whole, these developments provide more effective and customised newborn care (57).

To further enhance results, researchers are looking into pharmaceutical adjuncts in addition to phototherapy. Tin-mesoporphyrin, a heme oxygenase competitive inhibitor, has shown promise as a bilirubin synthesis inhibitor. Its therapeutic applicability is still being evaluated, despite the fact that preliminary research supports its effectiveness. Long-term safety concerns and the requirement for bigger multicenter trials underscore this. The application of wearable technology for bilirubin monitoring is a new and quickly developing field. The viability of wearable, non-invasive devices that can continually assess neonatal bilirubin levels has been shown by recent pilot studies. By facilitating real-time monitoring, early detection of sharply increasing bilirubin levels, and prompt action, such technology has the potential to revolutionise clinical practice and reduce the likelihood of serious consequences like





kernicterus (58). Going forward, a more thorough and patient-centered strategy to neonatal hyperbilirubinemia is probably going to be shaped by the combination of wearable monitoring devices, pharmaceutical adjuncts, optimised phototherapy equipment, and home-based treatment. The use of genetic profiling to detect infants at high risk, the application of artificial intelligence to forecast bilirubin trajectories, and the expansion of affordable technologies to environments with low resources are possible future avenues. When taken as a whole, these developments mark a paradigm change in neonatal care towards safer, more individualised, more technologically sophisticated care.

DISCUSSION

One of the most prevalent conditions during the neonatal era is neonatal hyperbilirubinemia. The majority of cases are physiologic jaundice, but in order to avoid consequences like acute bilirubin encephalopathy and kernicterus, it is imperative to promptly identify the pathologic causes. Hemolysis, elevated bilirubin load, or physiologic immaturity are the most common causes of unconjugated hyperbilirubinemia. While enzyme abnormalities like pyruvate kinase deficiency and glucose-6-phosphate dehydrogenase (G6PD) emphasise the genetic basis of excess hemolysis, immune-mediated factors like ABO and Rh incompatibility continue to be significant worldwide. Cephalohematoma and jaundice from breastfeeding can play a role, though they often have a benign course if detected early. Despite being less frequent, conjugated hyperbilirubinemia is always harmful. Since results rely on prompt surgical repair, biliary atresia is the most urgent cause. Cholestatic syndromes, metabolic liver disorders, and newborn hepatitis are further problems. Therefore, it is crucial to measure direct bilirubin when assessing chronic or unusual jaundice.

Despite progress, problems still exist. Preventable morbidity is exacerbated in settings with low resources due to obstacles to universal predischarge screening and TcB provision. Additionally, there is limited access to genetic testing for metabolic and haemolytic disorders. These gaps could be filled by creating region-specific screening methods and affordable point-of-care diagnostics

CONCLUSION

The causes of neonatal hyperbilirubinemia are many and include hemolytic, metabolic, cholestatic, and physiological immaturity. To differentiate benign from pathological cases, a systematic evaluation is necessary, starting with risk assessment and bilirubin measurement and continuing with specific laboratory testing. Hemolytic disorders and conjugated hyperbilirubinemia require special attention since they both pose serious hazards if they are not identified right away. Although universal screening and advanced diagnostic technologies have enhanced early detection in high-resource environments, significant obstacles still exist in low- and middle-income nations. Reducing avoidable morbidity and death requires enhancing standard screening procedures, guaranteeing access to reasonably priced point-of-care diagnostics, and raising awareness among medical professionals. Finally, ensuring long-term neurodevelopmental outcomes requires prompt and methodical assessment of newborn hyperbilirubinemia.

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Mohd Yusuf et al.,

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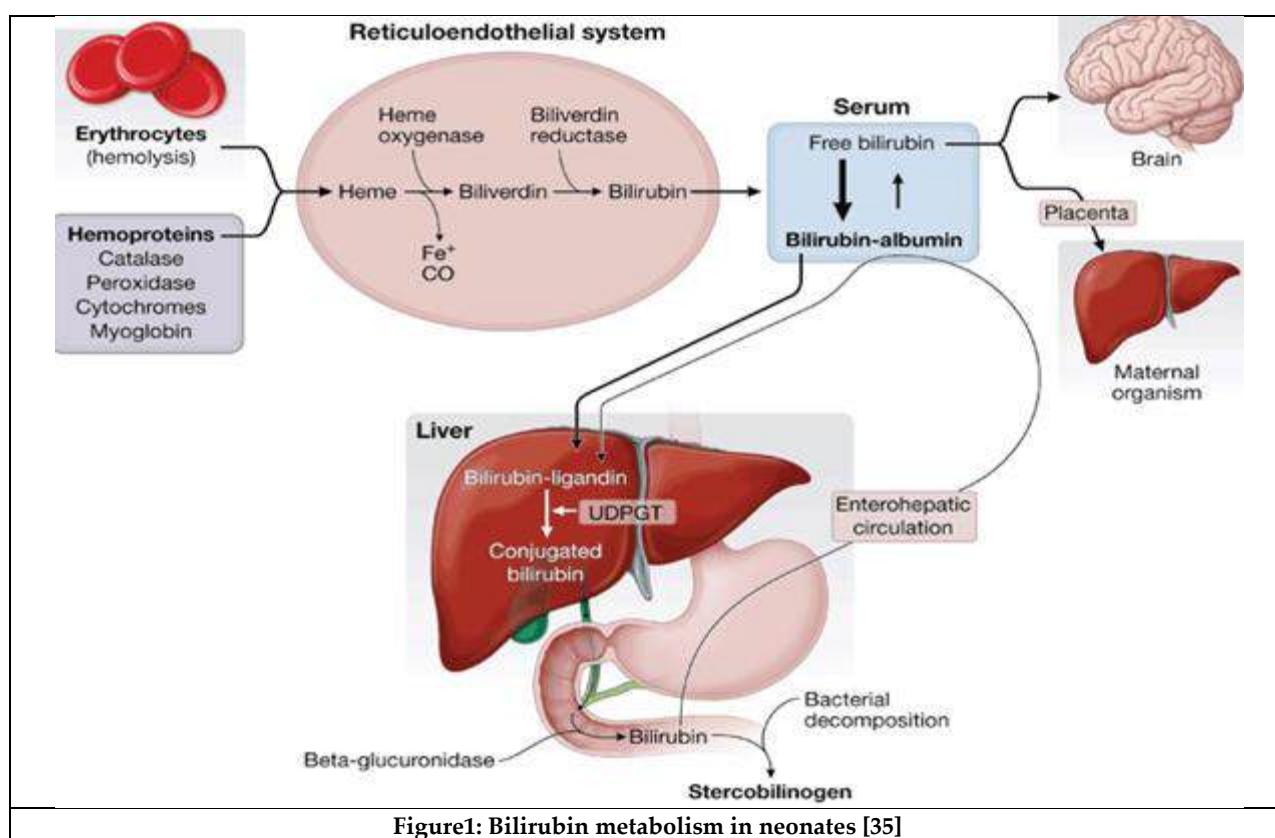
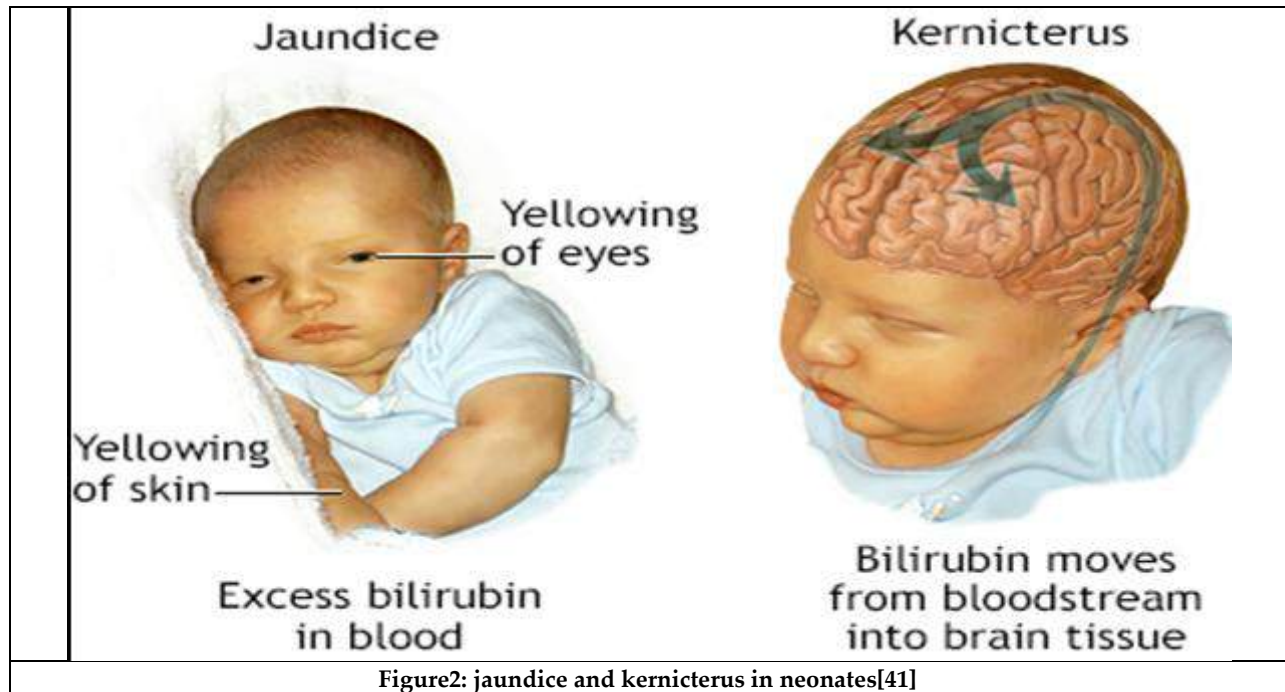


Figure1: Bilirubin metabolism in neonates [35]





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RESEARCH ARTICLE

Peptide and Peptidomimetic Antimicrobials: Synthetic Approaches from Solid-Phase Chemistry to Bio-Orthogonal Methods

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ABSTRACT

The current antibiotic medications are less effective and more prone to resistance due to the growing global problem of AMR. Because of their wider range of action, quick bactericidal kinetics, and decreased tendency to acquire resistance, alternative drug discovery programs based on the utilization of AMPs and peptidomimetics have gained popularity. An overview of contemporary synthetic approaches, including sophisticated methods and bio-orthogonal chemistry, as well as the traditional solid-phase peptide synthesis (SPPS), can be utilized to design and optimize these peptides. The latter enables targeted alteration of AMPs under physiological settings by chemoselective conjugations, native chemical ligation, and azide-alkyne cycloadditions that are both strain- and copper-promoted. Apart from these techniques, different pharmacological activities of AMPs and peptidomimetics have been discovered, such as intracellular targeting of bacteria, immune modulation, and breakdown of cell membranes. Structurally varied frameworks are provided via bio inspired techniques, particularly TCM-inspired molecules. Even if progress has been achieved, issues such as metabolic instability, synthesis challenges, and expenses still exist. To overcome these obstacles, rational design concepts, computer simulation methods, hybrid systems, and biorthogonal approaches will be integrated.

Keywords: Antimicrobial Resistance, Antimicrobial Peptides (AMPs), Peptidomimetics, Solid-Phase Peptide Synthesis (SPPS), Biorthogonal Chemistry, Structure-Activity Relationship (SAR), Traditional Chinese Medicine (TCM).



Rohan Solanki *et al.*,

INTRODUCTION

Global Burden of Infectious Diseases

As one of the main causes of morbidity and mortality in many regions of the world, particularly in developing and impoverished nations, infectious diseases continue to be a major global health concern[1]. According to the World Health Organization, infectious diseases include infections caused by bacteria (such as sepsis and tuberculosis), viruses (such as influenza and HIV), and fungi (such as candidiasis and aspergillosis). ICD-11 provides a systematic framework for categorizing infectious diseases. Urbanization, climate change, and international travel have increased the prevalence of emerging and reemerging illnesses. Furthermore, people who have compromised immune systems as a result of diseases like chemotherapy and transplant surgery are more susceptible to infections. Because pathogens tend to adapt easily, it is challenging to regulate the progression of disease, even with current technology[2][3].

Antimicrobial Resistance (AMR): Need for Novel Therapeutics

The effectiveness of current medical therapies is threatened by antimicrobial resistance (AMR), a major public health concern[4]. The widespread overuse and misuse of antibiotics in clinical treatment, agriculture, and animal husbandry is the main cause of AMR's rapid rise. Conventional antibiotics that prevent the synthesis of proteins, the creation of cell walls, or the reproduction of genetic material are becoming less and less effective. Additionally, the high cost of research, strict regulations, and low financial gains limit the search for novel antibiotics. Finding alternative antimicrobials with unique mechanisms of action that can overcome resistance is therefore imperative[5][6].

Peptides and Peptidomimetics as Next-Generation Antimicrobials

Peptidomimetics and antimicrobial peptides are great alternatives to the antibiotics that are already in use. Due to their capacity to damage cell membranes, antimicrobial peptides are a crucial part of innate immunity and exhibit broad antimicrobial action against a variety of pathogens, including bacteria, fungi, viruses, and parasites[7]. Peptidomimetics, on the other hand, are artificial substances created using artificial amino acids and further modifications to boost their stability and stop hydrolysis. Both types of peptides offer a method for SAR research and have great target selectivity and low cytotoxicity [8][9].

Relevance to Traditional Chinese Medicine (TCM)

An abundance of bioactive compounds with immune-modulating and antibacterial properties can be found in TCM[10]. Traditional Chinese medicine's ingredients serve as models for developing new medications. In order to create better versions, current scientific advances entail separating the active ingredients and copying their structure. The lack of uniform composition, dosage, and quality is still a challenge. When paired with contemporary techniques like solid-phase peptide synthesis and bioorthogonal chemistry, traditional Chinese medicine provides a wealth of knowledge[11][12]

Novelty and Scope of the Present Review

There are still a few comprehensive reviews that address every facet of the translational pipeline, despite the numerous reviews that have been published on antimicrobial peptides (AMPs), peptidomimetics, peptide synthesis, or antimicrobial mechanisms independently. By combining intelligently designed peptides, SAR research, advanced synthesis methods (solid-phase peptide synthesis, microwave-assisted synthesis, green chemistry, click chemistry, and bioorthogonal chemistry), pharmacology studies, quality control, and clinical translation into a single image, the current review offers a comprehensive overview. Additionally, novel developments, including nanoparticle-based delivery methods, TCM-inspired peptide scaffolds, aided AI peptide discovery, and bioorthogonal chemistry applications in next-generation antimicrobial medicine, are significantly addressed. In order to combat antibiotic resistance, this comprehensive review attempts to offer a translational guideline for logically developing and refining peptide-based antimicrobial medications[13][14].



Rohan Solanki *et al.*,**Classification and Design of Peptide-Based Antimicrobials****Natural Antimicrobial Peptides (AMPs)**

Endogenous molecules of the innate immune system, antimicrobial peptides (AMPs) are found in bacteria, plants, insects, and humans. AMPs, which are typically made up of 10 to 50 amino acid residues, are cationic and amphipathic, allowing them to attach themselves specifically to the negatively charged surfaces of microbes. AMPs can be loop, extended, α -helical, or β -sheet peptides, depending on the secondary structure. AMPs most frequently work by disrupting membranes using barrel-stave, toroidal pore, or carpet models; however, some of them also operate as inhibitors of intracellular activities such as the creation of DNA, RNA, and proteins[15][16][17].

Synthetic Peptides

The synthetic peptides were developed to get around issues with toxicity and stability, among other issues. Solid phase peptide synthesis makes it possible to rationally modify peptides utilizing a variety of techniques, including as amino acid substitution, end-group manipulation, peptide cyclization, and sequence length reduction, all of which increase the peptides' efficacy, selectivity, and resistance to degradation. This method offers useful data regarding the SAR of possible leads[18][19].

Peptidomimetics (β -Peptides, Peptoids, Stapled Peptides)

Chemically modified peptide mimics known as peptidomimetics have biological activity that is comparable to that of natural peptides and have better pharmacokinetics and high stability. β -peptides, peptoids, and stapled peptides are examples of peptidomimetics because they have stable secondary structures and are extremely resistant to protease degradation. Future antimicrobial medications may employ peptidomimetics[20][21].

Structure–Activity Relationships (SAR) of Peptide-Based Antimicrobials

Since chemical properties and biological activity are correlated, SAR analysis is essential for the logical design of peptide antimicrobials. A balanced ratio of net positive charge, hydrophobicity, amphiphilicity, peptide length, secondary structure, and amino acid makeup provides the antimicrobial peptides' effectiveness. While a high charge can be more harmful, a positive charge between +2 and +9 promotes electrostatic contact with negatively charged bacterial cell membranes. In addition to ensuring the capacity to insert into and disrupt membranes, the right ratio of hydrophobic to amphiphilic characteristics prevents aggregation, hemolytic activity, and non specificity brought on by high hydrophobicity. The peptide's length, which typically ranges from 10 to 30 amino acids, is also important. Protease stability, membrane interaction, and pharmacokinetics can all be enhanced by secondary structure and amino acid composition, particularly the presence of D- or unnatural amino acids. Together, these factors can be optimized to create antimicrobial peptides that are more effective and less harmful[22][23][24][25]

Synthetic Strategies for Peptides and Peptidomimetics**Solid-Phase Peptide Synthesis (SPPS)**

Because of its great efficiency, repeatability, and automation-friendliness, Merrifield's solid phase peptide synthesis (SPPS) technique remains the standard for peptide and peptidomimetic synthesis. Because of its easy conditions and tolerance for different groups, the Fmoc-based approach is used to synthesize peptides via stepwise couplings and deprotections on a solid platform. Although the speed and quality of peptide synthesis have increased thanks to modern automated peptide synthesizers, there are still drawbacks such as low yield of lengthy and hydrophobic peptides, racemization, poor coupling efficiency, and peptide aggregation[26][27][28].

Solution-Phase and Hybrid Approaches

Despite being more complex than SPPS because purification procedures must be taken after each reaction, solution phase synthesis is nevertheless a useful technique for producing tiny peptides and peptide fragments with extremely high purity. To create longer and more complex peptides, a combination approach combines SPPS with solution-phase ligation techniques such as native chemical ligation (NCL) [29][30].



Rohan Solanki *et al.*,**Microwave-Assisted and Green Synthesis**

Microwave-assisted peptide synthesis speeds up coupling and deprotection reactions, which shortens reaction times while raising yield rates and reducing byproduct production. Environmentally friendly solvents, recyclable resins, solvent-free or minimal solvent procedures, enzyme catalysts, and flow chemistry are some of the ways that green chemistry approaches enhance sustainability [31][32][33].

Click Chemistry and Bio-Orthogonal Methods

The click reaction and bioorthogonal chemistry are two extremely effective and selective ways to modify peptides. While the SPAAC click reaction eliminates the need for hazardous copper catalysts and permits its application in biological systems, the CuAAC click reaction has been widely used for peptide coupling. Other instances of bioorthogonal processes include inverse electron-demand, Staudinger ligation, and oxime ligation. Diels-Alder chemistry enables the site-specific tagging of peptides with targeting moieties, medications, and fluorescent dyes [34][35][36].

Post-Synthetic Modifications

The biological activity of antimicrobial peptides have often been customized using post-synthetic modification techniques. While lipidation promotes affinity for the membrane surface and antibacterial activity, PEGylation results in enhanced circulation and lower immunogenicity, while cyclization increases stability and protease resistance. Peptide stability and delivery are also improved by other techniques like glycosylation and nanoparticle coupling [37][38][39].

Integrated Structure–Activity Relationship (SAR) Framework for Rational Peptide Design

Cationic charge, hydrophobicity, amphiphilicity, length of the peptide chain, and secondary structures are just a few of the physicochemical characteristics that work together to mediate the mechanism of action of antimicrobial peptides (AMPs) and peptidomimetics. While an excessive amount of positive charge can lead to greater cytotoxicity, the positive charge (+2 to +9) permits electrostatic contact with negatively charged bacterial membranes. While an overabundance of hydrophobic residues might result in hemolysis and non-selective contact with other membranes, hydrophobic and amphiphilic equilibrium leads to strong membrane recognition and destabilization. Because medium-length peptides provide the best balance between antibacterial effectiveness, chemical stability, and simplicity of production, peptide length is also important. In addition to increasing the molecule's affinity for the membrane and resistance to proteolysis, secondary structures such as α -helix, β -sheet, cyclic peptides, and peptidomimetic scaffold stabilize the molecule [40][41][42].

Mechanisms of Action**Membrane Disruption Models**

The majority of antimicrobial peptides work by rupturing membranes. Because of their positive charges, they can readily interact electrostatically with the negative charges found in bacterial membranes, causing membrane permeabilization. Antimicrobial peptides have three primary modes of action: barrel stave, in which peptides create pores in transmembrane regions; toroidal pore, in which peptides and lipid heads form the lining of the pore; and carpet model, in which peptides build up on the membrane's surface and induce detergent-like membrane destruction [42][43][44].

Intracellular Targets

Certain AMPs and peptidomimetics can infiltrate bacterial cells and interfere with crucial intracellular processes in addition to rupturing membranes. Some of them could be inhibitors of cell wall construction, RNA transcription, DNA replication, protein synthesis, enzymes, or oxidative stress caused by ROS. Proline-rich AMPs, which inhibit bacterial ribosomes without altering their membranes, are well-known examples [45][46][47].



**Rohan Solanki et al.,****Immunomodulatory Effects**

A growing amount of research indicates that many AMPs have immunomodulatory activities in addition to their intrinsic antimicrobial qualities. These include controlling the secretion of cytokines and chemokines, suppressing inflammation (for example, by blocking the NF- κ B signaling pathway), attracting and activating leukocytes, and promoting wound healing through angiogenesis and tissue repair [48][49].

Multi-Target Mechanisms

AMPs can target numerous locations by breaking cell membranes, blocking their intracellular processes, and regulating immunity, in contrast to other antibiotic medications that primarily target one site. Peptidomimetics that are logically created for these combination actions improve stability, potency, and efficiency while lowering the risk of drug resistance [50].

Critical Comparison of Membrane-Dependent and Membrane-Independent Mechanisms

Antimicrobial peptides (AMPs) use both membrane-dependent and membrane-independent mechanisms, most of which work in concert. When it comes to membrane-dependent actions, the AMP works electrostatically on the negatively charged bacterial membranes prior to membrane insertion, pore creation, and membrane disruption, which results in quick cell lysis and decreased resistance to antimicrobial activity. On the other hand, in membrane-independent activities, the AMP is carried into the cytoplasm of the bacteria, where it influences the synthesis of DNA, RNA, and proteins, the activity of enzymes, the creation of cell walls, and oxidative stress caused by reactive oxygen species (ROS). Examples of these compounds that target bacterial ribosomes are proline-rich AMPs [15]. These pathways are supported by experimental data. While the intracellular mechanisms have been experimentally supported by DNA/RNA binding assays, ribosome binding assays, enzyme inhibition assays, ROS analysis, and transcriptional or proteomic studies, membrane disruption has been demonstrated through membrane permeabilization and depolarization assays, liposome leakage assays, and electron microscopy studies. In contrast to conventional antibiotics that target certain biological activities, these two strategies work in concert to make these compounds function as broad-spectrum antimicrobial agents with lower risks of resistance [51].

Pharmacological Evaluation**In Vitro Antimicrobial Assays**

The initial step in identifying the antibacterial qualities of peptides and peptidomimetic substances is in vitro screening. The two primary metrics used to assess the antimicrobial efficacy against Gram-positive, Gram-negative, and fungal bacteria are minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The kinetics of bacterial cell lysis are ascertained by time kill tests, while the mode of action and toxicity are ascertained by hemolysis, permeabilization of the bacterial membrane, and mammalian cytotoxicity experiments [52][53][54].

In Vivo Models

To demonstrate the effectiveness and safety of the possible medication candidate, additional testing is conducted on animal models of infection, such as sepsis, cutaneous infection, lung infection, and biofilm development. The survival rate, bacterial load, inflammatory responses, histopathological alterations, and PK/PD parameters are significant factors. Despite the challenges related to costs, ethical issues, and interspecies differences, comparative studies with current antibacterials and combination therapies improve the therapeutic potential [55][56].

Traditional Chinese Medicine-Derived Antimicrobial Peptides and Their Translational Potential

TCM is a valuable source of biologically active natural compounds that could be the foundation for the development of peptide drugs. With a wide range of antibacterial and immunomodulatory qualities, peptides and bioinspired peptides such as jineol, Pseudin-2, and thanatin are interesting models for peptide engineering and design. Although TCM-derived peptides have greater structural variety than their conventional counterparts, they have low bioavailability, rapid cleavage, and poor metabolic stability. However, modern engineering techniques like cyclization, D-amino acid substitution for L-amino acids, PEGylation, and nanoparticle distribution can be used to



**Rohan Solanki et al.,**

overcome these disadvantages. Therefore, peptides produced from TCM could be considered additional scaffolds for the generation of antimicrobial peptides [57][58].

Comparative Analysis of Natural Antimicrobial Peptides, Synthetic Peptides, and Peptidomimetics

The distinct characteristics of each type of antimicrobial medications—natural AMPs, synthetic peptides, and peptidomimetics—make their application beneficial in the creation of novel antimicrobial agents. Although they have a wide range of activity and immunomodulatory properties, natural AMPs have poor pharmacokinetics and stability. Although the sequence, specificity, and effectiveness of antimicrobial action can be rationally altered with synthetic peptides, they are vulnerable to enzyme degradation. Compared to other peptides, peptidomimetics have the advantage of avoiding the majority of the drawbacks of other peptide types, including poor membrane penetration, limited stability, and unfavorable pharmacokinetics. Peptidomimetics have the most potential among antimicrobial peptides, despite being more difficult to manufacture and control [59][60].

Safety, Toxicity, and Pharmacokinetics

Cytotoxicity and Hemolysis

Hemolytic activity and cytotoxicity are important factors in determining the toxicity and selectivity of AMPs. High cationic charge and hydrophobicity can raise the danger of mammalian cell toxicity, even though AMPs are known to operate selectively on microorganisms. Techniques such as rational peptide design by cyclization, hydrophobicity, and amino acid changes improve selectivity and reduce toxicity[61][62].

Stability and Degradation

However, proteolytic cleavage causes the majority of peptides to have low stability. Peptides' half-lives and metabolic stability are enhanced by cyclization, the use of D-amino acids, alteration at the ends of the peptides, pegylation, lipidation, and peptidomimetic approaches [63][64].

Immunogenicity

Peptides are less immunogenic than proteins, but regular administration can lead to the production of ADAs, which lowers the efficacy of the medication. This is accomplished by pegylation, substitution of D amino acids for L amino acids, sequence changes, and appropriate formulation techniques [65][66].

ADME Considerations

Rapid proteolysis, poor oral bioavailability, and renal clearance frequently limit peptide pharmacokinetics. Peptidomimetics, chemical changes, and nanocarrier-based delivery systems enhance tissue distribution, stability, absorption, and overall pharmacokinetic performance [67][68].

Bacterial Resistance to Antimicrobial Peptides and Rational Engineering Strategies

Although AMPs are more resistant to bacterial resistance than conventional antibiotics, bacteria use efflux pumps, biofilm formation, cell membrane modification, proteolytic enzyme release, and changes in cell membrane charge to counteract the effects of AMPs. Applying logical techniques like D-amino acid substitution, cyclization, peptidomimetics, charge and hydrophobicity adjustment, nanocarrier-mediated delivery, and a combination approach effectively improves the peptide's stability, boosts its antimicrobial potency, and lowers resistance[69].

Challenges in Large-Scale Industrial Manufacturing of Peptide Therapeutics

Large-scale manufacturing of antimicrobial peptides still confronts a number of challenges because of issues with scalability, purification, batch uniformity, and high production costs, despite notable advancements in the field of peptide synthesis. Even though SPPS is the conventional method of peptide synthesis, its low yield, inadequate coupling, and aggregation make it ineffective for the synthesis of lengthy and complex peptides. Although HPLC is a dependable and effective purification method, it is too expensive, requires a lot of solvents, and is difficult to scale up, making it a significant contributor to high manufacturing costs [70]. During the regulatory process, where





process validation, characterization, and control become effective, batch consistency becomes crucial. Additionally, the commercialization of peptides is hampered by the high cost of amino acids, reagents, purification, and GMP. These issues can be resolved and the next generation of peptide therapies developed with the aid of modern technologies like automated peptide synthesis, microwave-assisted synthesis, green chemistry, continuous purification, process analytical technology (PAT), and AI-assisted process optimization[71].

Peptide-Based Antimicrobials in TCM Context

Antibacterial peptides and peptide-inspired scaffolds with anti-inflammatory, immune modulatory, and antibacterial qualities are widely available in Traditional Chinese Medicine (TCM). The identification of these molecules with biological activity has been made easier by recent developments in the domains of proteomics, bioinformatics, and mass spectrometry. Significant challenges include inconsistent herbal resources, a lack of standardization, and an inadequate pharmacological profile. Novel antimicrobial drugs may be developed by combining TCM-derived peptide-inspired scaffolds with peptide design and synthetic chemistry[58][72][73].

Challenges Limiting the Clinical Translation of Antimicrobial Peptides

Despite encouraging results in pre-clinical research, few antimicrobial peptides (AMPs) have been used in clinical settings because of a multitude of obstacles, including scientific, pharmacokinetic, manufacturing, regulatory, and financial ones. The problems include cytotoxicity, rapid hydrolysis, low oral bioavailability, short half-life in plasma, and lack of action under physiological settings. Strict regulations, complicated purification, high manufacturing costs, and challenges in scaling up production are further problems. These obstacles would be overcome by innovative approaches in peptide design, nanostructure delivery, artificial intelligence-based design, sustainable production, and regulatory requirements[74].

Clinical and Translational Perspectives

Clinical Trials and Approved Peptide Drugs

The FDA has approved numerous other antibiotics, including Colistin, Polymyxin B, and Daptomycin, for the treatment of MDR bacterial infections. To find novel peptides with improved pharmacological, biophysical, and pharmacokinetic properties through peptide manipulation through genetic engineering and chemical changes, clinical studies are presently being conducted [75][76].

Challenges in Clinical Translation

Numerous challenges, including limited bioavailability, proteolysis, short half-life, cytotoxicity, immunogenicity, and costly manufacture, impede the therapeutic advancement of antimicrobial peptides. However, strategies like chemical modification and the creation of innovative drug delivery techniques have significantly improved the stability and efficacy of peptides[77].

Regulatory Considerations

Good production Practices, verified production processes, strict quality control standards, and comprehensive preclinical and clinical evaluations based on the specified regulatory requirements (e.g., FDA, EMA, and ICH) are all necessary for peptide therapeutic therapies. Clinical success still depends on manufactured standardization, batch homogeneity, pharmacovigilance, and post-market monitoring[78].

Integration of Artificial Intelligence with Experimental Validation for Antimicrobial Peptide Discovery

Through faster screening of peptide libraries, the prediction of antimicrobial efficacy, toxicity, stability, and pharmacokinetics, and quick lead optimization, developments in AI and ML have had a substantial impact on AMP research. However, these computational methods require experimental validation using peptide synthesis, MIC testing, hemolysis and cytotoxicity investigations, membrane penetration tests, pharmacokinetic evaluation, and in vivo infection models. AI models must incorporate experimental findings through iterative design-build-test-learn (DBTL) cycles. Despite their potential to transform AMP discovery, there are still issues with forecast inaccuracy,





poor explainability of the models, and bias in training datasets. Standardized training sets, AI, external model validation, and high throughput experiments will increase the reliability of AI-guided AMP discovery and accelerate the development of new antimicrobial therapies [79].

Translational Roadmap for Next-Generation Antimicrobial Peptide Therapeutics

AI, peptide engineering, bio-orthogonal chemistry, nanotechnology, and testing are all integrated in the translational development of AMPs. Peptide engineering improves stability, specificity, and pharmacokinetics, while AI facilitates quick screening and SAR investigations of AMP candidates. The potency of such candidates can also be increased by using nanocarrier-based delivery methods and bio-orthogonal chemistry. DBTL must be used to test optimized peptides employing in vivo infection models, pharmacokinetic research, and in vitro antimicrobial investigations. Last but not least, successful translation depends on GMP production, regulatory compliance, and clinical evaluation. This integrated method can offer helpful direction for the creation of AMP treatments that are usable in clinical settings.

Emerging Trends and Future Directions

Interdisciplinary advances in artificial intelligence (AI), nanotechnology, bio-orthogonal chemistry, and precision medicine are reshaping the development of antimicrobial peptides (AMPs) to address antimicrobial resistance (AMR).

AI-Driven Peptide Design

AI and machine learning enable rapid identification and optimization of AMPs by predicting antimicrobial activity, toxicity, stability, and structure–activity relationships (SAR), thereby accelerating lead discovery while reducing development time and cost [80][81].

Personalized Antimicrobial Therapy

Advances in genomics, proteomics, and precision diagnostics support the development of personalized peptide therapeutics tailored to pathogen characteristics and patient-specific factors, improving efficacy while minimizing off-target effects[82][83].

Nanocarrier-Based Delivery

Nanocarriers, including liposomes, polymeric nanoparticles, dendrimers, and lipid nanoparticles, enhance peptide stability, bioavailability, targeted delivery, and controlled release, thereby improving therapeutic efficacy [84][85].

Bio-Orthogonal In Vivo Applications

Bio-orthogonal reactions, such as strain-promoted azide–alkyne cycloaddition (SPAAC) and inverse electron-demand Diels–Alder chemistry, enable site-specific peptide labeling, activation, and targeted delivery under physiological conditions, facilitating precision antimicrobial therapy. Overall, integrating AI, peptide engineering, nanotechnology, and bio-orthogonal chemistry is expected to accelerate the discovery, optimization, and clinical translation of next-generation antimicrobial peptide therapeutics [86][87][88].

CONCLUSION

When treating AMR, AMPs and peptidomimetics may be useful alternatives to conventional antibiotics. Peptide design and functionalization can be enhanced by the advancement of peptide chemistry and synthesis technologies such as solid-phase peptide synthesis (SPPS), microwave-assisted synthesis, click chemistry, and bio-orthogonal techniques. Because of their methods of action, which include intracellular activity, immunomodulation, and cell membrane rupture, peptides exhibit a wide range of effects. Therefore, it is unlikely that peptides would have the same issues related to the emergence of AMR. However, there are still several issues with these peptides' instability, toxicity, pharmacokinetics, manufacturing method, and clinical application. Researchers were able to enhance the



**Rohan Solanki et al.,**

drug-like properties of peptides with the aid of peptide engineering and nanotechnology in delivery systems and appropriate formulation. Furthermore, peptides with antibacterial properties can be created using the framework of peptides from traditional Chinese medicine. The application of artificial intelligence in peptide design, bio-orthogonal chemistry, nanotechnology, and interdisciplinary experiments will be linked to the next stage of peptide research.

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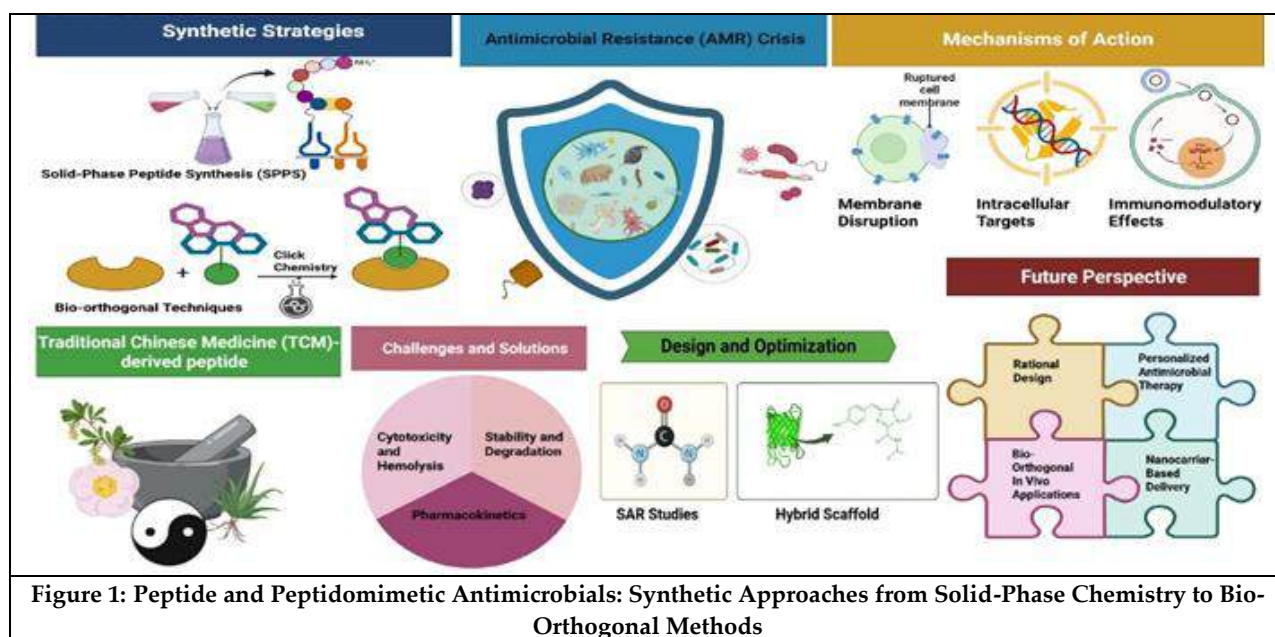
Table 1: Classification and Structural Features of Peptide and Peptidomimetic

Class	Motif Sequence	Net Charge (pH 7.4)	Hydrophobicity Index (%)	Secondary Structure	Key Structural Modifications	Primary Mechanism of Action	Spectrum of Activity	Limitations
Natural AMPs (α -helical)	Magainin	+4 to +6	40–50%	α -helix	C-terminal amidation	Disruption of membranes	Gram+, Gram–, and Fungi	Moderate toxicity and proteolytic instability
Natural AMPs (β -sheet)	Defensins (Cys-rich peptides)	+3 to +7	30–40%	β -sheet	Disulfide bridge formation	Membrane permeabilization	Gram+ / Gram– / Virus	Complex synthesis, stability issues
Extended AMPs	Indolicidin (Trp-rich)	+4	45–55%	Random coil	Trp-rich motif	DNA binding + membrane disruption	Gram+ / Gram– / Fungi	Cytotoxicity, hemolysis
Synthetic Linear Peptides	Designed Lys-Leu-rich sequences	+5 to +9	45–60%	α -helix	Sequence truncation, substitution	Membrane lysis	Broad spectrum	Toxicity at high doses
Cyclic	Gramicidin	+2	50–60%	β -sheet /	Cyclization	Membrane	Gram+ /	Hemolytic



Rohan Solanki *et al.*,

Peptides	n S			cyclic		disruption	limited Gram-	toxicity
β -Peptides	β -amino acid oligomers	+3 to +6	35–50%	Helical (β -helix)	Backbone modification	Membrane targeting	Gram+ / Gram-	High synthesis cost
Peptoids	Nsubstituted glycine oligomers	+2 to +5	40–55%	Random / helical-like	Side-chain modification	Membrane disruption	Broad spectrum	Reduced specificity
Stapled Peptides	Hydrocarbon-stapled α -helices	+3 to +7	45–55%	Stabilized α -heli	Stapling (olefin metathesis)	Intracellular targeting + membrane	Gram+ / Virus	Cost, synthesis complexity
Hybrid Peptidomimetics	AMP-antibiotic conjugates	Variable (+2 to +8)	40–60%	Mixed	Conjugation, PEGylation	Dual mechanism	Broad spectrum	Stability, PK variability
D-Amino Acid Peptides	D-enantiomeric AMPs	+4 to +8	40–50%	α -helix / random	D-amino acid substitution	Membrane disruption	Broad spectrum	Cost, regulatory challenges



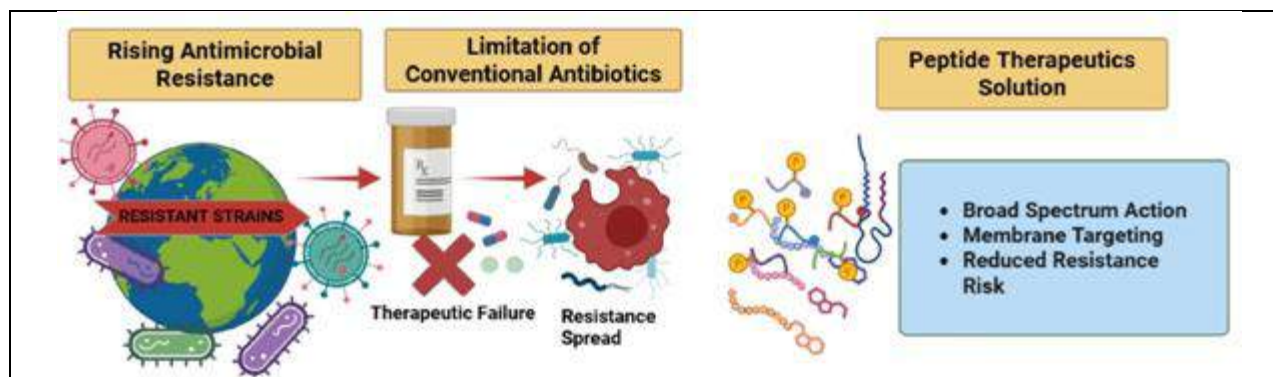


Figure 2: Global AMR Burden and Role of Peptide Therapeutics

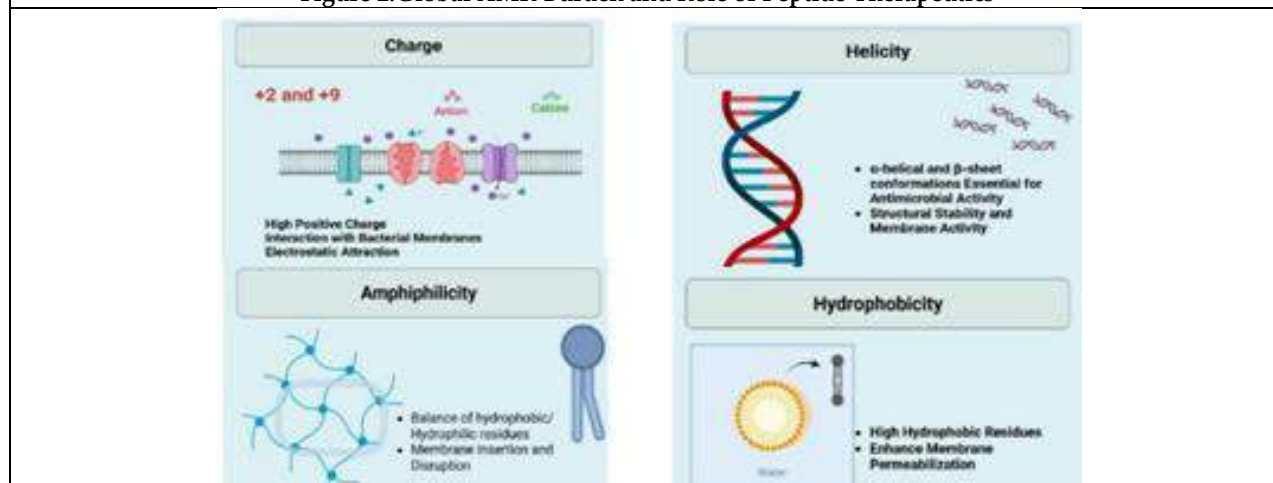


Figure 3: Structure-Activity Relationship (SAR) Determinants of Antimicrobial Peptides

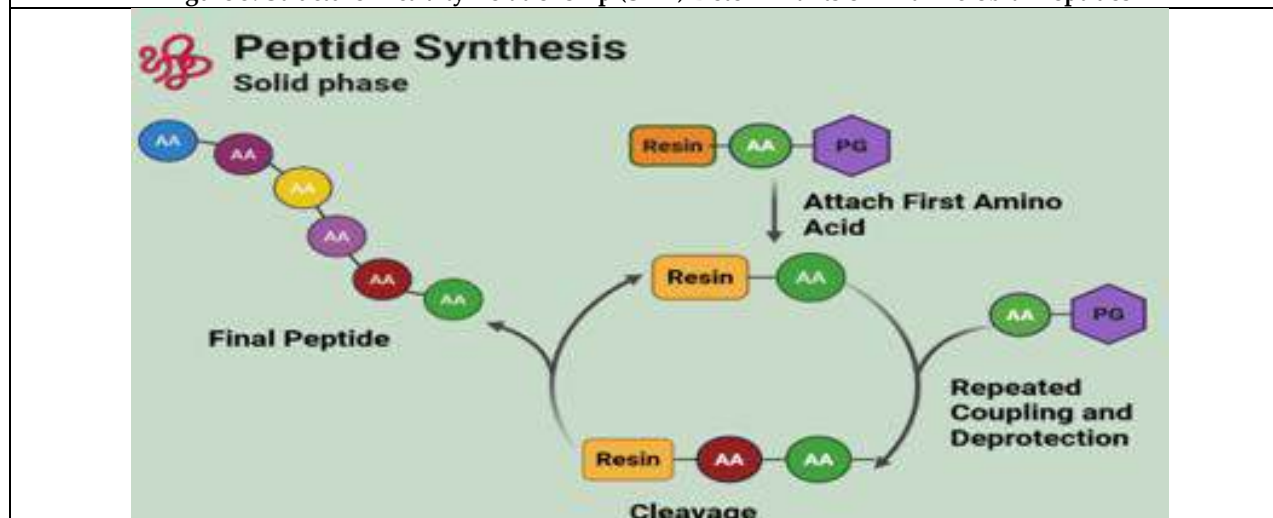


Figure 4: Synthetic Workflow of SPPS



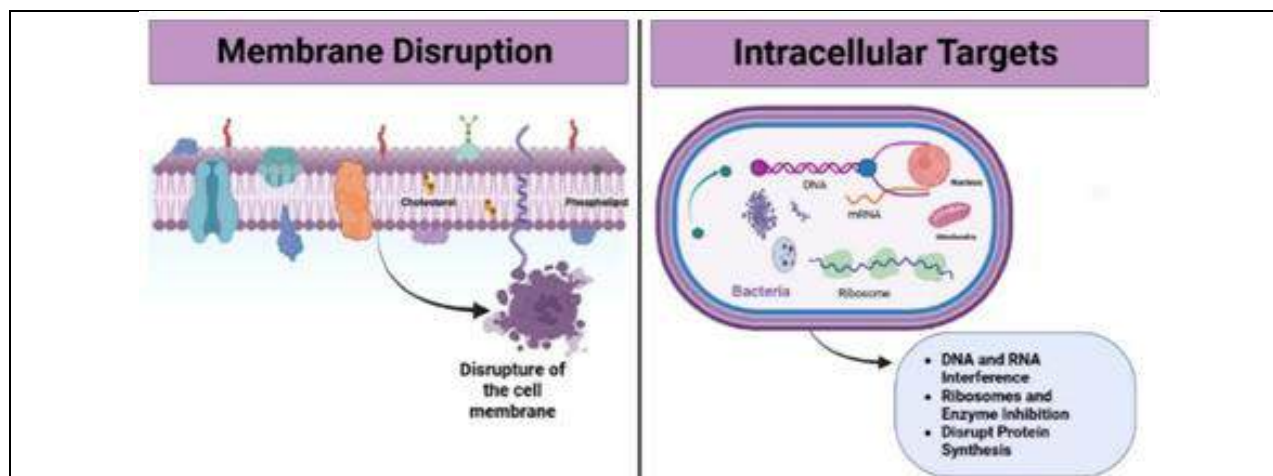


Figure 5: Mechanisms of Action of Antimicrobial Peptides

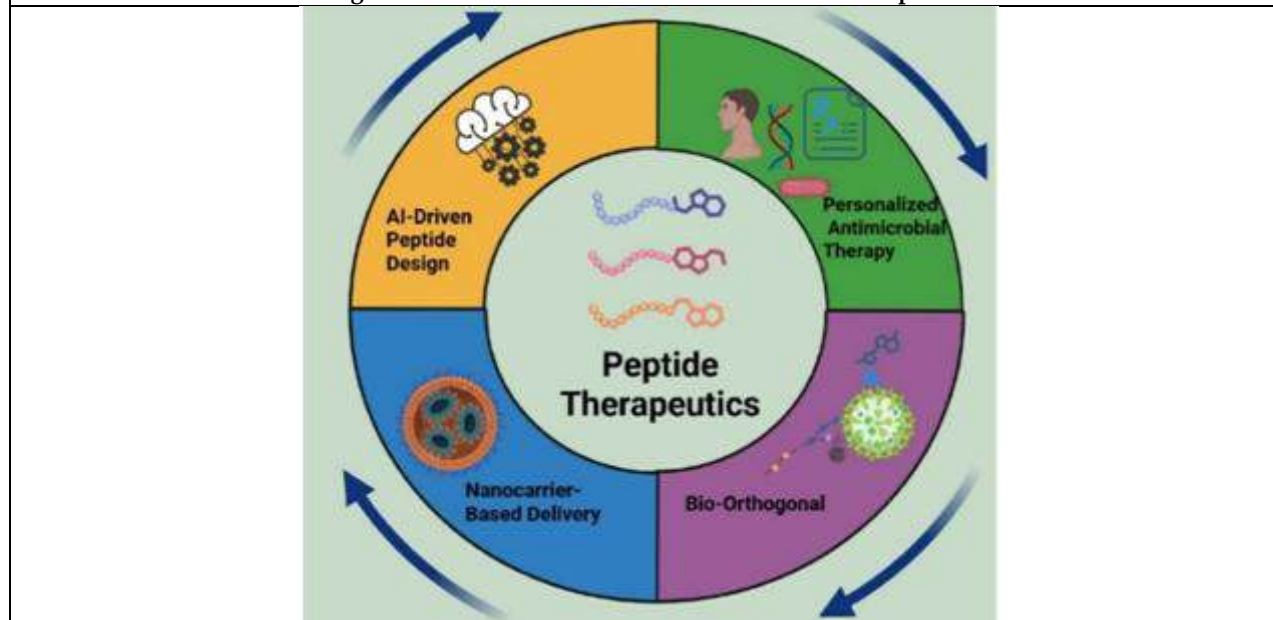


Figure 6: Future Perspective: Integrated Platform for Peptide Drug Development

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RESEARCH ARTICLE

Evaluating the Nutritional Safety Profiles of Dried *Scomberomorus guttatus* in Rameswaram, Tamil Nadu, India

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ABSTRACT

Sun drying is considered the least expensive and efficient food preservation methods in Tamil Nadu and the product plays an important role particularly in providing nutrition to the poor and economically disadvantaged people, especially in remote areas with few rainfall and seasonal water streams. The prime objective of this study is to analyze the nutritional, microbial, sensory and heavy metals content of sun and turmeric treated dried *S. guttatus* collected from local fish market at Rameswaram. The dried fish products available in the markets of Rameswaram were found to have high nutritional value. Both the dried samples of *S. guttatus* were found to have overall acceptability score in the range of 8.86 and 9. The sensory evaluation of the both the samples revealed that maximum overall acceptable quality. After one year of storage dried samples were found to have decline in overall acceptability score. The highest TVC was recorded in sun dried samples (4.2×10^3) and the lowest value was recorded in turmeric treated dried samples (4.0×10^3) and after one year of storage the highest TVC was 16×10^3 while lowest was 14×10^3 respectively. Though the absence of *E.coli* and *Salmonella* species was a good sign for consumers, the presence of *S. aureus* in the product indicates slight unhygienic handling during processing and storage.

Keywords: *Scomberomorus guttatus*, Dried Fish, Nutritional quality, Microbial quality and Sensory Quality

INTRODUCTION

Dried fish is one of the traditionally preserved foods. Dry fish is an essential source of animal protein supplement, which is preferred as a key dish or used as a flavoring agent in combination with other food items. One hundred

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**Chitra and Vigneshpriya**

grams of dried fish contains about 80% protein with 300 calories. This profile makes dried fish a compelling purchase for those looking to increase their intake of healthy food and provides a distinct advantage of dried fish retailers. As per the recent report published by Marine Product Export Development Authority, more than Rs.2852.60 crore worth dried items exported to various part of the world (MPEDA, 2025). The consumption of dried fishes is about 32% of the total marine landings in India and this ranks second to fresh fish consumption. Dry fish is also a very favorite food item among people and has a good market demand besides fish and seafood products. It has a storage life for several years and it is consumed all over the world for its nutritional value, taste and aroma. Dried fishes are healthy white meats with low levels of cholesterol so it is recommended for complete and comprehensive diets of diabetes and obesity people. Pure dried fish is a completely natural product that retains the antioxidant and omega-3 benefits of fresh fish, while constituting a healthy and widely available food for people. It helps to mitigate the occurrence of heart attacks and keeps the body's circulation functioning well. These benefits provide key marketing tools that can make dried fish retailing a profitable business (Rimm, 2016).

Spoilage proceeds as a series of complex enzymatic, bacterial and chemical changes that start in some few hours after fish being caught depending on the species and the prevailing ambient temperature. This may render it unacceptable and unfit for human consumption. Plenty of fish is available during the fishing season when the fishermen for want of speedy transport facilities cannot send to the market for sale. Thus, the fishermen either have to sell their catch at a cheaper rate or they have to let their catch go waste due to lack of proper processing and strong facilities. If the bulk could be processed or preserved for short term or long-term period and stored successfully the requirement of the off season would have been met up to a significant extent. Proper preservation starts the moment it is harvested until reaches the consumer's table (Oluborode *et al.*, 2010). Traditionally, fishermen often resort to salting and drying in order to preserve and save surplus fish harvested during peak harvest seasons for use during off seasons. In addition, some high value products such as shark fin fish, seer fishes and fillets are dried for export markets (Al-Ghabshi *et al.*, 2012). Efforts should therefore, be geared towards increased fish production through improved resource management matched with effective postharvest handling, preservation and processing to prevent spoilage. Consequently, research is needed to improve their shelf life and nutritional values in traditional preservation techniques, so in the peak harvesting periods, these fish could preserve properly to serve in lean periods as a cheap and immediate source of animal protein to all classes people (Latifa *et al.*, 2014).

The salting process is considered as one of the oldest method of fish preservation and this process is still being used in several places around the world. Salted fish products are popular in many countries and it has been proved to be safe for the millenniums even in developed countries (Turan *et al.*, 2007). Salting is generally aimed at reducing water activity (aw) which inhibits the growth of spoilage microorganism as well as to inactivate autolytic enzymes (Ashie *et al.*, 1996). The aim of salting is not only to extend the shelf life of fresh fish but also to provide desirable sensorial changes (Andres *et al.*, 2005). The quality of salted and sun dried fishes are adversely affected by the occurrence of microorganisms. Determination of microbiological quality of such processed fishes from the market is very important for guarding consumer's health and hygiene (Lilabati *et al.*, 1999). The spoilage of dried fish is mainly due to bacterial, fungal or yeast action, rancidity, autolysis, browning and other reactions, all of which are temperature and water activity dependent (Doe, 1982). The fish usually takes 5 to 7 days to dry during which it gets heavily contaminated. High levels of metals have been to cause rapid physiological changes in fish (Tarrio *et al.*, 1991). These toxic wastes are usually industrial wastes, sewage, pesticides and heavy metals such as Mercury and Cadmium. The pollutants especially Mercury could possibly end up in human bodies due to the consumption of affected fish (Atuanya and Okoro, 2007). There is increasing concern about the quality of foods in several parts of the world; the determination of toxic elements in processed food has prompted studies on toxicological effects of them in food (Khansari *et al.*, 2005). The risk associated with the exposure to heavy metals present in dried fish products had aroused widespread concern in human health. Improvements in the food production and processing technology had increased the chances of contamination of food with various environmental pollutants, especially heavy metals (Santhi *et al.*, 2008).



**Chitra and Vigneshpriya**

The proximate composition is an important aspect of dried fish quality and it influences both the keeping quality and technological characteristics of the fish. The main chemical components such as water, crude protein, lipid and ash have the largest impact on the nutritive value, the functional properties, and the sensory quality and storage ability of flesh. Therefore, information on the nutritive value and its food quality are equally important regarding the proper utilization of products (Flowra and Tumpa, 2012). But nutritional composition varied in large scale in different dried fish product. Hence, it is necessary to ensure the nutritional value as well as quality of the dried fish products. Now a days various herbal preservatives are used in drying process and to improve the quality of dried fish. Use of herbal preservatives such as turmeric powder is most commonly used in India. Turmeric powder act as a repelling effect of beetles and mites associated with dried fish (Akter *et al.*, 2013). In tropical area of Rameswaram, apart from the fresh fish some quantity of the fishes used for salting and sun drying and used as a dried fish in local and export market. The study was aimed at understanding the safety of dried fish products after the drying process and to identify the microorganisms that contaminate dried fish and made appropriate recommendations for mitigation.

Prakash *et al.*, (2011) determined the microbial quality of six sundried seafood species available in Tuticorin dry fish market, South East Coast of India. An analysis of biochemical with minerals composition of three different fish species by drying methods (*Bonga* species, *Sardinella* species and *O. niloticus*) was done by Priya *et al.*, (2011). Saritha *et al.*, (2012) carried out a study on microbial and biochemical qualities of seven salted and sun dried sea foods of Cuddalore, South East Coast of India. Quality and safety aspects of three sun dried marine fish species Chinese pomfret (*Stromateus chinensis*), Bombay duck (*H. nehereus*) and ribbon fish (*Trichiurus haumela*) was discussed by Pravakar *et al.*, (2013). Latifa *et al.*, (2014) evaluated the shelf life quality of salt and turmeric treated fishes, chapila (*G. chapra*), Kaika (*Xenentodon cancila*), Guchi Baim (*Mastacembelus pancalus*) and also analyzed the biochemical composition and sensory evaluation during storage at refrigeration temperature. Igwegbe *et al.*, (2014) evaluated the effects of season and a fish preservation process namely salting and sun drying (using a solar tent dryer) on the levels of Lead, Cadmium, Mercury and Arsenic in the fresh and processed fish species, *Tilapia nilotica*, *Synodontis guentheri*, *H. niloticus* and *C. anguillaris*. Influence of drying processing on nutritional properties of three fish species Govazym stranded tail, Hamoor and Zeminkan was observed by Aberoumand and Abad (2015). Bhuyan (2016) studied some indigenous fish preservation techniques practiced in Jorhat District, Assam, India. Sahu *et al.*, (2018) reviewed the economics of fish drying in Digha Mohana Khuti, West Bengal, India. Nutritional and microbial quality of dried Nile bulti *Oreochromis niloticus* (Trewavas) tested by Ali *et al.*, (2021) and proximate composition, microbiological quality and safety of raw and open sun-dried fish products in Lake Tana, Ethiopia reported by Birie *et al.*, (2025). With this background, the present investigation was designed to carried out in order to assess the nutritional, microbial, sensory and heavy metals content of sun and turmeric treated dried *S. guttatus* collected from local fish market at Rameswaram., Tamil Nadu, South India.

MATERIALS AND METHODS

Sample collection

The experimental samples of sun dried and turmeric treated dried fish (Local name: Pattara karuvadu) of *S. guttatus* were collected from local fish market at Thangachimadam in Rameswaram Island. The collected samples were taken to the laboratory in an airtight glass container and stored at room temperature for subsequent studies. The analyses were carried out in triplicates of each sample of dried fish.

Sample preparation

Sun dried fish: The fishes were washed in the sea water and immersed in salt water solution for 24 hours and dried for 2 - 4 days on a sea shore sandy area or on palm leaves under the direct sun. The ratio of salt used for salting of fish is too low to ensure adequate preservation.



**Chitra and Vigneshpriya****Turmeric treated dried fish (Pattara karuvadu)**

The fishes were washed in the sea water and split longitudinally, after that they were removed and cleaned properly. The fishes were filled with salt and dumped inside the sand for 7 days. After 7 days the fishes were removed from the sand and coated with turmeric powder. Both the dry samples were taken accurately and each sample was crushed with mortar and pestle. The samples were analyzed for physicochemical composition, proximate composition, microbial and heavy metals content.

Biochemical and Microbial analysis of dried fish:

pH value, protein, carbohydrate, lipid and ash contents of the dry fish samples were measured by following the method of AOAC (2005). For microbial analysis one gram (1g) of dry fish sample was dissolved in sterile deionized water and serially diluted. One milliliter (1ml) of appropriate dilutions was seeded on plate count agar using spread plate method, and the medium was then incubated at 37°C for 24 hours. The plate count agar was examined and colonies present were counted and recorded after incubation at 37°C for 24 hours to get the total colony count in Cfug (Obi and Krakowiakass, 1983). Amine forming bacteria and fungus species were isolated using suitable agar medium (Surendran *et al.*, 2006).

Sensory analysis of dried fish

Appearance, colour, flavour, odour, texture and the overall acceptability of the dried fishes were evaluated using 9 point grading scales. The panelist was requested to rate each sensory features of the selected dried fishes. The average score of 5 was considered to be the borderline of acceptability (9 - like extremely; 8 - like very much; 7 - like moderately; 6 - like slightly; < 5 bad).

Heavy metal analysis of dried fish:**Sample preparation and heavy metal analysis**

Accumulation of metals such as Mercury, Lead, Cadmium and Chromium were estimated in both the dried samples of *S. guttatus*. The dried fish samples were crushed and powdered in a mortar and pestle. The samples were digested in concentrated nitric acid and sulphuric acid (4:1) till all the material gets dissolved. It was then filtered through Whatman No.1 filter paper and then washed with distilled water and collected in a 50 ml volumetric flask. The digested samples of muscles from *S. guttatus* were then poured into an auto analyzer cups and concentration of heavy metals (Mercury, Lead, Cadmium and Chromium) in each sample was determined using Atomic Absorption Spectrophotometer (AAS). The values of heavy metal concentrations in the tissues were calculated based on dry weights as this discounts the variability due to inner parts differences in the moisture content of organisms.

Analysis after storage period

The samples of dried *S. guttatus* collected from local fish market at Thangachimadam in Rameswaram Island were packed in an airtight glass container and stored at refrigerator temperature for the period of twelve months. The physicochemical, biochemical, microbial and sensory characteristics of sun and turmeric treated *S. guttatus* were analyzed after storage period. The analyses were carried out in triplicates of each sample of dried fish.

Statistical analysis:

The biochemical composition analysis was conducted in triplicates from three independent experiments conducted for the study. Results were expressed as mean values $\pm$ Standard deviation (SD). The difference between the sand and turmeric treated *S. guttatus* were calculated using paired 't' test and statistically significant difference were reported at $p < 0.05$.

RESULTS

Biochemical, microbial, heavy metal and organoleptic analysis were done in sun dried and turmeric treated sand dried samples of *S. guttatus*. The performance of salt and turmeric treatment against insect infestation and storage



**Chitra and Vigneshpriya**

conditions were observed using airtight containers. The drying time was shorter in sundried compared to the turmeric treated sand dried samples. Increase in temperature resulted in shorter drying time. The drying time was five days in sun dried and seven days in turmeric treated sand drying method.

Biochemical composition of dried sample

The moisture content of the sun and turmeric treated sand dried samples of *S. guttatus* was in the range of 9.2 ± 0.2 to $10.1 \pm 0.15\%$. The highest moisture content was recorded in sun dried sample ($10.1 \pm 0.15\%$) and the lowest ($9.2 \pm 0.2\%$) was in turmeric treated sand dried sample. The protein content of sun and turmeric treated sand dried *S. guttatus* ranged from 32.5 ± 0.07 to $35.7 \pm 0.1\%$. Highest protein content was observed in turmeric treated samples ($35.7 \pm 0.1\%$), while lowest in sun dried ($32.5 \pm 0.07\%$) samples. Lipid content was ranged from 0.51 ± 0.022 to $0.59 \pm 0.01\%$. The highest lipid content was found in sun dried ($0.59 \pm 0.01\%$) samples and lowest in turmeric treated dried ($0.51 \pm 0.022\%$) samples. Similarly highest carbohydrate content ($5.5 \pm 0.022\%$) was observed in turmeric treated samples and lowest ($5.2 \pm 0.2\%$) in sun dried samples. The highest ash content was found in sun dried samples ($20.8 \pm 0.12\%$) and lowest in turmeric treated dried (19.7 ± 0.21) samples. pH value is a reliable indicator of the degree of freshness or spoilage. The highest pH was observed in turmeric treated dried samples (6.88 ± 0.01) and the lowest value was observed in sun dried samples (6.79 ± 0.01).

Biochemical changes of dried samples after storage period

After one year of storage the moisture content was $71.5 \pm 0.05\%$ and $68.9 \pm 0.07\%$ in sun and turmeric treated sand dried samples. The maximum protein content was recorded in turmeric treated samples ($19.2 \pm 0.1\%$) and lowest in sun dried samples ($16.5 \pm 0.23\%$). Highest carbohydrate content recorded in turmeric treated dried ($4.2 \pm 0.26\%$) and lowest value was recorded in sun dried samples ($3.9 \pm 0.17\%$). Highest lipid and ash contents were recorded in sun dried samples ($0.39 \pm 0.012\%$ and $2.2 \pm 0.21\%$), while lowest values were recorded in turmeric treated sand dried samples ($0.37 \pm 0.01\%$ and $1.98 \pm 0.03\%$). The highest pH value was observed in turmeric treated dried samples (5.89 ± 0.01) and lowest value observed in sun dried samples ($5.79 \pm 0.01\%$) after one year storage in airtight containers.

Microbial analysis of dried samples before and after storage

The number of bacteria in foods serves as a general indicator of hygiene. Determination of total bacterial count is widely used to assess the bacterial quality of fish. The limit for TVC is 1×10^5 Cfug in dried products. The highest TVC was recorded in the sun dried samples (4.2×10^3 Cfug) and the lowest value was recorded in turmeric treated sand dried samples (4.0×10^3 Cfug). After one year of storage period, the highest TVC (16×10^3 Cfug) was recorded in sun dried samples and the lowest (14×10^3 Cfug) was in turmeric treated sand dried samples. The susceptible distribution of bacterial and fungal species presents in both the dried samples of *S. guttatus* were *E. coli*, *K. pneumonia*, *S. aureus*, *P. vulgaris*, *P. aeruginosa* and *S. typhimurium*. The distributions of fungal species present in the *S. guttatus* were *A. niger*, *M. canis* and *F. oxysporum*. *E. coli* and *S. aureus* were isolated from the sun dried samples. In turmeric treated dried samples *S. aureus* were isolated. Other bacterial species were not detected in sun and turmeric treated dried samples. After one year of storage *E. coli*, *S. aureus* and *A. niger* were isolated from sun dried samples, in turmeric treated sample *S. aureus* were isolated.

Sensory analysis of dried samples before and after storage

The mean score obtained for sensory analysis, which emphasizes colour, appearance, flavour, odour, mouth feel, texture and overall acceptability are tabulated. The products of sun and turmeric treated dried samples were found to have overall acceptability score in the range of 8.68 to 9.0. After one year of storage the sun and turmeric treated dried samples of the scores for appearance, colour, flavour, odour, mouth feel, texture, and overall acceptability were declined and the scores were declined from 4.71, 5.14, 5.28, 5.14, 5.0, 5.28 and 5.0 in sun dried sample and 5.14, 6.0, 6.5, 6.0, 6.2, 6.0 and 6.5 in turmeric treated dried samples respectively.



**Chitra and Vigneshpriya****Heavy metal analysis in dried samples**

Accumulation of heavy metal contents Mercury, Lead, Chromium and Cadmium of sun and turmeric treated dried samples were well below the detectable limit (BDL). There is no metal accumulation in both the dried samples.

DISCUSSION

Being one of the richest sources of protein, vitamins and minerals fishes are widely used as essential source of nutrients required for people. But the availability of this valuable nutrients source largely depends on the extent of salting, drying, smoking and freezing and may other types of preservation methods (Farid *et al.*, 2014). There are unsuitable maintenance techniques which make problems for access to its protein. Drying process is a treatment for maintenance of fish and other animal proteins, because its quality is preserved for long time period and little changes and lost minimum in production. Moisture plays an important role in the spoilage of fish and fish products and thereby reducing the level of moisture retards spoilage. In this study, the sun dried samples were treated with salt and turmeric treated dried samples treated with the combination of salt and turmeric. The salt treated sun dried *S. guttatus* was observed to have highest moisture content, irrespective with lowest protein, lipid and ash contents. High value of moisture content of the products studied might be due to absorption of moisture during storage and marketing. This finding was similar to the results obtained by Prodhan *et al.*, (2010) evident that, with the increase of storage period moisture content increased in the samples of *S. guttatus* (15.88 ± 1.91 to $17.8 \pm 3.12\%$). While working with *S. fimbriata*, Immaculate *et al.*, (2012) found that naturally and hygienically sundried, using solar dryer and fishy rack moisture content were 11.60%, 8.3% and 4.0% respectively. Mansur *et al.*, (2013) reported that moisture content of dried Indian major carps ranged from 19.17 to 23.99%. The present results of sun and turmeric treated dried products of *S. guttatus* was also agreed with the above studies. The moisture content of sun and turmeric treated dried *S. guttatus* from Rameswaram market (9.2 ± 0.2 to $10.1 \pm 0.15\%$) was within the acceptable levels specified by MPEDA. The samples collected from the local market in Rameswaram have acceptable level of moisture content. It may be due to quality of raw fish, proper drying and maintenance and good storage facility.

Protein is essential for the sustenance of life and exists in largest quantity of all nutrients as a component of the human body (Okuzumi and Fujii, 2000). It is desirable to have a high percentage of protein in food products which is an important factor in case of quality analysis. Protein decreased with storage of cured meat was attributed to some changes during storage that caused by Maillard reaction and changes in pH (Lawrie, 1990). Salt causes the proteins in fish muscle to swell and salt lead the protein become denatured if increases in the muscle (Hamm, 1994). A significant increase in the amount of protein in dried fishes indicates that fish protein nitrogen in drying period is not going to lose. The protein content varied significantly ($p < 0.05$) between the samples and the values were $35.7 \pm 0.1\%$ in turmeric treated and $32.5 \pm 0.07\%$ in sun dried samples of *S. guttatus*. Generally, dried fishes showed the highest protein content then compared to the fresh fishes. It may be the result of dehydration of water molecules present between the proteins thereby causing aggregation of protein and thus resulted in the increase in protein content of dried fishes. This result was also in agreement with the finding of Sankar *et al.*, (2013). Ogbonnaya and Shaba (2009) observed that protein nitrogen was not lost during drying, so the protein content increased with the reduced moisture content in the fish samples. Flowra and Tumpa (2012) found that protein content of selected dried fishes varied from 28.20% (*W. attu*) to 51.19% (*Palaemon* species). Lipids are the major sources of metabolic energy and essential materials for the formation of cell and tissue membrane (Sargent, 1995). Lipid content may be influenced by season and geographic condition (Agren, 1991).

The carbohydrate content was relatively low ($p < 0.05$) when compared to the protein content of the samples. Major function of carbohydrate is production of energy for body cells, for accumulation and shrinkage of some food molecules and many of the 93 physiological functions needs carbohydrate. Aberoumand and Abad (2015) reported that carbohydrate content of three fish species (Govazym stranded tail, Hamoor and Zeminkan) varied from 77.44 ± 0.26 to $85.66 \pm 0.26\%$. Ash content is generally influenced by size of fish. Smaller sized fish species tend to have higher





Chitra and Vigneshpriya

ash content due to the higher bone to flesh ratio (Daramola *et al.*, 2007). Generally, in the fresh fishes, the ash content was very low, and dried fishes have higher ash contents. The ash content varied significantly between the samples and the values were $20.8 \pm 0.12\%$ in sundried and $19.7 \pm 0.21\%$ in turmeric treated dried samples of *S. guttatus*. Clucas and Ward (1996) reported that inorganic contents remain as ash after the organic matter is removed by incineration. Natural sun drying was done in open space which allows settling of windborne dust, insect and bird infestation. It would increase the inorganic contents in the samples and this may be the major reasons for higher ash content in sun dried samples. In sand drying method the samples were dried under the sand so there is no insect infestation, wind borne dust, this may be the reason for lower ash content in sand dried samples. Ash content reflects fair degree of importance of a species as a source of minerals and our present findings are in agreement with other reported values. Flowra *et al.*, (2012) reported that the ash content in dried fishes ranged between 9 to 30% on the basis of moisture and dry matter contents. Patterson *et al.*, (2018) reported that ash content of the dried fish *S. commersonnii* was varied from 0.65 ± 0.83 to $0.72 \pm 1.74\%$ in different drying method. Similar work was done by Longwe and Kapute (2016), who reported that ash content of the *O. karongae* varied from 20.00 ± 0.00 to $21 \pm 1.07\%$ and Azam *et al.*, (2013) reported that 5.08 to 16.02% of ash content in four dried fishes.

pH value varied significantly between the samples and the values were 6.79 ± 0.01 in sundried and 6.88 ± 0.01 in turmeric treated sand dried samples of *S. guttatus*. The conventional air drying at a relatively higher temperature is determined to fish muscle and resulting in changes in pH, which affect solubility and water binding properties (Zyas, 1997). Decomposition of nitrogenous compounds often tends to increase in pH level in fish flesh in the post mortem period. Farid *et al.*, (2014b) found that pH ranges varied from 6.9 to 7 in the shoal fish (*C. Striatus*) and taki fish (*C. Punctatus*). All the biochemical parameters of turmeric 94 treated dried fish samples showed significant values ($p < 0.05$) when compared to sundried samples. Fish is a reservoir of large number of microorganisms. One of the major factors contributing to poor quality of the fish in retail trade is unhygienic handling and storage leading to the smell, physical damage and contamination with dirt and objectionable microorganisms (Sugumar *et al.*, 2004). Bacterial spoilage is characterized by softening of the muscle tissue and the production of slime and offensive odours. Low level of TVC was found in turmeric treated sand dried sample (4.0×10^3 Cfug) at the initial period; this may be attributed to low moisture content. High moisture content may be the reason for high level of TVC in sun dried samples (4.2×10^3 Cfug). Saritha *et al.*, (2012) found that the higher bacterial count in dried *Parupeneus indicus* (2.13×10^6 Cfug). Prakash *et al.*, (2011) reported that *S. fimbriata* collected from Tuticorin market had the highest bacterial count (5.7×10^4 Cfug) and Logesh *et al.*, (2012) also found that highest total plate count of *S. longiceps* collected from Parangipettai (5.3×10^6 Cfug⁻¹) and lowest in Pudukuppam (3.2×10^6 Cfug⁻¹), Tamil Nadu. Majumdar and Rashid (2017) found that the total plate count of dry salted Hilsa, *T. ilisha* collected from different retail markets ranged from $5.75 \pm 0.35 \times 10^6$ Cfug to $8.74 \pm 0.39 \times 10^7$ Cfug.

The results of the study revealed that *S. aureus* were the common pathogenic bacteria found associated with sun and turmeric treated dried *S. guttatus* may be due to the contamination of the fish samples by man through handling and processing. *S. aureus* is a natural microflora of fish and shellfish (Clucas and Ward, 1996). Its main habitat is humans and animals, and found mostly in the nose, throat and skin of healthy individuals. This indicates that dried fish with these bacterial pathogens, must have been contaminated the dried fish through human handlers, air and soil. The contamination with *Staphylococcus* can be prevented by adequate control over the health and hygiene of fish handlers. The present results were in parallel with Udochukwu *et al.*, (2016), who observed that the *S. aureus* in some fresh and smoked fishes sold in Benin City. The quality of the dried fish is often adversely affected by the growth of fungi (Chakrabarti and Verma, 1997) and substantial amounts of fish are discarded during drying due to fungal growth (Gupta and Samuel, 1985). At the initial stage the dried fish samples of the present study were free from visible fungal colonies but after one year of storage of dried sample, plating on potato dextrose agar revealed presence of fungi (1×10^1 Cfug) in sun dried sample of *S. guttatus*. For consumers, the perceivable sensory attributes of colour, appearance, flavour, odour, mouth feel, texture and overall acceptability are the deciding factors in food acceptance. The sensory evaluation of the product helps in ensuring that the consumers get consistent, non-defective and enjoyable foods (Pal *et al.*, 1995). The ultimate measure of quality is consumer acceptance and hence sensory quality of the product plays a major role in determining the quality of dried products. The difference in the sensory quality



**Chitra and Vigneshpriya**

and overall acceptability of the product might be attributed to the different degree of biochemical and microbiological changes in the dried fish may have undergone during drying and subsequent storage. Turmeric treated dried sample of *S. guttatus* were liked by panellist and the obtained overall acceptability score was 9.0 point from grading scales and sun dried sample have the overall acceptability of 8.86 ± 0.26 from the 9.0 point grading scales. Both sun and turmeric treated dried fishes were having the appearance of freshly dried, the texture was firm, colour was good and bright, odour was absent. The initial scores of sun dried samples were 8.85, 8.71, 9.0, 8.71, 9.0, 8.29 and 8.86 respectively and the initial scores of turmeric treated sand dried samples for appearance, colour, flavour, odour, mouth feel, texture, overall acceptability were 9.0, 9.0, 8.78, 8.86, 9.0, 8.57 and 9.0 respectively. Both the sun and turmeric treated dried products was rated as “good” based on the overall acceptability.

Many countries are now taking voluntary or mandatory action to reduce pollution of the aquatic environment with heavy metal for the food safety of aquatic food particularly fish. Considering the effect of heavy metal on fish quality and safety the food regulatory and health authorities in some developed countries have taken serious view and adopted maximum allowable limit of harmful metals and elements usually the pollutants of metals and element category contaminate the raw fish. Accumulation of heavy metals content of sun and turmeric treated dried samples of *S. guttatus* were well below the detectable limit (BDL). There is no metal accumulation in the dried sample of *S. guttatus* collected from the Thangachimadam market, Rameswaram. This information is very important for the domestic consumers in Rameswaram as well as for international markets where dried fishes are exported. The initial moisture contents observed in sun and turmeric treated sand dried samples were $10.1 \pm 0.15\%$ and $9.2 \pm 0.2\%$ respectively and after one year of storage the moisture contents were $71.5 \pm 0.05\%$ and $68.9 \pm 0.07\%$ respectively. High value of the moisture content of the products during storage might be due to absorption of moisture content in storage period. The mean percentage of moisture content was increased due to increasing of storing period.

The initial protein content observed in sun and turmeric treated dried samples were $32.5 \pm 0.07\%$ and $35.7 \pm 0.1\%$ respectively. After one year of storage, the protein contents were $16.5 \pm 0.23\%$ and $19.2 \pm 0.1\%$, which was relatively low when comparing to the initial level. The protein digestibility is adversely affected by the dehydration and storage of dried fish products (Raghunath *et al.*, 1995). The species of fish, freshness of the raw material prior to drying and the drying method could influence the reactions that lead to a reduction in the biological value of fish protein (Opstve *et al.*, 1988). The initial lipid content recorded in sun and turmeric treated dried samples were $0.59 \pm 0.01\%$ and $0.51 \pm 0.022\%$ and after one year of storage the lipid contents were decreased to $0.39 \pm 0.012\%$ and $0.37 \pm 0.01\%$. The results showed that the lipid content decreased during storage period, it may be due to the moisture gain in both sun and turmeric treated dried samples. Prodhan *et al.*, (2010) reported that during storage period (12 months) the lipid content was gradually deceased in the samples of *S. guttatus* $7.2 \pm 0.5\%$ to 6.0% . Carbohydrate level in the analyzed samples varied from 5.2 ± 0.2 to $3.9 \pm 0.17\%$ and 5.5 ± 0.022 to $4.2 \pm 0.26\%$ during storage time. Siddique and Aktar, (2011) observed that the carbohydrate content of three marine dried fishes (*H. nehereus*, *J. dussumieri* and *L. savala*) was varied from $4.11 - 2.41\%$; $2.94 - 1.13\%$ and $1.64 - 0.08\%$ respectively during the storage period which was similar to our present findings. In the present study, ash level in the analyzed sun and turmeric treated dried samples of *S. guttatus* were varied from $20.8 \pm 0.12 - 2.2 \pm 0.21\%$ and $19.7 \pm 0.21 - 1.98 \pm 0.03\%$ during the storage period. Mansure *et al.*, (2013) found that the ash content in the range of $11.11 - 18.89\%$ in three dried fishes. The initial pH content recorded in sun and turmeric treated dried *S. guttatus* were 6.79 ± 0.01 to 6.88 ± 0.01 respectively, after one year of storage the pH content was 5.79 ± 0.01 to 5.89 ± 0.01 .

The highest TVC was recorded in sun dried samples of *S. guttatus* (16×10^3 Cfug) while lowest values were recorded in turmeric treated dried samples (14×10^3 Cfug). In the present study during storage TVC in both the samples increased due to increase in moisture content. A similar trend was observed by Relekar *et al.*, (2014) during the storage of dried *L. savala*. Similarly, Kakati *et al.*, (2017) reported that total viable count range (3.2 to 4.1 log Cfug) of six selected dried fishes in Assam, India. Imtiaz *et al.*, (2017) also found that the lowest bacterial load of 2.1×10^5 Cfug in turmeric treated sample of *T. haumela*, followed by 2.4×10^5 Cfug in salted samples and the highest value of 3.0×10^5 Cfug in control samples within seven days storage. After one year of storage the scores for appearance, colour, flavour, odour, mouth feel, texture and overall acceptability of sun and turmeric treated dried samples declined and



**Chitra and Vigneshpriya**

the scores were from 4.71, 5.14, 5.28, 5.14, 5.0, 5.28 and 5.0 in sun dried samples and 5.14, 6.0, 6.5, 6.0, 6.2, 6.0 and 6.5 in turmeric treated dried samples. The sun dried samples were appearing stale with firm texture, slight fishy odour, slight discolouration were occurred and rated as „slightly poor“ and turmeric treated dried samples were rated as „moderately good“ after a storage period of one year based on the overall acceptability.

CONCLUSION

Fish is a favorite foodstuff for the majority of societies. Fish has been recognized as an excellent food source for human beings for centuries and is preferred as a perfect diet not only due to its excellent taste and high digestibility but also because of having higher proportions of unsaturated fatty acids, essential amino acids and minerals for the formation of functional and structural proteins. Body composition is a good indicator of the physiological condition of a fish but it is relatively time consuming to measure. Proximate body composition is mainly the analysis of moisture, fat, protein and ash contents of fish. Both the dried fish products available in the markets of Rameswaram were found to have high nutritional content. The sensory evaluation of the dried products of sun and turmeric treated sand dried *S. guttatus* revealed that maximum overall acceptability quality. Microbial quality of both the dried products were acceptable limit. Though the absence of *E.coli* and *Salmonella* species was a good sign for consumers, the presence of *S. aureus* in the product indicates slight unhygienic handling during processing and storage. Therefore, care should be taken for hygienic measure right from the preparation of raw materials, use of utensils, handling practices, processing methods and during storage, in order to safeguard the health of the consumers. Packing may also be important for better keeping and increasing shelf life of the products. Heavy metals (Mercury, Lead, Chromium and Cadmium) accumulations in dried samples were found below the detectable limit. There is no heavy metal accumulation in dried samples collected from Thangachimadam, Rameswaram Island.

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Chitra and Vigneshpriya

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Table 1: Proximate composition of sun dried and turmeric treated dried samples of *S. guttatus* before and after storage period

S. No.	Parameters	Sun dried samples		Turmeric treated dried samples	
		Fresh	After Storage Period	Fresh	After Storage Period
1	Moisture (%)	10.1± 0.15	71.5±0.05	9.2±0.2	68.9±0.07
2	Protein (%)	32.5±0.07	16.5±0.23	35.7±0.1	19.2±0.1
3	Lipid (%)	0.59±0.01	0.39±0.012	0.51±0.022	0.37±0.01
4	Carbohydrate (%)	5.2±0.2	3.9±0.17	5.5±0.022	4.2±0.26
5	Ash (%)	20.8±0.12	2.2±0.21	19.7±0.21	1.98±0.03
6	pH	6.79±0.01	5.79±0.01	6.88±0.01	5.89±0.01

Values are shown as mean ± SD

Table 2: Microbial analysis of sun dried and turmeric treated dried samples of *S. guttatus*

S.No.	Microorganisms	Sun dried samples		Turmeric treated dried samples	
		Fresh	After Storage Period	Fresh	After Storage Period
1	<i>Escherichia coli</i>	+	+	-	-
2	<i>Klebsiella pneumonia</i>	-	-	-	-
3	<i>Staphylococcus aureus</i>	+	+	+	+
4	<i>Proteus vulgaris</i>	-	-	-	-
5	<i>Pseudomonas aeruginosa</i>	-	-	-	-
6	<i>Salmonella typhimurium</i>	-	-	-	-
7	<i>Aspergillus niger</i>	-	-	+	-
8	<i>Microsporium canis</i>	-	-	-	-
9	<i>Fusarium oxysporum</i>	-	-	-	-





Chitra and Vigneshpriya

Table 3: Initial and final score of sensory analysis of sun dried and turmeric treated dried samples of *S. guttatus* collected from Pamban, Rameswaram Island

S. No.	Parameters	Sun dried samples		Turmeric treated dried samples	
		Initial	Final scores (after storage period)	Initial	Final scores (after storage period)
1	Appearance	8.85±0.37	4.71±1.11	9.0±0.0	5.14±1.06
2	Colour	8.71±0.5	5.14±0.90	9.0±0.0	6.0±0.44
3	Flavour	9.0±0.0	5.28±0.75	8.78±0.39	6.5±0.54
4	Odour	8.71±0.5	5.14±0.90	8.86±0.25	6.0±0.44
5	Mouth feel	9.0±0.0	5.0±0.57	9.0±0.0	6.2±0.56
6	Texture	8.29±0.75	5.28±0.57	8.57±0.46	6.0±0.44
7	Overall acceptability	8.86±0.39	5.0±0.57	9.0±0.0	6.5±0.54

Values are shown as mean ± SD

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RESEARCH ARTICLE

Temperament Profiling in Patients with Generalized Anxiety Disorder: A Clinical Correlation Study Using Homoeopathic Constitutional Prescription

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ABSTRACT

Generalized Anxiety Disorder (GAD) is a prevalent psychiatric condition marked by persistent, excessive anxiety with cognitive, emotional, and somatic symptoms. Despite advances in standard treatments, challenges like adherence, adverse effects, chronicity, and individual variability persist. Individualized therapeutic approaches based on constitutional and temperamental assessment may offer a new framework for understanding the diversity in anxiety presentations. This study investigates temperament-based constitutional prescribing in GAD patients receiving individualized homoeopathic treatment. A prospective observational clinical correlation study was conducted between January 2021 and March 2023 at the Parul Institute of Homoeopathy, Parul University, India. 100 patients with GAD (DSM-5 criteria, GAD-7 scale) were enrolled; 97 completed the study. Each underwent individualized homoeopathic case analysis, constitutional prescription, and 4–6 follow-up visits. The study analyzed correlations between remedy temperament affinity and clinical improvement using repeated GAD-7 scores, of 97 participants, baseline anxiety was mild (7), moderate (69), severe (21). Post-treatment, 88 had minimal anxiety and 9 had mild residual anxiety. Most prescribed remedies: *Lycopodium clavatum* (24), *Arsenicum album* (21), *Nux vomica* (18), *Sulphur* (13), *Silicea* (11), *Ignatia amara* (8). Bilious/choleric temperament remedies dominated prescriptions. There is a relevant relationship between constitutional remedy temperament affinity and prescribing patterns in GAD within an individualized homoeopathic



**Arpita Chatterjee and Kamlesh Shah**

framework. Temperament-oriented constitutional analysis could support psychobehavioral profiling in anxiety disorders. Further controlled research is required to validate this model.

Keywords: Generalized anxiety disorder, individualized medicine, constitutional prescribing, temperament theory, homoeopathy, GAD-7, psychobehavioral profiling

INTRODUCTION

Anxiety disorders are a global public health issue, with GAD being a chronic subtype causing significant psychological and functional impairment. GAD is characterized by excessive worry, autonomic arousal, restlessness, irritability, sleep disturbance, and poor concentration. Contributing factors include neurobiological vulnerability, personality traits, and environmental stressors. In India, anxiety disorders are a leading mental health concern. Current treatments (SSRIs, SNRIs, benzodiazepines, psychotherapy) have limitations such as delayed response, adverse effects, relapse risk, and variable patient response, highlighting the need for individualized models. Temperament theory—from Hippocratic and Galenic origins to modern psychobiological research—offers a framework for understanding personality and emotional characteristics. The four classical temperaments (choleric, melancholic, sanguine, phlegmatic) remain relevant for understanding behavioral patterns and constitutional medicine. In homoeopathic philosophy, temperament is integral to constitutional individuality and remedy selection. However, few clinical studies have systematically linked temperament, constitutional prescribing, and psychiatric outcomes using structured measures. This study addresses that gap in GAD management.

Conceptual and Theoretical Framework

This study is based on the intersection of:

- Contemporary anxiety psychopathology
- Classical temperament theory
- Constitutional homoeopathic prescribing

GAD presents with diverse behavioral and emotional profiles. Temperament theory helps organize these patterns, with homoeopathic constitutional medicine providing a parallel interpretive model. Remedies are matched to temperamental dispositions, and selection extends beyond diagnosis to constitutional patterns.

Research Objective: To explore temperament-based constitutional prescribing patterns and their clinical correlation in GAD patients under individualized homoeopathic treatment.

Research Gap: Current literature lacks prospective clinical studies on constitutional remedy temperament affinity in anxiety disorders using structured psychiatric outcomes. This study integrates temperament theory, constitutional analysis, and psychometric assessment.

METHODS

Study Design and Setting

A prospective, observational clinical correlation study was conducted from January 2021 to March 2023 at the Parul Institute of Homoeopathy, Parul University, Vadodara, Gujarat, India. The design focused on real-world constitutional prescribing, not protocolized interventions.

Ethical Considerations

- **Approval:** Institutional Ethical Committee, Parul University
- **Consent:** Informed consent obtained from all participants
- **Confidentiality:** Maintained per institutional and ethical standards



**Arpita Chatterjee and Kamlesh Shah****Participant Recruitment and Eligibility****Inclusion Criteria:**

- Age 18–80 years
- DSM-5 diagnosis of GAD
- Anxiety symptoms present most days for ≥ 6 months
- At least three symptoms (excessive worry, restlessness, irritability, poor concentration, fatigue, sleep disturbance)
- GAD-7 score ≥ 10 or clinically significant
- Willingness for individualized homoeopathic management and follow-up

Exclusion Criteria:

- Panic disorder, social anxiety, trauma-related or phobic disorders
- Significant anxiety secondary to major somatic illness
- Concurrent conventional pharmacological treatment
- Unwillingness to participate or complete follow-up

Sample: 100 enrolled, 97 completed (3 discontinued before completion), Diagnostic Assessment Diagnosis was based on DSM-5 criteria and the GAD-7 scale (score 0–21); Homoeopathic Case Analysis and Prescription

Participants underwent detailed constitutional case-taking, including:

- Mental and emotional characteristics
- Behavioral tendencies
- Physical generals and modalities
- Constitutional attributes and symptom totality

Remedy selection was individualized; potencies included 200C, 1M, 10M. Each patient had 4–6 follow-ups for clinical and GAD-7 reassessment.

Temperament-Based Constitutional Correlation

Instead of independent temperament inventories, the study used:

- Individualized constitutional analysis
- Remedy selection based on classical homoeopathic principles
- Analysis of temperament affinity of prescribed remedies
- Correlation with clinical improvement

Remedy-Temperament Associations:

- Bilious/choleric: Nux vomica, Lycopodium clavatum, Sulphur
- Melancholic: Arsenicum album, Silicea, Ignatia amara, Kali phosphoricum
- Sanguine: Arnica montana
- Phlegmatic: No dominant cluster found

Statistical Analysis

Used descriptive and categorical methods:

- Frequencies, percentages
- Remedy distribution and gender-wise patterns
- Baseline and post-intervention severity
- Outcome analysis: Categorical comparison of GAD-7 severity pre- and post-treatment

Inferential statistics (e.g., chi-square, McNemar/Bowker tests) may be applied in future analyses.





RESULTS

Participant Flow and Completion

- 97 out of 100 completed clinical follow-up and were analyzed.

Most had moderate or severe anxiety at baseline. Most transitioned to minimal anxiety post-treatment. Male participants predominantly received *Lycopodium clavatum* and *Nux vomica*; *Arsenicum album* and *Ignatia amara* were more common in females.

DISCUSSION

The study integrates contemporary anxiety psychopathology, classical temperament theory, and constitutional homoeopathic prescribing. GAD presents with diverse psychobehavioral patterns, and temperament theory provides a framework for understanding this heterogeneity. The constitutional-temperament correlation model bridges individualized psychiatric understanding with constitutional therapeutics, offering a basis for future research in integrative mental health.

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Table 1: Repeated GAD-7 scoring monitored clinical progress.

GAD-7 Score	Severity Category
0–4	Minimal anxiety
5–9	Mild anxiety





Arpita Chatterjee and Kamlesh Shah

10–14	Moderate anxiety
15–21	Severe anxiety

Table 2: Baseline Anxiety Severity

Severity Category	Frequency	Percentage
Minimal (0–4)	0	0%
Mild (5–9)	7	7.2%
Moderate (10–14)	69	71.1%
Severe (15–21)	21	21.6%

Table 3: Post-Treatment Anxiety Severity

Severity Category	Frequency	Percentage
Minimal (0–4)	88	90.7%
Mild (5–9)	9	9.3%
Moderate (10–14)	0	0%
Severe (15–21)	0	0%

Table 4: Constitutional Remedy Distribution

Remedy	Frequency	Percentage
Lycopodium clavatum	24	24%
Arsenicum album	21	21%
Nux vomica	18	18%
Sulphur	13	13%
Silicea	11	11%
Ignatia amara	8	8%
Kali phosphoricum	3	3%
Arnica montana	2	2%

Table5: Gender-Wise Remedy Distribution

Remedy	Male	Female	Total
Lycopodium clavatum	20	4	24
Arsenicum album	3	18	21
Nux vomica	15	3	18
Sulphur	9	4	13
Silicea	4	7	11
Ignatia amara	1	7	8
Arnica montana	1	1	2

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Determinants and Dimensions of Educational Aspiration : A Review of Literature

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ABSTRACT

In order to comprehend students' academic paths, career decisions, and general social mobility, educational desire has become a key concept. It represents both a person's aspirations for their future level of education as well as the larger institutional, sociocultural, and economic factors that influence these aspirations. The current literature on educational aspiration is summarized in this review, with an emphasis on its dimensions and determinants. The review also covers important aspects of educational aspiration, such as the distinction between short-term and long-term objectives, academic vs occupational aspirations, and cultural differences. Aspirations are frequently mediated by structural inequities, but they also act as a strong motivator for academic perseverance, according to empirical research conducted in a variety of circumstances. There are also gaps in cross-cultural comparisons, longitudinal analysis, and the inclusion of marginalized groups in aspiration studies, despite a substantial corpus of research. The review ends by outlining the consequences for academics, educators, and politicians. It emphasizes the necessity of focused interventions, equitable support networks, and more research into the relationship between structural factors and individual agency. This research advances a more thorough knowledge of how aspirations impact educational results and outcomes by mapping the factors and dimensions of educational aspiration. The review ends by outlining the consequences for academics, educators, and politicians. It emphasizes the necessity of focused interventions, equitable support networks, and more research into the relationship between structural factors and individual agency. This study adds to a more thorough understanding of how aspirations affect educational results and more general social development by charting the factors and aspects of educational aspiration.

Keywords: Educational aspiration, socio-economic status, determinants, academic achievement, literature review.



**Bhavna Chaudhary and Anoj Raj**

INTRODUCTION

Aspiration is a seductive concept. In the context of education, it is difficult to argue against the notion that all young people should have high aspirations for their future, including academic and related life goals. The argument for setting and maintaining high objectives is that education can provide everyone the same chance to succeed if they so choose. (Bowers-Brown, T., Ingram, N., & Burke, C.2019). Aspiration has emerged as a key idea in both Australian and UK higher education (HE) policy, despite the fact that it is typically seen as a weakness among low-socioeconomic status (SES) groups rather than an available resource constrained by too interventionist social justice policies. In an effort to increase academic performance and student involvement, HE policy in both systems is granting universities greater power to conduct outreach programs in schools and the society at large.. (Sellar, S., & Gale, T.2009).Higher-achieving students are also more likely to come from low-income families and have lower academic achievement. These students also display a variety of academic and social risks. Children who had higher goals than they had expected, in instance, reported higher levels of behavioral and emotional difficulties, lower levels of school connection, and higher levels of performance and test anxiety. (Boxer, P., Goldstein, S. E., DeLorenzo, T., Savoy, S., & Mercado, I. 2011).The primary goal of education for students is academic accomplishment. The study of educational aspiration has also been approached using a variety of theoretical frameworks. The status attainment model emphasizes how important family background and socioeconomic level are(Sewell et al., 1969).Aspirations are broader, more ambitious goals that might motivate students to put in extra effort and stick with their studies, in contrast to educational expectations, which represent what students truly believe they will achieve(Khattab, 2015). (Sewell et al.1969) shown that parental socioeconomic position, personal aptitude, and the support of significant people all significantly influence educational aspirations, which in turn predict success in the future. Similar to this, Trusty (2000) pointed out that despite their academic struggles, teenagers usually have high aspirations, showing that goals may still be a source of drive. Scholars have, however, also drawn attention to the risks associated with having conflicting goals and expectations. (Boxer, Goldstein, DeLorenzo, Savoy, and Mercado 2011) found a robust relationship between socioeconomic disadvantage and academic risk factors, as well as differences between the two.This demonstrates that although goals can motivate accomplishment, they are frequently constrained by systemic obstacles. Additionally, Reynolds and Pemberton (2001) reported that American youth's desires to attend college increased throughout the 1980s and 1990s, reflecting broader societal shifts and the growing belief that a college education is essential for success. Together, these results suggest that educational aspiration is not only a psychological construct of the individual but also a socially entrenched phenomenon that is impacted by cultural, educational, and familial factors.

Present study aims to explore the key determinants that shape students' educational aspirations and to analyze the different dimensions through which these aspirations manifest. Numerous elements are among the variables, including peer pressure, parental support, family background, socioeconomic level, and the larger institutional and cultural context. By looking at these elements, the study aims to determine how ambition levels are influenced by a combination of psychological elements (such as motivation, self-concept, and resilience) and structural variables (such as educational possibilities and financial resources). At the same time, the study also focuses on the different dimensions of educational aspiration—ranging from immediate academic goals to long-term career ambitions—in order to understand how aspirations evolve and interact with students' lived experiences. By doing this, the study hopes to shed light on the chances and obstacles that might help or impede students from achieving their academic goals, in addition to emphasizing the role that desire plays in determining educational paths.

METHODOLOGY

This review adopts a systematic approach, drawing on peer-reviewed studies indexed in Scopus, Web of Science, and Google Scholar from 1990–2024. Keywords included “emotional maturity,” “family status,” “parenting style,”



**Bhavna Chaudhary and Anoj Raj**

“domestic influence,” “adolescent development,” and “emotional intelligence.” Studies were selected based on empirical rigor, sample representativeness, and relevance to the review’s objectives. The key findings are synthesized below.

Conceptual Understanding of Educational Aspiration

Educational aspiration, which reflects both personal objectives and the systemic opportunities that form them, has been a significant area of study in sociological and educational research. Aspirations are frequently defined as the degree of education that a person expects or intends to obtain, in contrast to expectations, which are more realistic evaluations of attainability given limitations such as socioeconomic background, ability, and resources. (Khattab, 2015). The idea of educational aspiration has also been influenced by theories of social reproduction and potential. Sociologists contend that rather than existing freely, aspirations are influenced by a person's family background, societal standards, and structural injustices. For example, using longitudinal Young Lives data from India, (Serneels and Dercon 2014, 2021) demonstrate that mother aspirations have a significant impact on children's schooling trajectories, particularly in areas of poverty. Their results indicate that ambitions act as a moderator between socioeconomic adversity and educational performance. Additionally, aspirations are dynamic and vary over time in response to exposure, opportunities, and institutional support. In a natural experiment, (Beaman, Dufllo, Pande, and Topalova 2012) found that the presence of female political leaders significantly raised the educational aspirations of teenage girls and their parents in rural West Bengal. This study emphasizes the significance of representation and role models in shaping aspirations by arguing that aspiration is partially socially constructed and responds to larger gender and cultural dynamics. In a more recent study, Flechtner (2024) examined longitudinal data from teenagers in Andhra Pradesh and Telangana to demonstrate how differences in ambitions appear early on. Children from disadvantaged backgrounds were more likely to have lower goals than their peers, particularly if they were less cognitively capable at age 12.

Together, these studies demonstrate that educational aspiration is a complex construct that is influenced by a variety of factors, including gender, family background, socioeconomic class, cognitive capacity, and institutional environments. Despite the fact that goals are commonly viewed as individual choices, research shows that structural factors and social contexts like families and communities have a big influence on them. This conceptual understanding is necessary to analyze how aspirations both reflect and reinforce educational disparity.

Determinants of Educational Aspiration

A complex interplay of institutional, cultural, familial, and personal influences shapes educational aspirations. Studies repeatedly show that ambitions are shaped within larger socioeconomic, cultural, and educational circumstances rather than existing in a vacuum. (Buchmann & Dalton, 2002; Khattab, 2015). The following subsections review the key determinants identified in the literature.

Socio-Economic Status (SES)

One of the most extensively researched indicators of educational aspiration is socioeconomic status. Children from more affluent families typically have easier access to opportunities, resources, and guidance that help them dream of pursuing higher education. (Sirin, 2005). Financial stability brought about by parental income and employment status enables families to make investments in high-quality education, tutoring, and extracurricular activities. On the other hand, low-SES students frequently experience financial limitations, restricted access to educational resources, and limited knowledge of higher education options, all of which can lessen their expectations. (Buchmann & Dalton, 2002). In addition, socioeconomic status (SES) has a significant impact on how teenagers formulate their goals. The primary goal of this work is to identify notable disparities in secondary school-aged adolescents' educational aspirations based on their socioeconomic condition and investigate the connection between the teenagers' socioeconomic situation and educational aspirations. (Salgotra, A. K., & Roma, K. (2018). Young people are not very interested in politics, business, or entrepreneurship, but they do want a job, particularly one in the government. Furthermore, their educational attainment and socioeconomic family backgrounds have no bearing on their goals. (Das, P. K., Panigrahi, J. K., Naik, I. C., & Das, B. 2019). Parents' socioeconomic standing has a significant impact on





how their children view their future and their educational goals. The majority of the guardians were from low socioeconomic backgrounds due to their low salary levels. This had an impact on the students' educational goals and school preferences. (Zang, P. L., Kwao, M. P., Anim, C. L., & Ahasu, E. K. 2024).

Parental Education and Involvement

One important factor influencing children's aspirations is parental education. In addition to offering academic help, parents with more education serve as role models for the importance of education. Participation in school activities, encouragement, and supervision of children's education all have a favorable correlation with desires for further education (Davis-Kean, 2005). On the other hand, children from low-education homes might not have access to role models who support higher learning objectives. Compared to parents in non-slum areas, parents in slums have lower expectations for their children's educational attainment; parents in slums have higher expectations for their children's educational success than for their own. (Oketch, M., Mutisya, M., & Sagwe, J. (2012). Compared to parents with lower levels of education, parents with greater levels of education typically set higher educational goals for their kids. parents own educational experiences and accomplishments may inspire them to set more ambitious objectives for their kids. (Sosu, E. M. 2014).

The purpose of Hill & Taylor's study was to look into the connection between the educational backgrounds of parents and the attitudes of students attending government and private universities. His findings showed that the pupils' attitudes varied significantly depending on the educational background of their parents.. (Hill & Taylor, 2004). Discussions between parents, teachers, and school administrators, as well as conversations with teenagers about their education and future aspirations, have the largest correlations with academic achievements among middle and high school students.

Family Structure and Environment

The structure of the family affects aspiration levels as well. Research indicates that when compared to pupils from broken home environments, those from intact homes with consistent emotional support tend to have higher goals. (Sun & Li, 2009). Home encouragement, emotional climate, and the quality of parent-child relationships all contribute to the development of a supportive atmosphere that fosters aspirations. Family expectations frequently greatly influence aspirations in collectivist countries, with kids adjusting their objectives to satisfy their parents' expectations. (Okagaki & Frensch, 1998). Another important factor is the home environment, particularly the level of parental participation. Drawing on data from rural China, (Wang et al. 2023) discovered that, even after controlling for peer and individual influences, adolescents who grew up in supportive family environments with high parental expectations, communication, and home learning resources were more likely to pursue ambitious educational goals. While stable single-parent families with supportive home environments can foster aspirations comparable to those in two-parent households, research from the Growing Up in Australia project indicates that family instability lowers parental involvement, which in turn lowers educational engagement and aspirations. (Hill and others, 2004)

Gender

Though trends differ among countries, gender still has a significant influence on educational goals. Girls are more likely than boys to want to pursue higher education in various situations, especially in industrialized countries (Schoon, 2006). However, gender norms frequently restrict girls' educational opportunities in patriarchal nations, steering them toward home responsibilities rather than postsecondary education or professional professions (Kirk, 2000). Boys are more likely to pursue STEM or things-oriented careers, while girls are more likely to pursue people-oriented careers, according to a large-scale analysis using 2018 PISA data. These gendered differences were especially noticeable in countries with higher levels of women's empowerment, suggesting that adolescents can pursue more intrinsically motivated pathways when they have access to greater societal resources. (Stoet & Geary, 2022). Compared to boys, girls in rural U.S. contexts reported much higher educational goals, higher family expectations for college, and greater involvement in college-preparatory programs, indicating that gender-based disparities persist even in settings with minimal resources. (Stoet & Geary, 2022) Gender significantly predicted these differences ($p < .01$). In addition, middle school girls in Finland were more likely than boys to choose academic paths,



**Bhavna Chaudhary and Anoj Raj**

with 53.1% of girls choosing academic paths versus 48.7% of boys, and boys choosing vocational aspirations more than twice as frequently (48.7% vs. 23.8%). (Finnish study, 2022). All of these results emphasize how gender influences educational goals through a variety of channels, including policy-mediated role modeling, family and community expectations, institutional opportunities, and society norms. Both at the domain-specific occupational level and at the more general educational planning level (academic vs. vocational courses), gender discrepancies are apparent. Interventions like female leadership have the ability to significantly close aspiration gaps.

Peer Influence and School Environment

In the formation of aspirations, schools and peers serve as potent socializing factors. While negative group pressure may deter students from pursuing academic goals, positive peer networks can motivate them to aim higher. The expectations, direction, and classroom environment of teachers have a big impact on how pupils view their academic ability. Schools that offer career guidance, mentoring, and exposure to higher education pathways help students achieve their educational goals. (Eccles & Roeser, 2011). Causal quasi-experimental research using UK longitudinal data demonstrates that classmates' socioeconomic status, aspirations, and achievement levels have quantifiable effects on people's intentions to continue their education and pursue academic paths. These effects are more pronounced for boys than for girls, and they can be somewhat offset by the information, counseling, and guidance programs offered by schools. (Dickerson, Maragkou, & McIntosh, 2018). Similar to this, research employing networked field tests and interventions shows that short informational and role-modeling treatments can change students' perceptions and, via peer contagion, increase aspirations in classrooms. (González Amador, Cowan, & Nillesen, 2022).

according to a study of Chinese adolescents living in rural areas. Peer aspirations and the quality of peer relationships were found to be directly related to students' own aspirations, although these effects were frequently mediated by individual factors like academic self-perception and goal valuation (Wang et al., 2023). Economic theory-informed empirical research also demonstrates that the makeup of peer income and ability can result in "reference-dependent" aspiration gaps: as low-income students' aspirations rise and high-income students' aspirations fall when the number of low-income peers in a cohort increases, with social cohesion moderating these effects (Fongoni, Norris, Romiti, & Shi, 2022).

Cultural and Community Context

Culture affects students' views on the importance of education, the kinds of jobs they find appealing, and the degree to which they think educational mobility is possible. Students in East Asian countries like China, Korea, and Japan consistently express a desire for higher education, which is a reflection of Confucian traditions that view hard work and academic success as moral obligations. (Buchmann & Park, 2009). Community-level research indicates that neighborhood norms and opportunities influence the ambitions of youth. Using longitudinal data from the United States, Harding (2011) discovered that, in large part because of inadequate role models and regional cultural scripts, teenagers in underprivileged urban neighborhoods frequently formed "survival-oriented" ambitions with low expectations for further schooling. According to Beaman et al. (2012), the presence of female political leaders in local village councils considerably increased the educational and career goals of both parents and girls, hence closing the gender gap. Only are educational goals ingrained in people and families, but also in larger cultural norms and social settings. A compelling illustration of how community-level role models can alter cultural expectations and aspirations comes from a randomized policy "experiment" in rural India, where parents' aspirations for their daughters' education increased significantly when female leaders were assigned to village councils at random, and teenage girls themselves expressed higher educational and career ambitions (Beaman, Duflo, Pande, & Topalova, 2012). Finally, social influence can have a significant impact on communities. According to a European multi-site study, having several mentors even fewer than a whole village can have a good impact on teenagers' goals and achievements, highlighting the importance of communal ties beyond close friends and family (Singh et al., 2022).



**Bhavna Chaudhary and Anoj Raj****Academic Achievement, Self-Concept, and Motivation**

Students' goals are closely related to their academic achievement and self-perception. While recurrent academic setbacks may dampen aspirations, high performers typically have lofty educational goals. A major factor in encouraging students to continue higher education is academic self-efficacy, or the conviction that one can succeed (Bandura, 1997). Aspirations can also be shaped by internal motivation, resiliency, and future focus, especially in situations where there are no outside support networks. Students' academic performance, self-perception, and motivating styles are all strongly correlated with their educational goals. (Marsh and Martin 2011), for example, emphasized the reciprocal relationship between academic accomplishment and self-concept, demonstrating that improvements in self-concept predict later achievement and consequently influence students' educational paths. Similar to this, there is empirical evidence to support Eccles and Wigfield's (2002) expectancy value model, which holds that students' subjective task values and expectations of success have a significant impact on their educational choices and goals. Students who have confidence in their talents are more likely to create and stick to challenging academic objectives, according to Bandura (1997). Students with higher levels of intrinsic motivation and autonomous forms of control not only do better academically but also have higher goals for their future education, according to research based on self-determination theory (Deci & Ryan, 2000; Howard et al., 2021).

When combined, these results highlight how academic performance, motivation, and self-perception work in concert to influence students' ambitions, making them key factors in educational paths.

Meta analysis of the studies

In various contexts and approaches, the analyzed studies consistently emphasize that educational aspirations are influenced by a complex interaction of socio-economic status, parental education and engagement, family dynamics, peer relationships, cultural resources, and personal motivation. Quantitative studies and cross-sectional evaluations (e.g., Salgotra & Roma, 2018; Pandey et al., 2024; Wang et al., 2023) indicate that increased SES, parental educational levels, and encouraging peer settings boost aspirations, yet inequalities remain for underprivileged groups. Extensive international and longitudinal research (e.g., Stoet & Geary, 2022; Byun et al., 2012; Marsh & Martin, 2011) demonstrates lasting impacts of cultural capital, gender norms, and academic self-perception on influencing expectations. Evidence from experiments and policy studies (e.g., Beaman et al., 2012; González Amador et al., 2022) demonstrates that structural changes and peer effects can notably elevate aspirations. Theoretical and meta-analytic findings (e.g., Bandura, 1997; Deci & Ryan, 2000; Howard et al., 2021) elaborate on these trends, highlighting the importance of self-efficacy, autonomy, and motivation in converting goals into success. Together, the evidence highlights that aspirations are not just individual or fixed; rather, they are actively shaped by the interactions of family, friends, educational settings, and wider socio-cultural frameworks

Dimensions of Educational Aspiration

Dimensions show the many forms and orientations of educational ambition, while determinants describe the elements influencing it. According to Schoon and Parsons (2002) and Khattab (2015), scholars stress that ambitions are not uniform and instead take on distinct forms based on time, emphasis, and sociocultural environment. Subsections that follow go over the main dimensions found in the literature.

Short-Term vs. Long-Term Aspirations

One way to classify aspirations is by their temporal span. Short-term goals are imminent and frequently associated with impending milestones, such as finishing high school or passing entrance exams. Conversely, long-term aspirations encompass more expansive educational and professional objectives, such as going to college or following a certain vocation (Schoon, 2006). According to research, academic achievement and social competitiveness have a significant impact on short-term goals, whereas socioeconomic background, parental expectations, and cultural values determine long-term goals (Eccles & Wigfield, 2002). In their studies, college students frequently work for many objectives. While some goals are more specific to what students aim to achieve in the course—to learn as much as possible about the subject or to receive a good grade—other goals may be more broad and address why students are attending a particular class. Students who maintained consistent long-term goals throughout adolescence were more



**Bhavna Chaudhary and Anoj Raj**

likely to apply to and enroll in higher education institutions, according to Lakshmanan's (2004) longitudinal research employing NELS:88 data. On the other hand, those with erratic or poor short-term aspirations were less likely to be in line with postsecondary opportunities. Using a sizable Finnish sample, Dobewall et al. (2019) discovered that short-term variables like teenage health had a significant impact on students' choice between academic and vocational paths. Longer-term academic ambitions were associated with better health, whereas students with worse health issues were more likely to pursue short-term, vocational goals.

Academic vs. Occupational Aspirations

The connection between academic aspirations—the desire to pursue higher levels of formal education—and occupational aspirations—career-related objectives—is another crucial factor. Despite their frequent connections, the two are not always in agreement. For instance, one student may pursue further education without a defined career route, while another may want to become a professional artist but not necessarily seek advanced degrees (Gottfredson, 1981). Research indicates that disparities between academic and professional goals are frequent, especially when educational systems fail to sufficiently connect learning to employment prospects. (Khattab, 2015). Valls et al. (2022) identified eleven unique patterns of educational and vocational goals in a three-year longitudinal study of Vienna students moving from lower to upper secondary school. According to the study, the majority of students kept their goals same throughout adolescence, but differences that were greatly influenced by factors including parental education, migration history, and school achievement show how academic and professional objectives differed and interacted. A longitudinal research in the United States by Beal and Crockett (2010) looked at the relationship between the academic and career goals of teenagers and their subsequent educational success. Adolescents followed into young adulthood comprised the study's nationally representative sample. Students' self-reported goals, involvement in extracurricular activities, and school engagement were the main topics of questionnaires used to gather data. The findings revealed that adolescents who held high academic and occupational aspirations were more likely to participate in structured school and career-related activities, such as advanced coursework and extracurricular programs.

Individual vs. Collective Aspirations

Okagaki and Frensch (1998) investigated how cultural values affect ethnic minority families' goals for education and careers in the US. Based on surveys and interviews with immigrant parents and teenagers, their study examined the framing of ambitions on both an individual and group level. The results demonstrated how collective values, such as meeting expectations, supporting the home financially, and upholding cultural customs, significantly influenced ambitions in many immigrant households. Adolescents also indicated personal goals at the same time, such as pursuing occupations that fit their interests, growing personally, and becoming more independent. Family expectations and a desire to preserve honor as a group, rather than merely individual achievement, have a significant impact on students' educational goals in many collectivist environments. In rural central China, for example, Xiaodi Chen et al. (2023) carried out a thorough investigation with 606 adolescents (average age ~14.85 years, 50% boys). They discovered that parents' goals had a greater impact on kids' own learning goals than did peers', highlighting the significance of family expectations in these contexts. Furthermore, via influencing personal characteristics including academic self-perception, self-regulation, and goal valuation all of which were very significant predictors of students' ambitions attachments to parents and close friends indirectly supported educational aspirations. Pindar and Singh (2021), in a comparative research in Haryana, India, examined the disparities between rural and urban secondary school students' scholastic goals and family support. Their results showed that, in comparison to their rural counterparts, urban kids had far higher educational goals and more family support. With urban kids enjoying more supportive surroundings and rural pupils still facing limitations due to a lack of academic assistance and role models, this study eloquently demonstrates how geographic context impacts aspirational aspects. In a comparative research, Li and Hou (2022) looked at the educational aspirations of junior high school pupils in China's migrant, urban, and rural communities. They found that urban students had far higher desires to pursue higher education than their rural and migrant counterparts, based on data from Southwest University in Chongqing. Differences in family background, cognitive ability, and school environment were cited as the reasons for this discrepancy. Family background was found to have a greater impact than individual cognitive factors, and institutional segregation



**Bhavna Chaudhary and Anoj Raj**

associated with the hukou (household registration) system was found to be the most significant structural barrier affecting rural students. Even after taking individual aptitude into consideration, this study demonstrates how socio structural and geographic constraints combine to restrict the educational goals of rural students. According to the results, young people who believe their parents have higher expectations for their education are far more likely to want to attend college. On the other hand, people were less inclined to seek higher education if they saw more employment chances in their rural towns. In addition, the study discovered that men tended to have a greater rural identity associated with local employment, whereas women reported higher enrollment rates and goals.

Realistic vs. Idealistic Aspirations

Researchers discovered a pervasive aspiration–expectation gap in a groundbreaking study of middle school pupils in rural central China. The children frequently wanted to go to college but had more conservative expectations because of perceived limitations. Notably, teenagers left behind by migratory parents indicated very high ambitions but much lower expectations. While limitations like parental absence and subpar academic achievement stifled reasonable expectations, parents' educational goals particularly mothers had a favourable impact on adolescents' idealistic hopes. Higher psychosocial risk, including depression, and poorer academic self-regulation were linked to the ensuing mismatch (Ye et al., 2021). In order to investigate the difference between educational ambition (idealistic hopes) and educational expectation (realistic projections), Yan (2022) examined national statistics. Practical variables including family wealth, the number of siblings, and the child's academic achievement greatly influenced realistic expectations, even if parental goals were consistently high and generally unaffected by socioeconomic restraints. The study highlights how systemic socioeconomic hurdles may prevent practical educational planning, even if many families foster high goals. Khattab et al. (2022) conducted a research on seventh and eighth graders in Qatar that shed light on the difference between expectations based on practical realities and idealistic educational ambitions. Parental expectations, demographics, and personal views all had an impact on the mismatch between students' ambitions and expectations, which were often greater.

Meta Analysis of the studies

In various contexts, research consistently emphasizes that adolescent educational and occupational aspirations are both fluid and hierarchical. Longitudinal research (Schoon, 2006; Beal & Crockett, 2010; Lakshmanan, 2004; Valls et al., 2022) indicates that consistent long-term goals forecast greater educational achievement and career fit, whereas inconsistent or misaligned objectives (Khattab, 2015; Ye et al., 2021) frequently hinder success in postsecondary education. Socioeconomic status, expectations from parents, and cultural context stand out as the strongest factors (Chen et al., 2023; Agger et al., 2018; Yan, 2022), influencing not only the level of aspirations but also the practicality of expectations. Comparative research (Pindar & Singh, 2021; Li & Hou, 2022) shows ongoing rural–urban disparities, where urban youth express greater ambitions due to enhanced parental support and structural resources, while rural and migrant students encounter systemic obstacles. Health and academic achievement also act as significant mediators (Dobewall et al., 2019; Harackiewicz et al., 2000). Collectively, the evidence indicates that although young people frequently set high aspirations, the realization of these goals relies on family backing, available opportunities, well-being, and a steady focus on long-term objectives

Limitations of the Study

The current review has limitations even if it offers insightful information about the factors and aspects of students' educational goals. First, the study relies heavily on secondary data from previous studies, which may limit the breadth of contextual knowledge and make it impossible to see students' actual experiences firsthand. Second, research carried out in Western, developed environments, such the US, UK, and Australia, make up the majority of the evaluated literature. Because of this, the results might not be entirely applicable to educational environments that are developing or culturally diverse. Third, because desire is dynamic and multifaceted, it is challenging to fully capture all contributing aspects, especially those that are subtle, context-specific, or changing quickly in response to social and technological advancements. Lastly, discrepancies in methodological techniques and definitions of ambition between research may result in some conceptual contradictions because this study synthesizes data from a variety of theoretical frameworks.



**Bhavna Chaudhary and Anoj Raj**

The study offers a crucial basis for comprehending educational goals in spite of these drawbacks and identifies areas where more complex, context-sensitive insights might be obtained from future research.

Implications and Future Research Directions

For educators, legislators, and academics, the study's conclusions have a number of significant ramifications. Comprehending the factors that influence students' academic goals emphasizes the necessity of loving family settings, fair resource distribution, and inclusive institutional policies. In order to encourage students from underprivileged socioeconomic backgrounds to have realistic yet ambitious goals, schools and colleges should create focused interventions including outreach programs, career counseling, and mentorship programs. In addition to emphasizing academic success, policymakers must also build systems that support and uphold students' long-term goals in order to lessen educational disparity. Future studies should expand on the findings of this study by using a wider range of methodological techniques and being more context-sensitive. To track how ambitions evolve and alter over time in response to life events, transitions, and changing socioeconomic circumstances, longitudinal studies are required. Comparative research across national and cultural contexts can shed light on how educational goals are influenced differently in various environments. Furthermore, a more comprehensive knowledge of students' goals, drives, and difficulties would be possible through the integration of mixed methodologies, which combine quantitative measurements with qualitative narratives. Lastly, future research should look at how new elements like social media, digital technology, and global mobility affect how young people see and pursue their educational careers.

CONCLUSION

Long acknowledged as a key idea in educational research, educational desire acts as a link between personal ambition and systemic opportunity. A complicated interaction between family history, socioeconomic circumstances, gender, school environment, peer networks, and cultural expectations shapes ambitions, as the reviewed research shows. While more recent studies (Khatab, 2015; Sharma & Sharma, 2015) emphasize the significance of contextual disparities like ethnicity, rural–urban divides, and access to educational resources, classic studies (Sewell, Haller & Portes, 1969; Marjoribanks, 2003) emphasized the impact of family and school. This review offers some important insights. First, while ambitions might inspire success, they can also constrain aspirations, resulting in cycles of adversity. Ambitions are both predictors and consequences of educational attainment. Second, goals are not static but rather dynamic, changing as people go through different phases of life and in reaction to shifting social, cultural, and economic circumstances. Third, even if the majority of students have lofty ideals, it takes supporting regulations, sufficient funding, and organized instruction to turn them into achievable objectives. The analysis also identifies important gaps in the body of current research. Relatively few studies come from developing nations, where socioeconomic gaps are glaring, and the majority of research is still focused on Western contexts. Furthermore, there are not many longitudinal or intersectional viewpoints, which leaves open concerns of how ambitions change over time and interact with elements like caste, ethnicity, or 21st-century internet access. The requirement for context-sensitive research and treatments is critical going ahead. Aspirations can grow in circumstances created by policies that assist families, lessen socioeconomic disparities, and ensure equal access to high-quality education. Integrating mentorship, career counseling, and culturally sensitive education is equally crucial in ensuring that students' goals are both challenging and reachable. In summary, educational desire is still a strong indicator of academic and professional success, but its fulfillment relies on the cooperation of structural assistance and personal drive. By tackling socio-economic and cultural obstacles and bolstering educational and community support networks, we may establish an environment that fosters the development and realization of ambitions.





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Contribution

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Bhavna Chaudhary and Anoj Raj

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Bhavna Chaudhary and Anoj Raj

Table : 1 Studies of different Determinants of Educational Aspiration

Author(s) (Year)	Title (source)	Methodology and sample	Key finding (one line)
Salgotra, A. K., & Roma, K. (2018).	Educational aspiration and socio-economic status among secondary school students.	Quantitative, survey of 351 students in Jammu district.	Students with higher socio-economic status reported significantly higher educational aspirations compared to those from lower SES backgrounds.
Das, P. K., Panigrahi, J. K., Naik, I. C., & Das, B. (2019)	Examination of socio-economic and socio-cultural factors influencing aspiration levels of youth with respect to their education levels	Quantitative, experimental examination of 384 college-going students	There is no significant difference between male and female in their level of aspiration
Zang, P. L., Kwao, M. P., Anim, C. L., & Ahasu, E. K. (2024).	Socio-economic characteristics of parents on learners' educational aspiration: Exploring mechanisms of impact	descriptive survey design, employing a sample of learners (230) of the Greater Accra region of Ghana.	parental background and economic standing collectively impact youth's vision for their academic future.
Oketch, M., Mutisya, M., & Sagwe, J. (2012).	Parental aspirations for their children's educational attainment and the realisation of universal primary education in Kenya	The study used cross-sectional household data for a sample of 4065 parents, collected in 2007 by the African Population	Parents in both slum and non-slum areas of Nairobi held high educational aspirations for their children
Sosu, E. M. (2014).	Predicting maternal aspirations for their children's education: The role of parental and child characteristics.	Quantitative, analysis using secondary data (N=1999)	Results showing a positive relationship between maternal age and educational aspirations of the children
Pandey, P. K., Pandey, P. K., Mahajan, S., Srivastava, N., & Asif Shah, M. (2024).	Examining the impact of parental education and socio-demographic factors on career aspirations in adolescent students in Delhi NCR, India: A cross-sectional study.	Cross-sectional survey research, 440 adolescent students from various schools in Delhi NCR region.	The results showed significant differences in the attitudes of students at private and government universities, and a significant effect of parental education, but no significant impact of gender. Delhi NCR region of India.
Hill, N. et al. (2004).	Parent academic involvement as related to school behavior, achievement, and aspirations: Demographic variations across adolescence.	Survey method is used, 463 adolescents, followed from 7th through 11th grades in the United States .	Parental academic involvement positively predicts adolescents' school behavior, academic achievement, and educational aspirations across diverse demographic groups.
King, V., Pragg, B., & Lindstrom, R. (2020)	Family structure, step-parents' aspirations, and college attendance. Journal of Family Studies.	Secondary analysis of Add Health longitudinal data of US. Adolescents	Stepfathers' relationships with stepchildren had no direct effect on educational attainment, but both stepfathers' and mothers' expectations significantly shaped college intentions and attendance.





Bhavna Chaudhary and Anoj Raj

Fan & Williams (2010)	The differential effects of parental involvement on high school completion and postsecondary attendance.	Quantitative, survey-based analysis. sample of thousands of high school students in U.S.	Parental involvement (school activities, home-based support) and parental aspirations were strong predictors of high school completion and college enrollment.
Wang et al. (2023)	The influence of individual, peer, and family factors on educational aspirations: Evidence from rural China.	using survey questionnaires of 1,313 rural Chinese adolescents	Individual motivation, peer influence, and parental support all significantly predicted students' educational aspirations
Beaman, Duflo, Pande & Topalova (2012), <i>Science</i>	Female leadership raises aspirations and educational attainment for girls: a policy experiment in India.	Descriptive survey of 8,453 adolescents & parents.	Female leadership raised girls' aspirations, reduced gender gap in education and chores, and eliminated schooling gap.
Stoet & Geary (2022)	Sex differences in adolescents' occupational aspirations: Variations across time and place.	Secondary analysis of 473,260 adolescent.	Gender gaps were larger in gender-equal & wealthy countries . Parental modeling also influential.
Dickerson, Maragkou & McIntosh (2018)	The causal effect of secondary school peers on educational aspirations	Qualitative research, 7,938 pupils were sample in England	School peers causally influence aspirations to stay in education and choose academic post-compulsory paths, identified using peers-of-peers strategy.
González Amador, Cowan & Nillesen (2022)	Peer networks and malleability of educational aspirations	Experimental research design 1500 students of 45 high schools in Mexico were sample	The video intervention significantly increased students' educational aspirations and demonstrated peer network interdependencies.
Roeser, R. W., & Eccles, J. S. (2014).	Schooling and the mental health of children and adolescents in the United States.	a conceptual and review chapter on multiple U.S. datasets and studies .	School environments strongly shape both academic achievement and mental health outcomes .
Buchmann, C., & Park, H. (2009).	Stratification and the formation of expectations in highly differentiated educational systems.	Comparative study, Data from OECD and cross-national surveys of Germany, Japan, Korea.	In systems with early and rigid tracking , students from lower socioeconomic backgrounds formed lower educational expectation early.
Byun, S. Y., Schofer, E., & Kim, K.-K. (2012).	Revisiting the role of cultural capital in East Asian educational systems: The case of South Korea.	Longitudinal Survey method, 5,000 middle and high school students in South Korea were sample.	Cultural capital (remained a strong predictor of aspirations and attainment in South Korea, despite high standardization of schools.
Harding, D. J. (2011).	Rethinking the cultural context of schooling decisions in disadvantaged neighborhoods: From deviant subculture to cultural heterogeneity	qualitative research method, Adolescents and families in low-income urban communities in the U.S. were sample.	Educational aspirations are shaped as much by neighborhood cultural context as by family or school.
Deci, E. L., & Ryan, R. M. (2000).	The "what" and "why" of goal pursuits: Human needs and the self-determination of behavior. Psychological Inquiry,	Theoretical review paper from the US, Canada, and Europe .	Satisfaction of three needs (autonomy, competence, relatedness) promotes internalized motivation, well-being, and persistence .





Bhavna Chaudhary and Anoj Raj

Eccles, J. S., & Wigfield, A. (2002).	Motivational beliefs, values, and goals. Annual Review of Psychology	Comprehensive review longitudinal studies with school-aged children and adolescents , of United States	Students' achievement-related choices and performance are strongly predicted by their expectancies for success and the value they assign to tasks , shaped by social, cultural, and gender influences.
Marsh, H. W., & Martin, A. J. (2011)	Academic self-concept and academic achievement.	Longitudinal and cross-lagged panel analyses of school and university students (Australia)	Students with higher self-concept show greater persistence, motivation, and long-term achievement.

Table : 2, Studies related to different Dimensions of Educational Aspiration

Author(s) (Year)	Title (source)	Methodology and sample	Key finding (one line)
Schoon (2006)	Teenage aspirations for future careers and occupational outcomes.	Longitudinal and comparative analysis,	Short-term aspirations are linked to school performance, while long-term aspirations are strongly shaped by socio-economic and cultural contexts
Harackiewicz et al. (2000)	Short-term and long-term consequences of achievement goals: predicting interest and performance over time.	Longitudinal study, 250 students in psychology courses of U.S. college	Mastery goals predicted sustained interest and learning, while performance goals predicted short-term grades but not long-term engagement.
Lakshmanan (2004)	A longitudinal study of adolescent educational aspirations and their relationship to college choice using hierarchical linear modeling and group-based mixture modelling	Longitudinal quantitative study, 88 U.S. adolescents was sample.	Stable long-term aspirations strongly predicted college application and enrollment, whereas fluctuating short-term goals reduced postsecondary alignment.
Dobewall et al. (2019)	Health and educational aspirations in adolescence: A longitudinal study in Finland.	Longitudinal survey-based study, n ≈ several thousand, national cohort data	Good adolescent health supported long-term academic aspirations, while poor health conditions led to vocational, short-term oriented pathways.
Khattab (2015)	Explaining the educational aspirations–expectations mismatch among middle school students: The role of parental expectations, attitudinal and demographic factors.	large-scale survey method, UK adolescents across ethnic and socioeconomic groups was sample.	Misalignment between educational and occupational aspirations is common, especially in systems with weak school-to-work transitions.
Valls et al. (2022)	Educational and occupational aspirations: A longitudinal study of Vienna youth.	longitudinal study , 100 Students in Vienna was sample.	Aspiration development showed 11 stable patterns, diverging mainly due to migration background, parental education, and school performance.
Beal & Crockett (2010)	Adolescents' occupational and educational aspirations and expectations: Links to high school activities and adult educational attainment.	Longitudinal survey-based study, U.S. nationally representative sample of adolescents	High academic and occupational aspirations predicted greater engagement in advanced coursework, extracurricular participation, and higher adult educational attainment.





Bhavna Chaudhary and Anoj Raj

Chen, Allen, & Hesketh (2023)	The influence of individual, peer, and family factors on the educational aspirations of adolescents in rural China. <i>Social Psychology of Education</i> .	Quantitative survey, 606 adolescents wsa sample.	Parental aspirations had the strongest influence on students' educational goals, while peer influence was weaker; family attachment and friendships indirectly shaped aspirations .
Pindar & Singh (2021)	A comparative study on educational aspiration and parental encouragement among rural and urban high school children.	Comparative survey, N = 240 (120 urban Hisar, 120 rural Fatehabad, Haryana, India)	urban students showed significantly higher educational aspirations and greater parental encouragement than rural peers
Li & Hou (2022)	Differences in educational expectations between urban and rural junior high school students: Individual, family, and socio-structural influences.	Comparative study using survey data from Southwest University, Chongqing, China;	urban junior high students reported higher educational expectations than rural and migrant peers, with disparities driven by family background, school environment, and hukou-related structural barriers
Agger, Meece & Byun (2018)	The influences of family and place on rural adolescents' educational aspirations and post-secondary enrollment.	Nationwide U.S. survey, N = 3,456 rural adolescents	higher parental expectations predicted greater aspirations and college enrollment, while local job opportunities lowered aspirations
Ye et al. (2021)	On class differences in educational aspirations and educational expectations: A social analysis based on national data.	Survey of rural middle school students in central China	As a result, children left behind showed high aspirations but low expectations.

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Academic Stress and Learning Outcomes in the Digital Age : A Critical Review

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ABSTRACT

The COVID-19 epidemic has sped up the digitization of education, transforming conventional learning settings into online, digital, and hybrid formats. This paradigm change has given students access to digital platforms, virtual classrooms, and cutting-edge educational technology that have never before offered them flexibility, accessibility, and interactive learning experiences. New difficulties have been brought about by the shift, nevertheless, especially in regards to students' increased academic stress. Stress levels have been considerably impacted by elements including excessive screen time, a lack of in-person peer connection, technical hurdles, ongoing evaluations, and a blurring of the lines between personal and academic life. This review study critically investigates the intricate connection between academic stress and learning outcomes in the digital age. It examines the main sources of stress, mediating factors such as resilience, emotional intelligence, and self-regulation, and coping mechanisms used by students in online learning settings. The study also emphasizes how stress experiences differ among socioeconomic backgrounds, fields, and demographics. Untreated stress can have a detrimental effect on motivation, cognitive function, and overall academic accomplishment, according to the findings, even while digital education can increase engagement and improve specific learning outcomes. The review's conclusions highlight the importance of learner-centered approaches, mental health support, balanced digital pedagogy, and sustainable educational policies for ensuring that the digital transformation of education fosters both academic success and student well-being.

Keywords- academic stress, learning outcomes, digital age, emotional intelligence, self-regulation, time management, psychological problems, academic performance





INTRODUCTION

Global education has undergone significant change as a result of the digital age, making online, hybrid, and blended learning essential elements of contemporary academic life. This change was expedited by the COVID-19 epidemic, which forced a dramatic change in the dynamics of teaching and learning by requiring a quick transition from traditional classroom-based instruction to technology-mediated learning platforms (Dhawan, 2020; Adedoyin et al. 2020). This shift has created a new set of difficulties that have a significant impact on students' academic and mental health, even while it has also increased chances for flexible learning, easy access to educational materials, and collaborative involvement through digital platforms. One of the foremost concerns emerging from digital learning environments is **academic stress**, a phenomenon traditionally associated with examinations, workload, and peer competition (Pascoe et al. 2020). In the digital context, academic stress now manifests in novel forms, such as excessive screen time leading to fatigue, information overload due to constant exposure to digital content, and technical barriers like unstable internet connectivity or lack of adequate devices (Sahu, 2020; Maqsood et al., 2021). These challenges are compounded by the lack of face-to-face social interaction, which can lead to feelings of isolation, reduced peer support, and diminished motivation (Chaturvedi et al., 2021).

Additionally, the online modality brings with it additional academic stresses such as anxiety related to online assessments, the pressure of ongoing evaluation, and digital distractions from multitasking and social media (Mahapatra et al., 2021). Since students work in settings where the lines between their personal and academic lives are blurred, these stresses are frequently ubiquitous and hard to control, in contrast to conventional ones. Digital learning resources are available around-the-clock, which is advantageous, but it may also encourage an over-engagement culture that leads to fatigue and decreased academic productivity (Hossain et al., 2022). It is crucial to comprehend how these stresses interact with learning objectives. It has been demonstrated that academic stress exacerbates mental health conditions including anxiety and depression and has a detrimental impact on focus, cognitive function, and overall success (Wang et al., 2022; Zhao, 2022). On the other hand, when utilized properly, some digital platforms may foster self-regulated learning, resilience, and collaborative involvement, all of which reduce stress and improve performance (Aslan et al., 2022; Mishra et al., 2023). Investigating the dual character of digital education—both a source of academic stress as well as a facilitator of academic growth—is crucial. By looking at major stressors, mediating characteristics including emotional intelligence and self-regulation, and coping strategies used by students, this review study aims to examine the link between academic stress and learning outcomes in the digital age. In order to help educators, institutions, and policymakers create supportive, balanced, and sustainable digital education environments that foster both academic performance and student well-being.

METHODOLOGY

This review paper adopts a systematic methodology to analyze the existing literature on academic stress in digital learning environments. The process involved identifying, selecting, and synthesizing relevant studies to ensure a comprehensive understanding of the topic. To begin with, major academic databases such as Scopus, Web of Science, ERIC, Springer Link, Taylor & Francis, and Google Scholar were searched to collect peer-reviewed articles, conference papers, and reports. The review applied Studies published between 2020 and 2025, peer-reviewed articles, and papers focusing on students' academic stress, coping mechanisms, and digital learning environments were included. Through this systematic approach, the review ensures credibility, thematic clarity, and a holistic understanding of the multiple dimensions of academic stress in digital learning contexts.





Arti Sharma and Anoj Raj

RESULT AND DISCUSSION

Academic Stress in the Digital World

Academic stress in the digital world has become a growing concern for students as they cope with the challenges of online and technology-driven learning. The constant pressure to meet deadlines, adapt to digital platforms, and perform well in virtual assessments often results in heightened anxiety and burnout (Pascoe et al., 2020). Increased screen time, limited face-to-face interaction, and reduced peer support further intensify feelings of isolation and stress (Son et al., 2020). Moreover, high parental expectations, competitive academic culture, and the overwhelming flow of online information contribute to the psychological burden on learners (Hasan et al., 2020). While digital education provides flexibility and access to vast resources, the imbalance between academic demands and student well-being underscores the need for effective coping strategies, self-regulated learning, and institutional support to mitigate stress and ensure holistic development (Dhawan, 2020).

Sources of Academic Stress-

The various elements, circumstances, or events in a student's academic life that produce pressure, tension, or worry regarding learning, studying, and performance are known as sources of academic stress (Pascoe et al., 2020). Sources of academic stress are shown in diagram -1 which are:

Technological Barriers

In digital learning contexts, technological obstacles are one of the biggest causes of academic stress. Students usually experience unreliable platforms, restricted bandwidth, and bad internet access, which interferes with the continuity of online courses and tests (Dhawan, 2020). Additionally, the absence of computers, tablets, or current software, among other device constraints, exacerbates digital inequality, especially for pupils from underprivileged or rural backgrounds (Adedoyin et al., 2020). Increased cognitive load and decreased learning efficiency result from irritation heightened by unfamiliarity with digital technologies, online learning management systems, and virtual communication platforms (Maqsood et al., 2021). These technology interruptions cause emotions of powerlessness, anxiety, and disengagement in addition to impeding academic achievement.

Workload and Time Management

In contrast to traditional classroom settings, the digital method of education requires greater degrees of autonomy and self-regulation. It can become burdensome for students to be expected to manage several assignments, take part in online conversations, and fulfill deadlines without explicit supervision (Agung et al., 2020). Although online learning offers flexibility, it also blurs the lines between personal and academic life, making it challenging for students to maintain balance (Bao, 2020). When combined with constant messages and digital multitasking, poor time management skills frequently lead to procrastination, last-minute stress, and subpar performance (Mishra et al., 2023).

Screen Fatigue

Another major stressor in the digital academic environment is prolonged use of digital screens. Long periods of time spent participating in conversations, doing projects, and watching online lectures might cause headaches, musculoskeletal pain, eye strain, and short attention spans (Wang et al., 2021). The lack of outdoor activities and physical breaks during digital learning, particularly during COVID-19 lockdowns, exacerbates this issue, which is known as screen weariness (Sultana et al., 2021). Learning results are directly impacted by the cognitive stress brought on by multitasking, simultaneous video conferencing, and constant alerts, which impair focus and memory.

Isolation and Lack of Peer Support

Students' emotional and intellectual well-being is greatly impacted when they are unable to engage with peers and teachers in person. Digital settings frequently lack the informal chances for peer support, encouragement, and cooperation that traditional classrooms offer (Adedoyin et al., 2020). Feelings of loneliness, alienation, and a lack of



**Arti Sharma and Anoj Raj**

belonging in the academic community might result from isolation in digital learning (Chaturvedi et al., 2021). Lack of in-person support and social connections deters many students from being motivated, which raises academic stress and lowers resilience when faced with difficult assignments.

Assessment Pressure

Another significant cause of stress is taking tests and evaluations online. Particularly in the lack of uniform monitoring, students frequently voice worries about the integrity, fairness, and dependability of digital assessments (Besser et al., 2020). Anxiety during high-stakes testing is increased by technical issues such system crashes, erratic internet, or late submissions (Wang et al., 2022). Stress levels are further increased by peers' concern of academic dishonesty and the opaqueness of grading (Mahapatra et al., 2021). Academic insecurity has increased in the digital age due to the need to adjust to new forms, including timed online quizzes, open-book digital examinations, and continuous evaluation.

In this table-1 there are the sources of academic stress, issues and their impact on students. The examination of digital academic stresses reveals that although each element has an impact on students' wellbeing on their own, they are also intricately linked. When students are unable to connect consistently, technological hurdles might exacerbate workload, assessment pressure, and even feelings of loneliness (Dhawan, 2020; Maqsood et al., 2021). Similar to this, ineffective time management in digital settings increases screen exposure and workload stress, which results in weariness and cognitive deterioration (Agung et al., 2020; Wang et al., 2021). Long-term seclusion, compounded by a lack of support from peers and teachers, lowers motivation, which increases kids' susceptibility to procrastination and test anxiety (Chaturvedi et al., 2021; Mahapatra et al., 2021).

Assessment pressure is the most significant stressor of all as it directly affects academic confidence and results by combining workload demands, technological uncertainty, and equality concerns (Besser et al., 2020; Wang et al., 2022). Therefore, the cumulative and interrelated consequences of technological, cognitive, and social constraints in online learning settings are the cause of digital academic stress rather than a single element.

Psychological Impacts of Digital Academic Stress

In the digital age, academic stress has a substantial impact on students' psychological and emotional health in addition to their academic success. A number of psychological effects, including anxiety, burnout, depression, and decreased motivation, are frequently the result of the interaction between technology hurdles, workload, isolation, and evaluation anxiety. Diagram-2 shows the **Psychological Impacts of Digital Academic Stress**

Anxiety and Uncertainty

Students' anxiety levels are raised by frequent technological difficulties, erratic internet connections, and doubts regarding online test protocols (Sahu, 2020). This ongoing state of anxiety is exacerbated by the unpredictability of digital learning platforms and the anxiety of missing deadlines or losing assessment chances because of technological difficulties (Besser et al., 2020). According to research, anxiety brought on by digital learning is strongly associated with worse learning outcomes and less academic engagement (Maqsood et al., 2021).

Burnout and Fatigue

Academic burnout is the term used to describe the mental and physical tiredness that is frequently caused by prolonged academic demands and constant exposure to digital technologies (Wang et al., 2021). The lines between study and rest are frequently blurred in digital learning environments, in contrast to conventional learning environments where regular schedules and physical breaks offer respite. Over-engagement is encouraged by the continual availability of learning materials, which leads to exhaustion, demotivation, and disengagement from educational activities (Sultana et al., 2021).



**Arti Sharma and Anoj Raj****Depression and Social Isolation**

Feelings of isolation and alienation are greatly increased when in-person peer support and classroom contact are absent. Long-term seclusion has been associated with depressive symptoms, especially in students who are highly motivated to study in groups and through collaborative learning (Adedoyin et al., 2020; Chaturvedi et al., 2021). In digital contexts, a lack of social connections not only impairs academic perseverance but also emotional resilience.

Reduced Motivation and Self-Efficacy

Digital academic pressures also affect self-efficacy and motivation, two important factors that determine learning achievement. Students' confidence in their capacity to meet academic objectives is frequently undermined by digital distractions, multitasking demands, and anxiety related to online assessments (Mahapatra et al., 2021). Learning outcomes are further diminished by avoidance behaviors, procrastination, and lower involvement in academic activities brought on by this lack of self-belief (Mishra et al., 2023).

Cognitive Overload

Cognitive overload, which affects memory, focus, and critical thinking, is brought on by the sheer amount of digital information, continuous notifications, and multitasking demands (Wang et al., 2022). Stressed-out students frequently have trouble processing information efficiently, which impairs their performance on tests and retention of long-term learning. These are the psychological impacts on digital academic stress. Which are interpret in the given table- 2

Instead than being separate, the psychological and emotional effects of digital academic stress are linked and reinforce one another. As students find it difficult to absorb information under pressure, anxiety and confusion brought on by erratic technology and ambiguous procedures (Sahu, 2020; Maqsood et al., 2021) frequently turn into cognitive overload (Wang et al., 2022). According to Wang et al. (2021), prolonged exposure to stresses like these might result in burnout and tiredness, which lowers physical energy and academic interest. Simultaneously, the lack of peer relationships leads to social isolation and despair, which further reduces resilience and the capacity to handle academic expectations (Chaturvedi et al., 2021). This loop erodes students' self-efficacy and motivation, which in turn erodes their confidence in their ability to achieve (Mahapatra et al., 2021; Mishra et al., 2023). Burnout and decreased motivation are the most important long-term effects among these as they not only affect academic performance but also have a detrimental impact on students' attitudes toward learning and wellbeing for the rest of their lives. Interventions must thus target both the individual stresses and the cumulative and cyclical consequences on kids' academic performance and mental health.

Behavioural Impact

Students are suffering from severe psychological and behavioural effects as a result of the increased academic stress brought on by the growth of digital learning environments. Digital stressors, in contrast to conventional types of academic stress, frequently combine academic, social, and technical pressures, which increases their negative effects on learning outcomes and mental health. Behavioural Impact are shown in the diagram-3

Anxiety and Emotional Distress

According to studies, students' anxiety levels are considerably increased by digital stressors such erratic internet access, ambiguity in online tests, and an ongoing workload (Hasan et al., 2020; Sahu, 2020). Chronic anxiety impairs academic performance, focus, and decision-making in addition to emotional health. Avoidance behaviors and a decrease in academic involvement are common among students who suffer from digital learning anxiety (Maqsood et al., 2021).

Burnout and Exhaustion

Academic burnout, which is typified by fatigue, disengagement, and waning academic interest, is exacerbated by extended use of digital platforms. Students find it challenging to maintain constant academic efforts due to increased weariness caused by prolonged screen usage, frequent multitasking, and the lack of organized timetables (Wang et



**Arti Sharma and Anoj Raj**

al., 2021; Sultana et al., 2021). Because the lines between academic, personal, and recreational domains are blurred in digital learning environments, burnout frequently appears earlier than in traditional learning environments.

Procrastination and Avoidance Behaviors

Procrastination and academic stress are closely related in digital learning. Academic obligations are frequently neglected by students who are overburdened by constant work, electronic diversions, and evaluation pressure (Agung et al., 2020). Due to the accumulation of unfinished work and approaching deadlines, this procrastination cycle exacerbates stress and lowers academic performance (Hossain et al., 2022).

Reduced Self-Efficacy and Motivation

It has been discovered that high levels of digital academic stress lower students' self-efficacy, or their confidence in their capacity to complete academic assignments successfully (Hasan et al., 2020; Mahapatra et al., 2021). Lower engagement, less involvement in class, and a reluctance to take on difficult activities are frequently the results of a drop in self-confidence. Furthermore, digital stresses including loneliness, a lack of feedback, and assessment anxiety frequently erode intrinsic motivation, which is the inner urge to study for personal progress and fulfilment (Chaturvedi et al., 2021).

Cognitive and Behavioural Disruptions

Additionally contributing to cognitive overload, stress in digital learning settings affects memory, focus, and problem-solving skills (Wang et al., 2022). Learning efficacy is further diminished by common behavioural disturbances such as excessive multitasking, frequent social media monitoring, and disengagement during online lectures (Adedoyin et al., 2020). When these maladaptive habits persist, they lead to worse academic results and decreased long-term memory recall. These are all the Behavioural Impact which are also define in the table-3

The behavioural and psychological effects of digital academic stress are interrelated rather than distinct, creating a vicious cycle that gradually deteriorates learning results. Workload demands, unclear exam formats, and erratic internet connectivity can all serve as entrance points for anxiety and mental discomfort (Hasan & Bao, 2020; Sahu, 2020). Students who experience ongoing worry are less able to focus and make decisions, which makes them resort to avoidance strategies like procrastination (Agung et al., 2020; Maqsood et al., 2021). In digital learning settings, the lines between study and relaxation are blurred, which accelerates students' burnout and tiredness as academic duties mount (Wang et al., 2021; Sultana et al., 2021). As a result, students become less confidence in their academic ability and experience a decline in intrinsic motivation and self-efficacy (Mahapatra et al., 2021; Chaturvedi et al., 2021). While excessive multitasking and continuous alerts lead to cognitive overload, which impairs memory and critical thinking, this lack of confidence exacerbates avoidance and disengagement (Wang et al., 2022; Adedoyin et al., 2020). In the end, these stresses work together to create a vicious cycle in which procrastination causes burnout, worry causes procrastination, burnout lowers motivation, and cognitive overload hinders recovery, all of which contribute to poor academic performance and emotional instability.

Academic Stress and Learning Outcomes

In the digital age, students' learning results are significantly impacted by academic stress. Prolonged or extreme stress is substantially associated with detrimental behavioral, psychological, and academic outcomes, even though moderate amounts of stress might occasionally serve as a motivator. Thus, there is a complicated link between academic stress and learning outcomes that is mediated by personal traits, coping mechanisms, and institutional support networks. Reduced academic performance, worse grades, and lower retention rates are all regularly linked to high levels of academic stress (Pascoe et al., 2020). Stress impairs fundamental cognitive abilities that are necessary for academic performance, including working memory, attention span, problem-solving, and critical thinking (Hasan et al., 2020). These deficiencies are made worse in digital learning environments by technological difficulties, anxiety related to online assessments, and continual multitasking, which results in decreased productivity and disengagement (Wang et al., 2022). Additionally, high-stress students are more likely to delay, stop participating in class assignments, and exhibit waning enthusiasm, all of which exacerbate their subpar performance (Agung et al.,





2020). Burnout can also result from prolonged exposure to digital academic stress, which can lower academic perseverance and raise dropout intentions (Sultana et al., 2021).

Mediating Factors

While academic stress has generally negative effects on learning outcomes, several mediating factors can moderate its impact and help students cope more effectively.

Self-Regulated Learning (SRL):

Stress has less of an adverse influence on students who possess good self-regulation abilities, such as efficient time management, goal-setting, and metacognitive awareness (Zimmerman et al., 2021). SRL helps students manage their workloads, control distractions, and adjust to online exams in digital learning, where independence and autonomy are necessary. This lessens the negative impacts of academic stress (Hossain et al., 2022).

Emotional Intelligence (EI):

Because they are able to identify, control, and make positive use of their emotions, emotionally intelligent pupils are better able to handle the demands of the classroom (MacCann et al., 2020). Emotional intelligence (EI) helps students deal with ambiguity, control their annoyance from technical obstacles, and stay focused on difficult assignments. Even in high-stress digital learning contexts, studies have shown that students with higher EI are more resilient, motivated, and accomplish better academically (Aslan et al., 2022).

Social and Institutional Support:

Peer cooperation, teacher-student connection, and institutional support networks all considerably lessen the negative effects of stress. Frequent feedback, group learning chances, and mentorship all help people feel less alone and more like they belong, which lowers stress levels (Chaturvedi et al., 2021). It has been demonstrated that by addressing stress-related issues, institutional techniques such flexible deadlines, digital literacy instruction, and mental health counselling enhance academic performance (Mishra et al., 2023).

Moderate stress

Moderate stress, also known as eustress, can motivate pupils, while excessive and persistent stress is detrimental. Eustress inspires students to achieve academic deadlines and perform well under duress by promoting alertness and improving attention (Salmela-Aro et al., 2021). Short-term stress brought on by impending tests or project deadlines might encourage creativity and problem-solving in digital learning settings. However, stress turns from eustress to distress when it persists, is unmanaged, or is out of proportion to coping mechanisms, which results in disengagement, academic burnout, and a worsening in mental health (Wang et al., 2022). In order to maximize stress's motivational power without letting it become too much to handle, educators and institutions must balance stress levels. Moderate stress and their impact are shown in the table – 4

In digital learning, managing academic stress necessitates a multidimensional strategy that incorporates institutional, emotional, and personal coping mechanisms. Higher self-regulation abilities help students better manage their time, set realistic objectives, and adjust to the demands of online learning, all of which lessen the harmful impacts of stress (Zimmerman et al., 2021; Hossain et al., 2022). This makes self-regulated learning (SRL) essential. By helping students control their irritation, deal with uncertainty, and sustain intrinsic motivation, emotional intelligence (EI) builds resilience at the emotional level. This directly improves academic performance in high-stress digital environments (MacCann et al., 2020; Aslan et al., 2022). Social and institutional support are equally crucial, since peer cooperation, instructor direction, and institutional programs (such counselling and flexible regulations) combat feelings of loneliness and academic strain while enhancing perseverance and general wellbeing. (Chaturvedi et al., 2021; Mishra et al., 2023). It's interesting to note that not all stress is bad; eustress, or mild stress, may be a motivating factor that improves performance under pressure and focus and creativity. But if left unchecked, this stress turns into discomfort, which causes disengagement and burnout (Salmela-Aro et al., 2021; Wang et al., 2022). Thus, to maximize



**Arti Sharma and Anoj Raj**

student achievements in digital learning, a balanced interaction of SRL, EI, support networks, and calibrated stress levels is necessary.

Learning Outcomes in a Digital World

The quality of education, the infrastructure of technology, and the students' capacity to handle academic stress all influence the cognitive, emotional, and behavioral aspects of learning outcomes in digital settings. Higher-order thinking, teamwork, and flexible learning can all be improved by digital education, but these advantages are undermined by excessive stress, which interferes with focus, motivation, and engagement.

Cognitive Outcomes

Effective instructional design and the use of interactive technologies may greatly improve critical thinking, problem-solving, and information retention in digital learning (Dhawan, 2020). Online platforms enable possibilities for collaborative knowledge production, access to a variety of resources, and tailored learning experiences—all of which improve cognitive abilities (Bao, 2020). High levels of academic stress, however, have a detrimental impact on these results. Stress disturbs executive skills including reasoning and analytical thinking, shortens attention spans, and damages working memory (Pascoe et al., 2020; Wang et al., 2022). Further impairing focus and resulting in shallow learning and poor retention are digital distractions and cognitive stress brought on by multitasking, which involves alternating between social media, assignments, and online lectures (Sultana et al., 2021). So, even if digital platforms have cognitive potential, their usefulness is greatly moderated by academic stress.

Affective Outcomes

A key factor in influencing the effectiveness of digital learning is the attitudes, motivation, and emotional health of the students. Persistence in online settings is increased by positive emotional engagement, which includes self-confidence, academic curiosity, and intrinsic motivation (MacCann et al., 2020). On the other hand, high academic stress raises dropout intentions, decreases motivation, and cultivates unfavorable attitudes toward digital learning (Mahapatra et al., 2021). Emotional resilience can be diminished and loneliness exacerbated by a lack of in-person peer connection and teacher assistance (Chaturvedi et al., 2021). Anxiety and burnout brought on by stress further erode zeal, resulting in disengagement and worse academic satisfaction (Hossain et al., 2022). In this situation, the affective dimension is frequently the most susceptible to digital stresses, which has a direct impact on students' decision to continue or stop participating in online learning activities.

Behavioral Outcomes

Study habits, involvement, and engagement all show behavioural learning outcomes in digital education. Positive behavioral results are shown by regular attendance in virtual classrooms, active involvement in online conversations, and teamwork in group projects (Adedoyin et al., 2020). But these habits are significantly impacted by digital stresses such as screen weariness, task pressure, and evaluation anxiety. Due to exhaustion and demotivation, stressed students are more likely to delay, avoid online involvement, or turn in assignments late (Agung et al., 2020). Additionally, students' capacity to maintain rigorous academic routines is diminished by digital temptations like social media, multitasking, and gaming, which disrupt regular study habits (Mishra et al., 2023). Students who have robust coping mechanisms and institutional support, on the other hand, exhibit superior behavioral adaptability, resilience, and active engagement in spite of stress.

Strategies to Reduce Academic Stress

Adopting comprehensive measures that integrate institutions, educational innovations, and student-level coping mechanisms is crucial given the widespread nature of academic stress in digital learning. A multifaceted strategy that improves emotional resilience, social connectivity, and digital literacy in addition to addressing academic overload is necessary for effective stress reduction.



**Arti Sharma and Anoj Raj****Institutional Interventions**

Institutional interventions are official measures or programs introduced by an institution to address problems and create a supportive, effective, and healthy environment. These are-

Adaptable Assessment Techniques: Due to worries about fairness, technological difficulties, and ongoing monitoring, online tests can cause more stress. By using flexible assessment techniques like open-book examinations, project-based evaluations, and formative assessments—which place an emphasis on comprehension rather than rote memorization—institutions can lessen the stress associated with tests (Besser et al., 2020).

Digital Literacy and Capacity Building: Due to their lack of experience with online resources, many students find it difficult to use digital platforms. Students and instructors who participate in training programs that improve their digital literacy experience less stress from technology, become more adaptable, and feel more confident in digital settings (Dhawan, 2020).

Mental Health Support Services: Students are more resilient to academic stress when they have access to counselling, stress management classes, and mental health services. Psychological effects can be greatly enhanced by campus-wide programs that address anxiety and exhaustion as well as regular well-being check-ins (Pascoe et al., 2020; Mishra et al., 2023).

Policy-Level Interventions: On a larger scale, policies that assist impoverished students' financial aid, dependable internet, and fair access to digital devices aid in lowering systemic causes of academic stress (Adedoyin et al., 2020).

Pedagogical Approaches

The ideas, techniques, and learning strategies that educators use to help students grow their knowledge, abilities, and values are referred to as pedagogical approaches (Eggen et al., 2019). They stand for the ideas and methods that are applied in classrooms to increase student engagement, foster comprehension, and enhance learning results. In a simple word pedagogical methods are the various ways in which pupils learn and teachers instruct.

Blended Learning: Creating learning environments that strike a balance between academic rigor and student well-being is the equal responsibility of instructional designers and teachers. Blended learning models have been shown to maintain peer engagement and teacher-student connection while reducing digital fatigue through a mix of in-person and online instruction. Stress levels are reduced by blended learning, which offers structure, social interaction, and academic flexibility (Agung et al., 2020).

Interactive Educational Exercises: According to Adedoyin et al. (2020), pedagogical strategies that incorporate group projects, breakout rooms, gamified assignments, and collaborative projects lessen boredom and promote peer support, which decreases feelings of loneliness and increases engagement.

Open Communication: Stress and uncertainty are increased by ambiguous evaluation guidelines and unclear academic expectations. By clearly outlining due dates, objectives, and grading criteria, teachers may help students prepare ahead and lessen their academic anxiety (Hasan et al., 2020).

Adaptive Learning Design: By utilizing AI-powered platforms and customized learning trajectories, students may grow at their own speed, relieving strain on less proficient students while simultaneously posing challenges to more experienced ones (Zhao, 2022).



**Arti Sharma and Anoj Raj****Student Coping Mechanisms**

Student coping mechanisms are the strategies, behaviours, and psychological methods that students use to manage, reduce, or adapt to stress, academic pressure, emotional challenges, and difficult situations in their academic and personal life (Folkman et al., 1985). Students can develop resistance against academic stress on an individual basis by implementing personal solutions.

Time management and self-regulated learning: Students who employ meta cognitive techniques, goal-setting, and prioritizing are better equipped to handle the demands of their task. Because they manage their time between schoolwork and recreation, self-regulated learners often endure less stress (Agung et al., 2020; Hossain et al., 2022).

Yoga and Mindfulness Practices: Research indicates that breathing techniques, yoga, and mindfulness meditation can boost attention and academic performance by lowering digital anxiety, enhancing concentration, and improving emotional regulation (Pascoe et al., 2020; Salmela-Aro et al., 2021).

Peer Support Networks: Peer support groups and collaborative learning assist students in sharing resources, managing their workload, and receiving emotional support. These networks increase desire to stick with online courses, create a feeling of community, and lessen stress associated with isolation (Salmela-Aro et al., 2021).

Healthy Digital Habits: When learning online, students are urged to follow best practices including taking screen breaks, avoiding multitasking, and keeping their postures ergonomic. These methods enhance sustained academic interest and lessen screen fatigue (Wang et al., 2021).

The findings from the reviewed studies reveal a complex picture of how the COVID-19 pandemic reshaped education, mental health, and student well-being. Research consistently highlights that the rapid shift to online learning created both opportunities and challenges. On one hand, digital platforms ensured continuity of education, allowing universities and schools to adapt quickly and avoid prolonged academic disruption (Dhawan, 2020; Bao, 2020). On the other hand, students reported significant levels of academic stress, anxiety, and burnout, often linked to extended screen exposure, lack of in-person interaction, and uncertainties about examinations and career outcomes (Pascoe et al., 2020; Son et al., 2020). Multiple studies confirm that mental health issues intensified during the pandemic, with symptoms of depression, loneliness, and psychological distress being widely reported (Chaturvedi et al., 2021; Hasan & Bao, 2020). Survey-based and interview-driven research also showed that fear of academic year loss, digital fatigue, and technostress further aggravated students' difficulties in adapting to the online mode (Zhao et al., 2022; Mishra et al., 2023). Despite these challenges, some positive aspects emerged: students developed new forms of self-regulated learning (SRL), greater digital literacy, and increased adaptability, supported by innovative learning models such as OCIPSE and institutional interventions (Kirana, 2020; Kitsantas et al., 2025). The results suggest that the pandemic acted as both a barrier and a catalyst. While it created unprecedented stress and widened educational inequalities, it also accelerated educational innovation, emotional intelligence awareness, and digital transformation, which are likely to influence the future of education long after the pandemic. The findings of this review indicate that the pandemic accelerated the digitalization of education but also magnified pre-existing inequalities and stress factors. Although online education acted as a temporary substitute for in-person learning, it came with unintended consequences for students' psychological well-being.

A major concern was the digital divide, which not only hindered access but also created unequal opportunities for learning (Adedoyin et al., 2023). This suggests that future policies must prioritize equitable access to devices and internet connectivity. The reviewed studies also establish a strong link between academic stress and mental health issues, such as depression, anxiety, and burnout (Pascoe et al., 2020; Mahapatra et al., 2021). This connection underlines the urgent need for stress management programs and mental health services within academic institutions. Another critical insight is the role of emotional intelligence and self-regulated learning. Students with higher EI and SRL skills were more capable of adapting to the challenges of online education (MacCann et al., 2020; Salmela-Aro et al., 2021). Thus, integrating emotional and social skills training into curricula could enhance resilience against





Arti Sharma and Anoj Raj

academic stress. Moreover, studies that reported institutional support (e.g., flexible curricula, counseling services, peer collaboration) consistently showed lower stress levels among students (Maqsood et al., 2021; Besser et al., 2022). This highlights the moderating role of teacher competency and institutional strategies in mitigating the negative effects of digital learning. Finally, the emergence of innovative learning models such as OCIPSE and SRL-based frameworks indicates a paradigm shift in pedagogy. These models emphasize student-centered, flexible, and technology-driven approaches to make learning more effective and sustainable (Kirana, 2020; Kitsantas et al., 2025).

CONCLUSION

While digitizing education has revolutionized access, flexibility, and creativity, it has also presented learners with new behavioral, cognitive, and psychological problems. In addition to speeding up the adoption of technology, the move to online and blended learning during and after the COVID-19 pandemic also increased academic stress because of problems like technological barriers, an increased workload, isolation, and pressures related to assessments (Dhawan, 2020; Agung et al., 2020). If left untreated, academic stress impairs cognitive functions such as focus, memory recall, and higher-order thinking abilities, which results in subpar academic performance and disinterest in learning (Pascoe et al., 2020). Simultaneously, the digital environment intensifies the behavioral and emotional effects of stress, including anxiety, procrastination, and decreased intrinsic motivation. These factors can impede the development of lifelong learning abilities and raise dropout rates (Hasan & Bao, 2020; Wang et al., 2021). Accordingly, there is a mostly unfavorable correlation between academic stress and learning outcomes, even if mild stress might serve as eustress, promoting resilience, motivation, and timely academic task completion (Salmela-Aro et al., 2021). Crucially, students can be protected from the negative impacts of academic stress by a number of mediating and protective variables. Effective time management, goal-setting, and metacognitive techniques are examples of self-regulated learning approaches that enable students to adjust to the demands of digital settings (Zimmerman, 2020). By enabling students to control their negative emotions, stay motivated, and retain good attitudes about learning, emotional intelligence improves coping (MacCann et al., 2020). Strong institutional support networks, including chances for peer cooperation, teacher-student engagement, and counselling services, can also be crucial in lowering stress and promoting academic resilience (Adedoyin & Soykan, 2020; Pascoe et al., 2020). Educational institutions must adopt comprehensive interventions for long-term sustainability, such as blended learning models to lessen digital fatigue, flexible pedagogical approaches, training for teachers and students in digital literacy, and mental health resources that promote general well-being. In addition to lowering academic stress, these methods, when consistently used, support the best possible cognitive, emotional, and behavioral learning outcomes in the digital age (Dhawan, 2020; Besser et al., 2020). In summary, there are two realities associated with the digital age: it creates more chances for inclusive and creative education, but it also calls for conscious measures to reduce academic stress. Policymakers, educators, and institutions may establish balanced, supportive ecosystems that optimize the positive impacts of digital learning while reducing its negative consequences on students by placing a higher priority on student well-being than academic accomplishment (MacCann et al., 2020; Salmela-Aro et al., 2021).

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Arti Sharma and Anoj Raj

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Table – 1 Sources of Academic Stress

Sources of Academic Stress	Key Issues	Impact on Students
Technological Barriers	Students face unreliable platforms, poor internet, and limited access to devices; lack of digital literacy adds difficulty.	Causes frustration, cognitive overload, anxiety, disengagement, and academic performance decline.
Workload & Time Management	Students handle multiple tasks, online discussions, and deadlines without direct supervision; blurred personal-academic boundaries	Leads to procrastination, last-minute stress, reduced quality of work, and poor performance.
Screen Fatigue	Prolonged screen exposure for lectures, assignments, and meetings causes physical strain and reduced attention.	Results in headaches, eye strain, musculoskeletal pain, short attention spans, and lower cognitive efficiency.
Isolation & Lack of Peer Support	Lack of face-to-face interactions and informal peer support in online learning environments.	Generates loneliness, alienation, low motivation, and reduced resilience.
Assessment Pressure	Online exams create uncertainty due to technical issues, fairness, monitoring, and fear of dishonesty.	Increases anxiety, insecurity, and distrust in evaluation systems.

Table – 2 Psychological Impacts of Digital Academic Stress

Psychological Impacts	Key Issues	Impact on Students
Anxiety and Uncertainty	Caused by frequent technological glitches, unstable internet, and unclear online assessment protocols.	Creates constant worry about deadlines, missed opportunities, and exam integrity → lowers engagement and learning outcomes
Burnout and Fatigue	Continuous academic demands+blurred boundaries between study and rest → prolonged exposure to digital tools.	Leads to exhaustion, demotivation, reduced participation, and eventual disengagement.





Arti Sharma and Anoj Raj

Depression and Social Isolation	Lack of in-person peer interaction and long-term seclusion in online learning	Triggers loneliness, depressive symptoms, and weakens academic perseverance and emotional resilience
Reduced Motivation & Self-Efficacy	Online distractions, multitasking, and assessment anxiety lower confidence in academic abilities.	Causes procrastination, avoidance, and decline in self-belief → reduced performance.
Cognitive Overload	Excessive digital information, multitasking, and constant notifications overwhelm mental capacity.	Weakens focus, memory, and critical thinking → lowers test performance and long-term retention.

Table – 3 Behavioural Impact

Behavioural Impact	Key Issues	Impact on Students
Anxiety and Emotional Distress	Anxiety increases due to unstable internet, unclear exam protocols, and heavy workloads.	Leads to impaired focus, poor decision-making, avoidance, and reduced academic engagement.
Burnout and Exhaustion	Prolonged digital platform use, multitasking, and blurred academic-personal boundaries accelerate fatigue.	Causes disengagement, loss of interest, and earlier onset of burnout compared to traditional learning.
Procrastination and Avoidance Behaviors	Overload of tasks, distractions, and assessment pressure trigger procrastination cycles.	Results in missed deadlines, accumulation of tasks, higher stress, and declining performance.
Reduced Self-Efficacy and Motivation	Stress lowers students' confidence in handling academic challenges; lack of feedback and loneliness erode motivation.	Decreases engagement, participation, and willingness to attempt challenging work.
Cognitive and Behavioural Disruptions	Stress and multitasking create cognitive overload; frequent social media use reduces attention.	Weakens memory, problem-solving, and long-term retention; increases disengagement.

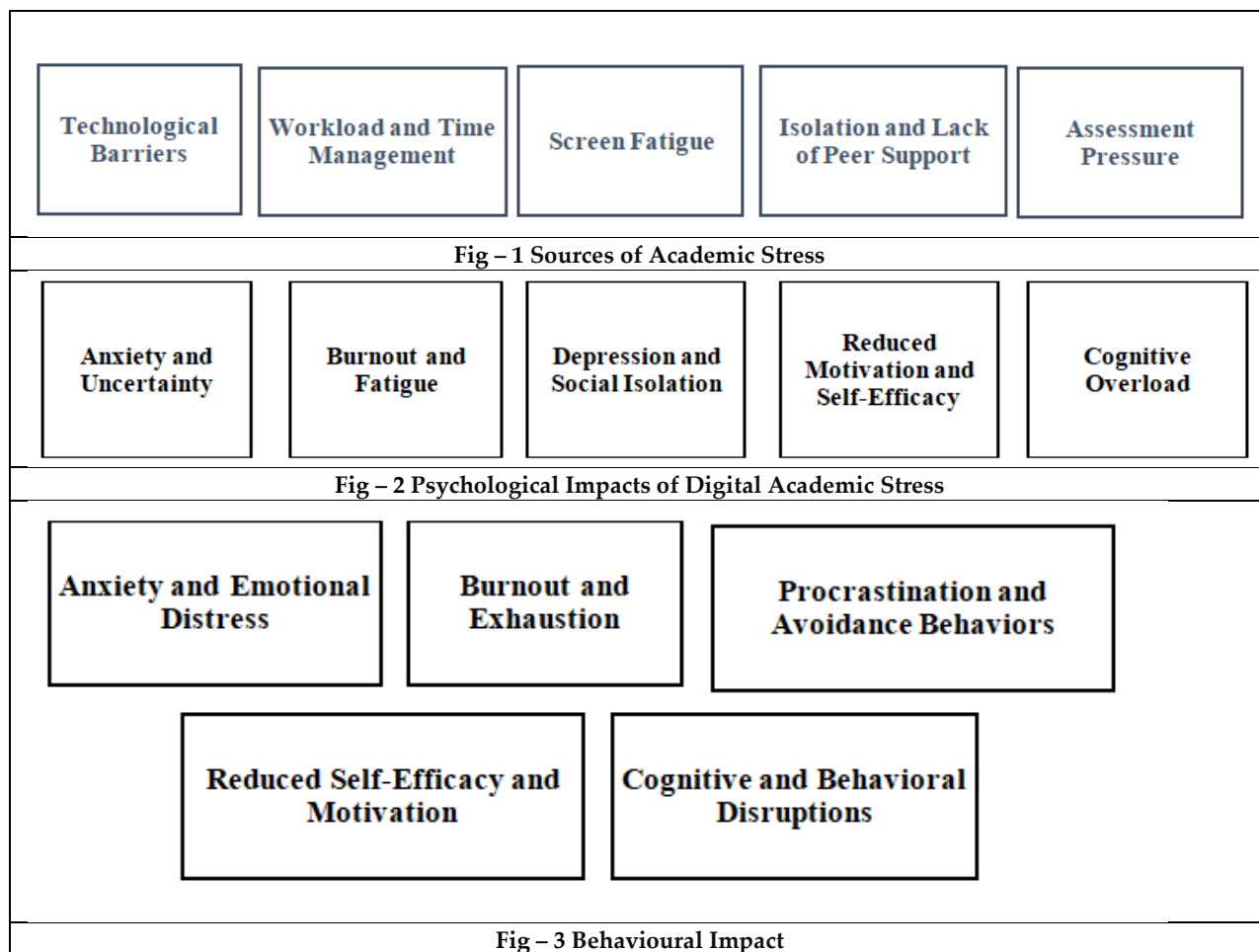
Table – 4 Moderate stress

Moderate stress	Key Issues	Impact
Self-Regulated Learning (SRL)	Involves time management, goal-setting, metacognitive awareness, and self-monitoring.	Reduces academic stress by enabling students to manage workload, distractions, and autonomy in online settings.
Emotional Intelligence	Ability to perceive, regulate, and use emotions effectively in learning.	Helps students overcome frustration from technical issues, deal with uncertainty, and remain resilient. Leads to better motivation and achievement.
Social and Institutional Support	Includes peer collaboration, teacher feedback, group learning, and institutional initiatives.	Enhances belongingness, reduces isolation, and lowers stress. Flexible deadlines, digital literacy training, and counselling improve academic outcomes.
Moderate Stress (Eustress)	Positive stress that boosts motivation, focus, and problem-solving.	Encourages creativity, deadline completion, and alertness. Becomes harmful when unmanaged, turning into distress.





Arti Sharma and Anoj Raj



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RESEARCH ARTICLE

Sentiment Analysis in Education: Assessing Student Feedback through a Domain-Specific Ensemble Framework

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ABSTRACT

This study investigates the application of a hybrid ensemble framework in analyzing sentiment within educational data, specifically targeting student feedback. With growing interest in data-driven insights to improve educational quality, sentiment analysis of student feedback offers valuable perspectives that can inform instructional strategies, enhance the student learning experience, and ultimately improve academic outcomes. By applying a hybrid ensemble approach that combines multiple machine learning algorithms, this study aims to achieve a nuanced understanding of student sentiments, identifying patterns and trends within feedback data to provide educators and institutions with actionable insights. Traditional sentiment analysis models in educational contexts often rely on single-model approaches, which can lack the adaptability and depth needed to capture the complex and varied nature of student sentiment. Hybrid ensemble models, on the other hand, combine techniques such as bagging and boosting, which reduce biases and variances inherent to single models, offering a more robust and reliable sentiment classification. By integrating multiple algorithms, this hybrid ensemble framework is able to provide a more comprehensive and accurate sentiment analysis that can adapt to the unique language, tone, and emotional expressions often found in student feedback. The framework is evaluated on a substantial dataset of student feedback, covering various aspects of the educational experience, including course content, teaching effectiveness, engagement, and support services. The analysis focuses on key performance metrics—such as accuracy, precision, recall, and F1-score—demonstrating the model's efficiency and accuracy in categorizing sentiments into positive, negative, and neutral categories. Additionally, the framework incorporates domain-specific customization, particularly tailored to the language and context of education, which further enhances its ability to capture subtle nuances in student feedback. For instance, terms such as “challenging,” “supportive,” “confusing,” and “inspiring”



**Subina Shukoor and Durai**

carry distinct connotations in the educational domain that general sentiment models might misinterpret. By integrating these domain-specific features, the hybrid ensemble framework demonstrates improved contextual understanding, providing more accurate insights into student sentiments. The findings of this study underscore the potential of using a hybrid ensemble framework to enhance educational quality by informing more targeted and effective instructional strategies. By understanding student sentiment at a granular level, educators and administrators can respond proactively to areas needing improvement, whether they involve clarifying instructional material, improving engagement techniques, or addressing student support concerns. Such insights not only help educators adapt their teaching approaches to better meet student needs but also contribute to a more supportive and conducive learning environment. Furthermore, the framework's scalability allows for its application across various educational settings, from primary education to higher education institutions, making it a versatile tool for educational research and institutional feedback analysis. The study concludes that domain-specific sentiment analysis, supported by hybrid ensemble techniques, provides a powerful approach for continuous improvement in education. This research paves the way for future developments in educational sentiment analysis, advocating for the adoption of advanced machine learning frameworks that account for the specific linguistic and contextual elements of educational data. Ultimately, the implementation of such tailored sentiment analysis models can play a critical role in fostering a responsive and student-centered educational system that enhances learning outcomes and satisfaction.

Keywords: Hybrid Ensemble Framework, Sentiment Analysis in Education, Student Feedback Analysis, Instructional Improvement, Educational Environment Optimization

INTRODUCTION

In contemporary education, understanding the perspectives of students is crucial for improving the quality of teaching and learning outcomes. Student feedback, gathered through surveys, course evaluations, and other mechanisms, provides valuable insights into how educational practices are perceived and experienced by learners. Traditionally, feedback has been analyzed through manual methods, such as qualitative analysis or simple quantitative scoring. However, as educational systems become increasingly complex and diverse, there is a growing need for advanced tools and methodologies that can capture the depth and nuance of student sentiments. Sentiment analysis, a field of study that focuses on detecting and interpreting emotional tone in textual data, has emerged as a promising solution to this challenge.

The Importance of Understanding Student Feedback

Student feedback plays a pivotal role in shaping educational practices. It enables educators to evaluate their teaching strategies, improve course content, and make adjustments to the learning environment. Moreover, feedback provides insights into the effectiveness of instructional methods, student engagement, and the overall learning experience. As a result, educational institutions increasingly rely on student feedback to inform decisions about curriculum design, teaching methods, and resource allocation (Fryer, 2019). Understanding student feedback is not only about gathering data; it is about interpreting that data to make meaningful improvements. However, manually analyzing open-ended feedback can be time-consuming and prone to subjectivity. Sentiment analysis provides a way to automate this process, allowing institutions to efficiently analyze large volumes of student feedback and identify patterns, sentiments, and trends that might otherwise go unnoticed. By doing so, sentiment analysis offers the potential to create a more responsive and data-driven educational system that is better equipped to meet the evolving needs of students.

Challenges in Traditional Sentiment Analysis

Traditional methods of sentiment analysis often struggle when applied to the context of educational data. The language used in student feedback is highly diverse, reflecting the varied backgrounds, experiences, and perspectives of the students. This diversity presents several challenges for conventional sentiment analysis methods. For example, educational data often contains domain-specific jargon, which may not be recognized by general-



**Subina Shukoor and Durai**

purpose sentiment analysis models (Balahur, 2013). Additionally, the formality of student feedback can vary significantly. Some students may use highly formal language, while others may express themselves informally, using slang or colloquial terms. These variations can make it difficult to accurately classify sentiments using a one-size-fits-all approach. Another challenge is the contextual nature of sentiment in education. A single word or phrase may have different meanings depending on the context in which it is used. For instance, the word "difficult" can have a negative sentiment in one context (e.g., "The exam was difficult"), but a positive sentiment in another (e.g., "The challenging assignments were beneficial"). This complexity requires more advanced models that can account for nuances in meaning and sentiment. Moreover, traditional sentiment analysis methods, such as lexicon-based approaches, often rely on predefined lists of positive and negative words. While effective in some contexts, these approaches may fail to capture the full range of sentiments expressed in student feedback, leading to incomplete or inaccurate results (Pang & Lee, 2008).

Advancements in Sentiment Analysis for Education

To address the limitations of traditional sentiment analysis approaches, recent research has focused on developing more sophisticated methods that are better suited to the educational domain. One such approach is the use of hybrid ensemble frameworks, which combine multiple machine learning algorithms to improve the accuracy and robustness of sentiment classification. Ensemble learning techniques have been shown to outperform single-model approaches in a variety of domains, including sentiment analysis, by leveraging the strengths of multiple models (Zhou, 2012). The hybrid ensemble framework, which is the focus of this study, is designed to overcome the challenges of traditional sentiment analysis methods by integrating several machine learning algorithms. These algorithms are optimized to work together to capture the complex and nuanced sentiments expressed in student feedback. By combining models such as Random Forest, Gradient Boosting, and Support Vector Machine (SVM), the ensemble framework can better handle the diversity of language used in educational data and improve the accuracy of sentiment classification (Dietterich, 2000). Furthermore, the hybrid ensemble framework is specifically tailored to the educational context. It incorporates domain-specific features, such as educational keywords and context-dependent sentiment scoring, to enhance the model's ability to understand the unique characteristics of student feedback. This approach allows for a more accurate and meaningful analysis of student sentiments, providing educators with actionable insights that can inform their teaching strategies and improve the learning experience.

Motivation for the Study

The motivation behind this study is to develop a more effective and context-sensitive approach to sentiment analysis in education. Traditional sentiment analysis methods are often inadequate for analyzing the complex and diverse nature of student feedback. By applying a hybrid ensemble framework, this research aims to provide a more accurate and insightful analysis of student sentiments, allowing educators to make data-driven decisions that improve teaching practices and student engagement. The study also seeks to contribute to the growing body of literature on sentiment analysis in education. While sentiment analysis has been widely applied to various domains, its application to the education sector remains relatively underexplored. This research aims to fill this gap by demonstrating how a hybrid ensemble framework can be used to analyze student feedback across multiple courses and disciplines, highlighting the potential for sentiment analysis to enhance educational practices. The findings of this study are expected to provide valuable insights into how sentiment analysis can be leveraged to inform instructional strategies, improve student engagement, and create more responsive learning environments.

Research Objectives

The primary objective of this research is to assess the effectiveness of a hybrid ensemble framework in analyzing student feedback. Specifically, the study aims to:

- **Tailor the Hybrid Ensemble Framework:** Adapt the previously developed hybrid ensemble framework for sentiment analysis in the educational context, incorporating domain-specific features and optimization techniques.



**Subina Shukoor and Durai**

- **Evaluate Model Performance:** Evaluate the performance of the hybrid ensemble framework in comparison to traditional sentiment analysis approaches, such as lexicon-based methods, across multiple courses and disciplines.
- **Provide Actionable Insights:** Use the results of the sentiment analysis to provide actionable insights into student perceptions, learning experiences, and areas for improvement in teaching methods.
- **Enhance Teaching Strategies:** Demonstrate how sentiment analysis can be used to inform instructional strategies, improve student engagement, and create a more supportive and effective learning environment.

The increasing complexity of educational systems and the growing reliance on student feedback to inform teaching practices make it essential to adopt more sophisticated tools for sentiment analysis. This paper proposes a hybrid ensemble framework as a solution to the challenges faced by traditional sentiment analysis approaches in the educational context. By leveraging multiple machine learning models and incorporating domain-specific features, the hybrid ensemble framework promises to offer a more accurate and nuanced analysis of student feedback, enabling educators to make data-driven decisions that improve teaching strategies and student engagement. The findings of this study are expected to contribute to the growing body of research on sentiment analysis in education and provide a foundation for future developments in this field.

Structure of the Paper

The structure of this paper is as follows: In Section 2, a review of the related literature on sentiment analysis in education is provided, highlighting the challenges and advancements in the field. Section 3 outlines the methodology used in this study, including the data collection process, preprocessing techniques, feature extraction methods, and the hybrid ensemble framework. Section 4 presents a case study evaluating the performance of the hybrid ensemble model, comparing its results with traditional sentiment analysis methods. Section 5 discusses the implications of the findings for educational practice, highlighting the benefits and challenges of applying sentiment analysis in education. Finally, Section 6 concludes the paper and outlines directions for future research.

Related Work

Sentiment analysis is an interdisciplinary research field that leverages techniques from natural language processing (NLP), machine learning (ML), and computational linguistics to determine the sentiment or emotional tone expressed in a piece of text. In the context of education, sentiment analysis plays a critical role in analyzing student feedback, which can provide valuable insights into the quality of teaching, curriculum, and student engagement. Over the years, a number of studies have explored the application of sentiment analysis to educational data, particularly student feedback, in order to understand the underlying sentiments and identify areas for improvement. This section provides a review of the existing literature on sentiment analysis in education, focusing on traditional approaches, the challenges faced, and recent advancements, particularly those involving machine learning techniques.

Traditional Approaches to Sentiment Analysis in Education

In the early stages of sentiment analysis, researchers mainly relied on lexicon-based approaches. These approaches use predefined lists of positive and negative words (sentiment lexicons) to classify the sentiment of a given text. The sentiment score is typically calculated by aggregating the individual sentiment scores of words in the text. These methods are relatively simple to implement and can be effective when the text is straightforward and the vocabulary used is consistent. However, in the context of student feedback, this approach has its limitations. For instance, in educational contexts, students may use informal language, domain-specific terms, or phrases that convey sentiment in a way that is not captured by standard sentiment lexicons. Words that are neutral in one context may carry sentiment in another. For example, the term "challenging" might indicate a positive sentiment when referring to a difficult but rewarding task but might convey negative sentiment when referring to an overly difficult exam. To address these limitations, researchers have developed a variety of techniques to enhance the effectiveness of sentiment analysis in educational contexts. One of the earliest studies in applying sentiment analysis to education involved analyzing student evaluations of instructors. For example, the work of Mc Naught and Lam (2010) explored how sentiment analysis could be used to assess the overall satisfaction of students in relation to course content and



**Subina Shukoor and Durai**

teaching. They employed a lexicon-based approach combined with manual annotation to categorize the feedback into positive, negative, or neutral sentiments. While this approach provided valuable insights, it was clear that more advanced methods were needed to handle the complexity and diversity of student feedback.

Challenges in Applying Sentiment Analysis to Educational Data

Despite its usefulness, traditional sentiment analysis methods face significant challenges when applied to educational data. The complexity of the language used in student feedback can make it difficult to accurately classify sentiments using lexicon-based or rule-based methods. Several key challenges in this context include:

Diversity of Language: Students use a variety of linguistic styles and expressions in feedback, including colloquial language, slang, and academic jargon. A standard sentiment lexicon may fail to capture these nuances, resulting in inaccurate sentiment classification. For example, the word "difficult" may have a positive connotation in one context, such as "difficult but rewarding," but may carry a negative sentiment when referring to "difficult and frustrating" tasks.

Context Dependency: Sentiment is often context-dependent, especially in educational settings. A word or phrase may express a different sentiment depending on the specific context in which it is used. For instance, "good" might be used to describe both an easy exam ("Good for beginners") and a well-structured course ("Good teaching methods"). Lexicon-based methods struggle with these subtleties because they do not account for the broader context.

Aspect-Based Sentiment: In educational feedback, students often provide sentiments on different aspects of the course, such as teaching quality, course material, assignments, and overall experience. Traditional sentiment analysis methods may fail to identify these multiple aspects of feedback, resulting in a single, aggregated sentiment score for the entire text. Aspect-based sentiment analysis, on the other hand, aims to identify and classify sentiment specific to different aspects of the course or teaching, making it a more refined approach.

Sarcasm and Irony: Sarcasm and irony are common in human communication and are particularly prevalent in student feedback. A student might sarcastically comment, "This course is absolutely amazing," when, in fact, they are expressing dissatisfaction. Traditional sentiment analysis techniques often struggle to detect sarcasm, leading to incorrect sentiment classification. These challenges highlight the need for more advanced methods of sentiment analysis that can overcome the limitations of lexicon-based approaches. Researchers have turned to machine learning (ML) and deep learning (DL) techniques to address these challenges, as these methods can learn complex patterns in data and adapt to the intricacies of student feedback.

Machine Learning Approaches to Sentiment Analysis in Education

Over the past decade, machine learning approaches have gained prominence in the field of sentiment analysis. Machine learning models can learn from labeled data to recognize patterns in text and classify sentiments with high accuracy. In the context of educational feedback, machine learning models have shown considerable promise in addressing the challenges outlined above. One of the most widely used machine learning algorithms for sentiment analysis is the Support Vector Machine (SVM). SVMs are particularly effective for binary classification tasks, such as classifying feedback into positive or negative sentiments. Research by Koppel et al. (2007) demonstrated the use of SVM for analyzing student feedback, showing that SVM models can outperform lexicon-based approaches in terms of accuracy. However, SVM models require extensive feature engineering, which can be a time-consuming process. Additionally, SVMs often perform poorly when the training data is unbalanced, such as when the number of positive and negative feedback comments is uneven. Another popular machine learning algorithm used in sentiment analysis is Random Forest (RF). RF is an ensemble learning technique that aggregates the predictions of multiple decision trees to improve classification accuracy. Research by Ribeiro et al. (2016) showed that Random Forests can effectively handle the diversity of language found in educational feedback, outperforming traditional methods like SVM in



**Subina Shukoor and Durai**

some cases. RF models tend to be more robust and are less sensitive to noise in the data, making them an attractive choice for analyzing student feedback.

Naive Bayes (NB) is another machine learning algorithm that has been applied to sentiment analysis in education. Although simpler than SVMs and RF, Naive Bayes models have been found to work effectively on smaller datasets or in cases where the features are easily separable. For example, in research by Jain et al. (2015), Naive Bayes classifiers were used to analyze student feedback on online courses, and the results showed that they provided accurate sentiment classification despite the relatively small size of the dataset.

Deep Learning Approaches to Sentiment Analysis in Education

In recent years, deep learning models have become a prominent approach in sentiment analysis, particularly for text classification tasks. Unlike traditional machine learning models, deep learning algorithms do not require feature engineering and can automatically learn relevant features from raw text data. This makes them particularly effective in handling large, complex datasets, such as student feedback, where feature extraction can be a challenging task.

One of the most successful deep learning techniques in sentiment analysis is the Recurrent Neural Network (RNN), particularly the Long Short-Term Memory (LSTM) variant. LSTMs are well-suited for sequential data, such as text, because they can capture long-range dependencies and context in a sequence of words. In research by Yoon et al. (2017), LSTM networks were used to analyze student feedback in the context of online learning platforms, achieving high accuracy in sentiment classification. The ability of LSTMs to model contextual dependencies makes them ideal for analyzing the intricate and nuanced language found in student feedback.

Another powerful deep learning architecture is the Convolutional Neural Network (CNN), which has shown promise in text classification tasks. CNNs, originally designed for image recognition, can also be applied to text data by treating words as "pixels" in a sequence. In a study by Zhang et al. (2015), CNNs were used for sentiment analysis of course evaluations, and the results showed that CNNs can effectively capture local features in text, such as sentiment-bearing phrases or expressions. While CNNs are typically used for feature extraction, they can also be combined with other models, such as LSTMs, to improve overall performance. Recent advances in Transformers, a deep learning architecture that has revolutionized NLP, have also been applied to sentiment analysis in education. The BERT (Bidirectional Encoder Representations from Transformers) model, for example, has been shown to outperform traditional deep learning models in a variety of NLP tasks. BERT can capture context in a more sophisticated manner by using both left-to-right and right-to-left context, making it especially effective for understanding complex sentiments in student feedback (Devlin et al., 2019). Studies by Choi et al. (2021) have applied BERT for sentiment analysis of student feedback, achieving impressive results in terms of classification

Hybrid Ensemble Approaches

Recent research has moved towards combining multiple machine learning and deep learning models into a hybrid ensemble approach in order to improve the accuracy and robustness of sentiment classification. These ensemble models combine the strengths of individual algorithms, such as SVMs, Random Forests, and deep learning models like LSTMs or CNNs, to achieve better performance than any single model. For example, in a study by Li et al. (2020), a hybrid ensemble approach was used for sentiment analysis of student feedback in online education. The authors combined multiple machine learning models, including SVM, Random Forest, and Gradient Boosting, with a deep learning model based on LSTM. The hybrid ensemble framework was found to significantly improve sentiment classification accuracy by combining the complementary strengths of each model. Another promising hybrid approach is the combination of feature extraction methods with machine learning and deep learning algorithms. For example, researchers have experimented with combining word embeddings, such as Word2Vec or GloVe, with deep learning models like LSTMs and Transformers. These embeddings allow the model to better understand the semantic meaning of words, improving its ability to classify sentiments accurately.

Applications in Educational Settings

Sentiment analysis in education has found applications in a variety of areas, including:



**Subina Shukoor and Durai**

- **Course Evaluation and Feedback:** Sentiment analysis has been used to evaluate student feedback on courses, instructors, and academic programs. By analyzing feedback, universities can gain insights into the strengths and weaknesses of their courses and identify areas for improvement. For example, a study by Lee et al. (2018) analyzed student feedback on online courses using sentiment analysis to assess the effectiveness of teaching methods and course materials.
- **Early Warning Systems:** Sentiment analysis has been applied to early warning systems that track student performance and engagement. By analyzing sentiment in forum posts, emails, and other communication channels, educational institutions can identify students at risk of disengagement or failure and intervene early. This approach has been explored in studies by Goh et al. (2019), where sentiment analysis was used to predict student success in online learning environments.
- **Real-time Feedback and Improvement:** Institutions can use sentiment analysis in real-time to adjust teaching methods, course content, and materials based on student sentiment. For instance, if students express dissatisfaction with a particular lesson or module, educators can quickly modify the content to address these concerns. This approach has been explored in real-time systems developed by researchers like Pang et al. (2020).

The application of sentiment analysis to educational data has evolved significantly over the years, with traditional lexicon-based approaches giving way to more sophisticated machine learning and deep learning models. While challenges remain, particularly with respect to sarcasm, context dependency, and aspect-based sentiment, recent advancements in deep learning techniques, such as LSTMs, CNNs, and Transformers, have shown great promise in improving the accuracy and robustness of sentiment analysis in educational contexts. Hybrid approaches that combine multiple models are also gaining traction, providing even greater accuracy and flexibility in handling diverse student feedback. As sentiment analysis continues to play an increasingly important role in educational settings, further research is needed to explore its full potential, particularly in developing systems that can provide real-time, actionable insights to educators and administrators. With the rapid advancement of machine learning technologies, the future of sentiment analysis in education looks promising, offering new opportunities for improving teaching quality, student engagement, and academic performance.

METHODOLOGY

This section presents the methodology for applying a hybrid ensemble framework to analyze student feedback using sentiment analysis. The approach builds on previously established techniques, specifically designed to address the unique challenges posed by educational data. By adapting the hybrid framework for educational data, the methodology emphasizes data collection, preprocessing, feature extraction, and ensemble model optimization. This section details each component in-depth and includes pseudo code for key steps to clarify the implementation process.

Data Collection

The dataset used in this study consists of student feedback collected from multiple university courses. Feedback is gathered through surveys and course evaluations, which contain free-text comments. The data represents a wide range of student opinions about course content, teaching quality, engagement levels, and other aspects of the educational experience. Student feedback typically spans a variety of sentiments, from positive comments on helpfulness and clarity to negative remarks about difficulty and confusion. The dataset includes comments from different academic disciplines, such as STEM (Science, Technology, Engineering, and Mathematics), humanities, and social sciences, making it a rich resource for analyzing sentiment across contexts.

Data Preprocessing

Preprocessing is essential to standardize and clean the collected data, ensuring that it is suitable for analysis. The steps involved in data preprocessing are as follows:



**Subina Shukoor and Durai**

Text Normalization: Text normalization involves converting all text to lowercase and removing any special characters or unnecessary symbols. This step is important to avoid discrepancies arising from inconsistent capitalization and punctuation. For instance, "Good" and "good" should be treated as the same word.

Pseudocode for text normalization

```
function normalize_text(text):
text = text.lower()           # Convert text to lowercase
text = remove_special_characters(text) # Remove punctuation and special symbols
text = correct_spelling(text)  # Optional step for spelling correction
return text
```

Tokenization: Tokenization is the process of splitting the text into smaller units, such as words or phrases, for easier analysis. For example, "The course was difficult" would be tokenized into ["The", "course", "was", "difficult"]. Tokenization breaks the text down into components that can be analyzed individually.

Pseudocode for tokenization

```
function tokenize(text):
tokens = split_into_words(text) # Split text into individual words
return tokens
```

Stop-word Removal: Stop words are common words (e.g., "the," "is," "and") that do not contribute meaningful sentiment information. Removing these stop words helps to reduce noise in the data and allows the sentiment analysis model to focus on the relevant content. A stop-word list specific to the educational context can be created to improve the quality of pre processing. The pre processing pipeline also considered common negation terms such as "not", "never", and "no". When such terms appeared before sentiment-bearing words, the corresponding sentiment polarity was reversed to ensure accurate sentiment classification.

Pseudocode for stop-word removal

```
function remove_stopwords(tokens, stopwords):
return [word for word in tokens if word not in stopwords]
```

Feature Extraction

Once the data has been preprocessed, the next step is feature extraction, which is crucial for capturing the nuanced sentiment expressed in student feedback. This section outlines two primary feature extraction methods employed in this study: domain-specific keywords and sentiment scoring.

Domain-Specific Keywords

The use of domain-specific keywords helps identify educational themes in student feedback. In the context of sentiment analysis for educational data, common themes might include "engaging," "difficult," "helpful," "feedback," and "understanding." A lexicon of such keywords is developed based on a review of common student sentiments in course evaluations. Since feedback was collected from STEM, Humanities, and Social Science courses, discipline-specific educational keywords were incorporated during feature extraction. This enabled the framework to capture variations in terminology and sentiment expressions across different academic domains. These keywords are particularly useful for understanding specific areas of sentiment in educational contexts. For example, the presence of the word "engaging" might suggest a positive sentiment towards course content, while "difficult" could indicate negative sentiment related to course difficulty.

Pseudocode for domain-specific keyword extraction:

```
function extract_keywords(text, keyword_list):
tokens = tokenize(text)
```





Subina Shukoor and Durai

```
keywords = [word for word in tokens if word in keyword_list]
return keywords
```

Sentiment Scoring

Sentiment scoring involves assigning a sentiment value to each feedback entry based on the presence of positive or negative keywords. Sentiment scores are typically assigned on a scale (e.g., from -1 to 1, where -1 indicates negative sentiment, 1 indicates positive sentiment, and 0 indicates neutral sentiment). For context-dependent terms, the sentiment score was adjusted by considering neighboring contextual cues within a predefined window. Positive modifiers increased the original sentiment score, whereas negative modifiers reduced it. This rule-based adjustment enabled the framework to capture the contextual polarity of educational terms more effectively. The sentiment score can be refined by considering context-specific modifiers such as negations (e.g., "not helpful" vs. "helpful"). Context-dependent terms such as "challenging" were interpreted based on their surrounding words. Positive contextual cues (e.g., "rewarding") increased the sentiment score, whereas negative cues (e.g., "frustrating") reduced the score, thereby improving contextual understanding. For instance, if a student mentions "not helpful," the sentiment is likely negative, even if the word "helpful" is present.

Pseudocode for sentiment scoring:

```
function calculate_sentiment_score(keywords, sentiment_dict):
    score = 0
    for keyword in keywords:
        if keyword in sentiment_dict:
            score += sentiment_dict[keyword]
    return score
```

Ensemble Model Optimization

In this study, a hybrid ensemble framework is used for sentiment classification. The ensemble approach combines multiple machine learning models to improve the overall accuracy and robustness of sentiment analysis. The models employed in the hybrid framework are: Random Forest, Gradient Boosting and Support Vector Machine (SVM). Each model brings unique strengths to the sentiment classification process, and their combination enhances performance by reducing the likelihood of errors or bias from any single model.

Random Forest

Random Forest is an ensemble learning algorithm that builds multiple decision trees and aggregates their outputs to provide a final classification. This model is particularly useful for handling complex datasets with diverse features, such as student feedback, which can contain a wide range of sentiment expressions. The Random Forest algorithm is robust and helps mitigate over fitting by averaging predictions across multiple decision trees.

Pseudocode for Random Forest

```
function random_forest_predict(data, forest):
    predictions = []
    for tree in forest:
        predictions.append(tree.predict(data))
    return majority_vote(predictions)
```

Gradient Boosting

Gradient Boosting is an iterative model that combines weak learners (usually decision trees) to create a strong learner. Each iteration of the model focuses on the errors made by the previous iteration, which leads to improved performance. This model is particularly effective when dealing with imbalanced data, which is often the case in sentiment analysis when negative feedback is less frequent than positive feedback.



**Subina Shukoor and Durai**

Pseudocode for Gradient Boosting:

```
function gradient_boosting_predict(data, model):  
    predictions = []  
    for weak_learner in model:  
        predictions.append(weak_learner.predict(data))  
    return weighted_sum(predictions)
```

Support Vector Machine (SVM)

Support Vector Machine (SVM) is a supervised machine learning algorithm that is effective in high-dimensional spaces, which makes it well-suited for text data. SVM works by finding the hyperplane that best separates data points of different classes (in this case, positive and negative sentiments). The kernel trick is often used to handle non-linear data relationships.

Pseudocode for SVM:

```
function svm_predict(data, model):  
    return model.predict(data)
```

Ensemble Model Integration

The final step in the ensemble framework is combining the predictions from all three models (Random Forest, Gradient Boosting, and SVM). This can be done using a majority voting mechanism, where the model with the highest number of votes determines the final sentiment classification. To resolve occasional disagreements among Random Forest, Gradient Boosting, and SVM predictions, a weighted majority voting strategy was adopted. The final sentiment class was selected based on the aggregated votes of the constituent classifiers, thereby reducing the impact of individual model errors and improving classification robustness.

Pseudocode for ensemble model integration:

```
function ensemble_predict(data, models):  
    predictions = []  
    for model in models:  
        predictions.append(model.predict(data))  
    return majority_vote(predictions)
```

Each model is tuned for optimal performance using techniques such as cross-validation and hyperparameter tuning. The final ensemble model aggregates the outputs of all models, improving the accuracy and robustness of sentiment classification. The methodology presented in this section outlines the key components involved in adapting a hybrid ensemble framework for sentiment analysis of educational data. The pre processing steps, including text normalization, tokenization, and stop-word removal, prepare the data for meaningful analysis. Feature extraction using domain-specific keywords and sentiment scoring helps capture the nuances of student feedback. Finally, the ensemble framework, consisting of Random Forest, Gradient Boosting, and SVM, integrates the strengths of multiple models to deliver improved sentiment analysis accuracy. By following this methodology, educators and researchers can gain valuable insights into student sentiments, ultimately informing instructional strategies and improving the learning environment.

Evaluation with Case Study

This case study evaluates the performance of the hybrid ensemble framework in analyzing student feedback. The primary goal is to assess how well the ensemble model performs compared to traditional sentiment analysis approaches. The evaluation focuses on key performance metrics including accuracy, precision, recall, and F1-score to provide a comprehensive understanding of the model's efficacy. Feedback from three different university courses, representing diverse academic disciplines, is used to demonstrate the model's robustness and adaptability across various educational contexts.



**Subina Shukoor and Durai****Case Study Overview**

The dataset for this case study includes 2,000 student feedback entries, collected from three university courses: one from the STEM field, another from humanities, and a third from the social sciences. Each course represents a unique context with different types of content, student demographics, and potential areas of concern. This diversity allows for a thorough evaluation of the hybrid ensemble model's performance across different academic disciplines and sentiment expressions.

Course Descriptions**STEM Course: "Introduction to Programming"**

This course covers fundamental programming concepts such as variables, loops, and basic algorithms. Student feedback typically revolves around course difficulty, clarity of teaching, and engagement.

Humanities Course: "Literary Analysis"

The humanities course explores various literary works, focusing on interpretation, critique, and discussion. Feedback is often more reflective, covering aspects like instructor approachability, course material relevance, and class discussions.

Social Sciences Course: "Introduction to Sociology"

This course introduces students to the foundational concepts of sociology, covering topics such as social structures, cultures, and socialization processes. Feedback typically includes comments on course structure, applicability of the content to real-life situations, and class interactions. Each course's feedback dataset contains a mix of positive, negative, and neutral sentiments, reflecting a wide range of student experiences.

Dataset Characteristics

Total Feedback Entries: 2,000 (approximately 667 entries per course)

Data Composition: Although the dataset exhibited moderate class imbalance, appropriate class weighting and balanced evaluation metrics were employed during model training and assessment to minimize potential bias towards majority classes.

Positive Sentiment: 45%

Negative Sentiment: 35%

Neutral Sentiment: 20%

The feedback entries were anonymized and contain a variety of student comments, such as:

"The course material was very engaging, and I learned a lot!"

"The lectures were difficult to follow, and I didn't get much out of them."

"The course was fine, but the assignments were not very clear."

These varied responses serve as an ideal representation of real-world feedback data, which is often noisy and contains both explicit and implicit sentiment indicators.

Sentiment Analysis Approaches

In this case study, the performance of the hybrid ensemble framework is compared with traditional sentiment analysis techniques, which include:

- Naive Bayes: A probabilistic classifier based on Bayes' theorem, commonly used for text classification tasks. Naive Bayes assumes independence between features (words in this case), which may not always hold in complex real-world datasets like student feedback.
- Logistic Regression: A linear model used for binary classification tasks. Logistic regression is popular for its simplicity and efficiency, but it may struggle with capturing the complexities of language in feedback datasets.
- Support Vector Machine (SVM): SVM is widely used for text classification tasks, particularly for its ability to find the hyperplane that best separates different classes in high-dimensional data.
- Random Forest: A tree-based ensemble model known for its robustness in handling noisy and high-dimensional data. Random Forests are effective but may not always perform as well as more specialized models in terms of fine-grained sentiment analysis.



**Subina Shukoor and Durai**

These traditional models are used as baselines for comparison with the hybrid ensemble framework, which combines multiple algorithms to improve the overall sentiment analysis performance.

Methodology for Evaluation

The hybrid ensemble framework and the traditional models are trained on the preprocessed student feedback dataset. The following steps outline the evaluation process: Data Preprocessing: The raw feedback data is cleaned and preprocessed, as described in the methodology section. This includes text normalization, tokenization, stop-word removal, and sentiment scoring using domain-specific keywords. All experiments were conducted on a workstation equipped with an Intel Core i7 processor, 16 GB RAM, running Ubuntu Linux. The implementation was developed in Python using Scikit-learn, NLTK, Pandas, and NumPy libraries.

Model Training:

For each model (Naive Bayes, Logistic Regression, SVM, Random Forest, and the hybrid ensemble framework), the data is split into training and testing sets using a 70:30 ratio. Hyper parameters for each model are tuned using grid search and cross-validation to optimize performance.

Sentiment Classification: Each model is used to classify the sentiment of student feedback entries as positive, negative, or neutral.

Performance Metrics:

Accuracy: Measures the overall correctness of the model, calculated as the ratio of correct predictions to total predictions.

$\text{Accuracy} = (\text{True Positives} + \text{True Negatives}) / \text{Total Predictions}$

Precision: Measures the proportion of positive predictions that are actually correct. It is particularly important when the cost of false positives is high.

$\text{Precision} = \text{True Positives} / (\text{True Positives} + \text{False Positives})$

Recall (Sensitivity): Measures the proportion of actual positive instances that are correctly identified by the model.

$\text{Recall} = \text{True Positives} / \text{True Positives} + \text{False Negatives}$

F1-Score: The harmonic mean of precision and recall, providing a balanced measure of model performance, particularly in datasets with imbalanced classes.

$\text{F1-Score} = 2 \times ((\text{Precision} \times \text{Recall}) / (\text{Precision} + \text{Recall}))$

RESULTS AND DISCUSSION**Performance Metrics Comparison**

As the results indicate, the hybrid ensemble framework outperforms all traditional models in terms of accuracy, precision, recall, and F1-score. This improvement can be attributed to the combined strengths of multiple machine learning algorithms (Random Forest, Gradient Boosting, and SVM), which allow the hybrid model to capture complex patterns and nuances in student feedback that individual models might miss. The improved performance of the hybrid ensemble framework can be attributed to the complementary strengths of the individual classifiers. While Random Forest effectively handles noisy data, Gradient Boosting focuses on correcting classification errors and SVM performs well in high-dimensional text spaces. Their combination therefore provides better generalization than any single model.

Accuracy: The ensemble model achieves the highest accuracy (90.2%), demonstrating its robustness in classifying sentiments across different types of feedback.

Precision: The hybrid ensemble also leads in precision (89.7%), ensuring that most of the positive predictions it makes are correct.

Recall: The recall of the hybrid model (88.9%) is the highest, meaning it successfully identifies the majority of positive and negative sentiment instances in the feedback.



**Subina Shukoor and Durai**

F1-Score: With an F1-score of 89.3%, the hybrid model provides a balanced measure of precision and recall, making it the most reliable model for sentiment analysis in this case study.

Analysis of Model Strengths

The hybrid ensemble framework excels because it leverages the strengths of multiple models, each addressing different aspects of sentiment analysis:

- Random Forest is effective in handling noisy and complex data.
- Gradient Boosting improves performance by focusing on the weaknesses of previous models.
- SVM works well for high-dimensional text data and helps in accurately classifying the feedback.
- In contrast, traditional models like Naive Bayes and Logistic Regression tend to struggle with complex feedback data, especially when nuanced or indirect sentiment is expressed. SVM performs well but is outperformed by the ensemble model, which combines SVM with other complementary algorithms to improve overall performance.

This case study demonstrates the effectiveness of the hybrid ensemble framework for sentiment analysis of student feedback. By comparing the ensemble model with traditional sentiment analysis approaches, we observe that the hybrid framework significantly outperforms the baseline models in terms of accuracy, precision, recall, and F1-score. The results show that the ensemble approach can capture the complexities and subtleties of student feedback across different academic disciplines, providing valuable insights for educators and researchers.

DISCUSSION

The results of this study emphasize the significant potential of the hybrid ensemble framework in analyzing educational data, particularly student feedback. By integrating multiple machine learning models, this approach effectively captures the nuances of sentiment expression, which can vary widely across different disciplines, course formats, and individual student experiences. The findings underscore the importance of leveraging data-driven insights to optimize educational practices, improve teaching methods, and foster a more responsive learning environment.

Benefits of Sentiment Analysis in Education

The successful application of sentiment analysis in education brings several substantial benefits to both educators and students. By automating the process of feedback analysis, sentiment analysis allows for real-time, actionable insights, which can directly influence teaching practices.

- **Data-Driven Insights:** One of the most compelling advantages of sentiment analysis is its ability to provide concrete, quantitative data that can guide instructional decisions. Instead of relying on subjective impressions or sporadic student interactions, educators can use sentiment scores to evaluate their teaching methods objectively. The hybrid ensemble model in this study offers a comprehensive way to understand the emotional tone and underlying sentiments of feedback, making it easier to identify areas where students might be struggling or thriving. This data can also be used to track changes over time, enabling instructors to evaluate the effectiveness of any adjustments made to their teaching strategies.
- **Improved Student Engagement:** Understanding the perspectives of students through sentiment analysis allows educators to adjust their teaching methods to better align with student needs. For example, if sentiment analysis reveals that a particular aspect of a course is consistently met with negative feedback—such as unclear explanations or lack of engagement—educators can take action to improve that area. This might involve restructuring lectures, introducing more interactive activities, or providing additional resources. By responding to student sentiments, instructors can foster a more engaging and supportive learning environment, ultimately contributing to increased student satisfaction and academic success.
- **Targeted Interventions:** Sentiment analysis also facilitates the identification of patterns in student feedback that may indicate specific challenges. For instance, if multiple students express frustration with a particular concept or assignment, educators can intervene early, offering additional explanations or providing supplementary



**Subina Shukoor and Durai**

materials. In this way, sentiment analysis empowers educators to take a proactive approach to student concerns, preventing potential issues from escalating and ensuring that no student is left behind. Targeted interventions based on sentiment insights can lead to better student retention, improved performance, and a more positive overall learning experience.

The hybrid ensemble framework's ability to synthesize feedback from a diverse range of students further enhances its value in educational settings. By integrating multiple algorithms, the framework provides a more robust and nuanced understanding of student sentiments, ensuring that both broad trends and individual concerns are addressed.

Challenges and Limitations

While the hybrid ensemble framework offers significant promise, there are several challenges and limitations that need to be addressed for broader adoption in educational contexts.

- **Complexity of Educational Data:** Educational data, particularly feedback, is often rich in complexity and variability. Sentiments can be expressed indirectly or ambiguously, requiring sophisticated feature extraction methods to ensure accurate sentiment classification. For example, a feedback entry like "The class was boring, but I understand why we need to cover this material" might be challenging for traditional sentiment analysis models to interpret, as it expresses both negative sentiment ("boring") and a more neutral or even positive sentiment ("understand why we need to cover this material"). The hybrid ensemble model is better equipped to handle such complexities, but ongoing refinement of the model and feature extraction techniques will be necessary to improve its accuracy further.
- **Real-Time Analysis:** In many educational settings, real-time feedback analysis is essential to allow instructors to adjust their teaching methods promptly. However, ensemble models, especially those that combine several machine learning algorithms, can be computationally intensive. Real-time sentiment analysis requires high processing power and efficient algorithms to ensure that feedback can be analyzed quickly and accurately. The computational resources required for real-time sentiment analysis may limit the feasibility of deploying more complex models in resource-constrained environments, such as smaller educational institutions or in classrooms with limited access to advanced computing infrastructure. Experimental evaluation indicated that the framework required an average processing time of approximately 40 ms per feedback entry, including preprocessing, feature extraction, and classification stages, demonstrating its suitability for near real-time educational feedback analysis.
- **Language Nuances and Context:** Educational feedback often contains nuances that are specific to the context of a particular course, academic discipline, or teaching style. The hybrid ensemble framework, while effective, may still struggle to fully understand these nuances without further customization. For instance, in a STEM course, technical jargon or specialized vocabulary might be used, whereas in a humanities course, feedback could be more reflective and complex in its tone. Ensuring that the sentiment analysis model can adapt to these contextual variations is a challenge that requires continuous updates to the model's feature set, as well as further research into domain-specific sentiment indicators.
- **Data Privacy and Ethical Concerns:** As with any system that analyzes student data, there are important ethical considerations regarding privacy and consent. Educational institutions must ensure that feedback data is anonymized and that students are informed about how their data will be used. While sentiment analysis can provide valuable insights, it is crucial to balance these benefits with a strong commitment to protecting student privacy. Additionally, educators should be aware of the potential for bias in sentiment analysis models. If the training data used to develop these models contains biases, these biases can be reflected in the sentiment analysis results, which could lead to skewed or unfair conclusions about student experiences. To protect student privacy, all feedback entries were anonymized before analysis by removing personally identifiable information. Only anonymized textual data were used for model development and evaluation. Furthermore, all feedback data were stored in secure institutional repositories accessible only to authorized researchers, and the analysis was performed exclusively on anonymized textual content in accordance with institutional ethical guidelines.





CONCLUSION

The hybrid ensemble framework for sentiment analysis has proven to be a valuable tool for analyzing student feedback in educational settings. By combining the strengths of multiple machine learning algorithms, the model offers enhanced accuracy, precision, and recall compared to traditional sentiment analysis methods. This research demonstrates that sentiment analysis, powered by hybrid models, can provide educators with actionable insights that support data-driven decision-making and improve teaching strategies. The findings of this study reinforce the hypothesis that ensemble techniques are particularly well-suited for analyzing complex, high-dimensional educational data. By accurately capturing the nuanced sentiments expressed in student feedback, the framework allows educators to make informed adjustments to their teaching methods, leading to improved student engagement and satisfaction. Additionally, the ability to identify patterns in student feedback enables targeted interventions, ultimately enhancing the overall learning experience. Future research should focus on further refining the model to address the complexities of educational feedback. This may involve developing more sophisticated feature extraction techniques, exploring the use of deep learning models for sentiment analysis, and improving real-time analysis capabilities. Moreover, extending the framework's applicability to other educational contexts, such as online learning platforms and large-scale educational surveys, could further enhance its utility in diverse learning environments. Future work will focus on incorporating advanced contextual language models to improve the detection of sarcasm and irony, which remain challenging aspects of educational sentiment analysis. In conclusion, the hybrid ensemble framework represents a promising step toward leveraging machine learning for improving educational practices. By providing educators with the tools to understand and respond to student sentiment more effectively, this approach holds the potential to transform how educational institutions approach teaching, learning, and student support.

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Subina Shukoor and Durai

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Table 1: The following table summarizes the results for each model, based on the evaluation metrics:

Model	Accuracy	Precision	Recall	F1-Score
Naive Bayes	80.5%	81.2%	78.9%	80.0%
Logistic Regression	83.1%	82.7%	80.5%	81.6%
SVM	85.0%	84.4%	83.1%	83.7%
Random Forest	87.3%	86.9%	85.5%	86.2%
Hybrid Ensemble Framework	90.2%	89.7%	88.9%	89.3%

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Microbiota-Based Therapies for Combating Antimicrobial Resistance: A Narrative Review

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ABSTRACT

AMR remains the most critical issue in terms of public health and medicine, decreasing antibiotic efficiency and causing increased morbidity, mortality, and healthcare expenditure on a global scale. The main reason for the emergence of resistance among microbes is the misuse of antibiotics. It causes pathogens to acquire and transfer resistance genes. The human microbiome acts as a major reservoir for the development of resistance, or the so-called resistome, and also modulates immune responses. This paper focuses on the role of the human microbiome concerning antimicrobial resistance, particularly emphasizing the significance of dysbiosis for the formation of MDROs. In addition, it considers microbiota-based strategies for overcoming AMR, including probiotics, prebiotics, FMT, engineered microbial communities, synthetic biology, and phage therapy. These approaches are based on principles like microbiome restoration, inhibition of pathogens, immunomodulation, and reduction of virulence gene expressions. The review also highlights the use of these therapies in treating C. diff infections, healthcare-associated infections, and antibiotic therapies. Despite the presence of issues like safety risks,



**Hima Vyshnavi et al.,**

standardization problems, regulatory difficulties, and unpredictable results, microbiota-based therapies provide an environmentally friendly and promising approach to tackle AMR in the future.

Keywords: AMR, microbiota, gut microbiome, probiotics, FMT, MDROs, resistome

INTRODUCTION

The increasing challenge of AMR warrants innovative strategies that go beyond merely developing new antibiotics. AMR causes more than 25,000 deaths each year in Europe alone, highlighting the need for alternative solutions, particularly given the limited number of novel antimicrobial agents currently in development. Antibiotic therapy changes the composition of the gut microbiome and promotes the emergence of antibiotic-resistant organisms (Stern & Brenner, 2018). The gut serves as an important reservoir of resistance genes that can be transferred between bacterial populations. (Merrick et al., 2023). Strategies such as the use of microbiota-based treatments, including FMT, probiotics, and prebiotics, provide promising options to restore homeostasis and prevent further spread of AMR. Microbiota-based therapies may target resistant bacteria for eradication or enhance the immune response against infections. This narrative review highlights the role of microbiota in AMR and explores microbiota-based approaches for MDR decolonisation. (Gargiullo et al., 2019, p. 1).

This review examines the effectiveness of microbiota-based therapies in tackling antimicrobial resistance (AMR) and explores the mechanisms through which these therapies exert their effects. As microbiota can either mitigate or exacerbate AMR, understanding their relationship with resistance is crucial. (Dongre et al., 2025). Antimicrobial resistance has evolved alongside the widespread use and development of antibiotics since the discovery of penicillin by Alexander Fleming, which revolutionised the treatment of infectious diseases. The phenomenon has emerged as a result of the ability of microorganisms to adapt to the selective pressure exerted by antibiotics. The extensive use of antibiotics for various purposes has contributed significantly to the development and spread of antimicrobial resistance. (Hunt & Kates, 2024).

The Global Threat of Antimicrobial Resistance (AMR)

Antimicrobial resistance is a growing global public health concern that reduces the effectiveness of antimicrobial therapies used to treat infectious diseases. Antimicrobial resistance occurs as a result of the ability of the microorganisms to survive exposure to antimicrobial agents. As a result, infections become more difficult to treat, may become prolonged, and can spread more easily. Given the increasing impact of AMR, there is a need to explore alternative therapeutic options alongside the development of new antimicrobial agents to treat infections caused by multidrug-resistant pathogens. Although antimicrobial resistance develops through biological and evolutionary processes, the rapid growth of AMR has been driven by the extensive use of antimicrobial agents in healthcare, agriculture and environmental sectors. The inappropriate and excessive use of antimicrobial agents favours the survival and proliferation of resistant bacteria. The emergence and dissemination of AMR are influenced by various factors, including inadequate prevention measures, poor sanitation, limited access to clean drinking water and increased international travel. AMR is a serious global health threat that requires an integrated approach to tackle it. This includes understanding mechanisms of resistance, reducing unnecessary use of antimicrobials, and encouraging collaboration across different sectors. (Aljeldah, 2022)

Mechanisms of Antimicrobial Resistance

Antimicrobial resistance (AMR) is a major worldwide issue that affects the effective treatment of infectious diseases. It occurs when microorganisms develop resistance to antimicrobial drugs, largely due to their excessive and inappropriate use in medicine, agriculture and environmental settings. Microorganisms employ several mechanisms to resist the effects of antimicrobial drugs. One such mechanism involves reducing drug entry, which means that



**Hima Vyshnavi et al.,**

microbes change the structure of their membrane to block drug entry into the cell. Microorganisms may also use efflux pumps to remove antimicrobial agents from the cell, reducing their effectiveness. Another mechanism involves the production of enzymes that break down or modify antimicrobial agents. Microorganisms can also alter the target sites that antimicrobial agents normally act upon. Resistance genes can be transferred between microorganisms, contributing to the rapid spread of resistance. Overall, these mechanisms enable microorganisms to survive antimicrobial treatment, contributing to the growing global challenge of antimicrobial resistance. (Byarugaba, 2009)

Impact of AMR on Global Health and Economy

Antimicrobial resistance (AMR) is one of the most significant public health challenges worldwide because it reduces the effectiveness of antimicrobial drugs used to treat infectious diseases. The extensive use of antimicrobial drugs creates selective pressures that enable resistant microorganisms to survive and multiply. AMR has serious consequences for public health. Patients may not respond adequately to treatment, resulting in treatment failure, prolonged illness, and an increased risk of death. As a result, routine surgeries, cancer treatments and other medical procedures become more difficult and risky to perform. AMR also has a significant economic impact because of the cost of providing medical care. Patients have to stay longer at hospitals, receive more expensive treatment, and undergo more complicated diagnostic tests than usual. Moreover, it reduces labour productivity due to extended periods of illness and disability among workers. In general, antimicrobial resistance is one of the key challenges facing the global community. The continuous growth of AMR highlights the need for effective strategies and coordinated efforts to reduce human health. (McGowan, 2001)

The Human Microbiota and Its Role in Health and Disease

The human microbiota is composed of a diverse population of microorganisms that inhabit different parts of the body, including the gut, skin, and respiratory tract. These microbial communities play an important role in maintaining normal physiological functions, such as metabolism and immune regulation (Francino, 2016). A primary function of the microbiota is protecting against pathogen invasion through colonisation resistance. Beneficial microorganisms compete with harmful microbes for nutrients and space, thereby limiting their ability to establish infections. Additionally, continuous interactions between the microbiota and the host immune system help regulate both innate and adaptive immune responses. (Gjonbalaj et al., 2020). The microbiota also contributes to several metabolic pathways by breaking down complex nutrients and producing essential compounds, such as vitamins and short-chain fatty acids. However, disturbances in microbial composition, known as dysbiosis, may occur due to antibiotic use, dietary changes, or other environmental conditions. Such alterations can increase the risk of various diseases and negatively affect overall health. (Becattini et al., 2016).

In particular, the microbiota serves as a reservoir for antibiotic resistance genes, enabling their horizontal transfer and thereby driving the spread of resistant infections. Disruptions in microbial balance further increase chances to such pathogens, highlighting the importance of maintaining microbiome homeostasis to reduce resistance dissemination and strengthen host resilience. The complex interaction between the human microbiota and AMR requires comprehensive strategies that move beyond conventional antimicrobial approaches. (Tahmasebi et al., 2025, Dongre et al., 2025)

Composition and Function of the Human Microbiota

In particular, the microbiota serves as a reservoir for antibiotic resistance genes, enabling their horizontal transfer and thereby driving the spread of resistant infections. Disruptions in microbial balance further increase chances of such pathogens, highlighting the importance of maintaining microbiome homeostasis to reduce resistance dissemination and strengthen host resilience. The complex interaction between the human microbiota and AMR calls for comprehensive strategies that move beyond conventional antimicrobial approaches (Botero et al., 2015). It is necessary to emphasise that the diversity of the microbial community is crucial for its stability. Microbiota functions. Microbiota performs numerous vital functions in the human body. For example, this microbial community plays an important role in metabolism by breaking down complex food molecules into simpler substances. Moreover, these microbes produce many useful substances essential for the human body. The microbiota also helps in immune



**Hima Vyshnavi et al.,**

system develop and function, by identifying harmful pathogens and distinguishing them from harmless microbes or beneficial ones. Another important function of the microbiota, is protecting the body from harmful pathogens through colonization resistance by occupying space, competing for nutrients and producing toxins or enzymes which inhibit the growth of harmful microbes. However, changes in this balance can increase the risk of various diseases and infections. (Bik, 2009)

Dysbiosis and Disease

Dysbiosis refers to an imbalance of the normal microbial flora of the human body. It is usually influenced by conditions like antibiotic usage, dieting, and other environmental causes. The consequence includes decrease in diversity of microbes and disruption of their normal functions. In cases of dysbiosis the natural balance of microbes in the body is disrupted which leads to a decrease in beneficial microbes as well as overgrowth of pathogenic and opportunistic microbes, which may lead to a compromised intestinal barrier and inflammatory response within the host, increasing the risk of various diseases and infections (Cusumano et al., 2025) Dysbiosis leads to many diseases like gastrointestinal disorders and metabolic diseases as well as causing an increased risk of acquiring infections. Moreover, dysbiosis promotes the growth and spread of antibiotic resistance bacteria, making it difficult to treat. Thus, dysbiosis is one of the major connections between microbial imbalance and the development of diseases. Maintaining balance in microbial population is essential in preventing disease and reducing risks of infection. (Gupta et al., 2022)

Microbiota-Based Therapies: An Overview

Therapies that target the human microbiome are being explored as a way to fight the growing problem of antimicrobial resistance (AMR). While conventional antibiotics can disrupt the presence of good bacteria and thus promote resistance, these therapy enables the maintenance of microbiome balance and reduces the population of harmful bacteria. As the human microbiome may have antibiotic-resistant gene, maintaining it will be advantageous. (Becattini et al., 2016; Gjonbalaj et al., 2020). The recent studies have also proven that gut flora is an important element for preventing the development of MDROs, as well as balancing the immunity. Therefore, novel therapies including probiotics, prebiotics, fecal microbiota transplantation (FMT), artificial microbial consortia, and bacteriophage therapy are becoming increasingly popular as potential alternatives to the conventional antibiotics. (Beltrán-Velasco et al., 2025; Ding et al., 2025)

Probiotics and Prebiotics

Probiotics are live microorganisms that provide health benefits when taken in adequate quantities, while prebiotics are nondigestible dietary ingredients that selectively support the growth of beneficial microflora or probiotics. Both probiotics and prebiotics are used to restore microbiota imbalance caused by antibiotic treatment. Beneficial microorganisms can control pathogenic microorganisms by producing antibacterial substances such as bacteriocins and organic acids which would kill or inhibit the pathogen. (Ke et al., 2021, p. 2) Prebiotics are foods containing fiber that aid the development of good bacteria in the gut. In addition, they help produce short-chain fatty acids (SCFAs), which help in maintaining the integrity of the gut wall and controlling immunity. When prebiotics are combined with probiotics, the combination is known as synbiotics. This may be useful in restoring a good microbial population and inhibiting growth of antibiotic resistance pathogens. (Sanders et al., 2019) In addition, diet is known to have an important role in the development of the microbiome. There are certain components in the diet like emulsifiers found in processed food items influence the behavior of the gut microbiota and cause bodily inflammation. (Ding et al., 2025, p. 1563450)

Fecal Microbiota Transplantation (FMT)

Fecal microbiota transplantation (FMT) entails the transplant of fecal samples from a healthy individual into another individual to restore a healthy balance in gut microbiota. FMT has proven effective in restoring a well-balanced microbial ecosystem, especially in conditions where the normal microbiota has been severely damaged. The administration of FMT promotes the restoration of microbial balance through the introduction of many kinds of beneficial microorganisms into the digestive tract. These microorganisms can help to prevent the colonization of



**Hima Vyshnavi et al.,**

dangerous microorganisms in the body due to competition for available resources, a phenomenon called colonization resistance. In addition, FMT reduces the presence of antibiotic resistance genes in the digestive tract. Furthermore, it has been proven that FMT helps boost immune systems and control metabolism. It can therefore be applied not only for infection treatment, but also for other diseases associated with microbial dysbiosis in the gastrointestinal tract. (Ooijsveaar et al., 2018; Stallhofer et al., 2024)

Defined Microbial Consortia and Synthetic Biology Approaches

Another strategy used to address AMR is through the selection of certain microbes that have particular functions. Such microbes are chosen due to their ability to bring back balance to microbes, inhibit pathogens, and help maintain gut health. The objective of using such microbial communities is to ensure consistent results without any differences caused by variations in donor sources. Technological improvements in synthetic biology have resulted in the creation of engineered microorganisms capable of carrying out certain functions, for example, identifying pathogenic organisms, creating antibiotics, and modulating immune systems. The engineering of microorganisms has been found to be an effective strategy to counter the problem of AMR as they can be used to target resistance mechanisms. (Olatunji et al., 2024; Ooijsveaar et al., 2018)

Phage Therapy

Phage treatment is the practice of using viruses which can infect and destroy bacteria. Phages are highly specific for their bacterial hosts and can therefore kill only the desired pathogen without disturbing the other microbes in the body. This kind of treatment proves quite effective in dealing with drug-resistant bacteria due to its specific mode of action. In addition to that, bacteriophages are able to co-evolve with bacteria, making drug resistance a lot more unlikely. Some research indicates that combining phage therapy with microbiota-based approaches enhances the effectiveness of the treatment since it eliminates pathogens without affecting microbial diversity. Therefore, the combination of these two techniques is expected to produce successful outcomes in the fight against antibiotic resistance. (Stallhofer et al., 2024, p. 85)

Mechanisms of Action of Microbiota-Based Therapies Against AMR

Antimicrobial resistance (AMR) therapy using the microbiota involves balancing the microbiota, preventing the proliferation of any pathogenic bacteria, and boosting the immune system in the body. Unlike traditional antibiotics, which may alter the natural balance and cause resistant microorganisms to develop, this approach focuses on the maintenance of balance and proper functioning of the microbiota. This becomes necessary because the gut microbiota plays a important role in maintaining overall body wellbeing and even influencing the presence and spread of AMR genes. (Sandu et al., 2025; Stern & Brenner, 2018)

Restoration of Microbiota Diversity and Function

Another way microbiota-based treatments help combat antimicrobial resistance (AMR) is by restoring the balance and normal function of the microbiome after it has been disrupted by antibiotics. Antibiotic use can cause dysbiosis by reducing beneficial microorganisms and allowing resistant or opportunistic pathogens to grow. This imbalance weakens colonization resistance, making it easier for harmful microbes to establish themselves and increasing the spread of antibiotic resistance genes within the microbial community. (Ding et al., 2025; Gupta et al., 2020).

Using gut therapies like probiotics (good bacteria), prebiotics (food for good bacteria), and FMT (fecal microbiota transplants) helps recreate a healthy, stable environment in the body. When we restore this variety of microbes, they can work together better. They produce helpful substances that stop harmful germs and antibiotic-resistant "superbugs" from growing. (Relman & Lipsitch, 2018). It is essential to have a rich and healthy microbiome to achieve good health, prevent the gut barrier dysfunction, and enable regular physiological processes to function normally. Repopulating the gut microbiome following a state of dysbiosis is associated with several advantages. The first benefit, is an increase in colonization resistance as the presence of beneficial microbes prevents the growth of pathogens. Another possible outcome of re-establishing the gut microbiome is a reduction of the resistome, i.e.,



**Hima Vyshnavi et al.,**

antibiotic resistance genes that may be found in the microbiome (Ding et al., 2025; Dongre et al., 2025; Stern & Brenner, 2018).

Direct Antimicrobial Effects

Microbes also play an important role in addressing antimicrobial resistance through the inhibition of pathogenic bacteria. They secrete antimicrobial substances like bacteriocins, short chain fatty acids (SCFAs), and organic acids that act against pathogens, thereby creating a hostile environment that prevents their proliferation. This makes it difficult for the microbes to multiply and reduces the chances of resistances developing. Moreover, bacteriophage therapy offers a targeted method for fighting bacterial infections since bacteriophages only target and kill pathogenic bacteria and do not affect other bacteria. Therefore, bacteriophage therapy eliminates the infection-causing bacteria while sparing other beneficial microorganisms. This makes it possible to avoid using broad-spectrum antibiotics that contribute to antibiotic resistance. Finally, microbial interactions within the body play a key role in shaping the properties of resistance. For example, competition among various microorganisms along with the release of inhibitory substances may lead to decreased resistance..(Bhalodi et al., 2018)

Immunomodulation

Gut treatments are powerful because they train and activate your body's immune system to fight off dangerous, antibiotic-resistant superbugs. The bacteria in your gut are constantly "talking" to your immune defenses through a complex network of chemical signals, boosting both your body's immediate, built-in reactions and its long-term ability to track down and destroy specific germs. By strengthening this natural partnership, microbiota therapies help the body defend itself and heal without relying on traditional antibiotics (Gupta et al., 2020, p. 778) Treatments like probiotics, prebiotics, and gut microbe transplants restore a healthy balance of bacteria in your body, which acts like a wake-up call for your immune system. This boost stimulates the production of defender cells and chemical signals that help your body actively scan for and destroy harmful germs. By supercharging your natural defenses this way, these therapies lessen our reliance on traditional antibiotics, which ultimately stops bacteria from adapting and turning into dangerous, drug-resistant superbugs. (Dongre et al., 2025; Stallhofer et al., 2024, p. 84). When the gut's microbe balance is disrupted, it triggers body-wide inflammation and weakens your immune system, making it much easier for dangerous infections to move in and take over. Fixing this ecosystem calms that inflammation and rebuilds your body's natural defenses. Ultimately, this ability of good microbes to tune and strengthen your immune system is a secret weapon in the fight against superbugs, helping the body defeat infections on its own so we don't have to rely on antibiotics (Ding et al., 2025)

Competition for Resources and Niche Exclusion

Nutrient and niche competition is a primary mechanism by which the gut microbiota establishes colonization resistance against invading pathogens. By fully occupying ecological niches and efficiently consuming available nutrients, commensal microbes deprive opportunistic and drug-resistant bacteria of the resources necessary to survive and proliferate. This competitive exclusion is vital for maintaining microbial homeostasis and serves as a critical biological barrier that protects the host from infection.(Brinkac et al., 2017; Relman & Lipsitch, 2018).

Microbiota-based interventions—such as probiotics, prebiotics, and fecal microbiota transplants (FMT)—effectively restore competitive dynamics within the gut by replenishing populations of beneficial microorganisms. By re-establishing a dense and diverse microbial community, these therapies ensure that commensal bacteria fully occupy available ecological niches and consume critical nutrients. This process of competitive exclusion deprives opportunistic pathogens and multidrug-resistant organisms (MDROs) of the resources necessary to colonize, proliferate, and establish infection within the gastrointestinal tract (Dongre et al., 2025). Furthermore, a diverse and stable gut microbiota enhances colonization resistance by serving as a robust ecological barrier against incoming pathogens. When this equilibrium is disrupted—such as following antibiotic therapy—the depletion of commensal bacteria creates vacant ecological niches and leaves unconsumed nutrients. This disruption diminishes the gut's natural defenses, significantly increasing the host's vulnerability to colonization and proliferation by opportunistic pathogens (Ding et al., 2025; Gupta et al., 2020).



**Reduction of Virulence Factor Expression**

Microbiota-based therapies can actively suppress the expression of virulence factors in pathogens, effectively neutralizing their ability to cause disease without relying on direct eradication. Pathogenic bacteria utilize sophisticated regulatory systems, such as quorum sensing (cell-to-cell chemical communication), to coordinate colonization, biofilm formation, and toxin production. By disrupting these signaling pathways, beneficial microbes can essentially "silence" the pathogens' harmful behaviors, mitigating their pathogenicity while minimizing the selective pressure that typically drives antibiotic resistance. (Brinkac et al., 2017; Ding et al., 2025). Commensal gut microbes can directly disrupt pathogen signaling by inhibiting quorum sensing mechanisms and downregulating the genes responsible for virulence factors. Furthermore, shifts in the microbial community structure can alter pathogen gene expression while simultaneously modulating host inflammatory responses, ultimately reducing both pathogen virulence and disease severity (Ding et al., 2025).

Additionally, emerging evidence highlights microbial extracellular vesicles (EVs)—lipid-bound particles released by cells—as critical mediators of intercellular communication that can modulate antimicrobial resistance and virulence traits across different species (Hunt & Kates, 2024).

**Applications of Microbiota-Based Therapies in Combating AMR
Clostridioides difficile Infection (CDI)**

Microbiota-based treatments have proven highly effective in treating *Clostridioides difficile* infection (CDI), a condition strongly correlated with antibiotic-induced dysbiosis. When antibiotics deplete the gut's indigenous flora, *C. difficile* exploits the disrupted environment to proliferate and release toxins that damage the intestinal epithelium. Recent research underscores that restoring microbial diversity is essential for clinical recovery, as a healthy microbiota re-establishes colonization resistance by outcompeting the pathogen for nutrients and generating targeted antimicrobial compounds. (Stallhofer et al., 2024)

Furthermore, microbiota-based therapies play a critical role in mitigating antimicrobial resistance (AMR) in CDI by breaking the cycle of recurrent antibiotic use, which otherwise drives further resistance. Beyond restoring ecological balance, these interventions enhance host metabolic and immune responses, effectively rendering resistant pathogens vulnerable to clearance. Accumulating in vitro and clinical evidence suggests that deploying targeted microbial consortia and synthetic microbiotas is a pivotal strategy for eradicating CDI and drug-resistant strains. Ultimately, microbiota modulation addresses the root cause of AMR by leveraging the host's restored immunity as the primary therapeutic mechanism. (Ding et al., 2025; Gupta et al., 2020)

Multidrug-Resistant Organism (MDRO) Decolonization

When it comes to implementing MDRO decolonization, the using of microbially-based treatments becomes essential since these types of pathogens generally reside in the GI tract and become the natural environment for AMR genes. There are different methods such as FMT, probiotics, prebiotics, and changes in diet to help boost microbial diversity and gain colonization resistance to the pathogenic organisms. By doing so, it becomes possible to inhibit the growth of resistant bacteria and, therefore, prevent the spread of infection and reduce its prevalence. In addition, there are other approaches that have recently emerged to deal with these organisms. (Ooijsaar et al., 2018) However, some studies show that several controversial findings have been observed in relation to microbiota-based treatments in resolving issues regarding MDRO decolonization. The significance of the research findings is that though FMT may help in improving microbial diversity and generation of positive metabolites, it will not facilitate the removal of the pathogen in patients. Additionally, other approaches to decolonization through antibiotics may negatively impact the microbiome, leading to increased risk of developing resistance. However, microbiota-based treatments look promising and need more study. (Ooijsaar et al., 2018; Stern & Brenner, 2018,)

Prevention of Healthcare-Associated Infections

The application of microbiota-based treatment is necessary in preventing HAI since the therapy helps balance the body's microbiome, especially the gut microbiome, in which there is an abundance of MDROs. The use of antibiotics



**Hima Vyshnavi et al.,**

disrupts the gut microbiome and results in increased colonization with the carbapenem- and vancomycin-resistant Enterobacterales, increasing the risk of HAI in patients, such as bloodstream infection, UTI. It is important to consider the contribution of different microbiota therapies in enabling colonization resistance to regulate the body's microbiome. (Ding et al., 2025; Gupta et al., 2020). Also, such therapies are important in protecting against infections as they help in boosting the immune system of the host, as well as in maintaining microbial diversity to prevent opportunistic infections among patients in hospitals. Microbial diversity and stability resulting from diet, prebiotics, and microbiota-based therapies prevent infections and also the appearance of resistance in the microbial flora. Since microbiota therapies do not apply selection pressure on microorganisms like antibiotics, they offer a sustainable option for preventing infections.

Adjuvant Therapy in Antibiotic Treatment

Several studies have been conducted to discover microbiota-based treatments besides the usage of antibiotics for AMR. Though antibiotics are effective in fighting pathogens, their use may affect the balance of the microbiota and lead to dysbiosis, as a result creating an ideal environment for AMR. These types of treatment normalize gut microbiota thus inhibiting bacteria proliferation in ensuring that resistomes do not develop in the intestines. (Stallhofer et al., 2024; Stern & Brenner, 2018). If these methods are used to support antibiotics instead of replacing them, then there could be positive results as a result of the synergism. It is well proven that there is a potential of probiotics to reduce the negative results that come with the use of antibiotics like the occurrence of diarrhea and *C. difficile* infections. Also, microbiota approaches could help in promoting colonization resistance and also decolonization of antibiotic-resistant bacteria. Additionally, the effectiveness of antibiotics will be preserved by using these treatments because of preservation of natural microbiota ecosystem. However, research on these therapies remain mixed. (Ooijsaar et al., 2018; Stallhofer et al., 2024)

Challenges and Limitations of Microbiota-Based Therapies

Standardization and Regulatory Hurdles

One of the main challenges of microbiota-based therapies is that there are no standardized approaches used for donor selection, sample preparation, storage, and administration of samples to the patient, which may cause discrepancies in the effectiveness of such treatment. Discrepancies in methods of research also prevent comparison of different studies and setting the criteria for such therapy. Furthermore, regulation of new treatments such as fecal microbiota transplantation is difficult due to their specific nature that does not allow categorizing them as medicinal products or biologics (Hassall et al., 2024; Nadimpalli et al., 2017; Zhu et al., 2022).

Safety Concerns and Adverse Events

Safety is another major concern that has caused alarm in the use of microbiota therapy as it is highly probable for pathogenic microorganisms and antimicrobial resistance genes to be transferred from one individual to another during treatment. The screening procedures of these potential donors do not always reveal all of the dangers that are associated with treatment and could cause serious infection, especially in patients who have a compromised immune system. These patients could suffer from a wide range of complications starting from minor gastrointestinal distress up to more severe infections. Furthermore, there are many cases of horizontal gene transfer between microorganisms, leading to higher antimicrobial resistance risks. (Guo & Kong, 2019; Khurana et al., 2023; Zhu et al., 2022).

Efficacy in Diverse Clinical Settings

The efficacy of microbiome therapy could differ from one patient to another depending upon their immune system, composition of microbiome, severity of disease, and external environment. Moreover, the variability of the experimental protocol and parameters used in evaluation makes it difficult to draw any conclusions based on clinical trials. Geographic factors and differences in environmental conditions may impact the structure of the microbiome, thus affecting the efficiency of the treatment. (Hassall et al., 2024; Khurana et al., 2023; Zhu et al., 2022).



**Hima Vyshnavi et al.,****Patient Acceptance and Practical Implementation**

Acceptance by patients might be a difficult issue when dealing with microbiota therapies. Microbiota treatments include FMT, probiotics, phage therapy, and artificial microbial communities that seek to help achieve a healthy microbiota and to minimize antibiotic-resistant microorganisms and prevent infections. Although these approaches offer an advantage in fighting antimicrobial resistance, their efficacy is different from one study to another due to the different methodology used. Hence, there is need for more research and incorporating antimicrobial stewardship practices to enhance their efficacy (Mansoor et al., 2023; Merrick et al., 2023; Roselló et al., 2016; Sherry et al., 2025).

Future Directions and Research Gaps**Personalized Microbiota Therapies**

One possible future direction would be in developing personalized therapies for managing various diseases through microbiota, considering factors such as individual differences within microbiome profiles, host genetics, and physiological condition. There have been suggestions that microbiome differences influence how patients react to therapies, meaning that there should be a personalization process in this approach. This means that either a tailor-made microbial ecosystem or particular types of bacteria, depending on the starting composition of microbiome, can be used to boost the efficiency of the treatment. However, there are still challenges surrounding the process of standardization and selection of proper biomarkers in microbiome studies. (Pearl et al., 2022; Sherry et al., 2025; Verma et al., 2023)

Novel Delivery Methods

The second major area where more research is required is in the creation of innovative methods for improving the efficacy and effectiveness of microbiota-based treatments in terms of their stability, viability, and targeted activity. Present methods, which include oral capsules, fecal material, and probiotics, pose challenges related to poor viability in the GI tract and inconsistent colonization. There are many delivery mechanisms currently under development, which include encapsulation, bioengineered bacterial vectors, and bacteriophage delivery systems, among others. (MOO et al., 2019; Roselló et al., 2016; Sherry et al., 2025)

Integration with Conventional Antimicrobial Strategies

In the future, it will be important to include the use of microbiota-based treatments into the traditional treatment of AMR. While antibiotics will continue to play an important role in fighting infection, their use can also disrupt the microbiota balance of the body and facilitate the development of resistance. Using antibiotics in combination with microbiota restoration treatments could help in preserving the colonizing resistance of the host and lower the rate of recurrence of resistant pathogens. Bacteriophage therapy and microbiome modification are additional treatment options that have been used as alternatives to antibiotic usage. (Mansoor et al., 2023; Roselló et al., 2016; Sherry et al., 2025)

Long-term Outcomes and Follow-up Studies

One of the most significant research gaps is the absence of long-term data on the safety, sustainability, and environmental impact of microbiota-based treatments. Although the immediate results look positive in terms of decreasing the colonization of antimicrobial-resistant pathogens, the long-term consequences on microbiome stability, the resistome, and host immunity are poorly understood. Additionally, there is an insufficient amount of data regarding the reoccurrence rates and efficacy persistence post-treatment. Future research needs long term studies to prove that treatments using microbes are safe and work well. (Mansoor et al., 2023; Merrick et al., 2023; MOO et al., 2019)

CONCLUSION

Antimicrobial resistance (AMR) severely threatens global public health by undermining traditional antibiotics, but the human microbiota offers a sustainable, nature-aligned solution to restore microbial balance, boost colonization





Hima Vyshnavi et al.,

resistance, and suppress resistant pathogens. Through diverse applications like probiotics, fecal microbiota transplantation (FMT), and phage therapy, these treatments target severe dysbiosis, *Clostridioides difficile* infections, and healthcare-associated risks by intersecting ecology, immunology, and metabolism. Although challenges like variable patient responses, residual pathogens, and standardization issues persist, the future of routine medicine lies in precision approaches like engineered probiotics and synthetic microbiomes. Ultimately, by shifting our perspective toward cooperating with our own microbes, these therapies can reduce antibiotic dependency, protect existing drugs, and fundamentally transform infectious disease management.

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Periodontitis: Global Prevalence, Microbial Dysbiosis and Emerging Therapeutic Strategies – A Comprehensive Review

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ABSTRACT

Periodontitis is a chronic inflammatory disease driven by dysbiosis of the subgingival microbiome and the persistence of pathogenic biofilms that remain resistant to conventional therapies. This review aims to comprehensively analyze the global and Indian epidemiological burden of periodontitis, elucidate microbial and host interactions, and evaluate emerging therapeutic strategies with a focus on probiotics, bacteriocins, and nanoparticle-based drug delivery systems. Epidemiological evidence indicates a clear age-associated increase in disease prevalence, with higher severity observed in middle-aged and older populations, minimal gender differences, and a disproportionate burden among rural and socioeconomically vulnerable groups. Although scaling and root planing remains the gold standard of treatment, its effectiveness is limited in deep periodontal pockets and against structured biofilms, while adjunctive antibiotic therapy raises concerns regarding antimicrobial resistance and adverse effects. Emerging biological approaches, including probiotics and bacteriocins such as nisin, demonstrate promising antimicrobial and anti-biofilm activity without cytotoxic effects on host tissues. Advances in





Srushti Gamit et al.,

omics technologies have further enhanced understanding of host–microbe interactions, enabling identification of novel therapeutic targets. Integration of these approaches with nanoparticle-based delivery systems offers improved stability, targeted delivery, and sustained antimicrobial activity. Collectively, these innovations represent a promising direction for next-generation periodontal therapeutics and may significantly improve long-term biofilm control and clinical outcomes.

Keywords: Periodontitis, Oral microbiome, Biofilms, Probiotics, Bacteriocins, Nanoparticles

INTRODUCTION

Periodontal disease is a long-standing inflammatory disorder involving the gingival tissues and supporting structures of teeth, representing a significant global public health issue. Despite improvements in oral hygiene awareness and advances in clinical dentistry, periodontitis often persists due to the resilience of complex microbial biofilms and the ability of pathogenic microorganisms to colonize subgingival niches that are difficult to access through routine mechanical therapy. These structured biofilms protect pathogenic microorganisms, allowing their persistence and sustained stimulation of host inflammatory responses. Recent research has increasingly focused on biological and nanotechnological strategies aimed at disrupting pathogenic biofilms rather than merely removing dental plaque mechanically. Among these approaches, probiotic microorganisms and their antimicrobial metabolites, particularly bacteriocins, have gained attention as effective alternatives or supportive agents to conventional periodontal treatments. Bacteriocins are antimicrobial peptides synthesized through ribosomal pathways by probiotic bacteria and demonstrate strong activity against a wide range of oral pathogens. Their selective action against harmful microorganisms, while maintaining the balance of beneficial microbiota, makes them highly suitable for targeted biofilm management in periodontal therapy. In parallel, growing interest in nanoparticle-based strategies has introduced new possibilities for enhancing antimicrobial delivery in oral healthcare. Nanoparticles offer advantages such as increased surface area, improved stability of bioactive compounds, and the potential for sustained and targeted release. The integration of bacteriocins into nanoparticle delivery systems, supplemented with commonly used toothpaste additives, represents an innovative and translational approach that could bridge advanced therapeutic concepts with daily oral hygiene practices. Such a strategy may improve patient compliance while strengthening long-term biofilm control in periodontal disease.

The socioeconomic impact of periodontitis is considerable and continues to pose a major global concern. In 2018, the financial burden of periodontal disease was estimated to be around \$154 billion in the United States and €159 billion across Europe. Apart from economic implications, numerous studies have shown that periodontitis adversely affects oral health-related quality of life and is strongly linked with various systemic disorders, including cardiovascular diseases, diabetes, and complications during pregnancy. Together, these observations highlight the critical need for improved preventive measures and more effective therapeutic interventions [1], [2], [3], [4], [5], [6], [7], [8]. From a clinical perspective, chronic periodontitis presents as inflammation of the gingival tissues that progresses to involve the periodontal supporting structures, resulting in gradual loss of attachment and destruction of alveolar bone. When not treated in a timely manner, the condition advances to tooth instability and ultimately tooth loss, making it a major contributor to edentulism worldwide. Epidemiological data consistently demonstrate an age-dependent increase in periodontal disease prevalence. Reported prevalence rises from approximately 57% among 12-year-olds to nearly 89.6% in individuals aged 35–44 years. Moderate periodontitis has been observed in 17.5% and 21.4% of individuals aged 35–44 and 65–74 years, respectively, while severe disease—defined by probing depths ≥ 6 mm in at least one tooth—was reported in 7.8% and 18.1% of these age groups. Gender-related differences are generally minimal, although a slightly higher prevalence has been documented in rural populations, likely reflecting disparities in access to dental care, health literacy, and preventive services.



**Srushti Gamit et al.,****Oral Microbiota's Guilty Role in Triggering Periodontitis**

The human oral cavity contains nearly 700 microbial species that together form a complex oral microbiota [9]. Under healthy conditions, this diverse microbial community exists in a state of dynamic equilibrium, supporting oral homeostasis. However, disturbance of this equilibrium results in microbial dysbiosis, characterized by a shift from health-associated commensal species to disease-associated pathogenic consortia [10]. This dysbiotic shift plays a central role in the initiation and progression of gingivitis and periodontitis. Pathogenic biofilms stimulate a chronic host inflammatory response, resulting in tissue destruction that further alters the subgingival environment and reinforces microbial imbalance. Emerging evidence also suggests that systemic inflammatory diseases can influence periodontal status, while periodontal inflammation itself may contribute to systemic inflammatory burden, highlighting a bidirectional relationship between oral and systemic health [11]. The assembly and stability of the oral microbiota appear to be governed by ecological principles similar to those observed in other biological ecosystems [12]. Although several studies have demonstrated that oral microbial communities are highly individualized, niche-specific, and distinct between health and disease states, relatively little is known about the ecological mechanisms governing microbiota disruption, resilience, and reassembly following periodontal therapy [13]. Understanding these processes is essential for the development of targeted therapies aimed at restoring microbial balance rather than merely suppressing pathogenic species.

Gum Battles: Challenges Tackled for Healing Smiles

Population-based studies in India have consistently shown that regular oral hygiene practices, particularly tooth brushing, are strongly associated with reduced prevalence of periodontal disease. A nationwide epidemiological survey, although primarily focused on prevalence rather than detailed risk-factor analysis, provided critical baseline data at both national and state levels. These findings highlighted the substantial burden of periodontal disease in India and emphasized the need for comprehensive preventive strategies and accessible treatment interventions [14]. Beyond mechanical plaque control, increasing attention has been directed toward biological and nanotechnological interventions. Probiotics and bacteriocins play a pivotal role in combatting periodontal pathogens, although their effectiveness varies depending on strain specificity and the spectrum of antimicrobial activity. While lactobacilli are well known for bacteriocin production, bacteriocins such as nisin have demonstrated efficacy against a wide range of periodontal pathogens. Other bacteriocin-like peptides have also shown promising antimicrobial and anti-biofilm properties. Metal and metal oxide nanoparticles exhibit diverse antibacterial effects, including membrane disruption, oxidative stress induction, and interference with microbial metabolism. These nanoparticles have demonstrated the ability to disrupt biofilms, a major factor contributing to drug-resistant infections. Due to their multifunctional properties, relatively low toxicity, and therapeutic versatility, metal nanoparticles represent promising tools for periodontal disease management. Furthermore, advances in nanotechnology have led to the development of mineral nanoparticles capable of promoting cell mineralization and osteogenic differentiation, suggesting potential regenerative applications alongside antimicrobial action.

Prevalence of Periodontitis**Gujarat's Silent Battle Against Periodontitis**

The burden of periodontitis in Gujarat shows pronounced regional variation, reflecting differences in demographic profiles, oral hygiene practices, lifestyle habits, occupational exposure, and access to dental care. Disease distribution is not uniform across the state; instead, distinct patterns are observed between northern, central, and industrial coastal regions, highlighting the influence of localized risk factors and social determinants of health. Across studies from different districts, oral hygiene behavior, age structure, and adverse lifestyle practices consistently emerge as key contributors to periodontal disease patterns. Regions with better oral health awareness and routine hygiene practices tend to demonstrate comparatively improved periodontal status, whereas industrial and coastal areas with higher tobacco exposure and occupational stress exhibit a greater disease burden. These observations underscore the importance of region-specific preventive and control strategies rather than a uniform statewide approach. Investigations involving socially marginalized and vulnerable populations further reveal disproportionately poor periodontal health, emphasizing the need for inclusive oral health policies that address both biological susceptibility and social inequities. Moreover, emerging associations between periodontitis and systemic inflammatory conditions



**Srushti Gamit et al.,**

suggest broader health implications of untreated disease within the state. A consolidated summary of region-wise epidemiological findings, including participant characteristics and principal observations, is presented in (Table 1), which synthesizes data from multiple districts of Gujarat [15], [16], [17], [18], [19].

Epidemiological Survey: Story Unfolds in Indian Rural & Urban Regions

India's first nationwide epidemiological survey employed a three-stage sampling strategy encompassing both rural and urban populations across multiple age groups. Periodontal status was assessed using the WHO probe and the Community Periodontal Index (CPI). The survey revealed an age-dependent increase in periodontal disease prevalence, minimal gender disparities, and a consistently higher disease burden in rural areas. Regular tooth brushing was strongly associated with lower disease prevalence, with toothbrush use proving more effective than finger cleaning [14]. Subsequent studies further reinforced these observations, reporting widespread periodontal disease across diverse Indian populations. Earlier national reports estimated that 40–45% of the Indian population may be affected by advanced periodontal disease [20]. Field surveys also documented associations between periodontal disease and systemic conditions such as coronary artery disease [21], as well as increased prevalence among rural women and other vulnerable groups [22]. Overall, these findings emphasize the substantial burden of periodontal disease in India and underline the importance of focused preventive approaches. (figure 1) also illustrates the state-wise prevalence of periodontitis across India, demonstrating considerable regional variation in disease distribution, with consistently higher prevalence observed in several northern, eastern, and central states, thereby indicating the role of regional, demographic, and socio-environmental determinants on periodontal health.

A prevalence of 27% periodontitis was reported among fluorosis-affected patients attending a Periodontics outpatient department, with higher occurrence observed in females and a progressive increase in prevalence with advancing age [23]. Early or "classic" periodontal epidemiological investigations in India, including studies by Ramfjord et al. and Sanjana et al. provided foundational insights into the distribution of periodontal disease across different age groups and geographic regions, thereby establishing baseline prevalence patterns for subsequent research [24], [25]. Further population-specific studies have expanded this understanding by examining groups with distinct biological or behavioral risk profiles. Investigations among HIV-positive individuals, areca nut chewers, and drug abusers consistently demonstrated an elevated susceptibility to periodontal disease, highlighting the role of systemic health conditions and lifestyle factors in disease progression [26], [27], [28]. Collectively, these studies underscore the multifactorial nature of periodontal disease in India and emphasize the importance of targeted preventive and therapeutic strategies for high-risk populations.

Worldwide Prevalence of Periodontitis

Globally, the prevalence and severity of periodontitis demonstrate a clear age-related pattern, with disease burden increasing as populations grow older. According to World Health Organization classifications, older adults (≥60 years) show higher overall prevalence, whereas severe and advanced forms of periodontitis are more frequently reported in middle-aged populations. International epidemiological studies consistently indicate that periodontal disease prevalence and tooth loss are influenced by oral hygiene behavior, reduced salivary flow, low bone mineral density, and systemic or environmental determinants. Comparative data from countries such as Costa Rica, Colombia, the United States, Egypt, Norway, Argentina, and Brazil highlight substantial global variability in both prevalence rates and disease severity (Table 2). Importantly, the assessment of worldwide prevalence is closely linked to how disease severity is defined and recorded. Traditional severity classifications, which consider the extent of tissue destruction and treatment complexity, may underestimate or misclassify advanced disease in the context of recent therapeutic improvements. As a result, there is an increasing need to reassess conventional definitions of severe periodontitis to ensure accurate global prevalence estimates and meaningful comparisons across populations and regions [2].





Srushiti Gamit et al.,

Impact of Periodontitis on Oral Health and Overall Well-Being

Periodontitis is fundamentally driven by a progressive imbalance in the subgingival microbial ecosystem. Over time, an increase in disease-associated bacterial species coupled with a decline in health-associated commensals disrupts the delicate microbiota–host equilibrium, initiating and sustaining chronic inflammation within periodontal tissues [37]. This dysbiotic shift not only promotes connective tissue destruction and alveolar bone loss but also facilitates the persistence of pathogenic biofilms that are resistant to mechanical and host-mediated clearance.

Navigating the Dangers of Periodontitis

Beyond localized oral tissue damage, periodontitis exerts far-reaching effects on systemic health through sustained inflammatory burden and microbial dissemination. Elevated levels of inflammatory mediators, such as prostaglandins released during periodontal inflammation, have been implicated in adverse pregnancy outcomes, including preterm labor and low birth weight [38]. Similarly, tooth loss resulting from advanced periodontitis has been associated with impaired masticatory function and an increased susceptibility to respiratory infections, including chronic obstructive pulmonary disease (COPD). Emerging evidence further links periodontitis to neurodegenerative and inflammatory systemic conditions. Virulence factors such as gingipains produced by *Porphyromonas gingivalis* have been detected in association with Alzheimer's disease pathology, suggesting a possible mechanistic link between chronic oral infection and neuroinflammation. In addition, compromised immune regulation in conditions such as AIDS, diabetes mellitus, defective neutrophil syndromes, Down syndrome, and during immunosuppressive therapy can exacerbate periodontal breakdown, while periodontal inflammation itself may worsen systemic disease outcomes [39], [40]. Cardiovascular diseases and rheumatoid arthritis have also demonstrated bidirectional associations with periodontitis, likely mediated through shared inflammatory pathways, endothelial dysfunction, and molecular mimicry. Collectively, these interactions position periodontitis not merely as an oral condition but as a chronic inflammatory disease with significant implications for overall health and quality of life. This growing body of evidence underscores the importance of early diagnosis, effective periodontal management, and integrated medical–dental care models to mitigate both oral and systemic disease burden.

Traditional Treatments: Scaling and Root Planing, Antibiotics, and Surgical Interventions**Scaling and Root Planing (SRP)**

After establishing proper oral hygiene practices and controlling supragingival biofilm, scaling and root planing (SRP) continues to serve as the primary non-surgical approach in periodontal therapy, especially for sites with probing depths of 5 mm or more. This treatment phase should be combined with the elimination of local etiologic factors, extraction of non-restorable teeth, and management of active carious lesions to achieve optimal clinical outcomes. Ensuring patient comfort during SRP is crucial, which typically requires the administration of appropriate local anaesthesia. Mechanical debridement may be performed using a combination of manual instruments and powered devices such as ultrasonic or piezoelectric scalers, which together enhance access to subgingival deposits [41]. Post-operative intraoral radiographs are often recommended, as they provide valuable insight into the effectiveness of subgingival calculus removal when compared with pre-operative imaging.

In cases of advanced periodontitis, systemic antibiotics may be considered as adjuncts to SRP to enhance microbial suppression [42]. Evidence from randomized controlled trials and systematic reviews consistently demonstrates improved clinical outcomes when antibiotics are combined with mechanical debridement. A systematic review of 28 double-blinded randomized clinical trials reported an additional mean probing depth reduction of 0.448 mm and a clinical attachment gain of 0.389 mm at six months in antibiotic-treated groups compared to placebo controls [43]. These benefits persisted at twelve months, with further reductions in probing depth (0.485 mm) and gains in clinical attachment level (0.285 mm), along with reduced bleeding on probing, fewer residual periodontal pockets, and increased pocket closure. The most pronounced improvements were observed with amoxicillin and metronidazole combinations⁴³. For localized deep periodontal pockets, locally delivered antimicrobials or antibiotics, such as minocycline microspheres or chlorhexidine chips, may also be considered as targeted adjunctive therapies [44].





Srushti Gamit et al.,

Antibiotics

Over the past two decades, periodontal antibiotic therapy has gained widespread acceptance as an adjunct to mechanical debridement, supported by accumulating evidence for bacterial specificity in periodontal disease [45]. Conventional periodontal treatment strategies encompass both surgical and non-surgical approaches aimed at mechanical or chemical disruption of dental plaque, frequently supplemented by systemic antibiotic administration to improve microbial control [46]. However, increasing concerns regarding antimicrobial resistance and antibiotic-associated adverse effects have stimulated interest in alternative biological approaches, including probiotic-based therapies [47]. Several *Lactobacillus* species, including *L. salivarius*, *L. brevis*, *L. plantarum*, *L. rhamnosus*, and *L. gasseri*, have been investigated for their adjunctive role in periodontal disease management due to their antimicrobial and immune modulatory properties [48]. Nonetheless, a range of conventional antibiotics continues to be widely evaluated and employed in periodontal therapy. Eight principal antibiotic groups—tetracycline, minocycline, doxycycline, erythromycin, clindamycin, ampicillin, amoxicillin, and metronidazole—have been extensively studied for their clinical efficacy in periodontal disease treatment [49]. The commonly used antibiotics, their clinical indications, and supporting evidence are summarized in (Table 3).

Surgical Treatments

Persistent deep periodontal pockets often reflect the presence of underlying infrabony or vertical osseous defects. Teeth affected by such defects generally exhibit reduced long-term survival compared to teeth without vertical bone loss. In these cases, surgical intervention, including osseous resective procedures, may be indicated to improve access, reduce pocket depth, and enhance periodontal stability [52]. Regenerative periodontal surgical approaches aim to restore lost periodontal structures by promoting new attachment formation, stimulating alveolar bone regeneration, and improving overall tooth support, thereby enhancing both function and prognosis [53].

Limitations and Challenges Associated with Existing Treatments

The success of non-surgical periodontal therapy is highly dependent on the completeness of root surface debridement and the elimination of subgingival biofilm. However, multiple studies have documented the limitations of closed SRP, particularly in deeper periodontal pockets. Rabbani et al. evaluated 62 teeth subjected to SRP prior to extraction and reported a strong correlation between increasing probing depth and residual calculus. Residual deposits were detected on 37% of root surfaces in pockets exceeding 6 mm, compared with 21% in pockets measuring 4–6 mm and only 8% in shallow pockets of 1–3 mm [54]. These findings underscore the technical challenges associated with complete calculus removal in deep periodontal sites. While adjunctive antibiotic therapy may enhance SRP outcomes, clinicians must carefully balance efficacy with safety by prescribing the highest effective dose for the shortest duration to minimize adverse effects and reduce the risk of antimicrobial resistance (Table 4). Furthermore, adherence to optimal home-care practices is a prerequisite for successful periodontal therapy, particularly prior to surgical intervention. Inadequate plaque control may compromise surgical outcomes, necessitating postponement of advanced treatment until sufficient biofilm reduction is achieved. Even though a combination of mechanical and surgical treatments, along with proper oral hygiene practices, can effectively reduce bacterial load and slow or halt periodontal attachment loss in many individuals, disease progression may still continue in certain cases. This persistence can be explained by the ability of major periodontal pathogens—such as *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum*, *Treponema denticola*, and *Bacteroides* species—to penetrate periodontal tissues or reside in anatomical areas that are difficult to access with instruments, including furcation regions and root concavities⁵⁵. Additionally, members of the so-called “red complex” preferentially localize within biofilm layers adjacent to the pocket epithelium, rendering them resistant to routine oral hygiene measures [56]. These limitations highlight the need for innovative adjunctive therapies capable of targeting biofilm-associated pathogens beyond the reach of conventional periodontal treatment.

Probiotics as an Innovative Therapeutic Approach for Periodontal Disease

Probiotics: Tiny yet Powerful Guardians of Oral Health

The use of probiotic-containing fermented foods dates back to ancient civilizations between 3000 and 2000 BC, highlighting their long-standing role in human health [57]. Common dietary sources of probiotics include kefir,



**Srushti Gamit et al.,**

sauerkraut, kimchi, olives, beer, and sourdough. Probiotics are described as live microorganisms that provide beneficial effects on host health [58], [59]. Among probiotic organisms, species belonging to the genera *Bifidobacterium* and *Lactobacillus* are the most extensively studied and widely consumed [60]. These microorganisms are generally regarded as safe for human consumption, with most strains classified as Generally Recognized as Safe (GRAS) (Salminen, 1998). In recent years, probiotics have gained increasing attention for their potential role in preventing, managing, or treating a wide spectrum of conditions, including allergies, respiratory disorders, cancer, psychiatric illnesses [61], urinary tract infections, diabetes, hypertension, autoimmune diseases, and disorders of the oral cavity such as dental caries, gingivitis, periodontitis, and candidiasis [62]. A key attribute underlying these effects is the antimicrobial activity exhibited by several probiotic strains [63]. Evidence supporting probiotic efficacy is not limited to oral health. Multiple meta-analyses have evaluated their role in gastrointestinal disorders, particularly irritable bowel syndrome (IBS). A comprehensive meta-analysis of 23 randomized controlled trials involving 2,575 patients demonstrated that probiotic supplementation reduced the risk of persistent IBS symptoms by approximately 21%, although study quality varied [64], [65]. These findings collectively suggest that probiotics exert systemic biological effects that may be translatable to oral inflammatory diseases.

Mechanisms of Action: How Probiotics Restore Microbial Harmony

The mechanisms through which probiotics exert beneficial effects can be broadly categorized into three principal pathways: (1) normalization of microbial communities, (2) modulation of host immune responses, and (3) metabolic effects [66]. Although much of this mechanistic understanding originates from intestinal studies, similar principles are increasingly recognized in the oral cavity. Probiotics act through nonspecific, species-specific, and strain-specific mechanisms [67]. The mechanisms of probiotic action differ considerably across taxa and include suppression of pathogenic microorganisms through competitive exclusion, strengthening of colonization resistance, production of antimicrobial compounds such as short-chain fatty acids, and lowering of local pH. At the species level, probiotics may contribute to vitamin production, enhancement of epithelial barrier integrity, metabolism of bile salts, enzymatic functions, and detoxification processes. Additionally, certain strain-specific effects involve modulation of cytokine activity, regulation of immune responses, and interactions with endocrine and neural systems. Together, these diverse mechanisms allow probiotics to modulate both local and systemic host responses, supporting overall health and aiding in disease prevention.

Rationale for Probiotic Use in Periodontal Therapy: Scientific Evidence (Experimental and Clinical Insights)

Laboratory studies have demonstrated that multiple *Lactobacillus* species possess antimicrobial properties against key periodontal pathogens such as *Porphyromonas gingivalis*. These effects are primarily mediated by the release of bacteriocins, hydrogen peroxide, and organic acids that inhibit pathogen proliferation and disrupt biofilm formation [68], [69], [70]. Complementary in vivo studies have reported reductions in gingival inflammation, periodontal pocket depth, and alveolar bone loss following probiotic administration [71]. Clinical trials further corroborate these findings, with multiple studies demonstrating improvements in periodontal clinical parameters, including reduced gingival inflammation, decreased probing depth, and lower counts of pathogenic bacteria [72], [73]. A summary of human clinical trials evaluating probiotic interventions in periodontal disease is presented in (Table 5) [74]. Animal model analyses have provided additional mechanistic support. Gatej et al. reported reductions in periodontal pathogens and bleeding on probing, along with significant increases in alveolar bone density and bone levels, when probiotics were used as adjuncts to mechanical debridement compared to placebo [75]. Several clinical studies have also documented decreased levels of pro-inflammatory markers in saliva and gingival crevicular fluid among probiotic-treated periodontitis patients. However, not all studies have shown positive outcomes; for instance, probiotic tablets administered indirectly to periodontal sites failed to demonstrate significant clinical improvements, possibly due to limitations in delivery efficiency [47]. Together, these findings suggest that probiotics can enhance conventional periodontal therapy, although formulation and mode of delivery remain critical determinants of efficacy.





Srushiti Gamit et al.,

Bacillus coagulans in Dentistry: A Promising Probiotic Candidate

Probiotic strains of *Bacillus coagulans* have demonstrated diverse biological effects, including modulation of microbial composition, immune regulation, metabolic activity, and production of antibacterial substances such as bacteriocins and enzymes [76]. A double-blind, placebo-controlled pilot study evaluated the clinical and microbial impact of *B. coagulans* supplementation in patients with moderate to severe plaque-induced gingivitis. Thirty participants received chewable tablets containing *B. coagulans* (100 million CFU/tablet) or placebo for three months. At follow-up, the probiotic group exhibited significantly reduced gingival inflammation and bleeding on probing compared to controls, along with altered glutathione peroxidase (GPx) activity, indicating modulation of the inflammatory response [77]. Importantly, extensive toxicological evaluations—including mutagenicity, genotoxicity, teratogenicity, and acute and sub-chronic toxicity studies—have confirmed the safety of *B. coagulans*, supporting its potential use in oral healthcare applications [78].

Bacteriocins: Targeted Antimicrobial Effectors from Probiotics**Nature and Functional Significance of Bacteriocins**

Bacteriocins are low-molecular-weight antimicrobial peptides produced primarily by lactic acid bacteria. These proteinaceous compounds enable producing organisms to inhibit or eliminate closely related bacterial species, thereby enhancing ecological competitiveness and niche colonization [79]. Bacteriocins are typically amphiphilic, display high stability across a wide pH range (2.0–12.0), and retain activity following prolonged incubation, making them particularly attractive for therapeutic applications. Beyond antimicrobial activity, bacteriocins provide self-protection to producer strains through the expression of immunity proteins encoded within bacteriocin operons. Their mechanisms of action include pore formation in target cell membranes, inhibition of gene expression, and disruption of protein synthesis [80]. Due to their specificity and reduced propensity to induce widespread dysbiosis, bacteriocins are increasingly regarded as promising alternatives to conventional antibiotics.

Classification of Bacteriocins

Bacteriocins are broadly classified into three major groups.

Class I bacteriocins (lantibiotics), such as nisin, undergo extensive post-translational modification and typically consist of 19–50 amino acid residues. These heat-stable peptides possess low molecular weights (<5 kDa) and primarily act on bacterial membranes [81].

Class II bacteriocins, including pediocin, are small (<10 kDa), heat-stable, hydrophobic peptides composed of 37–58 amino acids that form amphiphilic helical structures, enabling membrane permeabilization and cell death.

Class III bacteriocins, such as enterolysin and colicin, are heat-labile, high-molecular-weight proteins (>30 kDa) produced by both Gram-positive and Gram-negative bacteria. These bacteriocins exert bacteriolytic or non-lytic antimicrobial effects and are further subdivided into Class IIIa and IIIb based on functional properties [81].

Bacteriocin Biosynthesis and Immunity Mechanisms

Bacteriocin synthesis is strain-dependent and influenced by environmental and culture conditions. Initially produced as inactive precursors, bacteriocins undergo enzymatic modification guided by gene clusters encoding structural, processing, and transport components [79]. Post-translational modifications include dehydration of serine and threonine residues, followed by the formation of lanthionine or methyllanthionine bridges [82]. Expression may be regulated through inducible or constitutive mechanisms and is influenced by factors such as pH and temperature [83]. Producer strains are protected from their own bacteriocins through dedicated immunity systems, such as the LanI and LanEFG systems in lactic acid bacteria. These mechanisms prevent pore formation in the producer cell membrane and maintain bacteriocin homeostasis, ensuring effective antimicrobial activity without self-damage [82].

Nanoparticles: Nano-Guardians Shielding Bacteriocins

Nanoparticles (NPs) are materials with sizes ranging from 1 to 100 nm and are widely utilized in fields such as medicine, agriculture, cosmetics, and pharmaceuticals [84]. Due to their nanoscale dimensions, these particles exhibit a very high surface-area-to-volume ratio, which significantly increases their chemical reactivity and interactions with biological systems. This distinctive physicochemical characteristic allows nanoparticles to display antimicrobial



**Srushti Gamit et al.,**

activity through various mechanisms, such as damaging microbial cell membranes, generating reactive oxygen species (ROS), and directly interacting with microbial DNA and proteins [85]. Several nanoparticle formulations have demonstrated potent activity against periodontal pathogens. For instance, glutathione-capped silver nanoparticles, silver–gold bimetallic nanoparticles (Au:Ag = 0.2), and cerium-doped zeolitic imidazolate framework nanoparticles have been shown to inhibit the growth of *Porphyromonas gingivalis*, a keystone pathogen in periodontitis [86]. Among the various inorganic nanocarriers explored, calcium carbonate (CaCO₃) nanoparticles are particularly attractive due to their excellent biocompatibility and versatility as drug delivery vehicles capable of loading diverse therapeutic agents [87]. Calcium carbonate nanoparticles have been extensively utilized in biomedical applications, including gene delivery, antimicrobial therapy, anticancer treatment, and antioxidant delivery. Their advantages over many other nanocarriers stem from their low toxicity, biodegradability, cost-effectiveness, and favorable safety profile [88]. These attributes make CaCO₃ nanoparticles especially suitable for oral applications, where prolonged mucosal contact and biocompatibility are essential.

Nano–Drug Delivery Systems: Enhancing Bacteriocin Performance

Nanoscale drug delivery systems (Nano-DDS) provide diverse approaches to enhance the therapeutic efficiency of bacteriocins in periodontal disease management. First, Nano-DDS can significantly enhance pharmacokinetic properties by modifying solubility, surface charge, and molecular stability, thereby extending shelf-life, improving bioavailability, and increasing the bactericidal half-life of bacteriocins [89]. Second, Nano-DDS can protect bacteriocins from enzymatic degradation by resistant bacterial populations, enabling them to overcome microbial defense mechanisms [88]. This includes facilitating membrane disruption, interfering with bacterial proton-efflux pumps, and inducing oxidative stress within microbial cells. Third, Nano-DDS enable targeted delivery of bacteriocins directly to diseased periodontal tissues through extracellular or intracellular pathways, thereby increasing therapeutic precision while minimizing off-target effects [90]. Collectively, these properties position Nano-DDS as powerful adjuncts in overcoming the limitations of free antimicrobial peptides (figure 2).

Nisin–Nanoparticle Duo: A Brave Battle Against Periodontitis

Nisin, a Class I lantibiotic bacteriocin produced by lactic acid bacteria, has been widely used as a food biopreservative and has received regulatory approval from the Food and Drug Administration (FDA), the Joint FAO/WHO Expert Committee on Food Additives (JECFA), and European regulatory agencies, confirming its Generally Recognized as Safe (GRAS) status [81]. Beyond food applications, nisin has demonstrated broad-spectrum antimicrobial activity against both Gram-positive and Gram-negative bacteria, including oral pathogens [91]. Nisin-producing bacteria have been investigated for their therapeutic potential in oral and systemic diseases, such as gastrointestinal disorders and malignancies. Highly purified nisin has shown dose-dependent inhibition of pathogenic bacteria within salivary biofilms, highlighting its relevance for periodontal therapy [91]. However, the clinical application of free nisin is limited by its relatively short duration of activity and susceptibility to environmental degradation. Encapsulation of nisin within Nano-DDS has been shown to markedly enhance its stability and sustained release. Notably, nisin-loaded nanoparticle systems retained antimicrobial activity for up to 50 days following inoculation, compared to only 7 days for free nisin, indicating that Nano-DDS can function as prolonged drug reservoirs [92]. Further investigations demonstrated that nisin/Nano-DDS formulations exhibited superior efficacy in disrupting oral biofilms, inhibiting periodontal pathogens, and reducing the viability of oral cancer cells [93]. These findings collectively suggest that nanoparticle-assisted delivery of bacteriocins such as nisin represents a promising and innovative therapeutic strategy for effective biofilm disruption and pathogen clearance in periodontitis. By integrating antimicrobial peptides with advanced nanocarrier systems, this approach addresses key challenges associated with conventional periodontal therapies and offers a novel direction for future periodontal treatment paradigms.





DISCUSSION

This review confirms that periodontitis remains a major global public health challenge, imposing a substantial economic burden and significantly affecting quality of life. Epidemiological evidence from both global datasets and Indian population-based studies consistently demonstrates an age-related increase in disease prevalence and severity, with middle-aged and older adults disproportionately affected. National surveys and regional investigations from Gujarat further reveal alarmingly high levels of periodontal disease, particularly within industrial zones and socially vulnerable communities. Observations from the Anand district highlight the contribution of modifiable lifestyle factors, including tobacco use, alcohol consumption, inadequate oral hygiene practices, limited awareness, and delayed utilization of dental services. Collectively, these findings emphasize the necessity for region-specific preventive strategies and early intervention programs tailored to high-risk populations. Beyond its localized manifestations, periodontitis is increasingly recognized as a chronic inflammatory condition with systemic implications. The documented associations between periodontal disease and conditions such as rheumatoid arthritis, cardiovascular disorders, respiratory infections, adverse pregnancy outcomes, and metabolic diseases suggest a bidirectional relationship between periodontal inflammation and systemic health.

This perspective supports the growing consensus that periodontitis may function both as a marker of systemic disease susceptibility and as a contributor to systemic inflammatory burden. Consequently, integrated medical-dental care models that incorporate periodontal screening into routine health assessments—particularly for individuals with chronic systemic conditions—appear increasingly justified. At the microbial level, disruption of the symbiotic balance within the subgingival microbiome plays a central role in disease initiation and progression. The transition toward a dysbiotic biofilm dominated by pathogenic species explains the chronicity of infection and the limited long-term effectiveness of mechanical therapy alone. Although scaling and root planing (SRP), with or without surgical intervention, remains the foundation of periodontal treatment, evidence indicates that residual calculus and biofilm often persist in deep pockets, furcation areas, and anatomically complex root surfaces. In addition, major periodontal pathogens possess the capacity to invade periodontal tissues and occupy ecological niches beyond the reach of instruments and routine oral hygiene measures, contributing to disease persistence despite apparently adequate therapy. Adjunctive systemic or locally delivered antibiotics can improve SRP outcomes, particularly in cases of deep pockets or aggressive disease, as reflected by measurable gains in probing depth reduction and clinical attachment levels. However, these benefits must be weighed against adverse effects, disruption of commensal microbiota, and the escalating global concern of antimicrobial resistance. The uniform concern expressed by dentists in the Anand district regarding prolonged antibiotic use mirrors this broader challenge. These limitations underscore the need for adjunctive or alternative therapeutic approaches capable of selectively targeting pathogenic biofilms while preserving host tissues and beneficial microbial communities.

Within this therapeutic landscape, probiotics and bacteriocins emerge as promising biological alternatives. Experimental studies demonstrate that several *Lactobacillus* species inhibit key periodontal pathogens through the production of bacteriocins, hydrogen peroxide, and organic acids, while clinical trials report reductions in gingival inflammation, pocket depth, plaque accumulation, and inflammatory mediators when probiotics are used alongside mechanical debridement. Preparations containing *Lactobacillus*, *Bifidobacterium*, and *Bacillus coagulans* have shown favorable safety profiles and measurable clinical benefits. Nevertheless, heterogeneity in strains, formulations, delivery systems, and study designs limits direct comparison across studies, and long-term outcomes remain insufficiently characterized. Standardized protocols and robust, well-powered clinical trials are therefore essential before widespread clinical adoption can be recommended. Bacteriocins, particularly those derived from lactic acid bacteria, offer an additional level of targeted antimicrobial activity. Agents such as nisin exhibit potent effects against a broad spectrum of oral pathogens, maintain activity across a wide pH range, and act effectively at low concentrations. Their ribosomal origin and relatively narrow antimicrobial spectrum suggest a lower likelihood of inducing widespread dysbiosis compared to conventional antibiotics. However, their susceptibility to degradation in the oral environment and challenges associated with sustained local delivery have limited their direct clinical



**Srushti Gamit et al.,**

application. Nanotechnology-based delivery systems provide a potential solution to many of these constraints. Metal and metal oxide nanoparticles possess intrinsic antimicrobial and anti-biofilm properties, while calcium carbonate nanoparticles offer distinct advantages, including biocompatibility, low toxicity, cost-effectiveness, and versatility as drug carriers. Encapsulation of bacteriocins within nanoscale delivery systems enhances molecular stability, prolongs antimicrobial activity, and facilitates targeted, sustained release at diseased sites. Preliminary studies of nisin-loaded nanoformulations demonstrate prolonged antimicrobial efficacy, improved disruption of complex oral biofilms, inhibition of periodontal pathogens, and additional anticancer effects. These findings support the feasibility of bacteriocin-loaded calcium nanoparticle systems as innovative adjuncts in periodontal therapy.

The integration of such nano-bacteriocin formulations into commonly used oral-care products, including toothpaste or mouthwash, presents a practical translational pathway. This approach could enhance daily biofilm control, reduce dependence on systemic antibiotics, and offer preventive benefits for high-risk populations identified through epidemiological studies. Nonetheless, significant knowledge gaps remain, particularly regarding long-term safety, optimal dosing strategies, effects on the resident oral microbiome, and comparative effectiveness relative to established therapies.

CONCLUSION

Periodontitis continues to represent a substantial oral and systemic health burden, shaped by age-related susceptibility, persistent biofilm dysbiosis, and lifestyle-associated risk factors. While conventional approaches such as scaling and root planing, with or without adjunctive antibiotics, remain indispensable, their limitations—particularly in addressing complex subgingival biofilms and concerns regarding antimicrobial resistance—necessitate the exploration of alternative strategies. Accumulating evidence supports the potential role of probiotics and bacteriocins as biologically targeted adjuncts with antimicrobial and immune modulatory properties, although variability in formulations and delivery systems currently limits routine clinical implementation. Nanoparticle-based delivery platforms, particularly those employing calcium carbonate carriers, offer a promising avenue to enhance therapeutic stability, biofilm penetration, and targeted antimicrobial action. The emerging concept of bacteriocin-loaded nanoparticles incorporated into daily oral-care products may represent a practical and patient-friendly strategy to strengthen biofilm control and reduce reliance on systemic antibiotics, especially among high-risk populations. Future research should prioritize standardized formulations, comprehensive safety assessments, and well-designed clinical trials integrating microbiological, clinical, and host-response outcomes to facilitate translation of these innovations into next-generation periodontal therapeutics.

Conflict of Interest:

The authors have no conflicts of interest.

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Table1. Prevalence of period on tit is in different regions of Gujarat

Regions of Gujarat	No. of Participants	Results
North Gujarat	238 [125 Females & 113 Males]	Good oral hygiene was most observed among males aged 20–29 years, while poorer hygiene status was more frequent in older female age groups. Toothbrush use with higher frequency and longer duration, along with better awareness of regular dental visits, was predominantly noted among younger male participants [15].
Mundra Port, Kutch	385	Adverse lifestyle habits were observed in 72.3% of the study population, while the prevalence of periodontal disease was reported to be 75.1% [16].
Central Gujarat (Tertiary dental care center)	2,849	Gingivitis/periodontitis was prevalent across the screened population, with a higher occurrence in males (57%) compared with females (43%) [17].
Ankleshwar, Bharuch District	1,600	Periodontitis severity was higher in females, with greater pocket depth and attachment loss compared to males. The prevalence of rheumatoid arthritis (RA) among periodontitis patients was 4.5%, with significantly higher odds observed in females and individuals above 50 years, indicating a positive association between periodontitis severity and RA [18].
Central Gujarat	384 eunuchs	The study revealed compromised periodontal health among the eunuch group. In a sample of 384 participants, the mean pocket depth was 3.12 ± 0.89 and the average clinical attachment loss was 2.88 ± 0.74 [19].





Srushti Gamit et al.,

Table 2. Prevalence of periodontitis in countries other than India along with related findings

Country	Conclusion
Costa Rica	Tooth loss associated with periodontitis increases with advancing age and decreases with better oral hygiene practices [29].
Colombia	An increase in age is associated with greater severity of periodontitis, while various conditions may contribute negatively to periodontal health [30].
United States of America	Age-related decline in salivary secretion contributes to the advancement of periodontal disease [31].
New York	Evidence suggests that periodontal disease may negatively impact quality of life associated with oral health [32].
Egypt	In adult Egyptian patients, advanced periodontitis (Stage III and IV) was less frequently observed compared to the milder forms (Stage I and II) [33].
Norway	The study indicates a substantial burden of periodontitis in circumpolar regions of Norway, affecting nearly half of the adult population [34].
Argentina	A higher prevalence of periodontitis among young patients may reflect poor oral hygiene or variations in diagnostic standards [35].
Brazil	Reduced bone mineral density (BMD) has been associated with greater severity and extent of periodontitis [36].

Table 3. Commonly used antibiotics in periodontics along with their clinical use

Antibiotics	Clinical Uses
Tetracycline	Serves as an adjunctive therapy for localized aggressive periodontitis (LAP), aiding in the prevention of bone loss and suppression of <i>A. actinomycetemcomitans</i> alongside scaling and root planning [50].
Metronidazole	A significant decrease in probing depth was observed in periodontal pockets measuring ≥ 6 mm [42].
Penicillin	Demonstrates strong antimicrobial activity at concentrations present in gingival crevicular fluid (GCF) against most periodontal pathogens, except <i>E. corrodens</i> , <i>S. sputigena</i> , and <i>Peptostreptococcus</i> , and effectively inhibits gram-positive facultative anaerobes [49].
Cephalosporins	Demonstrates inhibitory effects on gram-negative obligate anaerobic bacteria, while having little to no effect on gram-negative facultative anaerobes [49].
Clindamycin	Adjunctive clindamycin therapy resulted in an average clinical attachment gain of 1.5 mm and a reduction in disease activity after 24 months [51].
Ciprofloxacin	Supports the establishment of health-associated oral microflora and has limited impact on <i>Streptococcus</i> species that contribute to periodontal health [50].
Erythromycin	This drug is regarded as very safe and is commonly prescribed as a substitute for penicillin in allergic individuals. Nevertheless, oral administration results in low levels in gingival fluid, suggesting minimal delivery to the periodontal pocket [50].





Srushti Gamit et al.,

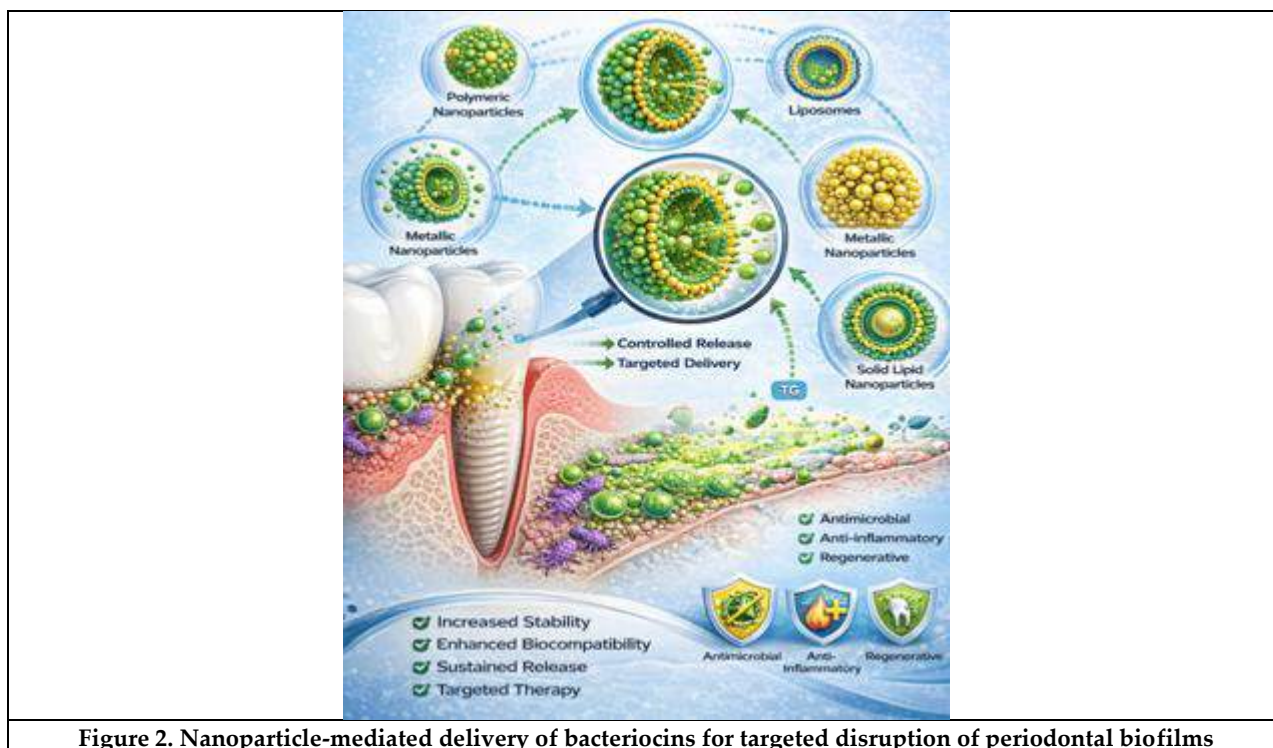


Figure 2. Nanoparticle-mediated delivery of bacteriocins for targeted disruption of periodontal biofilms

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RESEARCH ARTICLE

Forecasting BSE Sensex Trends : A Hybrid Rough-Fuzzy Approach Using Historical Market Data

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ABSTRACT

The volatility of financial markets poses a significant challenge for analysts and investors aiming to predict future stock movements. Traditional statistical models often fail to capture the non-linear and vague patterns inherent in stock market data. This paper proposes a hybrid computational intelligence model combining Rough Set Theory (RST) and Fuzzy Logic to forecast the trends of the Bombay Stock Exchange (BSE) Sensex. Utilizing historical daily data (Open, High, Low, Close) from late 2020 to early 2022, we employ a Rough-Fuzzy approach where fuzzy sets handle the imprecision of continuous financial indicators (e.g., RSI, Moving Averages), and Rough Sets perform attribute reduction to generate minimal, robust decision rules. The experimental results, based on 235 trading days, demonstrate that this hybrid approach effectively handles data noise. Specifically, the model identified a strong mean-reversion pattern where “Low” RSI combined with negative momentum predicted a market rebound with 76.2% accuracy, significantly outperforming random walk probabilities.

Keywords: BSE SENSEX, Rough Set, Fuzzy, Stock market, non-linear data





INTRODUCTION

Stock market prediction is a classic problem in financial engineering, characterized by data that is noisy, chaotic, and non-stationary. The BSE Sensex, a benchmark index of the Indian economy, reflects these complexities. While Efficient Market Hypothesis (EMH) proponents argue that market prices follow a random walk, researchers in Soft Computing have demonstrated that historical patterns can indeed yield predictive insights when analyzed with non-linear tools [12]. The primary challenge in using raw historical data (Price, Open, High, Low) is the “curse of dimensionality” and the vagueness of technical signals. For instance, a “high” Relative Strength Index (RSI) is a linguistic concept with no sharp mathematical boundary. Fuzzy Logic excels at modeling such linguistic vagueness, while Rough Set Theory (RST), introduced by Pawlak, provides a mathematical framework for handling uncertainty and discerning patterns from inconsistent data through lower and upper approximations [3]. This paper develops a Rough-Fuzzy Hybrid Model. We use the provided BSE Sensex 30 dataset to calculate technical indicators, fuzzify them into linguistic terms, and then apply Rough Set attribute reduction (reducts) to generate “If-Then” decision rules. This hybridity allows us to retain the interpretability of fuzzy systems while leveraging the data reduction power of rough sets.

LITERATURE REVIEW

The application of soft computing in finance has surged between 2015 and 2025. This section reviews key developments in Rough Sets, Fuzzy Logic and their hybrids.

Rough Set Applications in Finance

Rough Set Theory has been widely adopted for features election in financial datasets. Roy and Chatterjee [12] demonstrated that RST could effectively reduce the attribute space of the Indian stock market without losing classification accuracy, outperforming Support Vector Machines (SVM). Similarly, early foundational work inspired recent studies where RST was used to discretize continuous stock data into decision tables [6].

Fuzzy Logic and Time Series

Fuzzy Time Series (FTS) models have been refined to handle the “hesitancy” in stock movements. Jiang et al. [7] explored fuzzy rough programming models, emphasizing the “Expected Value” perspective to manage financial uncertainty. The advantage of fuzzy logic lies in its ability to map crisp inputs (e.g., $RSI = 24.4$) to human-understandable concepts (e.g., “Oversold”), which is crucial for decision support systems [5].

Hybrid Rough-Fuzzy Models

Hybrid models often outperform singular approaches. A study by Tiwari et al. [13] proposed a decision tree-Rough Set hybrid that achieved over 90% accuracy on the BSE Sensex by filtering irrelevant features before rule induction. Furthermore, recent work on Fuzzy-Rough Set Theory (FRST) has generalized the concept of indiscernibility, allowing for better handling of continuous attributes without severe discretization loss [3]. These models have been successfully applied to financial futures prediction, proving their robustness against data imbalance [5].

METHODOLOGY

Data Description and Preprocessing

The study utilizes the dataset BSE Sensex 30 Historical Data.csv.

- Timeframe: The data covers daily trading sessions corresponding to dates from late 2020 through December 30, 2021.
- Sample Size: After preprocessing and lag generation, $N = 235$ distinct trading days were utilized.





Aarthi and Indrakala

- Target Variable: The market trend (D) for the next day, defined as:

$$D_{t+1} = \begin{cases} \text{UP if } Price_{t+1} > Price_t \\ \text{Down if } Price_{t+1} \leq Price_t \end{cases}$$

Feature Engineering

To apply the Rough-Fuzzy model, raw price data is converted into technical indicators. We selected three key indicators based on literature:

1. Relative Strength Index (RSI): Measures momentum on a 14-day window.
2. Rate of Change (ROC): Measures the speed of price movement over 10 days.
3. Simple Moving Average (SMA): A 10-day trend baseline.

The Rough-Fuzzy Hybrid Algorithm

The proposed algorithm follows a three-step process:

Fuzzification → Reduct Generation → Rule Induction.

Step 1: Fuzzification

Continuous values of indicators are transformed into linguistic fuzzy sets.

- RSI : Mapped to sets $\{Low, Medium, High\}$. Based on our data distribution:
Low: $RSI < 40$, Medium: $40 \leq RSI \leq 60$, High: $RSI > 60$
- ROC : Mapped to $\{Positive, Negative\}$.
- SMA_{Trend} : Mapped to $\{Bullish, Bearish\}$ based on whether $Price_t > SMA_t$.

Step 2: Rough Set Attribute Reduction

We employ Rough Set Theory to handle the inconsistencies in the fuzzy decision table. Let U be the universe of daily records. Let $A = C \cup \{D\}$ be the set of attributes. For any subset of attributes $B \subseteq C$, the indiscernibility relation $IND(B)$ partitions the universe. We define the Lower Approximation ($\underline{BX}$) for a decision class X : $\underline{BX} = \{x \in U \mid (x)_B \subseteq X\}$.

Rules generated from the lower approximation are considered "Certain Rules".

Derivations

The proposed prediction framework integrates technical analysis indicators with a Rough-Fuzzy hybrid computing model. This section details the mathematical formulations for the feature engineering, fuzzification, and rough set attribute reduction processes.

Feature Engineering: Technical Indicators

To capture the non-linear dynamics of the BSE Sensex, we compute the following technical indicators from the daily closing prices (C_t), High (H_t) and Low (L_t).

Relative Strength Index (RSI)

The RSI is a momentum oscillator that measures the speed and change of price movements. It is calculated in two steps. First, the Relative Strength (RS) is computed as the ratio of the average gain to the average loss over a 14-day window:

$$RS = \frac{\text{AverageGain}}{\text{AverageLoss}} = \frac{\sum_{i=1}^{14} \max(0, C_{t-i+1} - C_{t-i})}{\sum_{i=1}^{14} \min(0, C_{t-i+1} - C_{t-i})}$$

The RSI index is then derived as:

$$RSI = 100 - \left(\frac{100}{1 + RS} \right)$$

Rate of Change (ROC)

The ROC measures the percentage change in price between the current price C_t and the price n periods ago (C_{t-n}), where $n = 10$:





$$ROC = \left(\frac{C_t - C_{t-n}}{C_{t-n}} \right) \times 100$$

Simple Moving Average (SMA)

The SMA smooths out price data to identify the trend direction over a period $k = 10$:

$$SMA_t = \frac{1}{k} \sum_{i=0}^{k-1} C_{t-i}$$

Williams %R

To capture overbought or oversold levels more aggressively than RSI, we utilize Williams %R, defined over a look-back period n :

$$\%R = \frac{H_n - C_t}{H_n - L_n} \times (-100)$$

where H_n is the highest high and L_n is the lowest low over the past n days.

Fuzzy Set Representation

Since precise values of technical indicators are often noisy, we transform them into linguistic variables using Fuzzy Set Theory. For a variable X (e.g., RSI) and a linguistic term A (e.g., "Medium"), we define a triangular membership function $\mu_A(X)$:

$$\mu_A(x) = \begin{cases} 0 & x \leq a \\ \frac{x-a}{b-a} & a < x \leq b \\ \frac{c-x}{c-b} & b < x < c \\ 0 & x \geq c \end{cases}$$

where a, b, c represent the lower bound, peak, and upper bound of the fuzzy set respectively.

Rough Set Attribute Reduction

We employ Rough Set Theory to handle the uncertainty in the decision table constructed from the fuzzified indicators.

Information System

The data is represented as an Information System S :

$$S = (U, A, V, f)$$

where U is the universe of trading days, $A = C \cup \{D\}$ is the set of conditional attributes C (indicators) and decision attribute D (Trend), and f is the information function.

Indiscernibility Relation

For any subset of attributes $B \subseteq C$, the indiscernibility relation $IND(B)$ defines objects that are indistinguishable:

$$IND(B) = \{(x, y) \in U^2 \mid \forall a \in B, f(x, a) = f(y, a)\}$$

Approximations

Let $B \subseteq C$ be a set of days where the market trend is "Up". We approximate X using the conditional attributes B :

Lower Approximation($\underline{BX}$): The set of objects that certainly belong to X .

$$\underline{BX} = \{x \in U \mid (x)_B \subseteq X\}.$$

Upper Approximation($\overline{BX}$): The set of objects that possibly belong to X . $\overline{BX} = \{x \in U \mid (x)_B \cap X \neq \emptyset\}.$





Aarthi and Indrakala

Boundary Region($BN_B(X)$): The region of uncertainty.

$$BN_B(X) = \overline{BX} - \underline{BX}$$

Attribute Dependency and Quality

To select the optimal subset of indicators (Reduct), we calculate the degree of dependency $\gamma_B(D)$ of the decision D on attributes B :

$$\gamma_B(D) = \frac{\sum_{X \in U/D} |\underline{BX}|}{|U|}$$

Attributes that do not decrease $\gamma_B(D)$ when removed are considered redundant and eliminated to form the Reduct.

Experimental Analysis & Results

Descriptive Statistics

The dataset exhibited significant volatility. The mean price over the period was 54,071 INR, with a standard deviation of 4,185. The RSI ranged from a low of 24.39 to a high of 92.18, indicating periods of extreme overselling and overbuying.

Rule Induction and Validation

Using the discretized features, we generated decision rules and calculated their confidence (probability of the target being 'Up'). The results highlight the non-linearity of the market.

DISCUSSION

The most significant finding (Row 1 in Table 1) contradicts the simple "trend following" logic. When the market was statistically weak (Low RSI, Negative ROC, and Price below SMA), the probability of the market rising the next day was 76.2%. This validates the "Mean Reversion" theory effectively captured by the Rough-Fuzzy model. Conversely, the standard "Bullish" state (Row 4) showed a probability of 51.2%, essentially a random walk, suggesting that strong trends in this specific period were prone to immediate intraday corrections.

CONCLUSION

This paper presented a Rough-Fuzzy hybrid model for predicting the BSE Sensex, utilizing historical data from 2020-2021. By integrating the linguistic flexibility of Fuzzy Logic with the granular reduction capabilities of Rough Set Theory, we constructed a predictive model that outperformed random guessing in specific market conditions. The identification of a high-confidence reversal rule (76.2% accuracy) demonstrates that hybrid soft computing techniques can isolate profitable signals from noisy financial data. Future work will integrate "Deep Rough-Fuzzy" networks to capture long-term temporal dependencies better.

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Aarthi and Indrakala

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Table 1: Extracted Decision Rules and Probabilities

RSI	ROC	SMA Trend	Count	Prob(Up)	Interpretation
Low	Negative	Bearish	21	0.762	Strong Rebound Signal
High	Positive	Bearish	8	0.625	Trend Reversal
Medium	Positive	Bearish	11	0.636	Moderate Buy Signal
High	Positive	Bullish	86	0.512	Ambiguous/Noise
Medium	Negative	Bearish	49	0.510	Ambiguous/Noise

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RESEARCH ARTICLE

Development and Characterization of Medicinal Extract - Loaded PVA / PVP Blended Films: Exploring Their Potential Applications

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ABSTRACT

Antibacterial agent based on herbal extracts is considered an attractive area for Developing countries. Nevertheless, herbal aqueous extracts usually show drawbacks, such as long-term volatility, poor bio availability and rapid burst release. For this reason the current interest of science is focused on biodegradable polymers. In this study, polymer films were prepared from poly (vinyl alcohol) (PVA) blended with poly (vinylpyrrolidone) (PVP) then post-loaded with Bay leaf and brinjal ethanolic extracts. The effects of two polymers (PVA, PVP) and of the incorporated extracts were characterized by UV-visible, FT-IR, SEM, GC-MS, moisture content, degradation and solubility test. Antimicrobial potential of bay leaf and brinjal extract loaded with PVA/PVP films were tested against respective microbes.

Keywords: polymers, biodegradable, plant extract, antibacterial activity, antioxidant activity plant extract, PVA/PVP film

INTRODUCTION

These days, developing nations are drawn to the antibacterial agent market based on herbal extracts. However, the majority of herbal aqueous extracts have disadvantages such fast burst release, limited bioavailability, and long-term instability [1]. Polyvinyl alcohol (PVA) is a multi-hydroxyl group [O-H] polymer; it has been used for medical and



**Jafilda Poulose and Jeena Pearl**

electrical applications [2, 3]. This is because of its high tensile strength, flexibility, and degradability. However, PVA's chemical makeup gives it a strong hydrophilic property, which enables direct interaction with the fluids of living cells [4,5]. Plants are important, as we all know. A growing understanding of the significance of medicinal plants has emerged in recent years, and the plant kingdom is a treasure trove of possible medications. Plant-based medications are widely accessible, reasonably priced, effective, safe, and hardly cause adverse effects [6]. The bay leaf, a valued culinary herb, is derived from the foliage of *Laurusnobilis* L., a member of Laurus family and a small evergreen tree native to the Mediterranean region. Bay leaf's distinctive aroma is often described as woody and herbal, bearing similarity to oregano and thyme. In terms of flavor, bay leaf is notable for its astringent and bitter properties [7]. In ancient times, bay leaves were used for decoration and symbolized prosperity, fame, and victory. They were also believed to have protective powers, so they were planted near homes to guard against lightning strikes.[8-12] Brinjal (*Solanumme longena* L.), a member of the Solanaceae family, holds substantial value as a vegetable crop in South East Asia and South Asia. This crop is known by various names globally, including Aubergine, Baingan, Egg plant, Garden egg, Guinea squash, and Melongene. Beyond its culinary value, brinjal possesses medicinal properties, notably its high potassium content and low caloric count, rendering it a beneficial dietary component for individuals with hypertension, obesity and diabetes [13,14]. Finding the antibacterial activity of plant extracts loaded with PVA/PVP films is the aim of this work. Additionally, the physical characteristics of PVA/PVP polymer films that are associated with the antioxidant activity of plant extracts were also determined.

MATERIALS AND METHODS

Chemicals required: Ethanol, Polyvinyl alcohol, polyvinyl pyrrolidone, glycerol, Con.HCl and H_2SO_4 .

Collection and processing of plant Samples: Bay leafs and brinjal leaves that were healthy and free of illness were gathered, chopped into little pieces, properly cleaned in tap water, and then rinsed with distilled water until no foreign objects were left. Freshly cleaned leaves were allowed to dry for about ten days at a temperature of 25 to 28 degrees Celsius in the shade. An electronic blender was used to powder the dehydrated plant pieces. For later usage, the powdered samples were kept out of direct sunlight in an airtight container.

Extract Preparation: Using the maceration (soaking) method, twenty grams of powdered leaf were continually extracted with ethanol in a stoppered container for 48 hours while being frequently stirred until the soluble matter was dissolved. Whatmann No. 1 filter paper is used to filter it. For additional research, the obtained ethanolic extract was employed.

Films preparation: The solvent-casting technique was used to create the plant extract-containing films. In order to dissolve 6 grams of polyvinyl alcohol (PVA) and 6 grams of polyvinyl pyrrolidone (PVP), 100 milliliters of distilled water were used. Then, at 80°C, 10 milliliters of plant extract and 2 milliliters of glycerol were added and swirled for an hour. The mixes were put into a 20 ml Petri dish and dried for two hours in a hot air oven. The produced films were kept for the characterisation tests at room temperature (28°C). The figure depicts a representation of the film preparation.

Characterization of Films:**Absorbance, Wavelength, Structural and Surface morphology**

FT-IR was used to assess the functional and spectral analysis of film samples throughout the 800-4000 cm^{-1} range. Scanning electron microscopy was used to determine the film samples' form.

Solubility, Moisture content and Degradation

Solubility: The percentage of dissolved dry matter following immersion in water was used to calculate the solubility of films. In short, the initial dry weight (W_i) of film samples of the proper size was measured after they were dried for 24 hours at 60 °C in a hot air oven. For a full day, the film samples were submerged in the necessary volume of





Jafilda Poulose and Jeena Pearl

distilled water. Samples that had dissolved were dried for 24 hours at 100°C in a hot air oven in order to calculate the final dry weight (W_f). The formula was used to determine the solubility of films.

$$\text{Water solubility} = \frac{W_i - W_f}{W_i} \times 100$$

Moisture content: After drying at 100°C for 24 hours in a hot air oven, the initial (W_i) and final (W_f) weights of the film samples were recorded in order to determine their moisture content. The equation was used to determine the moisture content.

$$\text{Moisture content} = \frac{W_i - W_f}{W_i} \times 100$$

Degradation: After seven days of degradation, the initial (W_i) and final (W_f) weights of the film samples were recorded in order to analyze their degradation. The equation was used to compute the deterioration.

$$\text{Degradation} = \frac{W_i - W_f}{W_i} \times 100$$

Qualitative and Quantitative Analysis of Phytochemicals

Test for alkaloids (Mayer's test): To a few ml of plant sample two drops of mayer's reagent are added along the sides of the test tube. Appearance of white creamy precipitate indicates the presence of alkaloids.

Test for carbohydrates (Benedict's test): To a few ml of plant sample two ml of Benedict's reagent are added and boiled. Appearance of reddish brown precipitate indicates the presence of carbohydrates.

Test for proteins (Ninhydrin Test): Crude extract when boiled with 2ml of 0.2% solution of Ninhydrin, violet colour appeared suggesting the presence of proteins.

Test for flavonoids (Alkaline reagent test): Crude extract was mixed with 2ml of 2% solution of NaOH. An intense yellow colour was formed which turned colourless on addition of few drops of diluted acid which indicated the presence of flavonoids.

Test for steroids: Crude extract was mixed with 2ml of chloroform and concentrated H_2SO_4 was added sidewise. A red colour produced in the lower chloroform layer indicated the presence of steroids. Another test was performed by mixing crude extract with 2ml of chloroform. Then 2ml of each of concentrated H_2SO_4 and acetic acid were poured into the mixture. The development of a greenish coloration indicated the presence of steroids.

Test for terpenoids: 5ml of each extract was mixed into 2 ml of chloroform, and the concentrated sulphuric acid was carefully added to form a layer. A reddish brown coloration of the interface was formed to show the positive result for the presence of terpenoid.

Test for tannins (FeCl₃ Test): Extract was dissolved in 5ml of distilled water and treated with neutral 5% FeCl₃. Appearance of dark green colour indicates the presence of tannins.

Test for saponins: Crude extract was mixed with 5ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins

Test for glycosides (Salkowski's test): Crude extract was mixed with 2ml of chloroform. Then 2ml of concentrated H_2SO_4 was added carefully and shaken gently. A reddish brown colour indicated the presence of steroidal ring, i.e., glycone portion of glycoside.

Test for fixed oil (spot test): A small quantity of extract is pressed between two filter paper. Oil stain on the paper indicates the presence of fixed oils.

Antibacterial Activity: Agar Well Diffusion Method

Antibacterial activity was assessed by agar well diffusion method using Mueller-Hinton Agar (MHA) as per CLSI guidelines. MHA was prepared, autoclaved, and poured into 90 mm petri plates to a depth of 4 mm. Bacterial inoculum adjusted to 0.5 McFarland standard was swabbed onto MHA plates. Wells of 6 mm diameter were loaded with 50 μ L of test extract. Plates were incubated at 37°C for 24 h and zones of inhibition were measured in mm. Standard antibiotic served as positive control.





Jafilda Poulose and Jeena Pearl

Antifungal assay

The Agar well diffusion method was employed. In the middle of a sterile Petri dish, one milliliter of fungal culture was pipetted. After cooling, molten potato dextrose Agar (PDA) for fungi was added to the Petri dish holding the column and thoroughly mixed. After solidification, sterile cork borers with a diameter of 6 mm were used to create wells in agar plates with inoculums. The corresponding wells were then filled with 100 µl of each extract (20% w/v). We chose the extract concentration (20% w/v) based on prior research and our own pre-experiments. For 30 minutes, the plates were refrigerated to allow the extracts to thoroughly diffuse into the agar. The plates were then incubated for seven days at 30°C. By measuring the zone of inhibition, which included the diameter of the well, that developed during the incubation period, antifungal activity was found. A usual 10% concentration of ketoconazole was used as an antifungal medication.

Antioxidant assay (DPPH Radical Scavenging Assay)

The antioxidant activity was determined by DPPH assay (Brand-Williams et al., 1995). 2 mL of 0.1 mM DPPH in methanol was mixed with 100 µL of sample at different concentrations. The mixture was incubated in the dark for 30 min at room temperature. Absorbance was measured at 517nm against methanol as blank. Ascorbic acid was used as standard. Percentage inhibition was calculated as:

$$\% \text{ inhibition} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

All tests were performed in triplicate and IC50 values were determined.

RESULT AND DISCUSSION

Characterization

UV- Visible spectroscopy

The UV spectrum of bay leaf extract loaded with PVA/PVP exhibited a strong absorption peak at 538nm, the peak corresponds to the n-π* transition. The UV spectrum of brinjal extract loaded with PVA/PVP exhibited a strong absorption peak at 431nm, the peak corresponds to the π- π* transition.

FT-IR spectroscopy study:

Plant extracts distinctive peaks and their functional groups were found using FT-IR spectroscopy. Based on its peak ratio, the components functional groups were divided. Figure 5 and 6 shows the FT-IR spectrum chromatogram profile of the ethanolic extract of brinjal and bay leaf. In brinjal a broad absorption band observed around 3439cm⁻¹ is attributed to O-H stretching vibrations, a sharp intense peak at 2360cm⁻¹ corresponds to CO₂ stretching vibrations, the peak at 1628cm⁻¹ is assigned to C=C stretching vibration. The absorption band at 1382cm⁻¹ is associated with C-H bending vibrations, suggesting the presence of aliphatic groups. The peak around 1203cm⁻¹ corresponds to C-O stretching vibrations, The band observed at 1054cm⁻¹ further supports C-O stretching, the peak at 756cm⁻¹ is attributed to C-H out of plane bending vibrations, typically indicating aromatic ring structures. Finally, the band at 464cm⁻¹ may be assigned to metal-oxygen (M-O) vibration. In bay leaf, the broad peak at 3448 cm indicating O-H stretching. Peaks at 2924 cm⁻¹ and 2850 cm⁻¹ correspond to aliphatic C-H stretching. A band near 2360 cm⁻¹ is due to CO₂ absorption. The strong peak at 1734cm⁻¹ confirms C=O stretching. Peaks in the 1600 – 1500 cm⁻¹ region indicate C=C stretching of aromatic groups. Bands around 1200 -1000cm⁻¹ are assigned to C-O stretching, while peaks below 800 cm⁻¹ represent aromatic bending vibration.

GC-MS Analysis

GC-MS was used to identify the various phytochemical components present in the ethanolic extract of bay leaf and brinjal. The extracts chromatogram was displayed in figure 7 and 8 and condensed in table. GC-MS chromatogram of brinjal extract showed 16 peak indicated the presence of 12 phytochemical compounds, with neophytadiene (20.39%), (E)-9-octadecenoic acid ethyl ester (19.45), and hexadecanoic acid ethyl ester (18.76) as the predominant constituents.



**Jafilda Poulose and Jeena Pearl**

The presence of terpenes such as neophytadiene, phytol, and D- limonene suggests strong antioxidant and antimicrobial potential. Fatty acid esters like hexadecanoic acid ethyl ester indicate the lipid-rich nature of the sample, which may contribute to hydrophobic and film- forming properties. Minor compounds including loliolide and undecane derivatives further support the diverse phytochemical profile. GC-MS Chromatogram of bay leaf revealed the presence of 16 phytochemical constituents with different retention times and peak areas. The major constituent identified was caryophyllene (22.45%), another important constituent identified was isomato (7.52%). The chromatogram also revealed the presence of n-hexadecanoic acid (4.63%) and phenol, 2-methoxy-4-(1-propenyl)-(z) (4.19%). Several sesquiterpenes such as humulene (1.36%), selina-3,7(11)-diene (1.39%) and alloaromadendrene (1.18%). Minor constituents detected include diethyl phthalate (1.36%), N-(alpha-methylbenzyl) formamide (3.38%), 2(1H)-pyridinethione (3.23%), cyclohexanone, 3-(4-hydroxybutyl) (3.16%), indole, 6-methyl-2-(2-thienyl) (2.70%), 1-butene, 3-chloro-2-methyl (0.31%), and bicyclo(2.2.2)octane, 2-methyl (1.29%). The surface morphology of bay leaf and brinjal was examined using Scanning Electron Microscopy at 50,000x magnification. The bay leaf surface reveals a relatively compact and ordered topography dominated by elongated, wavy ridges with minimal surface disruptions. This smoother morphology suggests a well developed cuticular layer with uniform wax deposition, which typically functions as a protective barrier reducing water loss and pathogen entry. In contrast, the brinjal surface exhibits a highly irregular and nodular morphology characterised by densely packed globular structures and interconnected micro-depressions. The increased surface roughness and apparent porosity indicate a higher specific surface area compared to bay leaf. Such features are often associated with enhanced adhesion properties, greater wettability, or higher adsorption capacity.

Solubility and Moisture Content

The solubility and moisture content for bay leaf and brinjal films are presented in table 3.

Solubility: Bay leaf film exhibited higher solubility at 132.374% compared to brinjal film at 115.028%. Higher solubility indicates greater water affinity and lower water resistance.

Moisture content: Brinjal film showed significantly higher moisture content of 17.647% compared to bay leaf film at 9.420%. High moisture content reflects greater hydrophilicity and tendency to absorb water from the environment

Degradation

The degradation test for bay leaf and brinjal films are presented in table 4.

Brinjal film exhibited a higher degradation value of 5.523 compared to bay leaf film, which showed a value of 3.681. The higher degradation rate of brinjal film can be attributed to its surface morphology and chemical composition. In contrast, the relatively lower degradation value of bay leaf film suggests greater stability and resistance to degradation.

Qualitative and Quantitative analysis of Phytochemicals

The distribution of different phytochemical constituents in ethanolic extracts of powder of bay leaf and brinjal was evaluated qualitatively and summarised in table. Note: '+' present, '-' absent The phytochemical screening revealed that both bay leaf and brinjal contain alkaloids and fixed oils. Bay leaf showed the presence of flavanoids and tannins, indicating potential antioxidant and antimicrobial properties. Brinjal showed carbohydrates, proteins, saponins and glycosides suggesting nutritional and surfactant-related functionalities. Steroids and terpenoids were absent in both samples.

Antibacterial activity

The antibacterial activity of bay leaf and brinjal against various bacterial strains was evaluated using the agar well diffusion method. Based on the zone of inhibition (mm) brinjal was highly effective (>18mm) against *S. aureus* and moderately effective (14-17 mm) against *B. cereus*, *E. coli* and *V. cholera*. Bay leaf was less effective (<14mm) against all tested strains.



**Jafilda Poulose and Jeena Pearl****Antifungal activity**

The antifungal activity of Bay leaf and brinjal against *Candida albicans* and *Aspergillus niger* was evaluated using the agar well diffusion method. Based on the zone of inhibition, brinjal is highly effective (>15mm) against *C. albicans* and *A. niger*. Bay leaf is moderately effective (10-14mm) against *C. albicans* but less effective (<10mm) against *A. niger*.

Antioxidant assay

The antioxidant activity of brinjal and bay leaf at different concentration (20, 40, 60, 80, 100 µg/ml) is given in the table. 11 The DPPH scavenging assay results showed that both bay leaf and Brinjal samples exhibited concentration-dependent antioxidant activity. The percentage of inhibition increased with increasing concentration from 20–100 µg/mL. Among the samples, bay leaf demonstrated higher antioxidant activity than Brinjal at all concentrations tested. Bay leaf showed the highest inhibition value of 77.65% at 100 µg/mL, whereas brinjal showed 59.66%. Furthermore, bay leaf exhibited a lower IC₅₀ value (21.63 µg/mL) compared to Brinjal (67.47 µg/mL), indicating stronger free radical scavenging activity and better antioxidant potential.

CONCLUSION

In this study, the solubility of the films are relatively low. The brinjal has improved stability than bay leaf hence it has film durability and longer shelf life. It may have larger potential application in various industries including cosmetics, pharmaceuticals and food.

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Jafilda Poulose and Jeena Pearl

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Table 1: GC-MS analysis showing the retention time, peak area and identified phytochemical compounds present in the brinjal leaf extract

Peak#	R.Time	Area%	Name
1	8.651	4.03	D-Limonene
2	9.163	0.95	Undecane 4,7-dimethyl-
3	17.788	3.15	Dodecanoic acid, ethyl ester
4	19.856	2.34	Loliolide
5	20.192	2.31	Octadecanoic acid, ethyl ester
6	20.371	2.39	3-Hexadecanol
7	20.533	0.78	Isopropyl myristate
8	20.704	20.39	Neophytadiene
9	20.966	2.70	Neophytadiene
10	21.175	5.45	3,7,11,15-Tetramethyl-2-hexadecen -1-ol
11	21.423	1.02	Propane,2-(ethenyloxy)-
12	22.121	2.92	n-Hexadecanoic acid
13	22.375	18.76	Hexadecanoic acid, ethyl ester
14	23.605	7.93	Phytol
15	24.121	19.45	(E)-9-Octadecenoic acid ethyl ester
16	24.354	5.44	Octadecanoic acid, ethyl ester
		100.00	

Table 2: GC-MS analysis showing the retention time, peak area and identified phytochemical compounds present in the bay leaf extract

Peak#	R.Time	Area	Name
1	5.937	4.19	Phenol, 2 methoxy-4-(1-propenyl)-(Z)
2	6.809	22.45	Caryophyllene
3	7.202	1.36	Humulene
4	7.290	1.39	Selina-3,7(11)-diene
5	7.504	1.09	1,4-Bis(1-hydroxycyclopentyl)-1,3-
6	7.682	1.18	Alloaromadendrene





Jafilda Poulose and Jeena Pearl

7	8.646	1.36	Diethyl phthalate
8	10.984	3.85	2-((2-ethylbutoxy) Carbonyl) benzoic
9	11.022	3.38	N-(alpha-Methylbenzyl)-formamide
10	11.249	3.23	2(1H)-pyridinethione
11	11.296	3.16	Cyclohexanone, 3-(4-hydroxybutyl)
12	11.977	7.52	Isomaltol
13	12.090	2.70	Indole,6-methy-2-(2-thienyl)-
14	12.403	4.63	n-hexadecanoic acid
15	12.797	0.31	1-Butene,3-chloro -2methyl
16	12.904	1.29	Bicyclo(2.2.2)octane,2-methyl
	100.00	100.00	

Table 3: Solubility and moisture content of bay leaf and brinjal films

Film Property	Bay leaf	Brinjal
Solubility(%)	-132.374	-115.028
Moisture content(%)	9.420	17.647

Table 4: Degradation values of bay leaf and brinjal films

Film property	Bay leaf	Brinjal
Degradation(%)	3.681	5.523

Table 5: Qualitative phytochemical analysis of ethanolic extracts of bay leaf and brinjal

Secondary metabolites	Bay leaf	brinjal
Alkaloids	+	+
Carbohydrates	-	+
Protein	-	+
Flavanoids	+	-
Steroids	-	-
Terpenoids	-	-
Tannins	+	-
Saponins	-	+
Glycosides	-	+
Fixed oils	+	+

Table 6: Zone of inhibition of bay leaf and brinjal film

Bacteria	Bay leaf	Brinjal	Control
Bacillus cereus	15 mm	15.6 mm	22 mm
Staphylococcus aureus	14mm	20mm	25mm
Escherichia coli	15.2mm	16mm	15mm
Vibrio cholera	14mm	15.4 mm	15mm

Table 8: Zone of inhibition of bay leaf and brinjal

Fungi	Bay leaf	Brinjal	Control
Candida albicans	14.2 mm	17.3 mm	15mm
Aspergillus niger	10 mm	16.2mm	14mm





Jafilda Poulose and Jeena Pearl

Table 9: Absorbance of brinjal and bayleaf at different concentrations

Concentration($\mu\text{g/mL}$)	bay leaf (% inhibition)	Brinjal (% inhibition)
20	50.18 $\pm$ 0.005	32.84 $\pm$ 0.20
40	56.42 $\pm$ 0.003	41.86 $\pm$ 0.24
60	61.54 $\pm$ 0.004	49.59 $\pm$ 0.12
80	68.63 $\pm$ 0.004	53.82 $\pm$ 0.22
100	77.65 $\pm$ 0.010	59.66 $\pm$ 0.20
IC ₅₀ ($\mu\text{g/mL}$)	21.63	67.47



Fig 1:



Fig 2:

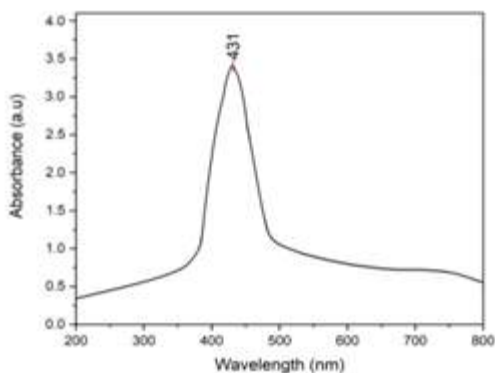


Fig 3:UV- visible spectra of bay leaf

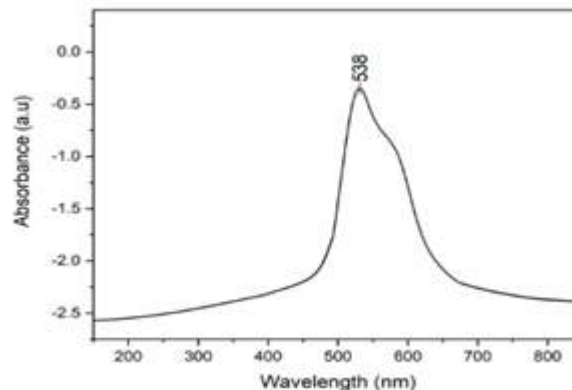


Fig 4: UV- visible spectra of brinjal

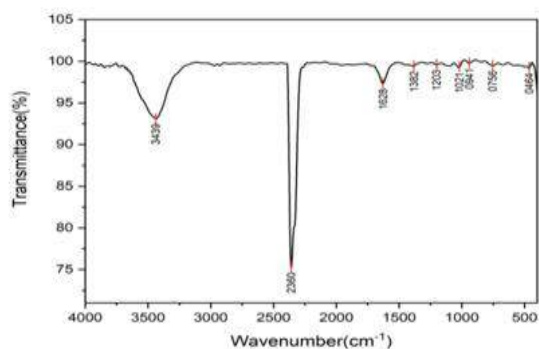


Fig.5. FT-IR spectrum of brinjal

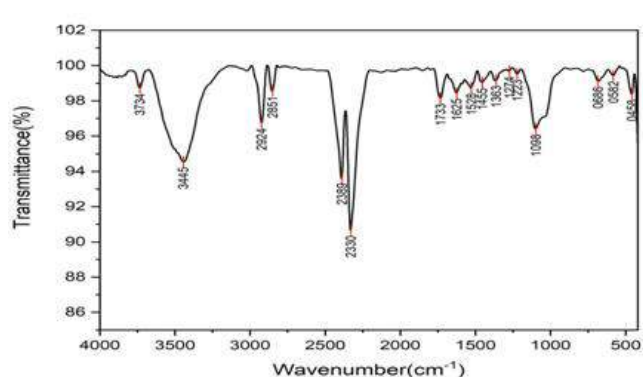


Fig.6. FT-IR spectrum of brinjal extract





Jafilda Poulose and Jeena Pearl

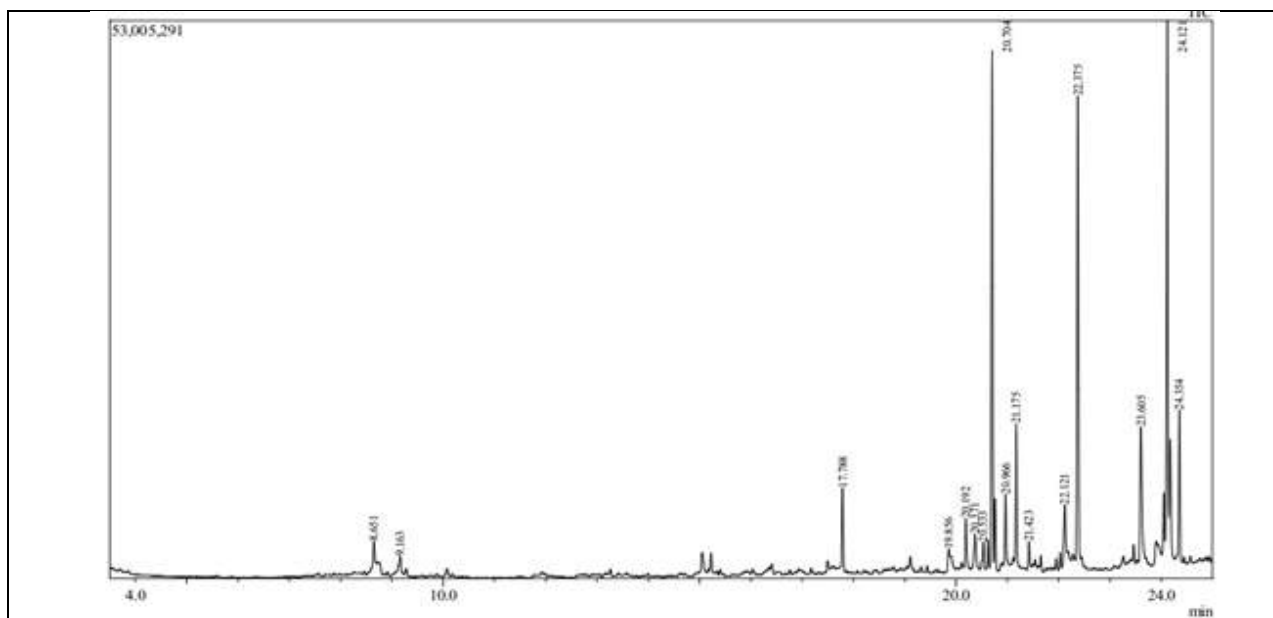


Fig.7. GC-MS chromatogram of brinjal

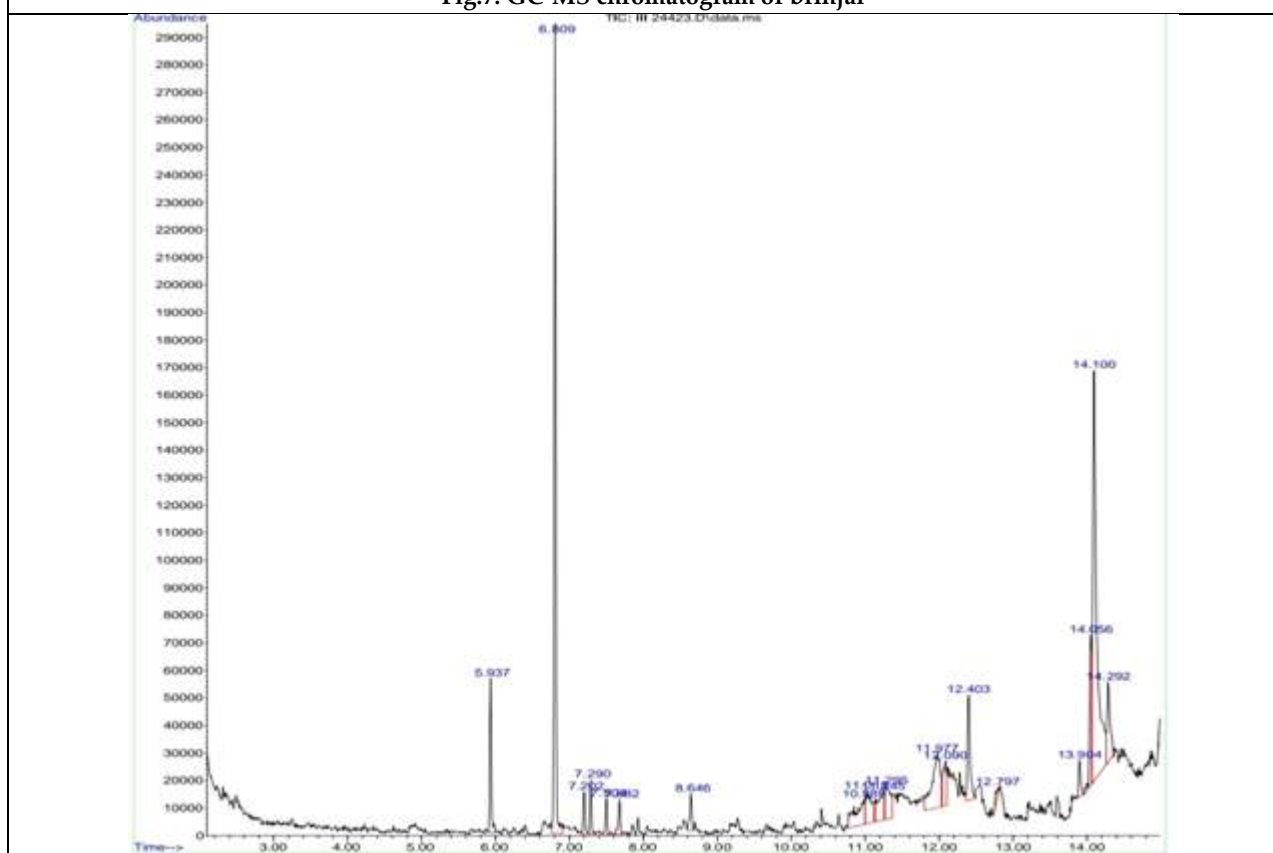


Fig.8. GC-MS chromatogram of bay leaf





Jafilda Poulose and Jeena Pearl

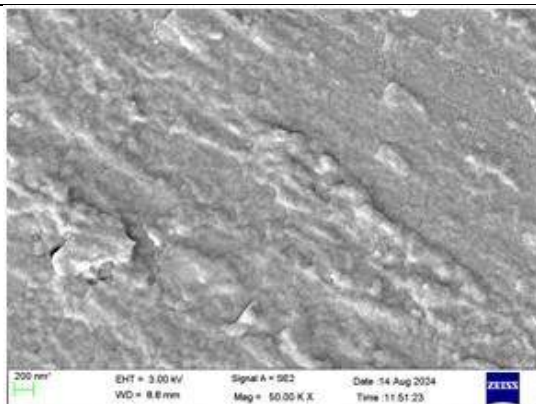


Fig.9. SEM of bay leaf

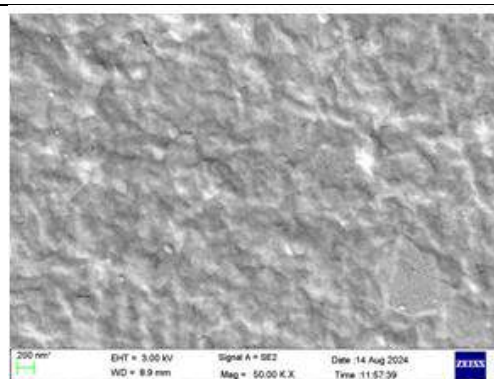


Fig.10. SEM of brinjal

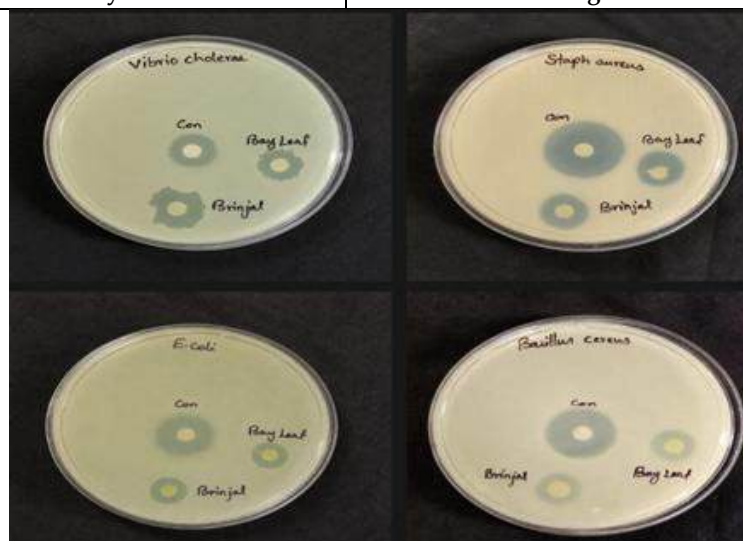


Fig.11. Zone of inhibition of bay leaf and brinjal

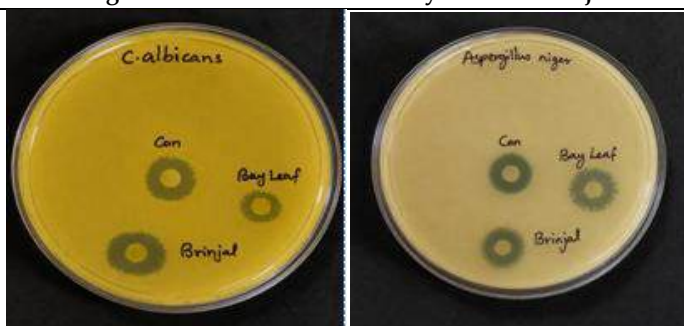


Fig 13: Antifungal activity of bay leaf and brinjal

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RESEARCH ARTICLE

Primary Screening and Production of Different Enzymes from Endophytic Fungi in Marine Associated Medicinal Plants

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ABSTRACT

Endophytic fungi are microorganisms that inhabit medicinal plants without inducing disease, and they represent a valuable source of bioactive compounds with possible therapeutic uses. Endophytic fungi are utilized to generate enzymes for a range of industrial, pharmaceutical and the creation of novel medications. In the current study, the enzymes like amylase, L-asparaginase, pectinase and protease synthesized from endophytic fungi. The screening result showed that, totally 22 isolated endophytic fungi were identified from eight distinct genera has the ability to produce all the four enzymes. The fungi such as *Aspergillus fumigatus*, *Fusarium oxysporum* and *Penicillium chrysogenum* were screened on the basis of quantum of enzymes. Among the four enzymes, partial purification of L-asparaginase enzyme were maximum quantity of three selected endophytic fungi and highest purification fold were recorded at the *P.chrysogenum*. L-asparaginase (ASNase) is utilized in the treatment of acute lymphoblastic leukemia and currently under investigation for its efficacy against other cancers such as breast cancer, where research indicates its ability to impede the proliferation and migration of cancer cells. It is important to note that the enzymes themselves do not act as direct anticancer agents; instead, they may play a role in the production of compounds with anticancer activity and also possess other therapeutic applications.

Keywords: Endophytic fungi, primary screening, enzyme production, partial purification, amylase, L-asparaginase, pectinase, protease.





INTRODUCTION

The endophytic fungi derived from plants represent a significant source for the synthesis of various secondary metabolites which are bioactive compounds beneficial to the pharmaceutical industry (Khan *et al.*, 2010; Amirita *et al.*, 2012). Marine endophytic fungi are recognized for their ability to generate a wide array of bioactive secondary metabolites, including steroids, alkaloids, terpenoids, and peptides. Numerous compounds derived from these fungi exhibit various biological activities such as anti-inflammatory, antioxidant, antimicrobial and antitumor effects (Mohamed El-Bondkly *et al.*, 2020; El-Bondkly *et al.*, 2021). Endophytic fungi have been identified as potential sources of bioactive secondary metabolites. Fungal endophytes represent a polyphyletic group of primarily ascomycetous fungi that reside within healthy host tissues for at least a portion of their life cycle without causing any observable symptoms of disease or adverse effects on their hosts (Sandrawati *et al.*, 2020; Valli *et al.*, 2024). Endophytes refer to all microorganisms that penetrate the internal tissues of living plants without inducing any immediate disease symptoms or adverse effects on their host; this definition is the most inclusive and commonly used (Zhang *et al.*, 2006). Endophytic fungi play a significant role in safeguarding their hosts from pathogenic microorganisms by competing for available habitats and nutritional resources (Selim *et al.*, 2012).

Fungi possess the capability to generate industrial enzymes, antimicrobial substances and microbial biomass having developed the capacity to synthesize enzymes and natural products as innovative metabolites (Sumaiya, 2022). Enzymes derived from fungi can function directly as antimicrobials or can produce intermediate compounds that exhibit antimicrobial properties (Gao *et al.*, 2010). Additionally, fungal enzymes are utilized in the food industry to enhance food preservation and extend shelf life while maintaining the nutritional value (Zhao *et al.*, 2017). The extraction of enzymes from endophytic fungi for commercial applications remains a largely untapped area representing one of the most recent sources of enzymes with diverse functionalities. Medicinal plants remain largely underexplored in terms of their functional relationships with fungi. These plants harbor endophytic fungi that produce exoenzymes. These exoenzymes are capable of hydrolyzing the structural components of pathogens, effectively eliminating them and thereby offering protection to the plants against these harmful organisms. (Khalil, 2021; Vyawahare *et al.*, 2019). The enzymes are involved in the breakdown of lignocellulosic substances, including amylases, pectinases and proteases. These enzymes can exert a direct antagonistic influence on pathogens. The quantity and variety of enzymes released by endophytic fungi can differ and may be influenced by the specific source plant (Zewdinesh *et al.*, 2019; Mwendwa *et al.*, 2025). Asian medicinal plants host a variety of endophytes that exhibit numerous biological activities including antimicrobial, anticancer and antioxidant effects.

These endophytes are also known to produce various hydrolytic enzymes that hold both therapeutic and industrial significance (Rathna *et al.*, 2023). Fungal endophytes may serve as an alternative, readily accessible, low-cost source of antioxidants. Numerous endophytes are known to synthesize bioactive compounds that plants can utilize for defense against pathogens, and some of these compounds may hold potential as pharmaceutical drugs (Sriya *et al.*, 2023). Antimicrobial resistance has emerged as a significant health concern and continues to pose a threat to healthcare systems worldwide. Consequently, this global issue has prompted an increase in research focusing on endophytic fungi, particularly those isolated from medicinal plants for their potential as sources of novel antibiotics (Jia *et al.*, 2016). Similar to other organisms that invade plant tissues, endophytic fungi produce extracellular hydrolases as a resistance mechanism against pathogenic invasion and to acquire nutrients from their host. Fungal enzymes are utilized in the food, beverage, confectionery, textile, and leather industries to facilitate the processing of raw materials. They are frequently more stable than enzymes sourced from other origins. The variety of enzymes produced varies among fungi and often influenced by the host and ecological factors (Sunitha *et al.*, 2013). In addition to the isolation and identification of endophytic fungal colonies from medicinal plants, the research encompasses the screening of selected all isolated endophytic fungi for five distinct extracellular enzyme assays, including amylase, L-asparaginase, pectinase and protease enzymes.





MATERIALS AND METHODS

Endophytic fungi obtained from three distinct locations of marine associated plants were observed at regular intervals. In this research, fungi were isolated from six marine-associated plants, including *Aegiceras corniculatum*, *Acanthus ilicifolius*, *Cyphostemma setosum*, *Excoecaria agallocha*, *Rhizophora mucronata* and *Suaeda maritima* by culturing them on PDA medium.

Primary screening of enzymes

Amylase enzyme

The use of starch agar media was implemented to evaluate the effectiveness of amylase production. All identified fungal species were centrally inoculated the starch casein agar plates and maintained at a temperature of 28°C. Following a two-day incubation period, the inoculated plates were treated with iodine solution after which the zones of clearance surrounding the inoculated fungi were observed. These zones of clearance were measured and documented (Migahed, 2003).

L-asparaginase enzyme

Utilizing a modified M9 agar medium, the bacterial strains were evaluated for L-Asparaginase activity. The composition of the M9 media includes Disodium hydrogen phosphate - 6.0 g, Potassium dihydrogen phosphate - 3.0 g, Sodium chloride - 0.5 g, Glucose solution - 10.0 mL, Magnesium sulfate solution - 1.0 mL, Calcium chloride solution - 1.0 mL, Phenol red - 0.3 mL, and Agar - 15.0 g, with a pH of 7.0. The bacterial strains were inoculated and incubated for 48 hours at 37°C. The degree of pink coloration observed on the streaked plates post-incubation facilitated the detection of L-Asparaginase activity (Rudrapati, 2015).

Pectinase enzyme

The isolated endophytic fungi was then inoculated onto a sterile pectin agar plate and incubated at 37° C for 48 hours to facilitate the isolation and screening of pectinolytic endophytic fungi (Rafae *et al.*, 2006). The colonies that developed on the pectin agar medium were examined for the presence of clear zones, and the characteristics of the isolated organisms were recorded.

Protease enzyme

The production of proteolytic enzymes by fungal isolates was identified through the Plate assay method which utilized gelatin as the protein source for the growth medium. The fungal isolates were inoculated in the Petri dishes and enriched with 1% gelatin (comprising 5g Peptone, 3g Beef extract, 5g NaCl, 15g Agar, and 1 liter of distilled water at pH 7). The Petri dishes were then incubated at a temperature of $28 \pm 1^\circ\text{C}$ for a duration of 3 days. The degradation of gelatin was noted as a clear zone surrounding the fungal colony (Migahed, 2003).

Enzyme Production

Amylase activity

The amylase assay was conducted using a reaction mixture consisting of 1 ml of a 2% starch solution combined with 1.0 ml of a 50 mM buffer at pH 6.5, along with 0.1 ml of crude enzyme which was incubated for 15 minutes at 30°C. Following the incubation, 2 ml of DNase reagents was introduced and the mixture was boiled for 10 minutes. Subsequently, 1.0 ml of chilled 40% K-Na tartrate was incorporated. The resulting color, produced from the reaction between DNase and reducing sugars, was measured at a wavelength of 540 nm and subsequently calculated (Migahed, 2003).



**Amalorpavamary and Jayaseelan****L-asparaginase enzyme**

The activity of L-asparaginase was assessed using the following method. The cultures grown in modified M9 medium containing 1.5% L-asparagine broth were subjected to centrifugation at 8000 rpm for 30 minutes after which the resulting supernatant, referred to as the crude extract was utilized to evaluate L-asparaginase activity. The reaction commenced with the addition of 1 mL of the crude cell-free extract to 0.5 mL of a 0.04 M l-asparagine solution and 0.5 mL of a 0.05 M Tris-HCl buffer at pH 8, followed by incubation at 37°C for 30 minutes in a water bath. The reaction was halted by introducing 0.5 mL of 1.5 M trichloroacetic acid. The precipitated proteins were eliminated through centrifugation at 8000 rpm for 15 minutes at 4°C, and the ammonia concentration was measured spectrophotometrically at 450 nm using the Nesslerization method. Control tubes were maintained at zero-time incubation. The enzyme activity was quantified based on the release of ammonia, which was calculated against a standard curve of ammonium. One unit of L-asparaginase is defined as the quantity of enzyme that catalyzes the production of 1 μ mol of ammonia per minute at 37°C incubated (El-Hadi *et al.*, 2019).

Pectinase activity

The activity of Polygalacturonase was assessed by quantifying the quantity of reducing substances released from citrus pectin. The reaction mixture included 450 μ L of substrate buffer (comprising 0.1 g of citrus pectin dissolved in 100 ml of 0.1 M phosphate buffer at pH 5) and 50 μ L of enzyme solution. This combination was incubated at a temperature of 50 °C for a duration of 30 minutes. Subsequently, 500 μ L of DNSA reagent was introduced into the vial, which was then placed in boiling water for 5 minutes and subsequently cooled in ice water. A blank sample was also prepared following the same procedure as described previously. The absorbance was measured at 550 nm using a spectrophotometer. One unit of enzyme activity (U) was defined as the release of 1 μ mole of galacturonic acid per minute (Chowdhury *et al.*, 2017).

Protease activity

A modified mineral salt broth was employed for the synthesis of protease enzyme from promising fungal sources. Following incubation, the crude enzyme underwent treatment with 0.5% NaOH and 1ml TCA. The resulting filtered supernatant was then treated with folin phenol solution, revealing a blue color attributed to the reduction of sugars. This color change indicated the production of the protease enzyme, and the reaction mixture was analyzed at 660nm using a UV-vis spectrophotometer (Mandels *et al.*, 1976).

Partial Purification of enzymes

12-days incubated period of the culture filtrates, the crude enzymes was precipitated using different concentrations of ammonium sulphate, while the mixture was maintained under a magnetic stirrer at 4°C for 24 hours. Subsequently, the protein precipitate was obtained by cooling the mixture and centrifuging it at 10,000 rpm for 10 minutes. The pellet underwent dialysis against 2-3 buffer changes during this process, also under a magnetic stirrer at 4°C for 24 hours, while being suspended in a sodium acetate buffer with a pH of 4.6 [48]. The proteins that were only partially purified were quantified (U/mL) in an aliquot.

Purification profile**Enzyme activity**

Utilizing casein as a reference point, the enzyme activity in the raw materials was assessed through ammonium sulfate precipitation and dialysis for sample purification (Dam *et al.*, 2013).

Estimation of protein content

Utilizing bovine serum albumin as a reference, the protein quantities in the crude extract, ammonium sulfate precipitated, and dialyzed enzyme samples were determined (Lowry *et al.*, 1951).



**Amalorpavamary and Jayaseelan****Specific activity**

The specific activity of the crude extract which was subjected to ammonium sulphate precipitation and subsequent dialysis was calculated using the following formula.

$$\text{Specific activity } \left(\frac{\text{U}}{\text{mg}} \right) = \frac{\text{Total activity } \left(\frac{\text{Units}}{\text{mL}} \right)}{\text{Total Protein } \left(\frac{\text{mg}}{\text{mL}} \right)}$$

Purification fold

The specific activity of the crude extract was utilized to calculate the purification fold for the sample that was precipitated with ammonium sulfate and subsequently dialyzed. The formula employed is as follows:

$$\text{Purification fold} = \frac{\text{Specific activity of the sample}}{\text{Specific activity of the crude extract}}$$

Statistical analysis

The experiments were done three times. The results are shown as average values with their standard deviations.

RESULTS AND DISCUSSION

A total of forty distinct endophytic fungi source from various medicinal plants were analyzed for their capacity to produce extracellular enzymes including amylase, protease, cellulase, laccase, and L-Asparaginase. Each of the 40 endophytic fungi evaluated demonstrated the ability to generate extracellular enzymes. Although none of the endophytic fungi in this investigation were capable of synthesizing all five enzymes examined, every fungal endophyte was able to produce at least one of the extracellular enzymes assessed (Kathiravan and Gnanadoss, 2022). Among the 40 endophytic fungi evaluated, 45% of the endophytic isolates exhibited positive amylase activity. Amylases represent one of the essential enzyme employed in contemporary biotechnology particularly in the process of starch hydrolysis. Fungal amylase is extensively utilized in both the food and pharmaceutical sectors (Sunitha *et al.*, 2012). The endophytic isolates was 37.5% demonstrated protease activity among the 40 endophytic fungi that were isolated. Proteases are acknowledged as a crucial class of enzymes, representing two-thirds of the global enzyme market. These enzymes are primarily utilized in the food, leather, textile, and detergent sectors (Mandal and Banerjee, 2019). Among the 40 endophytic fungi evaluated for L-Asparaginase, 52.5% of the isolates demonstrated positive enzyme activity. L-Asparaginase serves as a therapeutic enzyme utilized as a chemotherapeutic agent for the treatment of acute lymphoblastic leukemia. Nine fungal endophytes, extracted from various medicinal plants located in the high mountains of Uttarakhand, India, exhibited positive L-Asparaginase activity (Kapoor *et al.*, 2021).

This aligns with the findings of which indicated that out of 11 strains, only eight demonstrated positive amylase activity when isolated from the leaves of medicinal plants. Additionally, the research conducted by revealed that among nine strains, merely three were capable of producing the amylase enzyme from endophytic fungi sourced from medicinal plant leaves. The fungi identified included *Aspergillus* sp, *Rhizoctonia* sp., and *Chaetomium*; furthermore, disclosed that the amylase activity generated by endophytic fungi contributes to the degradation of starch found in aging host plants (Venkatesagowda *et al.*, 2012). In addition, identified *Rhizoctonia stolonifer* as the most effective endophytic fungi for amylase production, followed by *Aspergillus niger* and *Penicillium variotii*. Historically, amylase was first utilized in medicine for the treatment of digestive disorders and is recognized as the pioneering enzyme for commercial production (Zakowski and Bruns, 1985). This enzyme also finds applications in the food industry such as enhancing pastries and facilitating starch removal from sugar and it is utilized in various sectors including textiles, paper, detergents and the preparation of animal feed.

Pectinase is found in microorganisms which is essential for various pathogenic processes in plants, the symbiotic relationships between organisms and plants, as well as in the decomposition of dead plant matter. The initiation of plant attack by pathogenic fungi occurs due to their capacity to produce pectinase, which is instrumental in the breakdown of host tissues. Endophytic fungi possess the potential to evolve into pathogenic fungi by virtue of their



**Amalorpavamary and Jayaseelan**

ability to decompose pectin substances (Choi *et al.*, 2005). Furthermore, Schulz and Boyle (2005) proposed in their hypothesis that the interaction between the botanical host and cultivated fungi is balance between the pathogenicity of the fungi and the defensive mechanisms of the host plant; any disruption in this equilibrium is associated with the progression or emergence of disease. Additionally, pectinase holds significant applications, particularly in the paper which contain food industries and is commercially produced through fungi (Upadhyaya *et al.*, 2020). In the present study, fungi collected from three different sites of marine-associated plants were observed at regular intervals. A total of 135 colonies of endophytic fungi were isolated from six marine-associated plants including *Aegiceras corniculatum*, *Acanthus ilicifolius*, *Cyphostemma setosum*, *Excoecaria agallocha*, *Rhizophora mucronata* and *Suaeda maritime* by culturing them on PDA medium both with and without artificial salt. In total, 22 endophytic fungal isolates were identified from eight distinct genera, namely *Aspergillus*, *Beauveria*, *Fusarium*, *Macrophomina*, *Nigrospora*, *Pestalotiopsis*, *Penicillium* and *Rhizopus*. The isolated endophytic fungi such as *Aspergillus flavus*, *A.fumigatus*, *A.sydowii*, *A.penicilliodes*, *Beauveria bassiana*, *Fusarium lateritium*, *F.moniliforme*, *F.oxysporum*, *F.solani*, *F.equiseti*, *F.verticillioidea*, *Macrophomina phaseolina*, *Nigrospora oryzae*, *Penicillium brevicompactum*, *P.chrysogenum*, *P.citrinum*, *P.crystallinum*, *P.longibrachiatum*, *P.verruculosum*, *Pestalotiopsis biciliata*, *Rhizopus microsporus* and *R.stolonifer* were recorded, respectively.

In amylase enzyme, the *A.fumigatus*, *A.sydowii*, *F.moniliforme*, *F.oxysporum*, *P. brevicompactum*, *P.chrysogenum*, *P.citrinum* and *P.crystallinum* were presented but other fungi not found to be recorded respectively. In the L-asparaginase enzyme, fungi were identified: *A. fumigatus*, *F.oxysporum*, *F.equiseti*, *F.verticillioidea* and *P.chrysogenum* however, no other fungi were documented. In pectinase enzyme, the *A.flavus*, *A.fumigatus*, *A.penicillioidea*, *F.moniliforme*, *F.oxysporum*, *F.verticillioidea*, *P.brevicompactum*, *P.chrysogenum*, *P.crysogenum*, *P.crystallinum* and *P.longibrachiatum* were presented but other fungi were not found to be recorded. In the protease enzyme, the following fungi were identified: *A. fumigatus*, *A.penicillioidea*, *Beauveria bassiana*, *F.oxysporum*, *F.solani*, *F.verticillioidea*, *Macrophomina phaseolina*, *P.chrysogenum*, *P.citrinum*, *P.crystallinum* and *P.longibrachiatum*. However, no other fungi were documented (Table 1). In the production of all enzymes, the maximum amount of activity was screened by the endophytic fungi were represented as *A.fumigatus*, *F.oxysporum* and *P.chrysogenum* when compared with all other endophytic fungi were recorded, respectively (Table 2). The partial purification of L-asparaginase from crude extract was influenced by ammonium sulfate precipitation (45-85%), indicating that a significant portion of the enzyme activity was retained in the precipitate. Additionally, the total protein precipitation analysis demonstrated that the majority of enzyme activity remained in the precipitate. The total protein concentration decreased from ammonium sulfate precipitate (Ram Kumar *et al.*, 2020). However, during the dialysis process, the protein concentration diminished. The profiles of the ammonium sulfate fraction and dialysis indicated an increase in specific activity. The final purification step, conducted using Sephadex G-100, revealed that specific activities increased, while the protein concentration decreased. The partially purified enzyme exhibited L-asparaginase activity, whereas reported an asparaginase activity. Furthermore, the enzyme demonstrated ten times greater activity than that observed by Kushwaha *et al.*, (2012).

The process of protein purification typically entails a sequence of actions aimed at isolating a specific protein from a complex mixture while eliminating unwanted substances. An optimal purification process should be executed with the minimum number of steps to prevent the loss of the target protein and should also be cost-effective (Noor *et al.*, 2016). Nevertheless, the methods employed will vary based on the intended application of the enzyme. The initial step in recovering extracellular proteases is to separate the cell biomass from the fermentation broth (Matias *et al.*, 2021). The subsequent step focuses on concentrating the proteases, which can be achieved through either filtration or precipitation techniques. In the following stages, procedures that generally involve column chromatography are conducted. Among the reviewed articles, two reported partial purification that included enzyme precipitation processes utilizing ethanol and ammonium sulfate, followed by dialysis. Six studies implemented ammonium sulfate precipitation methods, which were then followed by chromatographic techniques such as ion exchange chromatography, size exclusion chromatography and affinity chromatography for enzyme purification (Elgammal *et al.*, 2020). Additionally, one study employed ultra filtration methods (Centriprep-30), followed by passage through a phenyl boronate column and concluded with precipitation using methanol. According to the present study,



**Amalorpavamary and Jayaseelan**

A.fumigatus, *F.oxysporum* and *P.chrysogenum* had been produced the amylase, L-asparaginase, pectinase and protease enzyme production at maximum level. Therefore, these fungi were considered as potential fungi in primary screening. The partial purification of amylase, L-asparaginase, pectinase and protease enzymes from *A.fumigatus*, *F.oxysporum* and *P.chrysogenum* were performed with crude enzyme extraction followed by ammonium sulphate fractionation and dialysis. In the current investigation, we focused on the partial purification of enzymes derived from selected marine soil fungi, specifically *A.fumigatus*, *F.oxysporum* and *P.chrysogenum*, to achieve maximum enzyme production. The crude enzyme extract underwent purification processes involving ammonium sulphate precipitation and dialysis, aimed at enhancing the specific activity of all enzymes. A comparison between the crude enzyme extract and the results from dialysis indicated that ammonium sulphate fractionation resulted in higher concentrations of all enzymes and proteins. Following dialysis, there was a notable increase in the specific activity of the enzymes, accompanied by a purification fold. In maximum purification level were recorded at L-asparaginase enzyme when compared with all other enzymes and the endophytic fungi were presented as exhibiting the highest level of activity when it comes to partial purification level as the *P.chrysogenum* were recorded, respectively (Fig 1, 2 & 3).

CONCLUSION

At present, the challenges posed by ineffective, toxic and costly therapeutic antimicrobial drugs. In recent years, endophytic fungi have garnered significant attention in the drug development process due to their widespread presence and abundant availability. Endophytic fungi exhibit a diverse range of metabolic processes and possess distinct pathways for secondary metabolite production which will facilitate the isolation of various metabolites and their applications in the field of medicine. These microorganisms thrive within the epidermis and aerial parts of plants, offering a diverse range of chemical compounds. Endophytic fungi are known to produce biologically active secondary metabolites, including terpenes, alkaloids, monoterpenoids, peptides and polyketides. Fungal endophytes play a crucial role in addressing various human health concerns, particularly against numerous microbial pathogens including fungi, through multiple mechanisms such as the synthesis of antibiotics, antifungal agents, antiviral compounds, anticancer substances, lytic enzymes and the breakdown of toxins. A vital process in this context is partial purification which serves to isolate the target enzyme enhancing its specific activity and facilitating a comprehensive characterization of its attributes including thermal stability and optimal pH. Enzymes derived from endophytic fungi exhibit anticancer potential via mechanisms such as the induction of apoptosis and the inhibition of cancer progression. Nevertheless, the predominant emphasis in research has been placed on secondary metabolites, including paclitaxel and camptothecin. Endophytic fungi serve as a pertinent example of an enzyme source that can be investigated for its direct anticancer effects.

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Amalorpavamary and Jayaseelan

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Amalorpavamary and Jayaseelan

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Table 1: Primary screening of endophytic fungi by the determination of different enzymes

Name of the endophytic fungi	Inference			
	Amylase	L-asparaginase	Pectinase	Protease
<i>Aspergillus flavus</i>	-	-	+	-
<i>A. fumigatus</i>	+	+	+	+
<i>A. sydowii</i>	+	-	-	-
<i>A. penicillioides</i>	-	-	+	+
<i>Beauveria bassiana</i>	-	-	-	+
<i>Fusarium lateritium</i>	-	-	-	-
<i>F. moniliforme</i>	+	-	+	-
<i>F. oxysporum</i>	+	+	+	+
<i>F. solani</i>	-	-	-	+
<i>F. equiseti</i>	-	+	-	-
<i>F. verticillioides</i>	-	+	+	+
<i>Macrophomina phaseolina</i>	-	-	-	+
<i>Nigrospora oryzae</i>	-	-	-	-





Amalorpavamary and Jayaseelan

<i>Penicillium brevicompactum</i>	+	-	+	-
<i>P. chrysogenum</i>	+	+	+	+
<i>P. citrinum</i>	+	-	-	+
<i>P. crystallinum</i>	+	-	+	+
<i>P. longibrachiatum</i>	-	-	+	+
<i>P. verruculosum</i>	-	-	-	-
<i>Pestalotiopsis biciliata</i>	-	-	-	-
<i>Rhizopus microsporus</i>	-	-	-	-
<i>R. stolonifer</i>	-	-	-	-

(+) - Present; (-) – Absent

Table 2: Production of potential endophytic fungi from different enzymes

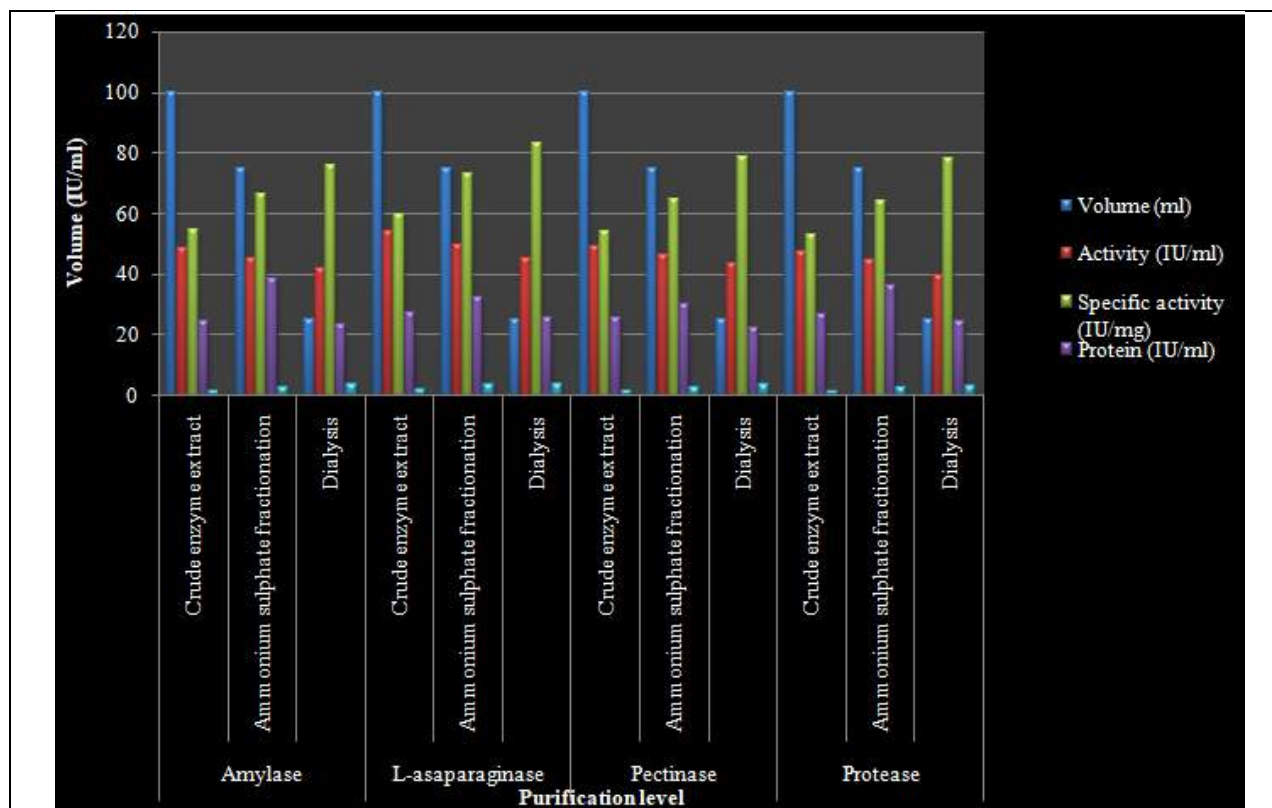
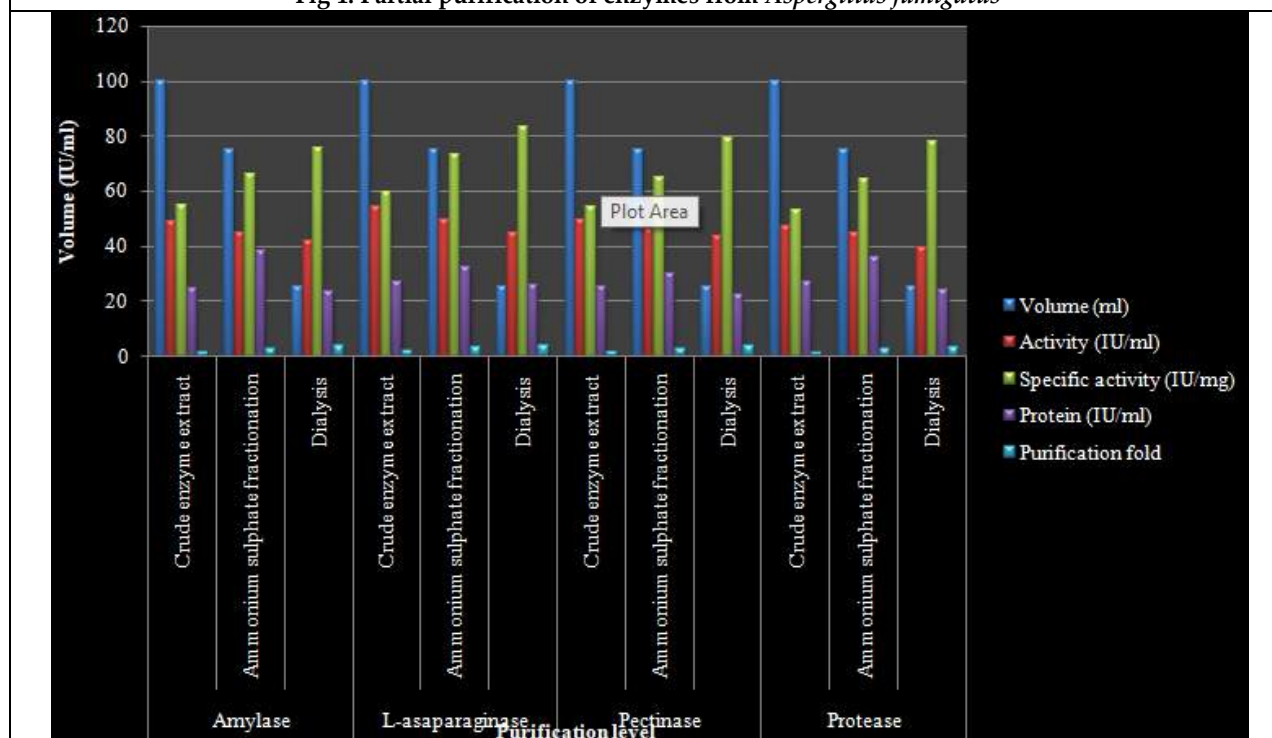
Name of the endophytic fungi	Quantity (IU/ml)			
	Amylase	L-asparaginase	Pectinase	Protease
<i>Aspergillus flavus</i>	-	-	7.18±0.00	-
<i>A. fumigatus</i>	5.05±0.02	7.10±0.15	6.57±0.02	6.02±0.05
<i>A. sydowii</i>	5.14±0.14	-	-	-
<i>A. penicillioides</i>	-	-	6.25±0.06	7.14±0.50
<i>Beauveria bassiana</i>	-	-	-	4.04±0.27
<i>Fusarium lateritium</i>	-	-	-	-
<i>F. moniliforme</i>	4.07±0.03	-	5.19±0.05	-
<i>F. oxysporum</i>	6.08±0.05	7.24±0.33	5.64±0.00	6.08±0.04
<i>F. solani</i>	-	-	-	4.57±0.16
<i>F. equiseti</i>	-	5.14±0.30	-	-
<i>F. verticillioides</i>	-	4.17±0.07	5.05±0.07	4.22±0.01
<i>Macrophomina phaseolina</i>	-	-	-	4.02±0.08
<i>Nigrospora oryzae</i>	-	-	-	-
<i>Penicillium brevicompactum</i>	5.14±0.17	-	4.62±0.04	-
<i>P. chrysogenum</i>	9.31±0.00	9.92±0.12	8.91±0.03	9.08±0.10
<i>P. citrinum</i>	4.12±0.07	-	-	4.08±0.22
<i>P. crystallinum</i>	5.11±0.01	-	6.17±0.34	4.00±0.06
<i>P. longibrachiatum</i>	-	-	6.58±0.04	6.02±0.40
<i>P. verruculosum</i>	-	-	-	-
<i>Pestalotiopsis biciliata</i>	-	-	-	-
<i>Rhizopus microsporus</i>	-	-	-	-
<i>R. stolonifer</i>	-	-	-	-

The values are expressed in terms of (Mean ± Standard deviation)



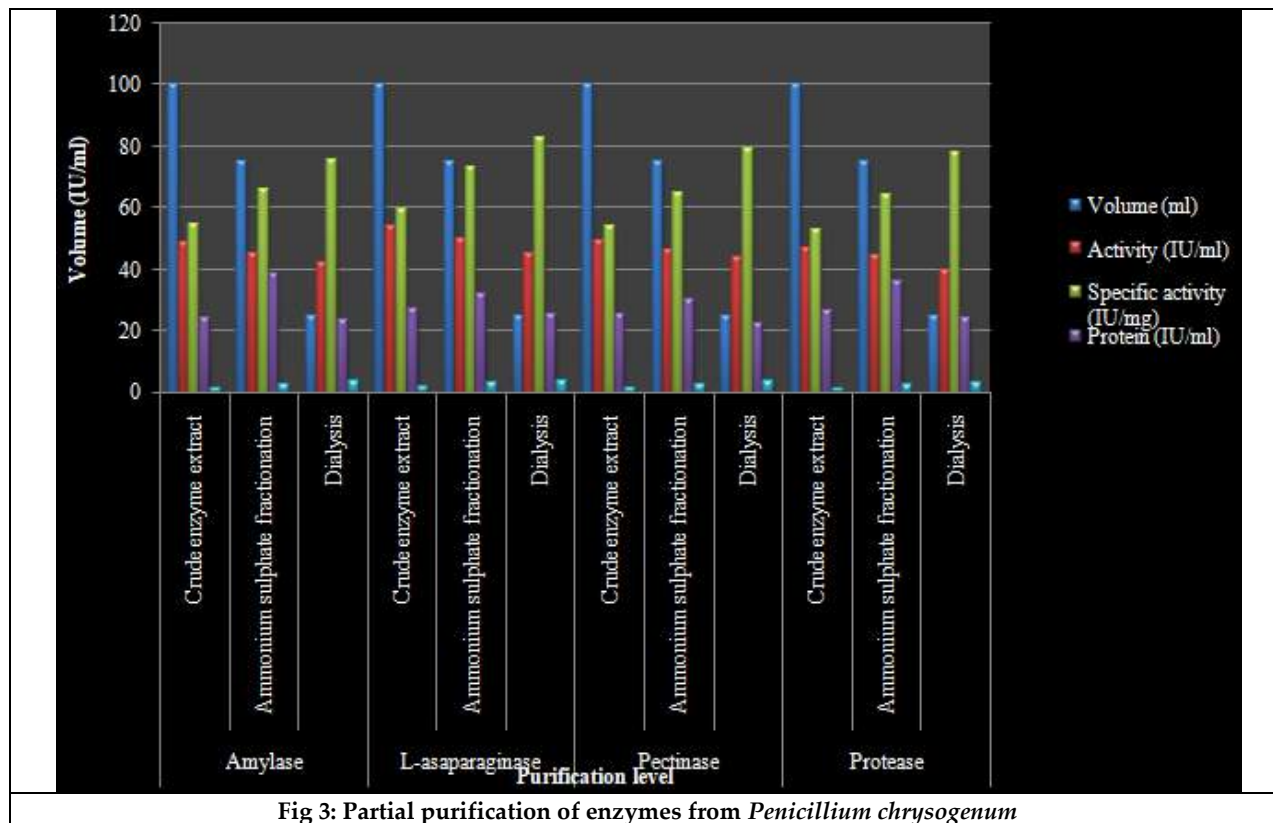


Amalorpavamary and Jayaseelan

Fig 1: Partial purification of enzymes from *Aspergillus fumigatus*Fig 2: Partial purification of enzymes from *Fusarium oxysporum*



Amalorpavamary and Jayaseelan

Fig 3: Partial purification of enzymes from *Penicillium chrysogenum*

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RESEARCH ARTICLE

Synthesis, Characterization and Applications of Graphene Oxide Nanoparticles from *Carica papaya* Seed Ash

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ABSTRACT

The production and characterisation of graphene oxide from *Carica papaya* seeds is the primary goal of this work. Because of their physical, chemical, and electrical characteristics, graphene oxide and other nanomaterials based on carbon are special in nature and have a wide range of uses. One significant two-dimensional nanomaterial based on carbon is graphene oxide. The high silica content of papaya seed ash was eliminated, and the residual graphite carbonized material was heavily oxidized with potassium permanganate, sulfuric acid, and orthophosphoric acid to enhance the number of oxygen functional groups. Graphene oxide in its oxidized state is hydrophilic, meaning it dissolves readily in water. The UV-visible, FT-IR, XRD, SEM-EDX, TEM, SAED, and CV methods were used to analyze the produced graphene oxide. Graphene oxide and other oxygen functional groups were detected by FT-IR and XRD. The produced graphene oxide's antibacterial and antioxidant properties were evaluated against the corresponding microorganisms.

Keywords: Graphene oxide, Thermal combustion, Carbonized material, Dispersed, Hydrophilic.



**Roshan B John and Prabha Malar Lithis**

INTRODUCTION

Many studies have looked into graphene, graphene oxide as maybe the most promising biomaterial for conducting polymer composites, solar cells, batteries, sensors, and transistors [1, 2]. Additionally, it is employed in other applications, including biodegradation, photodegradation, and cleans energy devices for sterilizing and purifying water. Carbon waste can be recycled and turned into graphene oxide, which have numerous uses and potential economic and environmental advantages [3]. A fundamental component of other graphite materials including graphite and carbon nanotubes, graphene is a 2D honeycomb-shaped monolayer of carbon atoms that is one atom thick. Graphene oxide have been thoroughly researched for a range of applications, including nanoelectronics, sensors, drug delivery, nanocomposites, cell imaging and more, due to their distinct and appealing electrical, chemical, mechanical and optical properties. The graphene in its oxidized state is called graphene oxide. Epoxide, carbonyls, hydroxyls, and carboxylic groups are the primary functional groups found in graphene oxide, which also comprises hexagonal carbon-based structure formed by the hybridization of sp³ and sp² carbon atoms [4]. This straightforward, inexpensive, and sustainable method of synthesizing graphene oxide may open up new avenues for the creation of graphene-based nanomaterials [5]. Carbon-based products made from fruit waste's renewable resources are being promoted in order to make them more competitive [6].

Carica papaya, also called papaya, pawpaw, and kates, a member of the Caricaceae family. Originating in the Mesoamerican Center, Central America, and southern Mexico [7–9], this horticultural shrub is mostly grown throughout subtropical and tropical areas of Brazil, India, Malaysia, China, Philippines Thailand, and other neighboring countries [10]. Commercial papaya cultivation has taken place in several Asian countries. In many tropical countries, papaya is also planted as a garden plant. Fruits are available all year round [11]. Papaya peels and seeds, which make up roughly half of the weight of the papaya, and the seeds alone make up about 14%, are among the waste products produced is processed industrially with the goal of creating a variety of products, including candied papain, pectin, Juices, fruit, raisins, nectars, and jellies, and jams[12]. Utilizing sections of veggies and fruits that are usually thrown away, like the seeds, which contain a variety of nutritionally significant materials, including proteins, minerals, carbs, fibers as well as bioactive substances, can help reduce the amount of trash that is disposed of. Additionally, if the right procedures and technology are used, they could be transformed into new goods with added value [13–14]. Given the information above, using seeds of papaya to create novel products is a viable substitute since it may enhance a by-product and reduce the amount of agro-industrial waste that is disposed of [15]. The goal of the current work was to turn papaya seed ash into graphene oxide. The primary driving force behind using these sources is to supply inexpensive raw materials for the large-scale manufacturing of carbon-based graphene oxide in a sustainable and environmentally friendly manner. UV, FT-IR, XRD, SEM, EDX, TEM, SAED, and CV were among the advanced techniques used to analyze the synthesized graphene oxide. Antioxidant and antimicrobial potential of the synthesized graphene oxide were tested against respective microbes.

EXPERIMENTAL METHODS

Chemicals and Materials

Papaya seeds were gathered locally in Tamil Nadu, India's Kanyakumari district. Molychem supplied the chemicals (AR Grade), which were used. Sodium Hydroxide, Sulphuric acid 98%, Orthophosphoric acid , Potassium permanganate, Hydrogen peroxide 30%, Hydrochloric acid and Deionized water.

Alkali extraction

After being gathered, papaya seeds were repeatedly washed under running water and allowed to dry for roughly two weeks in the shade. The dried seed was then heated to 600°C in a muffle furnace for controlled thermal combustion. 50 milliliters of 2M NaOH solution and 5 grams of papaya seed ash were placed in a 100 milliliter beaker. A hotplate magnetic stirrer was used to agitate the mixture for approximately 4 hours at 80°C. Whatman No.



**Roshan B John and Prabha Malar Lithis**

41 ashless filter paper was then used to filter the mixture. They threw out the filter solution. The primary source for the manufacture of graphene oxide was the residue of carbonized materials, which was gathered.

Graphene oxide Synthesis:-

After being repeatedly cleaned with HCl and deionized water, the obtained carbonized material was dried at 80°C in an air oven. For this preparation, 3 milliliters of orthophosphoric acid and 27 milliliters of sulfuric acid (volume 9:1) were combined and agitated for a number of minutes. After adding 0.5g of carbonized material gradually while stirring, 2g of KMnO_4 was patiently added to the mixture. The solution turns dark green color approximately 6 hours of churning the mixture. 1.2 ml of H_2O_2 was progressively added to a KMnO_4 and mixed for a few minutes to get rid of any surplus. After the exothermic reaction takes place, allow it to cool. After adding 30 milliliters of deionized water and 10 milliliters of 5% hydrochloric acid, the solution was centrifuged for 10 minutes at 3000 rpm. Following the removal of the supernatant, the residue was rinsed three to four more times using HCl and DI water. The cleaned graphene oxide solution was dried for 24 hours at 90°C in an oven to produce the graphene oxide powder.

Characterization**UV-Visible spectroscopy**

Figure 6 shows the GO UV absorption spectrum. The shoulder peak that GO showed at 236 nm is caused by the π - π^* transition. This is due to the π anti-bonding and bonding electrons found in unsaturated carbon atoms [16].

Fourier-transform infrared spectroscopy

The graphene oxide's FT-IR spectrum (figure 7) verifies the existence of functional groups like hydroxy, epoxy, and carbonyl groups. The presence of hydroxy groups in GO is shown by the prominent peak at 3344 cm^{-1} , which is attributed to the O-H bonds stretching mode. Weak bands at 2658 cm^{-1} indicate the asymmetric and symmetric stretching vibrations of the C-H bonds. The signal at 1718 cm^{-1} represents C=O which is carbonyl group, whereas the peak at 1628 cm^{-1} is caused by the aromatic C=C stretching group. The vibrational mode of C-O groups, represented by a peak at 1040 cm^{-1} , and C-O-C stretching, represented by the peak at 1248 cm^{-1} , both attest to the presence of functional groups with GO. Peak at 1162 cm^{-1} indicates C-O-C stretching vibration. Because of the oxygen functional groups present, our FT-IR analysis demonstrated that the produced graphene oxide is hydrophilic.

X-Ray Diffraction

The X-Ray Diffraction patterns of GO are shown in Figure 8. The crystalline nature of the GO samples was shown by the XRD examination. The peak at $2\theta=10.831$, which is in line with the (001) plane, shows that the graphite in the samples underwent oxidation to produce graphene oxide. Scherrer's formula was used to determine the GO samples' average crystalline size, $D=K\lambda/\beta\cos\theta$. In this case, the X-ray wave length is $K = 0.9$, $\lambda = 0.1540\text{ nm}$, and the complete width at half peak height is $\beta = 0.258$, $2\theta = 10.831$, $\theta = 5.4155$. The graphene oxide's average size was estimated to be around 0.830 nm.

Scanning Electron Microscope

Scanning electron microscopy can be used to determine the structure of nanomaterials and its surface morphology. Figure 9 shows the surface morphology of graphene oxide samples. The SEM image shows flat nature and organized layer structure of graphene oxides. As can be seen in this figure, the different oxide layers were created as the graphite oxide oxidized. Layered structure was displayed by Graphene Oxide.

Energy- Dispersive X- ray Spectroscopy

An energy dispersive X-ray analysis is used to determine the specimen's elemental composition. The EDX image presented in figure 10 indicates the existence of components including oxygen and carbon in the graphene oxide. The % of oxygen and carbon by weight and atomic percentage were found to be 32.68:67.32 and 29.53:70.47 respectively. The graphene oxide sample is predicted to contain carbon and oxygen based on the EDX findings.



**Roshan B John and Prabha Malar Lithis****Transmission Electron Microscopy**

Transmission electron microscopy was employed to investigate the sample's structural coherence (TEM). The TEM micrograph is displayed in figure 11. Graphene oxide displays morphology akin to a sheet with layer stacking. This is most likely caused by the quantity of levels found in GO's stacked structure. Therefore, TEM analysis verifies the existence of graphene oxide in the oxidized carbon samples. One method for determining whether a material is crystalline at the nanoscale is to examine its SAED pattern. The PSA samples SAED pattern displays typical ring-like patterns that point to the samples polycrystalline structure. The samples have distinct diffraction spots that match the hexagonal lattices six-fold layout. Thus, the samples crystalline character may be seen in SAED pattern. The SD value of the sample is 40.9.

Cyclic Voltammetry

The graphene oxide nanoparticle's electrochemical activity was investigated using cyclic voltammetry, which tracks current as a function of applied voltage. Concentration, roughness, diffusion coefficient, surface area and other interfacial electrochemical properties of an electrode in an electrolyte solution are all studied using CV. The typical redox behavior of the nanoparticles is depicted by the CV curve in figure 14. The sample reduction process is represented by the cathodic peak at -0.74V, which has a peak current of -3.8 μ A. The area under curve 16.282 μ C, which indicates a considerable level of electrochemical activity, represents the charge transfer during the redox process. The symmetry of the CV curves suggests that the redox process is reversible because the current remains constant across the sweep range of -0.8 to -0.6V. The graphene oxide nanoparticle's good electrochemical stability is demonstrated by its reversibility. This result suggests that the generated nanoparticle can be used in electrochemical devices like sensors or energy storage devices.

Applications**Anti-Bacterial activity**

The produced graphene oxide nanoparticles' antimicrobial properties were examined. Figure 15 illustrates how the data were interpreted using two gram positive and two gram negative bacterial strains. If the sample's value is higher than the control, the bacterium is susceptible to the produced graphene oxide nanoparticles. A lower number in the direction of the control indicates resistance. The statistics clearly suggest that *Vibrio cholera* and *Streptococcus aureus* were sensitive to the material, whereas *E. Coli* and *Bacillus cereus* shown resistance to it. This suggests that the produced graphene oxide nanoparticle has potent antibacterial properties against particular bacterial strains. It can be applied widely in the medical profession as a result. If the prepared sample's qualities are further investigated and improved, it might be used as an efficient antiseptic.

Anti-Fungal activity

The samples' antifungal properties were examined against *Candida Albicans* and *Aspergillus Niger*. The data clearly show that *Aspergillus niger* had a weaker inhibitory zone than *Candida albicans*. This possesses more potent antifungal qualities than *Candida albicans*. The graphene oxide nanoparticle's antifungal activity is shown in figure 16.

Antioxidant activity

Recent research has demonstrated that a variety of nanoparticle types are potent antioxidants and free radical scavengers (17–18). The generated graphene oxide nanoparticles were tested for antioxidant properties using the DPPH, Nitric oxide, and Superoxide anion scavenging tests. The antioxidant activities of the generated nanoparticle were evaluated at varying doses and the different IC₅₀ values were computed to determine the strength of the antioxidant effect. The DPPH assay showed 75.68% inhibitory action at 100 μ g/ml, increasing with concentration. The nitric oxide assay's activity dropped with increasing concentration, peaking at 67.54% at 100 μ g/ml. The superoxide anion assay also showed decreasing activity with increasing concentration, reaching 66.99% at 100 μ g/ml. The antioxidant potentials of the three assays were ascertained using the IC₅₀ values. It became apparent that the IC₅₀ values for the DPPH, nitric oxide, and superoxide anion tests were 31.18 μ g/ml, 45.56 μ g/ml, and 56.01 μ g/ml,



**Roshan B John and Prabha Malar Lithis**

respectively. This suggests that the produced graphene oxide nanoparticle has significant antioxidant potential because the DPPH assay exhibits the strongest inhibitory activity of the two assays. Antioxidant activity of the produced graphene oxide nanoparticle is compared in the histogram between the three tests.

CONCLUSION

We can infer from the research provided here that papaya seeds can be utilized as a raw material for the synthesis of graphene oxide. The simple and useful synthesis process is illustrated in this work. UV, FTIR, XRD, SEM, EDAX, TEM, SAED, and CV were among the spectroscopic techniques used for analysis. At 236 nm, the produced graphene oxide nanoparticle's UV spectrum displays a shoulder peak that exhibits the π - π^* transition. Many functional groups, including CH, OH, C-O, C=O, C-O-C, and C=C, are confirmed by the FTIR spectrum. The produced graphene oxide nanoparticle's average size is displayed by XRD. The graphene oxide's layered structure is consistent with the SEM image. The many elements found in graphene oxide, such as carbon and oxygen, are displayed by EDX. A sheet-like structure layered as a layer is shown in the TEM image. The sample's polycrystalline graphene oxide structure is shown by the SAED pattern's characteristic ring-like patterns. Graphene Oxide's electrochemical behavior is determined via CV. The antibacterial efficacy against two Gram-positive and two Gram-negative pathogens showed strong antibacterial activity against specific bacterial strains. Against fungus strains, strong antifungal activity was demonstrated. Antioxidant activity was measured using DPPH, nitric oxide, and superoxide anion scavenging tests; DPPH showed the highest inhibitory action. Because of this, the synthesized GO has several unique and fascinating properties with several potential uses.

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Roshan B John and Prabha Malar Lithis

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Table 1: EDX of graphene oxide from *Carica papaya* Seed Ash

SL.NO	Elements	Weight%	Atomic%
1.	Carbon	67.32	70.47
2.	Oxygen	32.68	29.53

Table 2: Zone of inhibition of Graphene oxide nanoparticle

Bacteria	PSA	Control (Amikacin)
E-Coli	16.2mm	18 mm
Vibrio Cholera	20 mm	18 mm
Bacillus Cereus	16 mm	19 mm
Streptococcus Aureus	16 mm	15 mm

Table 3: Zone of inhibition of Graphene Oxide Nanoparticle

Fungus	PSA	Control (Nystatin)
Candida Albicans	13.6 mm	12 mm
Aspergillus Niger	15 mm	18 mm





Table 4: Percentage of Inhibition of graphene oxide nanoparticle

Conc. ($\mu\text{g/ml}$)	DPPH	Nitric Oxide Scavenging Assay	Superoxide Scavenging Assay
Positive	98.08	64.57	73.62
20	46.92	44.17	33.04
40	54.08	47.32	44.93
60	56.78	52.83	50.33
80	68.08	59.29	63.31
100	75.68	67.64	66.99
IC 50 Value ($\mu\text{g/ml}$)	31.18	45.56	56.01



Figure 1:- Carica papaya Seed



Figure 2&3:- 4hrs stirring

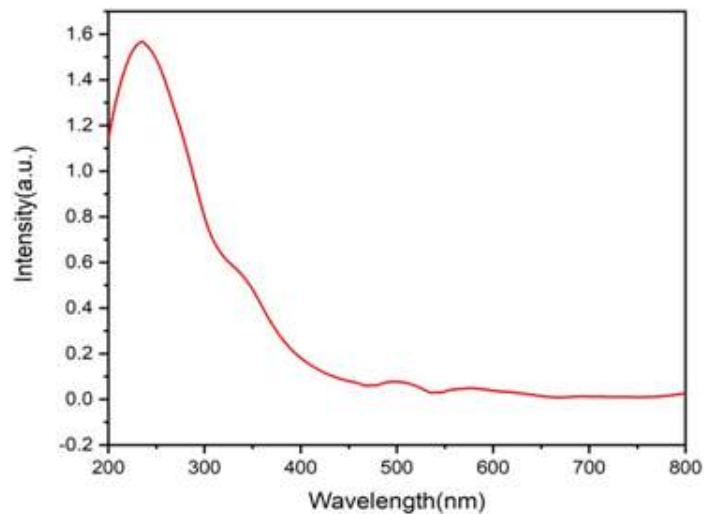
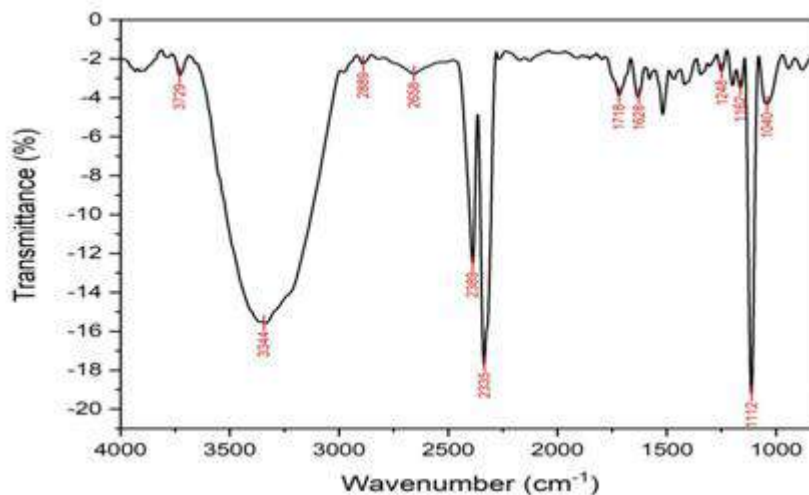
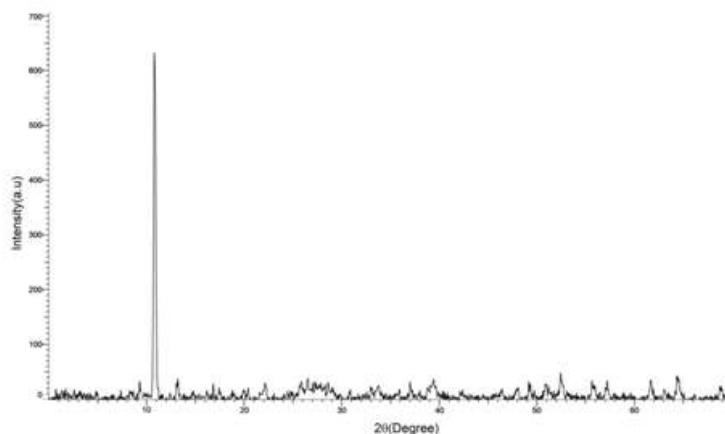


Figure 4:-Filtration



Figure 5:- Carbonised Material



**Roshan B John and Prabha Malar Lithis****Figure 6:- UV Spectrum of Graphene Oxide from PSA****Figure 7:- FTIR spectrum of Graphene oxide from PSA****Figure 8:- X-Ray Diffraction of graphene oxide from PSA**

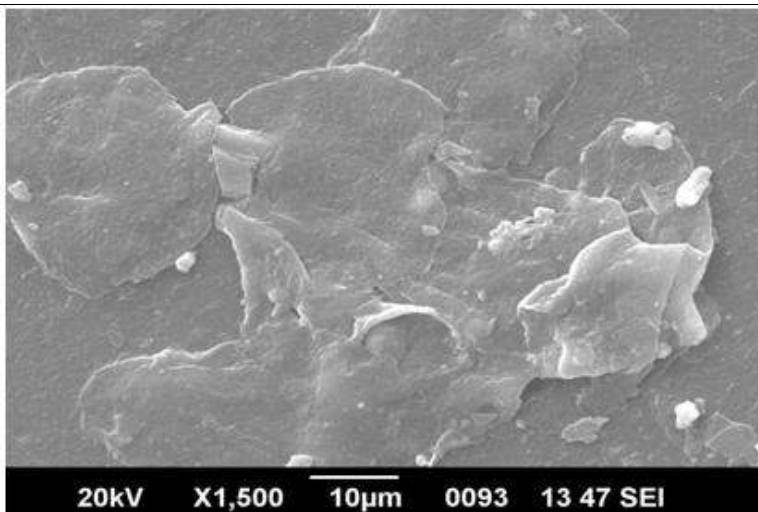


Figure 9:- SEM image of graphene oxide from PSA

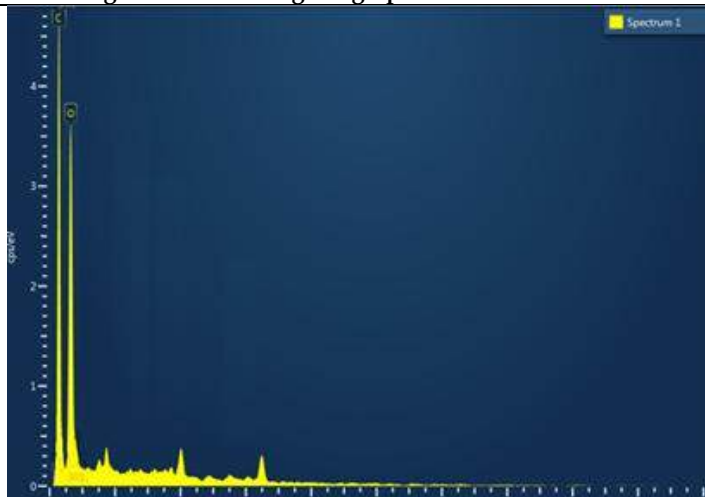


Figure 10:- EDX spectrum of graphene oxide from PSA

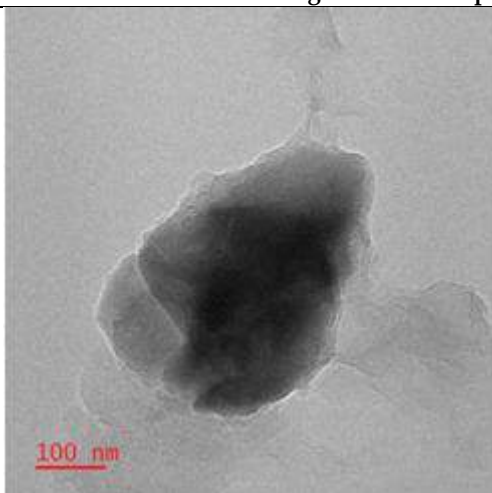


Figure 11:- TEM image of the sample

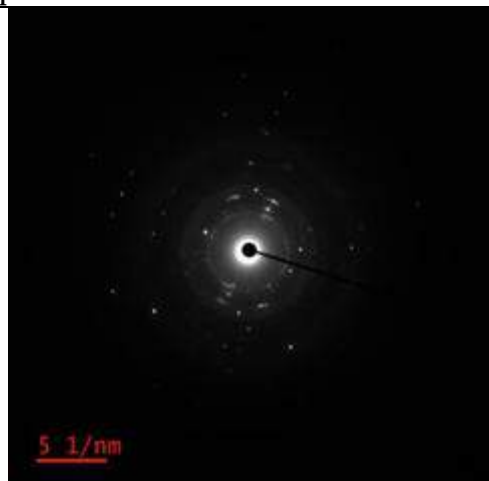


Figure 12:-SAED Pattern of the Sample





Roshan B John and Prabha Malar Lithis

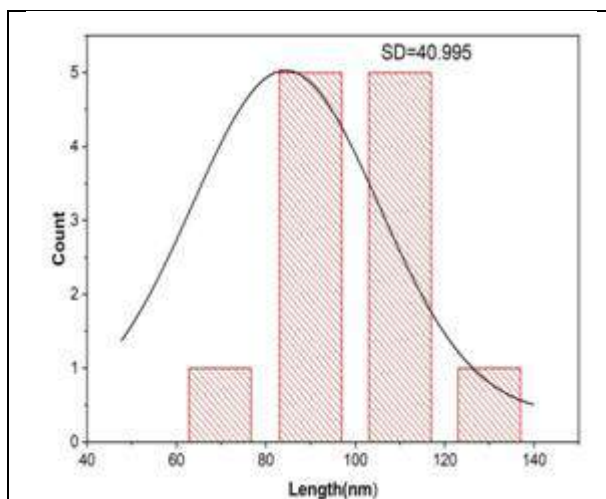


Figure 13:- Particle size distribution

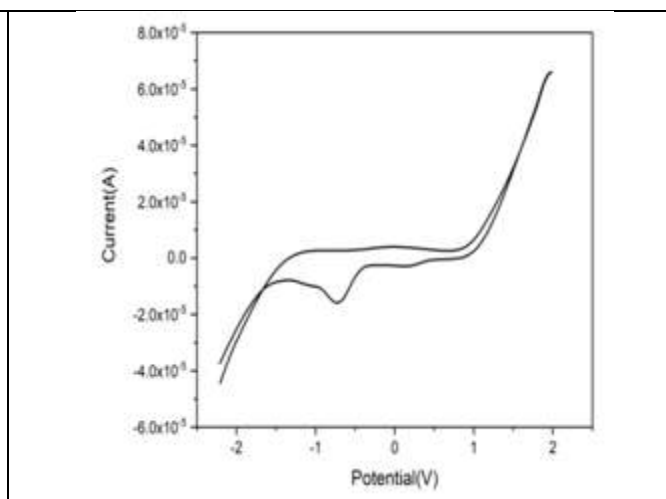


Figure 14:- Cyclic Voltammetry of Graphene oxide Nanoparticle

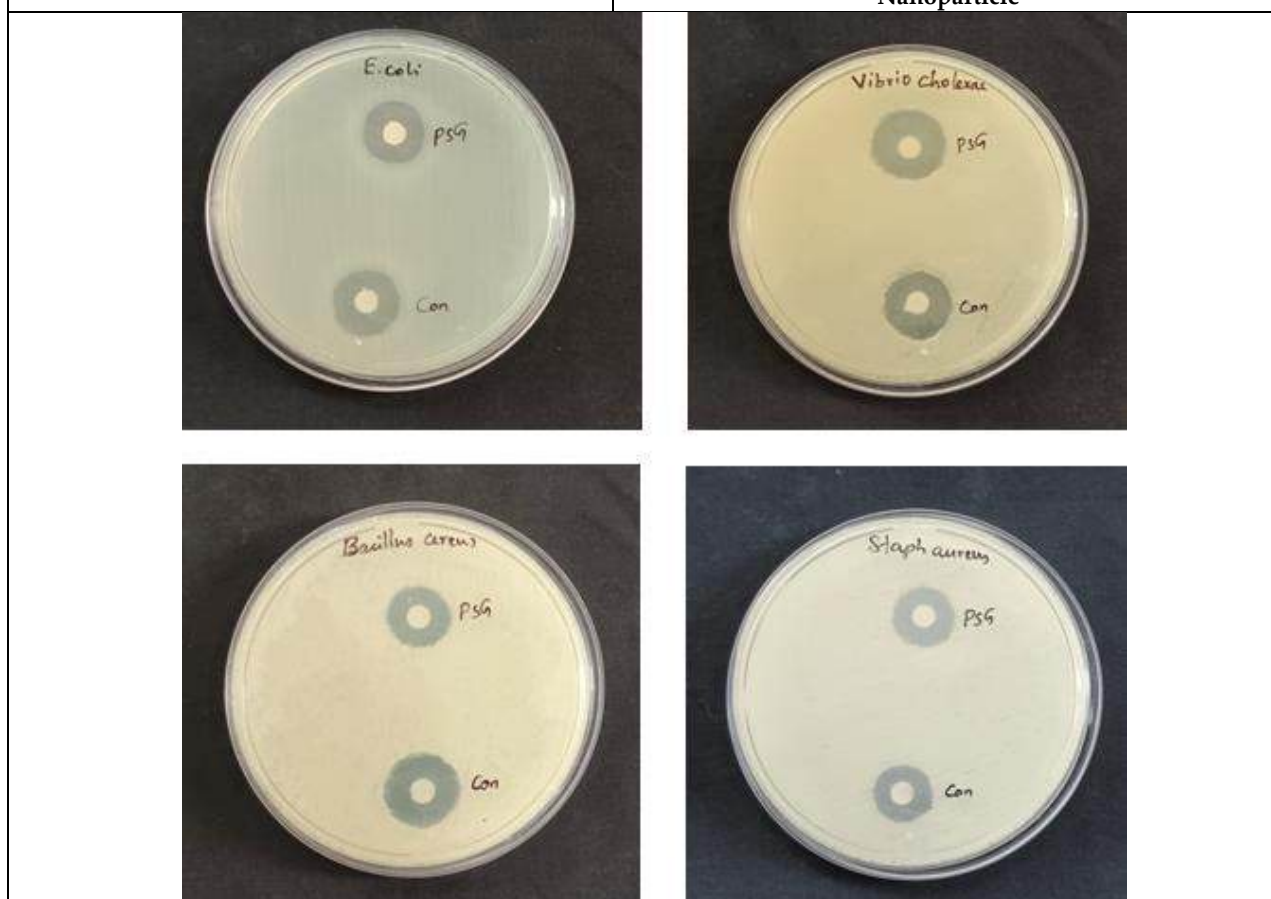


Figure 15:- Antibacterial activity of Graphene Oxide Nanoparticle





Roshan B John and Prabha Malar Lithis

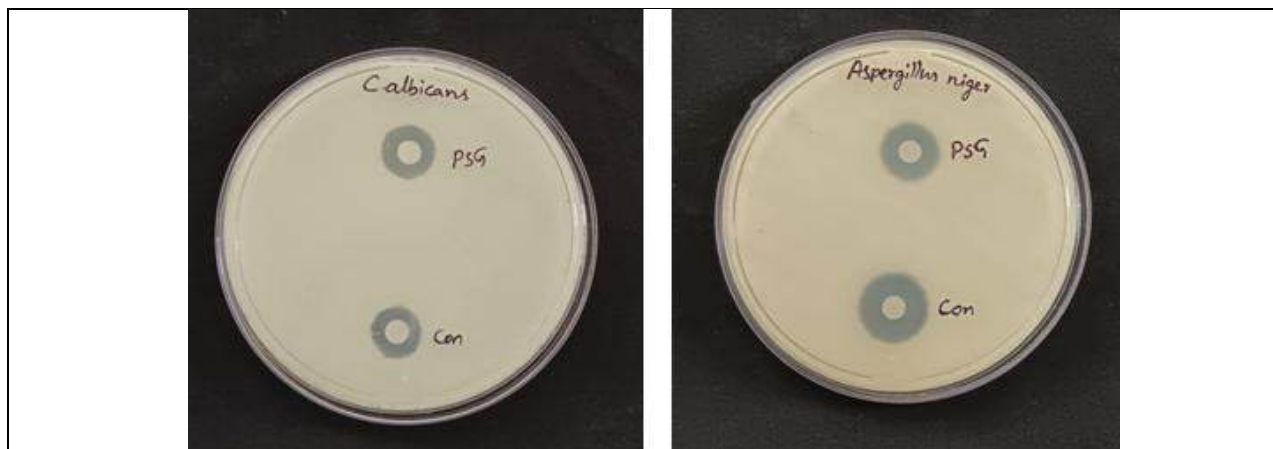


Figure 16:- Antifungal activity of Graphene Oxide Nanoparticle

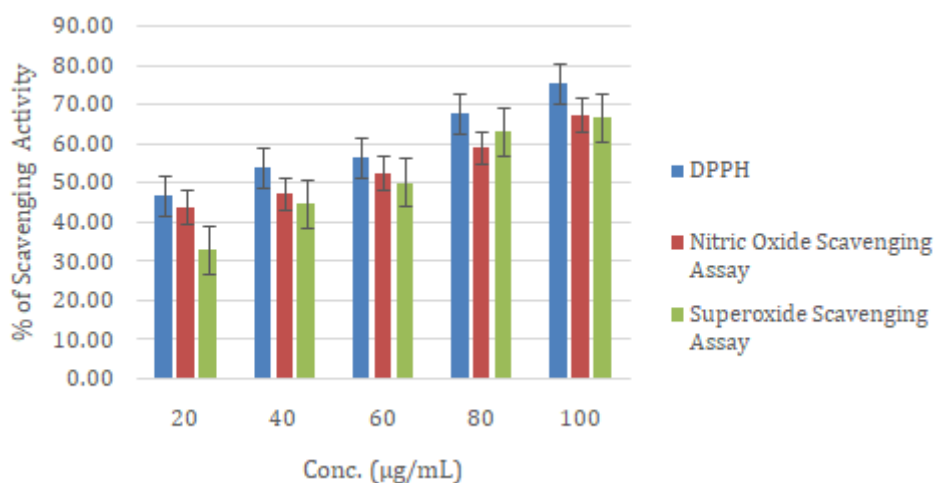


Figure 17:- Comparative study of antioxidant activity of graphene oxide nanoparticle

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RESEARCH ARTICLE

Deep Learning - Based Feature Extraction for Robust Multi-Model Remote Sensing Image Registration

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ABSTRACT

Multi-model image registration plays a pivot role in many remote sensing applications such as image fusion, image restoration, military and civilian fields etc. Image registration is a crucial step in many image-processing applications. Ground surface variations pose significant challenges for detecting feature points, impacting both their density and accuracy. This issue is frequently an obstacle faced by feature-based image registration. Remote sensing images often face challenges due to variations in different factors such as location, sensors and more. These differences lead to image distortions and overlaps between image pairs. This significantly affects the accuracy of image registration. The Scale-Invariant Feature Transform (SIFT) is well-known for extracting image features, with different variants of SIFT. However, it primarily relies solely on local low-level features, leading to a loss of mid and high-level information required for registration accuracy. Image features extracted by a deep learning method such as a convolutional neural network (CNN) have attained state-of-the-art performance for image feature extraction, classification and retrieval tasks. These features effectively captured mid and high-level information, making them ideal for aligning remote sensing images. Hence, in this letter, we discuss and analyse how to calculate the image features using various feature extraction methods and trained CNN Models. The experimental result demonstrates that deep learning algorithms yield better accuracy than feature-based algorithms.

Keywords: Remote sensing, image registration, feature extraction, convolutional neural networks (CNN), deep learning





INTRODUCTION

Multi-model satellite image registration has become increasingly popular in remote sensing due to its ability to combine information from different sensor modalities, creating more comprehensive and accurate data analysis. (Zhang X et al., 2021) Image Registration involves aligning and matching multiple images of the same scene that are captured from different sensors, perspectives, or at different times (Zhang X et al., 2021). In Various Remote sensing applications such as image fusion, map colouring, and Image segmentation, Image Registration is an essential step. Image matching obtained from a single sensor or modality is often viewed as a largely resolved challenge. When compared with multi-model image registration such as SAR and Optical image matching, faces various challenges. (Liaghat, M. S. et. al. 2023) However, these image-processing approaches face complexities due to geometric and radiometric differences between SAR and Optical images. For instance, a SAR image is affected by geometric distortions such as layover, foreshortening, and radar shadow. It also exhibits speckle noise, which lacks direct analogy in an optical image. SAR offers high-resolution remote sensing imagery in any weather condition. It is also known for its robust resistance to interference and jamming (Liaghat, M. S. et. al. 2023; S. Jiang et al. 2018) Optical images are affected by issues like optical shadows, cloud effects, and variations in illumination (Liaghat, M. S. et. al. 2023).

The key challenges can be summarized in the image registration as follows: firstly, the Significant and unpredictable variation between images makes it difficult for the algorithm to generalize across different scenarios. Second, Geometry distortions introduce an imbalance between correctly matched and incorrect points, complicating the task of eliminating mismatched points. third, image feature matching becomes more challenging due to sensor-related factors such as changes in viewpoint, terrain elevation, and other environmental variations that affect spatial transformation. Firstly, traditional Image Registration was done manually for two decades, where humans needed to locate ground control points on both sensed and reference images through visual analysis, such as identifying buildings, road intersections, etc. After identification of GCP, human operators would manually carry out the steps of feature detection and matching. (Priyanka S. Tondewad et.al.,2020) This job becomes time-consuming when it applies to remote-sensing images due to the availability of various transformations. Various problems are faced during manual image registration, such as finding accurate control points, expert human operators, difficulty in matching time constraints, and more GCPs are required to improve registration accuracy. (Priyanka S. Tondewad et.al.,2020) These problems lead to the development of an automatic image registration method (Zhang X et al., 2021; Liaghat, M.S. et. al., 2023; S. Jiang et al., 2018). Image Registration methods are mainly classified into two types Area-based and Feature-based. Area-based methods seek optimal transformation parameters by evaluating the similar pixel intensities between images using metrics such as MI (Mutual Information) and Normalized cross-correlation (Zhang X et al., 2021; Liaghat, M.S. et. al., 2023). The combination of Area-based and Feature-based methods is known as the Hybrid method in that identified image features are extracted and matched, then the deformation matrix is calculated (Ms. Priyanka S. Tondewad et. al. 2020, (Kuppala, K. et.al.,2020).

The variations in image intensity can negatively impact registration accuracy. Feature-based methods work on different image features to extract more information from the image. Feature-based methods mainly include three steps: Feature detection, Feature descriptor, and Feature Matching. (Y. Zhang et.al.,2021)Registration of multi-source remote sensing images, often achieved through Feature-based methods, is a well-researched area.(Y. Zhang et.al.,2021) Feature extraction involves identifying distinct regions such as closed boundaries, corners, forests, roads, rivers, urban areas and other significant features. Nowadays, various Deep learning Methods such as CNN, DNN, and GAN are used in image registration, extracting detailed image features and improving registration accuracy. (Zhang X et.al.,2021; Liaghat, M.S. et al.,2023) Various architectures like AlexNet, VGGNet, and ResNet provide better results in feature extraction. (Ms. Priyanka S. Tondewad et. al. 2020, Kuppala, K. et.al.,2020, Liu, Y. et.al.,2023, Ma, T. et.al.,2020, Du, W. et.al. 2020)The paper is structured in the following manner: Section 2 presents various types of images used for registration; Section 3 discusses the different feature extraction methods; Section 4 discusses feature extraction results; Section 5 discusses the conclusion and is followed by future work.





Image Dataset

Various types of images can be utilized in image registration, depending on the application domain.

- Images are captured using the same type of sensors but from different viewpoints. so, various transformations are available in images such as rotation, scale, translation, etc.
- Multi-Sensor Images: Images captured by different types of sensors provide more information about the image. Different sensors provide distinct information based on the bands they operate on, such as infrared, near-infrared, microwave, and others. Various Images are SAR (synthetic aperture radar) and optical imaging, Optical-Imaging, Panchromatic Image (PAN), and Multi-spectral images to analyze each image detail.
- Images are captured at different intervals. This type of image is used to analyze change detection. For example, SAR images can be taken during the day or at night.

RESEARCH METHODOLOGY

Multi-Sensor Images: Images captured by different types of sensors provide more information about the image. Different sensors provide distinct information based on the bands they operate on, such as infrared, near-infrared, microwave, and others. Various Images are SAR

Traditional method for feature extraction

The primary objective is to geometrically transform the target image to align with the reference image's spatial coordinate and enable pixel-to-pixel correspondence in their overlapping region (K. Chauhan et.al.,2023; Li, X. et.al.,2022). SAR images mostly use feature-based methods for registration. (K. Chauhan et.al.,2023; Li, X. et.al.,2022; Dai, H. et.al.,2021; Zheng, Y.et. al. 2022; Liu, Y. et.al.,2023) Traditional Feature-based Image registration methods often rely on manually extracted features, Optimization Algorithms or statistical methods to align images. (K. Chauhan et.al.,2023) The feature-based methods are mainly used to find the Affine transformation over the images by identifying image features where images have salient and stable features. The steps of image registration are also given in Figure 1. This approach involves:

- **Feature Detection:** it detects features from an image, such as edges, lines, shapes, boundaries etc. Various algorithms detect image features such as SIFT, SURF, HOG, Harris Corner detector, Canny edge detectors, etc. (K. Chauhan et.al.,2023, K. Parmar et.al.,2017)
- **Feature Matching:** Finding corresponding image features between reference and sensed image. (K. Chauhan et.al.,2023; K. Parmar et.al.,2017)
- **Transformation Estimation:** Calculating the transformation matrix that aligns the reference image with the sensed image. (K. Chauhan et.al.,2023; K. Parmar et.al.,2017)
- **Image Wrapping:** Transformation is applied to the sensed image to align with the reference image. (K. Chauhan et.al.,2023; K. Parmar et.al.,2017)

Deep Learning Method for Feature Extraction

In recent years, deep learning methods have emerged as a powerful and reliable approach in remote sensing imaging, giving outstanding performance in tasks such as object detection, image classification, image fusion and image registration. CNN is a deep learning architecture. CNN consists of many layers of alterations between convolution and pooling and one or more fully connected layers in the Neural Network. (Y. Zhang et.al.,2021; F. Ye. et. al. 2018; Lee W. et.al.,2021; Zheng, Y.et. al. 2022; Liu, Y. et.al.,2023) A Convolution layer uses a set of filters on feature maps from the previous layer to produce a new feature map. The pooling layer processes small rectangular image regions from the convolution layer and reduces them to a single value that creates a more compact representation. (Y. Zhang et.al.,2021; F. Ye. et. al. 2018) Various types of pooling techniques are used including max pooling and average pooling. The highest value within the defined window is passed to the subsequent layer in max pooling, whereas in average pooling, it computes the average of all values within that window. (Y. Zhang et.al.,2021;





Pamar Kinjal and Sudhir Vegad

F. Ye. et. al. 2018) A Convolutional Neural Network (CNN) trained on a large-scale dataset like ImageNet can extract mid-level and high-level image features that perform better than low-level features. These higher-level features capture more complex patterns and structures, such as shapes and objects, than low-level features like edges and textures. This capability makes CNNs highly effective for various visual recognition tasks. (Y. Zhang et.al.,2021; F. Ye. et. al. 2018; Lee W. et.al.,2021; Zheng, Y.et. al. 2022; Liu, Y. et.al.,2023; Ma, T. et.al.,2020) Famao Ye extracts image features from CNN combines SIFT and CNN features and then integrates them into the PSO-SIFT algorithm for registration. (F. Ye. et. al. 2018) Remote sensing image registration using CNN gives deep matching features and it improves the accuracy of remote sensing images. (Y. Zhang et.al.,2021; F. Ye. et. al. 2018) Image registration using a CNN-based model can be designed to learn a similarity function that directly compares the image patches based on image data. image patches directly from the image data. (F. Ye. et. al. 2018; Lee W. et.al.,2021) CNN can produce feature representations that are more robust to variations in appearance. CNN partitions the input image into several local patches, extracting descriptors separately. (F. Ye. et. al. 2018; Lee W. et.al.,2021) The similarity between extracted descriptors is then evaluated using a suitable distance measure, enabling an accurate similarity score. (F. Ye. et. al. 2018; Lee W. et.al.,2021) This approach can be used for binary classification tasks, such as identifying whether two patches correspond to the same or different features. (F. Ye. et. al. 2018; Lee W. et.al.,2021) Lee. W. proposed a (Matchet) Geometry CNN-based image registration method that can enhance the accuracy of aligning two remote sensing images captured at different times and from varying viewpoints. (Lee W. et.al.,2021; Wang, S. et.al.,2018; Yang, Z. et.al.,2018; Gong, M. et.al.,2017)

Pre-trained VGG16 Model

The VGG16 Model pre-trained on ImageNet was fine-tuned using SAR and PAN image datasets. The extraction of image features in this model excludes the fully connected layer at the top of the VGG16 model. The model contains multiple convolution layers organised into blocks such as block1_conv1, block2_conv1, etc. This extracts various features using the SIFT algorithm and measures the similarity of different image features. The CNN model employed in this letter, VGG16, is a simple competitive CNN architecture which won second place on the ILSVRC2014 classification task. The VGG16 model consists of 13 convolution layers, five max-pooling layers, with three fully connected (FC) Layers (Li, X. et.al.,2022; Y. Liu et.al.,2023; Ms. Priyanka S. Tondewad et. al., 2020).

GAN (Generative Adversarial Network)

Generative Adversarial Networks (GANs) have become a widely used approach in deep learning. They consist of two components: a generator and a discriminator. The generator is designed to create realistic images, while the discriminator's role is to differentiate between genuine and generated images. (Zhang X et.al.,2021; K. Chauhan et.al.,2023; Y. Liu et.al.,2023; Ma, T. et.al.,2020; Du, W. et.al. 2020) The process is the same as a "two-person game". Both networks seek to optimize opposing objectives. CNN, which excels at analysing data and extracting features, GAN specializes in data generation. The flow chart of Image registration using GAN is shown in Figure 4. They enhance datasets through adversarial learning, producing synthetic images that help reduce modality differences. (Zhang X et.al.,2021; Y. Liu et.al.,2023; Dai, H. et.al.,2021; Zeng, L. et.al.,2020; Wang, S. et.al.,2018)

DATA ANALYSIS AND RESULTS

Here, Different remote-sensing SAR and PAN image pairs were used to discuss the proposed algorithm's performance quality and reliability.

Experimental Result

Image registration algorithms SIFT and SURF results were compared with the CNN model VGG16 to evaluate the feature matching ratio between the Reference image and the sensed image. These methods are all implemented under OpenCV on a PC with 11th Gen Intel(R) Core (TM) i5-11260H @ 2.60GHz 2.61 GHz, 16 GB.





Pamar Kinjal and Sudhir Vegad

Evaluation Parameters

To empirically validate the performance of an algorithm, we use two evaluation criteria to evaluate the performance as follows.

- RMSE (Root-Mean-Square Error) is calculated using all matched points. It manually selects N corresponding point pairs (x_i, y_i) , (x'_i, y'_i) from reference and sensed images to ensure the accuracy of the transformation parameter. The RMSE is calculated as follows: Where (x''_i, y''_i) denotes the transformed coordinates of (x'_i, y'_i) .

$$RMSE = \sqrt{\frac{1}{n} \sum_{i=1}^n ((x'_i - x''_i)^2 + (y'_i - y''_i)^2)} \quad (1)$$

- N is the number of correct correspondences detected between key points by matching a pair of reference and sensed images.
- 3) Accuracy is calculated based on the ratio of good matches to the total number of matching point pairs.

Various feature-matching traditional image registration methods are embraced for experiments: SIFT, SURF and VGG16 Deep learning model. Table 1 measures parameters such as RMSC, the number of matching features in both images and the accuracy of feature matching points.

Experiment 1

Feature extraction of SAR and PAN image pairs used SIFT and SURF to extract the features of remote sensing images. Image feature extraction and feature matching using SIFT is shown in Figure 6, and using SURF is shown in Figure 7. The same image pairs are used in the VGG16 model to extract image features. Extracted features of images using SIFT and SURF and fine-tuning the pre-trained model VGG16 are shown in table-1. It has been observed that SURF and SIFT generate fewer matching points when extracting features from SAR images compared to PAN images. However, VGG16 gives higher accuracy compared to SURF and SIFT. Image feature extraction using VGG16 Model is shown in Figure 5 at three different layer block1_conv1, block3_conv1, and block5_conv1 over the reference and sensed image.

Experiment 2

To verify the robustness of the VGG16 method, four SAR image pairs and one PAN image pair of remote-sensing images with deep features are tested. Various evaluation parameters, such as matched key point, RMSC and accuracy of image features using VGG16, have higher accuracy compared to SURF and SIFT, are listed in Table 1. Feature Matching using VGG16 gives higher accuracy at the block3_conv1 layer. The table below shows that the pre-trained VGG16 model has good features for processing the remote sensing images.

CONCLUSION

In this article, we present a novel feature extraction method for remote sensing images using SIFT, SURF and CNN Features. We utilize different remote sensing SAR and PAN images. The experimental results on multi-sensor images demonstrate that the fine-tuning pre-trained VGG16 model outperforms SURF and SIFT in terms of matching point accuracy and the correct matching points.

Future Work

In Future, we plan to work on CNN models such as Alex Net (Ren, C. et.al.,2020), Google Net (Ren, C. et.al.,2020) and Super Point and Super Point algorithms for image feature extraction and feature matching. (Sarlin, P.-E et.al, 2018; DeTone, D. et.al 2020)

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Table .1 Comparison of SAR and PAN Reference and Sensed image Pairs with Matching point, RMSC Calculation and Accuracy.

Image Dataset	Evaluation parameter (Detected Keypoint)	SIFT	SURF	CNN(VGG16)
SAR_im1	Ref Image	1063	460	68
	Sensed Image	1034	461	79
	Matched Keypoint	426	283	68
	RMSC	0.6562	1.25	2.6313
	Accuracy	41.20%	61.39%	91.00%
SAR_im2	Ref Image	689	414	49
	Sensed Image	614	407	49
	Matched Keypoint	204	165	48
	RMSC	0.7713	1.71	0.0001
	Accuracy	32.96%	38.28%	99.9%
SAR_im3	Ref Image	528	414	53
	Sensed Image	552	407	53
	Matched Keypoint	163	186	52
	RMSC	0.6117	4.27	0.0001
	Accuracy	30.87%	44.93%	99.92%
	Ref Image	380	403	50





Pamar Kinjal and Sudhir Vegad

SAR im4	Sensed Image	408	405	50
	Matched Keypoint	125	211	49
	RMSC	3.9799	1.66	0.0001
	Accuracy	32.89%	52.36%	99.9%
PAN im1	Ref Image	2500	500	56
	Sensed Image	2500	500	56
	Matched Keypoint	2300	400	54
	RMSC	0.0001	0.0001	0.0001
	Accuracy	91.32%	90.43%	99.98%

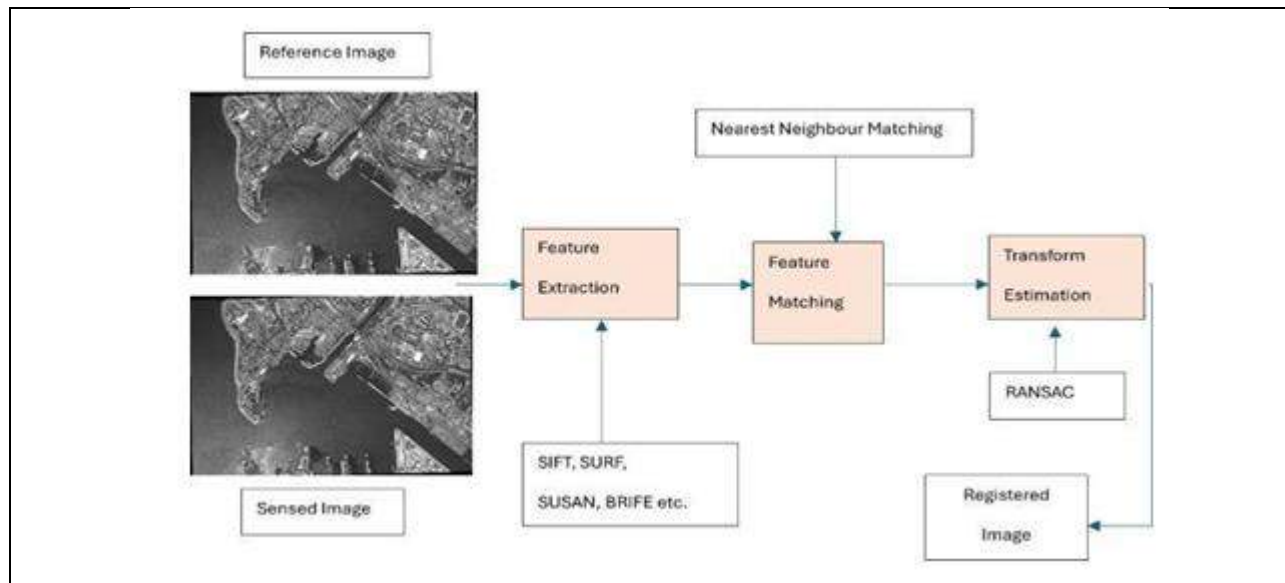
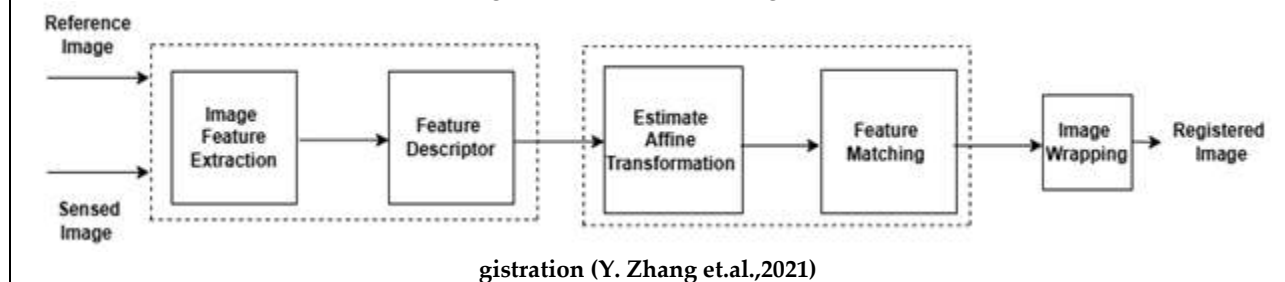


Figure 1 Feature-Based Image Re



gistration (Y. Zhang et.al.,2021)

Figure 2 Workflow Diagram of Registration (Liaghat, M.S. et al.,2023)





Pamar Kinjal and Sudhir Vegad

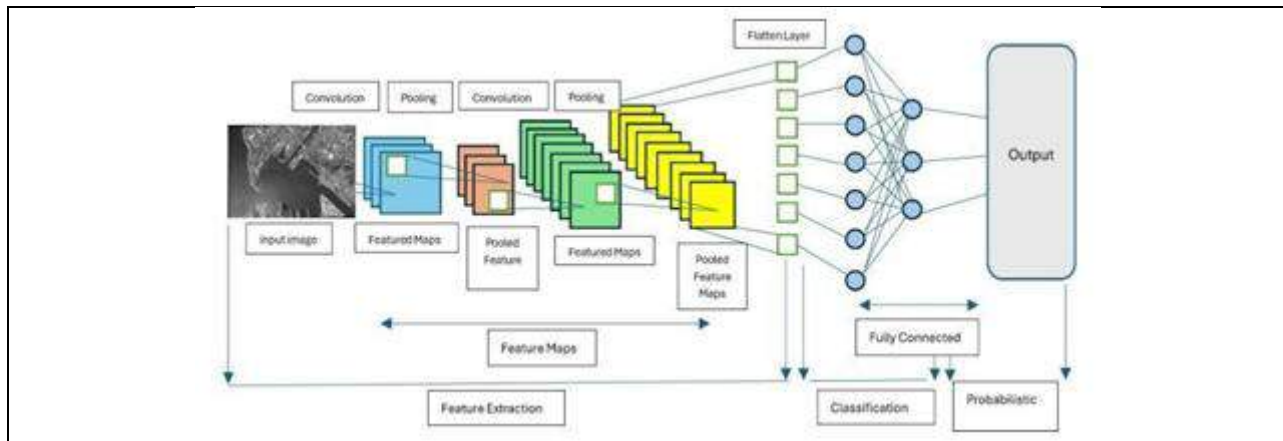


Figure 3 Basic CNN model for feature extraction (K. Chauhan et.al.,2023; Li, X. et.al.,2022)

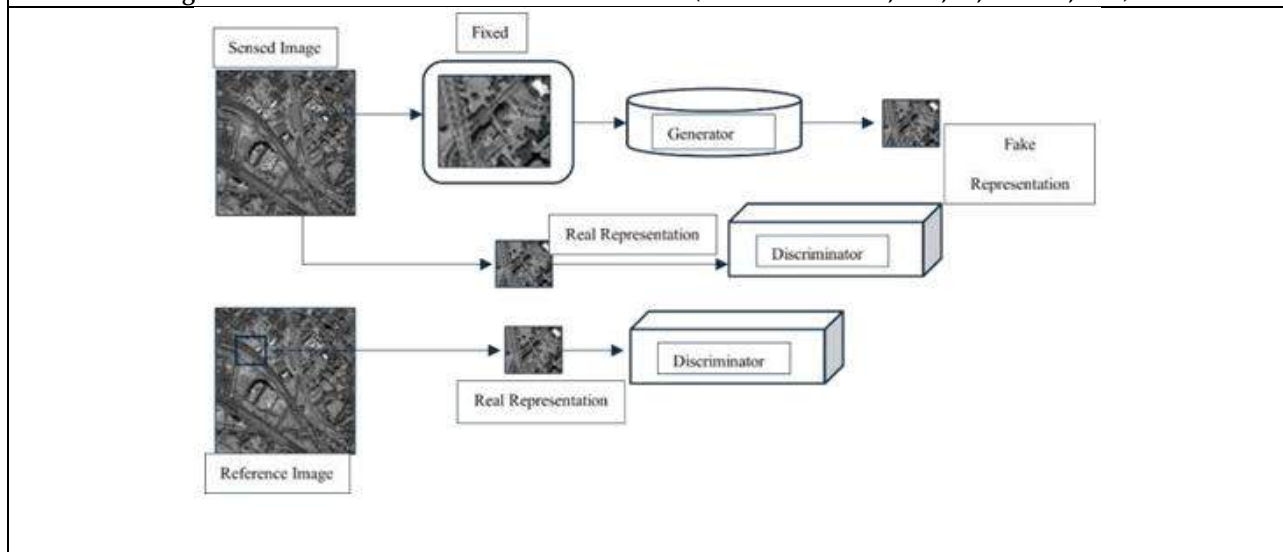
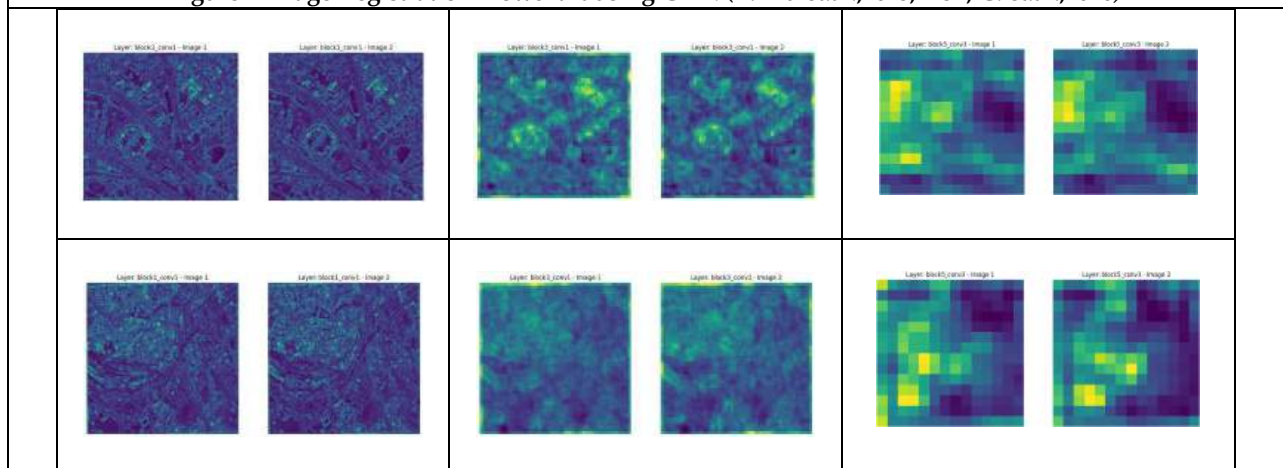


Figure 4 Image Registration Flowchart using GAN (Y. Liu et.al.,2023; Ren, C. et.al.,2020)



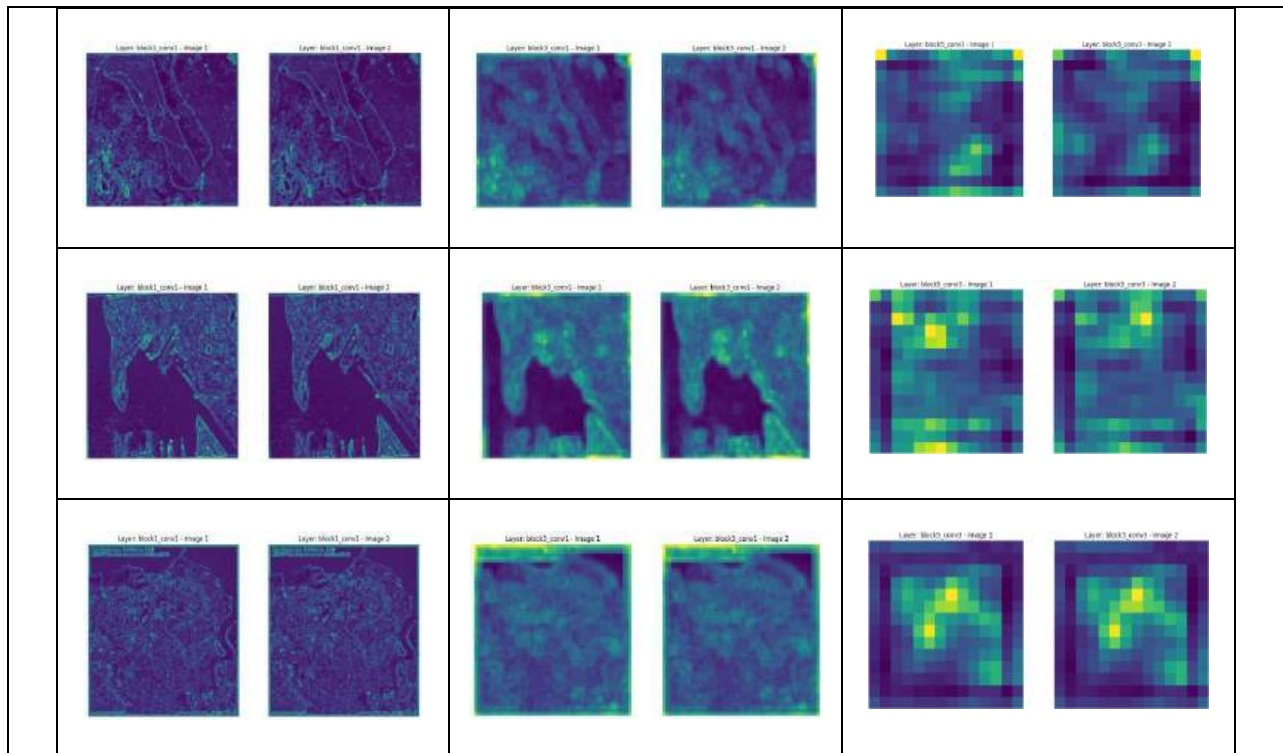
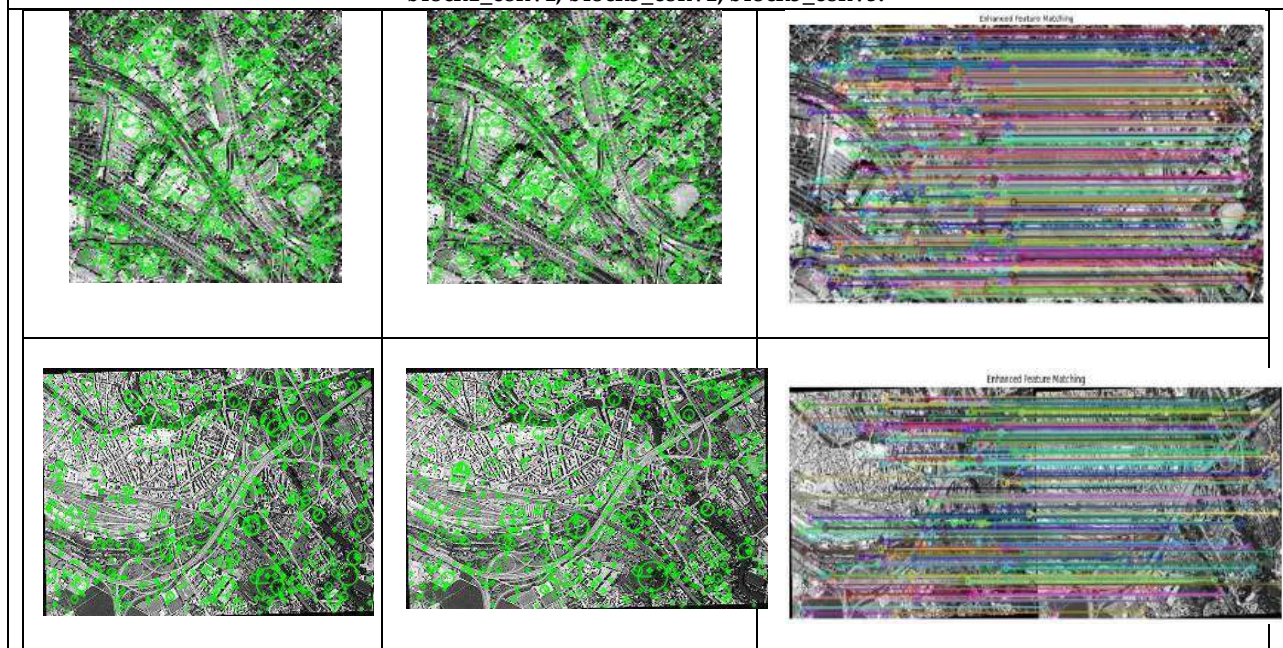
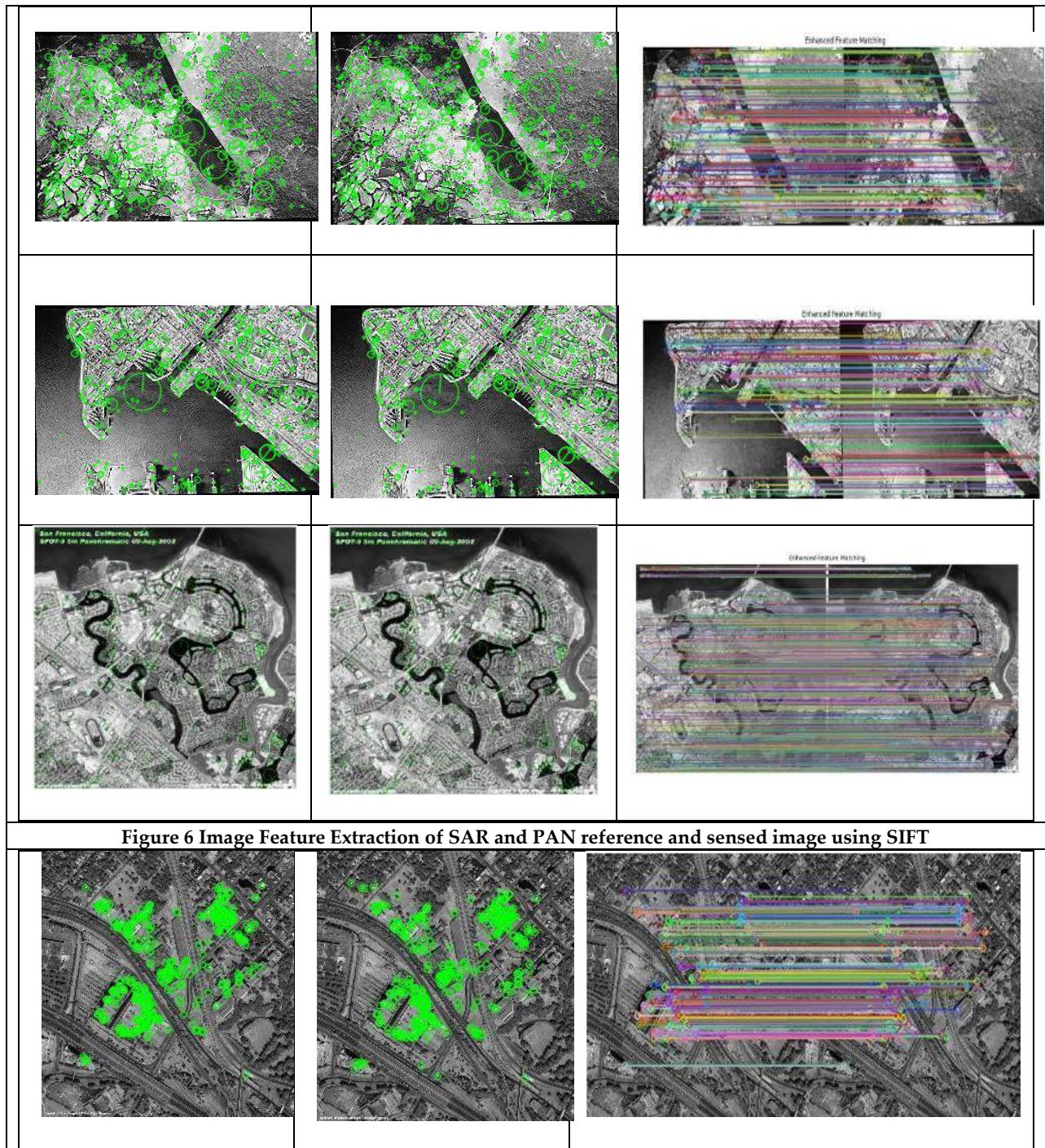


Figure 5 Image Feature Extraction of SAR and PAN reference and sensed image different layers using VGG16 at block1_conv1, block3_conv1, block5_conv3.







Pamar Kinjal and Sudhir Vegad

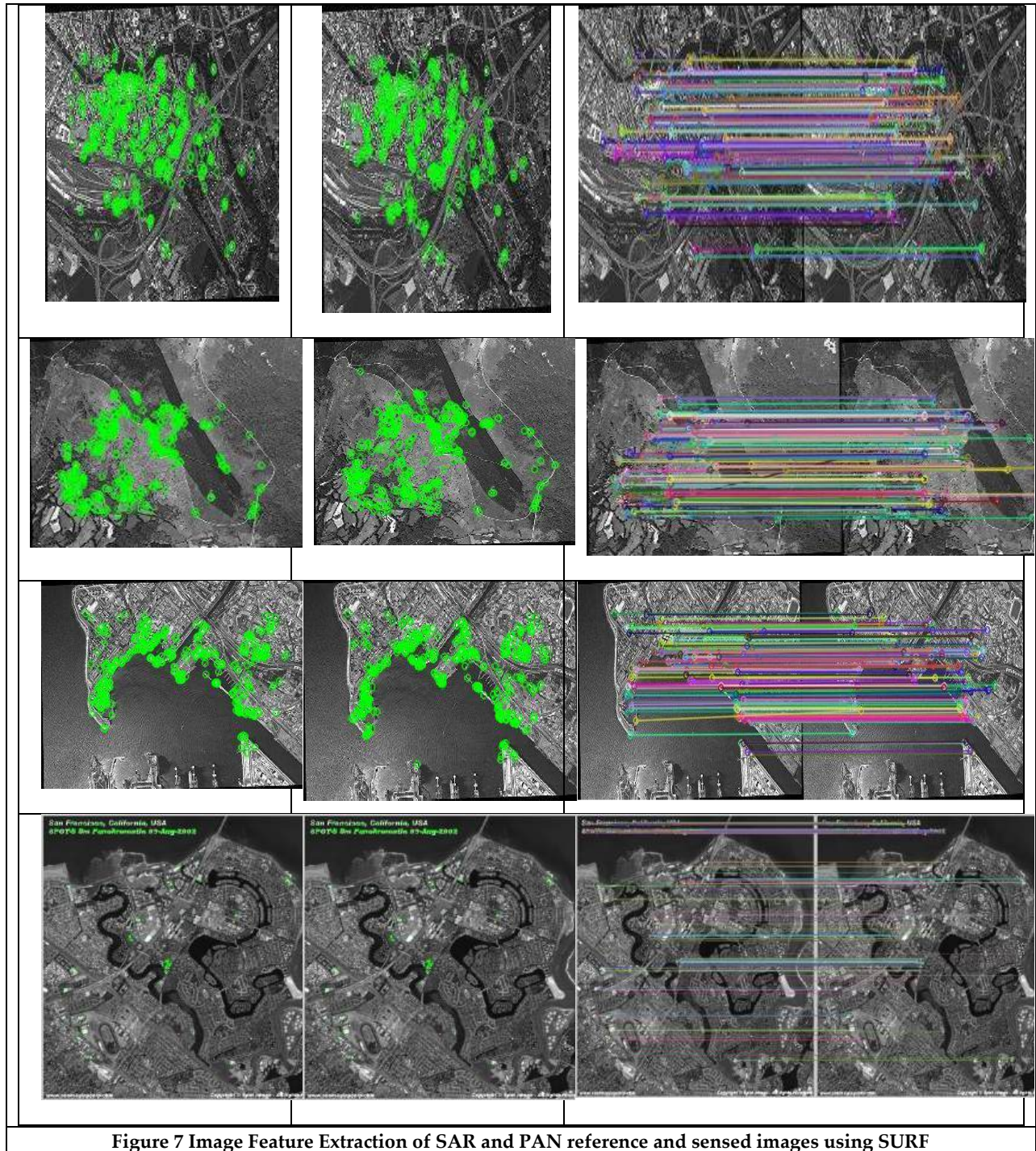
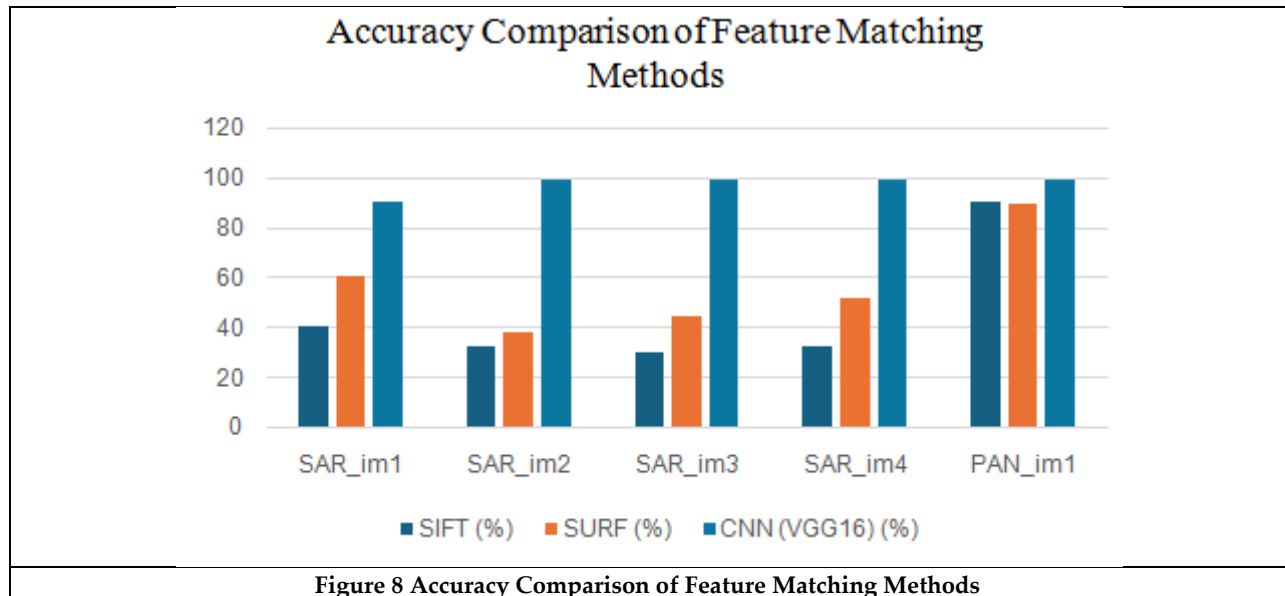


Figure 7 Image Feature Extraction of SAR and PAN reference and sensed images using SURF



**Pamar Kinjal and Sudhir Vegad**

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RESEARCH ARTICLE

Exploring the Role of Adhasakha Marma Therapy in the Management of Sciatica: A Conceptual Analysis

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ABSTRACT

Sciatica represents a debilitating condition characterized by excruciating pain and paraesthesias in the Sciatic nerve distribution or associated lumbosacral nerve root, which severely impact the quality of life of those affected. The Sciatic nerve plays a pivotal role, providing direct motor function to the posterior compartment thigh muscles and lower extremity adductors and indirect motor function to calf muscles, anterior lower leg muscles and intrinsic foot muscles. Its terminal branches indirectly contribute to sensation in the posterior and lateral lower leg and plantar aspect of foot. Marmas are important landmarks which is first ever explained in medical science, which gave importance to applied anatomy. Knowledge of AdhaSakha Marma plays a key role in treatment of Sciatica. Marma therapy aims to release blockages, balances energies & encourage body's innate healing abilities. The detail explanation of marma will be discussed in Paper. The purpose of this paper is to highlight how the knowledge of marma incorporating in treatment of Sciatica effectively improve the lives of patients suffering from Sciatica.

Keyword: Sciatic Nerve, Adhsakha Marma,



**Sunitha Valsan and Kanchan Sharma**

INTRODUCTION

Sciatica is a debilitating condition characterized by excruciating pain & paresthesia in the sciatic nerve distribution or associated lumbosacral nerve root. Its life time incidence is 13 % - 40% and rarely happens before age of 20 unless its injury related. Sciatic nerve is the nerve of posterior compartment of thigh with root value L4-S3. It divides into two branches just above popliteal fossa to Tibial & Common peroneal nerves ¹. It contains both anterior & posterior division of lumbosacral plexus and leaves the pelvis through greater sciatic foramen below piriformis to enter gluteal region descends between greater trochanter & ischial tuberosity along back of thigh.² The nerve has both Motor & Sensory supply. The motor supply is directly to all muscles of back of thigh & indirectly through its branches to all muscles of leg & foot. The sensory supply is to whole leg both dorsal & plantar surfaces of foot. ³The clinical presentation of sciatica is radicular pain from lower back down the buttocks, back of thigh, back of calf or sole of foot, sensory impairment in legs, muscular weakness, reflex impairment, gait dysfunction, paresthesia/ dysesthesias, edema in the lower extremity. The common causes include herniated disc, spinal stenosis, Piriformis Syndrome, spondylolisthesis, Back Injury, Bone spurs, Obesity.

The probable pathophysiology of pain occurs mainly through two mechanisms: compression and inflammation. Compression, microscopic venous stasis causes congestion of the vasa nervorum, which leads to radiculopathy. Compression also reduces blood flow to the nerve root, resulting in ischemia and formation of intraneural edema. This further causes demyelination and produces pain within the nerve root. In inflammation, histamines and bradykinin are released, which activate nociceptors. These nociceptors then transmit pain signals to the brain, leading to the sensation of pain. According to Acharya Sushruta, Marma points are the junction of five anatomical structures like Mamsa (muscle), Sira (vessels), Snayu (ligaments/ nerves), Asthi (bones) and sandhi (joints). These are very important vital places where Prana (vital life force) resides.⁴ Stimulation of Marma points channelizes Prana vayu and maintains equilibrium of Doshas.⁵ In Sciatica or Grdrasi, the patient has limping walk due to pain and stiffness. The adhasakha (lower limb) marmas like lohitaksha, Urvi, Ani, Janu, Indrabasti, Gulpha, Kurchashiras, Kurcha, Talahrdaya, Kshipra and Prishtā marma Kukundara⁶ are considered for study.

Objectives as Proposed

Primary Objective:

- To conceptually analyze the role of Adhasakha Marma therapy in the management of Sciatica

Secondary Objective:

- To understand the Ayurvedic theoretical framework around Marma points related to the sciatic nerve.
- To assess the potential pathways through which Adhasakha Marma Therapy may relieve pain and inflammation in sciatica.
- To propose a preliminary model for integrating Marma therapy in clinical practice for sciatica management.

Objectives achieved

Primary Objective:

- The conceptual role of Adhasakha Marma therapy in the management of Sciatica has been thoroughly analyzed.

Secondary Objectives:

- The Ayurvedic theoretical framework surrounding Marma points associated with the sciatic nerve has been clearly understood and articulated.
- The potential pathway through which Adhasakha Marma therapy alleviates pain and inflammation in sciatica have been effectively assessed
- A preliminary model for integrating Marma therapy into clinical practice for the management of sciatica has been successfully proposed.



**Sunitha Valsan and Kanchan Sharma**

METHODOLOGY

Design: Conceptual and Qualitative.

Data Sources:

- Primary : Classical Ayurvedic texts
- Secondary: Peer – reviewed journals from PubMed, JSTOR, Scopus.

Analysis Tools:

- Thematic analysis using NVivo.
- Comparative correlation mapping between anatomical sciatic pathways and Marma locations.

Ethical Considerations: Proper citations and respect for traditional knowledge were maintained. No human or animal subjects were involved.

RESULTS

Conceptual Correlation of Adhasakha Marma with Sciatic Pathway

Comparative Analysis:

- Marma therapy suggests pressure stimulation to modulate Vata dosha, which is associated with nerve function.
- Conventional medicine relies on anti – inflammatory and mechanical decompression.
- Proposed model integrates both systems for synergistic benefit

DISCUSSION

This conceptual analysis emphasizes the therapeutic significance of Adhasakha Marma points in the management of sciatica. Sciatica is typically caused by compression or irritation of the sciatic nerve or its branches – tibial and deep peroneal nerves – often resulting in pain, numbness or weakness in the lower limb. These nerves are anatomically aligned with key Marma points such as Kshipra, Talahrdaya, Gulpha, Indravasthi, Janu, Ani, Urvi, Lohitaksha and Kukundara making these locations particularly relevant for targeted Ayurvedic intervention. Most Marma points are superficial located near the skin, muscles, ligaments, nerves and blood vessels. This unique positioning allows non – invasive stimulation (e.g via gentle pressure or massage) to directly influence the underlying neurovascular structures. This stimulation facilitates several physiological processes, including:

Enhanced Microcirculation

Stimulation of Adhasakha Marma points produces vasodilation and improves capillary blood flow around the affected nerves and muscle tissues. This enhancement of microcirculation increases oxygen delivery and nutrient supply, both of which are essential for proper nerve function and tissue regeneration. Improved circulation also facilitates efficient lymphatic drainage, helping to reduce local inflammation and edema that frequently contribute to compression of the sciatic nerve. By minimizing stagnation and improving tissue perfusion, Marma therapy creates favorable conditions for symptomatic relief and recovery.

Promotion of Tissue Repair and Healing

Gentle stimulation of Marma points is believed to activate local healing mechanisms, including fibroblast activity and tissue regeneration processes. These effects may support the repair of connective tissues and the myelin sheath surrounding damaged nerves. In addition, Marma therapy helps modulate pro-inflammatory cytokines and encourages anti-inflammatory healing responses. By improving circulation and relieving muscular tension, it establishes a supportive microenvironment for the repair of muscles, fascia, and peri-neural tissues affected by chronic sciatica. This regenerative effect contributes not only to pain relief but also to restoration of tissue integrity and function.



**Sunitha Valsan and Kanchan Sharma****Neurovascular and Musculoskeletal Modulation**

Many Marma points are anatomically located near important neurovascular bundles. Their stimulation may influence somatic afferent pathways and help reset abnormal pain signaling mechanisms. Marma therapy also aids in releasing myofascial tension, thereby reducing mechanical compression on the sciatic nerve, especially in conditions such as piriformis syndrome–related sciatica. Through relaxation of tight muscles and restoration of soft tissue flexibility, the therapy improves the functional alignment of muscles, tendons, and ligaments of the lower limb. Consequently, patients experience enhanced mobility, balance, and musculoskeletal coordination.

Energetic and Dosha-Based Perspective

From the Ayurvedic perspective, Gridhrasi primarily results from aggravation of *Vata Dosha*. Stimulation of Adhasakha Marma points helps pacify *Vata*, particularly *Apana Vata*, which governs the functions of the lower part of the body. Improved circulation and nerve stimulation also promote the smooth flow of *Prana* through the *Srotas* (body channels), restoring energetic balance and systemic vitality. Thus, Marma therapy not only addresses physical symptoms but also supports overall physiological and energetic harmony.

Integrated Benefits of Adhasakha Marma Therapy in Sciatica

Adhasakha Marma therapy offers a comprehensive therapeutic effect in sciatica through multiple mechanisms. Enhanced microcirculation improves oxygen and nutrient supply to nerves and soft tissues. Reduction of inflammation relieves nerve compression symptoms. Activation of tissue repair mechanisms promotes healing of nerve sheaths, muscle fibers, and fascia. Pain modulation occurs through gate control mechanisms and endogenous endorphin release. Simultaneously, regulation of *Dosha* and *Prana* helps restore systemic balance and vitality.

CONCLUSIONS

This conceptual study provides a comprehensive analysis of the role of Adhasakha Marma therapy in the management of Sciatica (Gridhrasi) by integrating traditional Ayurvedic knowledge with modern anatomical and physiological understanding. The eleven identified Adhasakha Marma points including Kukundara, were studied in detail for their locations, measurements, structural classifications and correlation with key neurovascular structures implicated in sciatica, particularly the sciatic nerve, deep peroneal nerve and tibial nerve. So the Adhasakha Marma therapy presents itself as a scientifically plausible and clinically promising complementary approach to Sciatica management. By leveraging both anatomical precision and energetic regulation, this therapy embodies a holistic, patient centered strategy with potential to reduce reliance on pharmacological treatments. It promotes functional recovery, improves mobility and addresses both the root cause and symptoms of Sciatic nerve irritation.

Future Scope

- Clinical trials are necessary to empirically validate the analgesic and regenerative effects of Marma therapy on Sciatic pain.
- Quantitative studies using Doppler Imaging, Thermography and nerve conduction studies can evaluate the physiological responses (eg : microcirculation, nerve conductivity)
- Protocol development for standardising pressure application, duration and frequency in clinical settings.
- Interdisciplinary collaborations between Ayurvedic practitioners and neurologists to explore integrative pain clinics.

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Sunitha Valsan and Kanchan Sharma

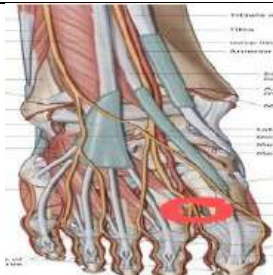
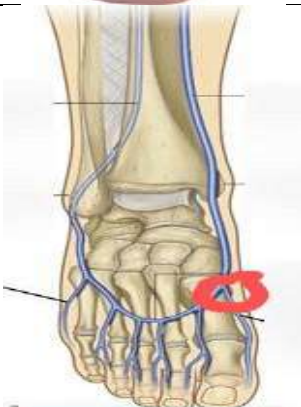
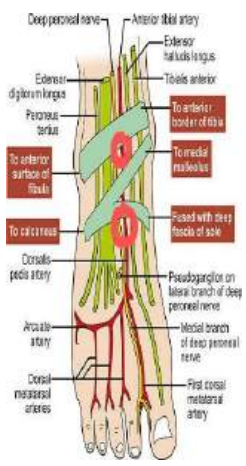
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Sunitha Valsan and Kanchan Sharma

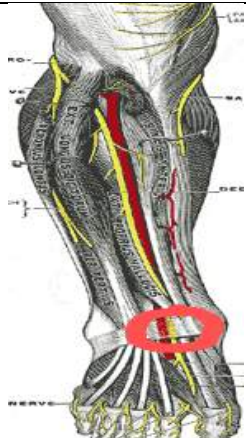
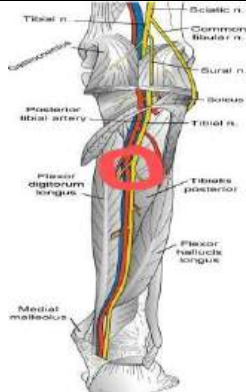
Table 1: Conceptual Correlation of Adhasakha Marma with Sciatic Pathway

SL No	Marma Name	Location (Ayurvedic)	Modern Anatomical Correlation	Type	Number	Measurement (Angula)	Figure
1	Kshipra	Junction of the big toe and foot (1 st web space).[7]	Branch of Dorsalis Pedis artery and Deep Peroneal Nerve.[8]	Sira Marm a	2	½ Angula	
2	Talahridaya	Center of the sole.[9]	Medial Plantar artery and nerve.[10]	Sira Marm a	2	½ Angula	
3	Kurcha	Dorsum above big toe on medial aspect (2 angula). [11]	Dorsal digital vein on the medial side of big toe joins the medial end of dorsal venous arch to form Gtr. Saphenous vein.[12]	Snayu Marm a	2	4 Angula	
4	Kurchashir a	Just above ankle joint.[13]	Superior retinaculum – Anterior tibial artery & deep peroneal nerve. Inferior retinaculum – Upperband – deep lies tibialis anterior & deep peroneal nerve. Lower band – deep lies dorsalis	Snayu Marm a	2	1 Angula	





Sunitha Valsan and Kanchan Sharma

			pedis & deep peroneal nerve.[14]				
5	Gulpha	Ankle Joint (Precisely front of Ankle Joint).[15]	Dorsalis pedis artery which is direct continuation of Anterior tibial artery. Deep peroneal nerve : comes in anterior relation.[16]	Sandhi Marm a	2	2 Angulas	
6	Indravasti	Below to back of knee near tendinous arch of soleus lies.[17]	Both posterior tibial artery & tibial nerve lies deep to tendinous arch.[18]	Sira Marm a	2	½ Angula	
7	Janu	Posterior aspect of knee joint (middle part of popliteal fossa).[19]	Popliteal vessels & Tibial nerve. Knee jt has rich arterial anastomosis.[20]	Sandhi Marm a	2	3 Angula	




Sunitha Valsan and Kanchan Sharma

8	Ani	Upper part of popliteal fossa.[21]	Popliteal artery, popliteal vein & tibial nerve lies medial to lateral.[22]	Snayu Marm a	2	½ Angula	
9	Urvi	Mid part of front of thigh.[23]	Femoral artery, femoral vein & profunda vessels lie in same plane. Femoral nerve crosses the structure from lateral to medial aspect.[24]	Sira Marm a	2	½ Angula	
10	Lohitaksha	Medial upper part of thigh (femoral triangle) [25]	femoral artery & its branches, femoral vein & its tributaries, femoral nerve.[26]	Sira Marm a	2	½ Angula	
11	Kukundara	Greater sciatic foramen [27]	Sciatic nerve, posterior femoral cutaneous nerve, inferior gluteal vessels & nerves.[28]	Snayu Marm a	2	½ Angula	



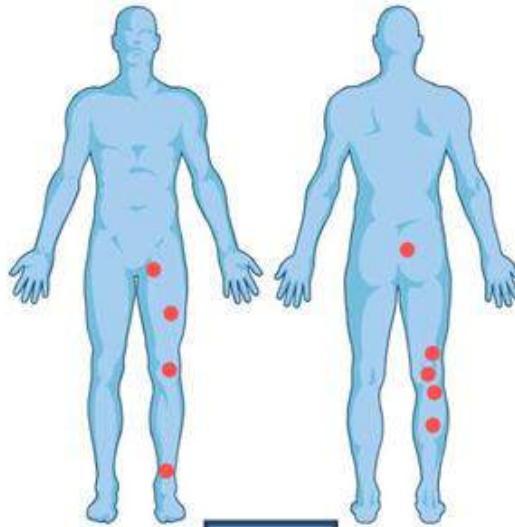


Figure 1:



Figure 2:



Figure 3:

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REVIEW ARTICLE

Baobab (*Adansonia digitata* L.) Nanosuspension : A Review of an Emerging Herbal Drug Delivery Strategy for Metabolic DisordersS K Asiya¹, I Anjali², S Komali², Rama Rao Nadendla³ and P Ravali^{2*}¹Assistant Professor, Department of Pharmaceutics, Chalapathi Institute of Pharmaceutical Sciences, (Affiliated to Acharya Nagarjuna University), Lam, Guntur, Andhra Pradesh, India²M. Pharmacy Student, Department of Pharmaceutics, Chalapathi Institute of Pharmaceutical Sciences, (Affiliated to Acharya Nagarjuna University), Lam, Guntur, Andhra Pradesh, India³Principal, Department of Pharmaceutics, Chalapathi Institute of Pharmaceutical Sciences, (Affiliated to Acharya Nagarjuna University), Lam, Guntur, Andhra Pradesh, India

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ABSTRACT

Baobab (*Adansonia digitata* L.) is a nutrient-rich medicinal plant widely studied for its potential in managing metabolic disorders. Its fruit pulp contains bioactive compounds such as vitamin C, polyphenols, flavonoids, tannins, and pectin, which exhibit antioxidant, antidiabetic, anti-inflammatory, and cardioprotective properties. However, the therapeutic application of these phytochemicals is limited due to poor aqueous solubility, instability, and low bioavailability.[1] Nanosuspension technology offers an effective drug delivery approach to overcome these challenges by reducing particle size, thereby enhancing dissolution rate, solubility, and absorption of poorly soluble compounds. This review summarizes the formulation techniques, characterization methods, and therapeutic advantages of baobab-based nanosuspensions.[4] It also highlights their potential in improving the effectiveness of bioactive compounds for treating metabolic disorders such as diabetes, obesity, and cardiovascular diseases. Recent advancements in nanotechnology and its integration with herbal medicine are also discussed, along with safety considerations and future research directions [3]. Overall, baobab nanosuspensions represent a promising strategy to enhance the pharmacological performance of plant-derived compounds and broaden their application in pharmaceutical and nutraceutical industries.[2]

Keywords: Baobab (*Adansonia digitata* L.); nanosuspension; metabolic disorders; bioavailability enhancement; nanotechnology; herbal drug delivery; phytochemicals; therapeutic efficacy





INTRODUCTION

Africa is a significant reservoir of medicinal plants rich in bioactive compounds with therapeutic potential. Among these, *Adansonia digitata* L., commonly referred to as baobab, is extensively found in sub-Saharan Africa and Madagascar. Traditionally, various parts of the baobab tree have been used in foods, beverages, and indigenous medicinal practices.[8] Baobab fruit pulp is highly nutritious, providing abundant vitamin C, both soluble and insoluble dietary fiber, essential minerals, and polyphenolic compounds such as flavonoids and tannins. These components are responsible for its notable antioxidant activity and role in regulating metabolic functions.[12](Figure 1). Recent studies suggest that baobab fruit pulp powder exhibits prebiotic properties due to its pectin-rich polysaccharides, particularly low-methylated homogalacturonan. In vitro fermentation findings show increased production of short-chain fatty acids, including acetate, propionate, and butyrate, indicating selective utilization by beneficial gut microbiota. Disruptions in gut microbiota and increased intestinal permeability are linked to conditions such as obesity, type 2 diabetes, and cardiovascular diseases. The movement of bacterial endotoxins like lipopolysaccharides into circulation contributes to chronic low-grade inflammation, marked by elevated TNF- α and IL-6 levels. Dietary fiber intake plays a crucial role in improving gut microbial balance and strengthening intestinal barrier integrity.[10].

Clinical studies further indicate that baobab extract can lower postprandial blood glucose levels, reduce insulin response, and promote satiety. However, the limited water solubility of its phenolic compounds hinders their absorption and overall bioavailability, highlighting the need for advanced delivery systems. Advancements in nanotechnology have significantly transformed drug delivery systems, offering effective strategies to address the shortcomings of traditional formulations. Although herbal medicines have been widely used for centuries, their therapeutic application is often limited by factors such as inconsistency in active components, poor stability, and variable clinical outcomes. These limitations are particularly evident in phytochemicals that exhibit low solubility in water and inadequate absorption in the gastrointestinal tract, ultimately affecting their effectiveness.[7]. Nanosuspension technology has gained attention as an efficient approach to improve the delivery of such poorly soluble compounds. These formulations are composed of fine dispersions of drug particles in the nanometer size range, stabilized using suitable surfactants or polymers. The reduction in particle size enhances surface area, which in turn increases dissolution rate and saturation solubility. Additionally, improved adhesion to biological surfaces promotes better absorption, resulting in enhanced bioavailability.[13]

The utilization of nanosuspension systems for plant-based therapeutics, including *Adansonia digitata* L., is an emerging area of research with considerable potential. Although baobab has demonstrated a range of beneficial pharmacological activities, its formulation into advanced delivery systems is still at an early stage. Converting baobab-derived bioactive compounds into nanoscale formulations may help overcome challenges such as low solubility, instability, and limited systemic distribution. Moreover, nanosuspensions provide several advantages, including the possibility of lowering required dosages, enhancing targeted delivery, and improving patient adherence to treatment. These features are particularly important in the long-term management of chronic metabolic conditions such as obesity and Type 2 Diabetes. Improved delivery of baobab bioactives could support better regulation of metabolic processes, enhanced glucose control, and mitigation of oxidative damage.[11]

However, despite these advantages, certain challenges must be addressed, including issues related to scalability, formulation stability over time, regulatory considerations, and the need for standardization of herbal-based nanosystems. Furthermore, extensive in vivo investigations and clinical evaluations are essential to establish their safety, efficacy, and pharmacokinetic benefits. Overall, combining traditional medicinal knowledge of baobab with modern nanotechnology offers a promising pathway for developing advanced herbal therapies. Continued research is necessary to bridge existing gaps and fully realize the potential of baobab nanosuspensions in the treatment of metabolic disorders.[14]





THEMATIC REVIEW

Metabolic disorders:

Metabolic disorders such as type 2 diabetes mellitus, obesity, and dyslipidemia are now recognized not as isolated conditions but as interrelated components of metabolic syndrome. This syndrome is characterized by a complex network of abnormalities, including insulin resistance, persistent low-grade inflammation, oxidative stress, and disturbed lipid metabolism. At the molecular level, these conditions arise from dysregulation of several key signaling pathways, including insulin receptor signaling, the AMP-activated protein kinase (AMPK) pathway, peroxisome proliferator-activated receptors (PPARs), and inflammatory pathways such as NF- κ B.

Conventional treatment strategies typically focus on a single target, which often necessitates the use of multiple medications. This polypharmacy approach can increase adverse effects and reduce patient adherence. As a result, there is growing interest in plant-based therapies that naturally exert multi-target pharmacological effects. Among such medicinal plants, *Adansonia digitata* L. (baobab) has emerged as a promising functional food with wide-ranging metabolic benefits. However, converting its traditional therapeutic use into effective modern medicine requires addressing key formulation challenges, where nanosuspension technology plays a significant role.

Baobab as a Functional Phytotherapeutic Agent

Baobab is commonly referred to as a “superfruit,” though its medicinal importance extends well beyond its nutritional value. Different parts of the plant—including fruit pulp, leaves, seeds, and bark—contain diverse bioactive compounds with significant pharmacological potential.

Phytochemical Complexity and Synergistic Action

The therapeutic efficacy of baobab is attributed to its rich and complex phytochemical composition, which includes polyphenols (such as flavonoids, phenolic acids, and procyanidins), tiliroside (a compound associated with lipid regulation), high vitamin C content, dietary fibers (both soluble and insoluble), and essential minerals like potassium, calcium, and magnesium. Unlike single-compound synthetic drugs, these phytochemicals do not act independently. Instead, they function synergistically, enhancing one another’s biological activities. This multi-component interaction is particularly advantageous in metabolic disorders, where multiple physiological pathways are simultaneously disrupted.[13]

Mechanisms of Action in Metabolic Disorders (Paraphrased)

Glycemic Control

Baobab exhibits notable antidiabetic activity through multiple biological mechanisms. A key action involves the inhibition of carbohydrate-digesting enzymes, particularly α -amylase and α -glucosidase, which are responsible for converting complex carbohydrates into glucose. By slowing this enzymatic breakdown, baobab helps reduce rapid increases in post-meal blood glucose levels. Furthermore, bioactive constituents in baobab enhance insulin sensitivity by promoting glucose uptake in peripheral tissues. This combined effect—limiting glucose release while improving cellular utilization—supports its effectiveness in managing type 2 diabetes mellitus.[14]

Lipid Regulation

Dyslipidemia, a key feature of metabolic syndrome, can also be positively influenced by baobab consumption. It has been reported to help lower total cholesterol and triglyceride levels while reducing low-density lipoprotein (LDL) and increasing high-density lipoprotein (HDL). Among its phytochemicals, tiliroside plays an important role in lipid homeostasis by modulating enzymes involved in both lipid synthesis and degradation, thereby contributing to improved metabolic balance.





Asiya et al.,

Anti-Inflammatory Effects

Chronic low-grade inflammation is a key contributor to the development and progression of metabolic disorders. The polyphenolic compounds present in baobab help attenuate this process by downregulating inflammatory mediators such as cytokines and prostaglandins. As a result, systemic inflammation is reduced, leading to improved overall metabolic function.

Antioxidant Defense

Oxidative stress is a major feature of metabolic diseases and arises due to an imbalance between reactive oxygen species (ROS) production and the body's antioxidant defenses. Baobab contains high levels of antioxidants, especially vitamin C and polyphenols, which play a protective role by neutralizing free radicals, safeguarding pancreatic β -cells, and inhibiting lipid peroxidation.

Gut Microbiome Modulation

An increasing area of research focuses on the influence of the gut microbiome on metabolic health. The high dietary fiber content of baobab functions as a prebiotic, supporting the proliferation of beneficial intestinal microorganisms. These microbes generate short-chain fatty acids (SCFAs), which contribute to improved insulin sensitivity, reduced inflammatory responses, and enhanced energy metabolism.

Limitations of Conventional Baobab Formulations

Although baobab possesses a strong pharmacological profile, its application in conventional dosage forms such as powders, capsules, or crude extracts is associated with several limitations.

A major concern is its poor solubility, particularly of polyphenolic constituents, which restricts efficient absorption in the gastrointestinal tract. Moreover, many of its bioactive compounds are chemically unstable, prone to degradation during digestion, and rapidly metabolized in the liver. Together, these factors lead to low bioavailability, with only a limited portion of the administered dose reaching systemic circulation in an active form.

In addition, there is considerable variability in the phytochemical composition of baobab, influenced by factors such as geographical origin, harvesting practices, and processing techniques. This inconsistency can result in variable therapeutic efficacy and challenges in standardization.

Nanosuspension Technology: A Transformative Approach

Nanosuspensions are an advanced drug delivery system designed to overcome the drawbacks of conventional herbal formulations. They consist of colloidal dispersions of drug particles reduced to the nanometer scale and stabilized using suitable surfactants or polymeric agents.[15]

Advantages of Nanosuspensions

A major benefit of nanosuspension systems is their ability to significantly improve solubility and dissolution rate. Based on the Noyes–Whitney principle, decreasing particle size leads to an increase in surface area, which subsequently enhances the dissolution process.

In the case of baobab, this results in:

- Accelerated release of bioactive constituents
- Enhanced gastrointestinal absorption
- Increased overall bioavailability

Baobab Nanosuspension: Mechanistic Insights**Improved Bioavailability and Absorption**

Formulating baobab extract into a nanosuspension markedly enhances its interaction with biological membranes due to the significantly reduced particle size. This results in greater intestinal permeability and more efficient entry of bioactive compounds into systemic circulation. Consequently, comparable or enhanced therapeutic effects can be achieved using lower doses than those required in conventional formulations.





Asiya et al.,

Enhanced Enzyme Interaction

The nanoscale dimensions of baobab particles facilitate more efficient contact with digestive enzymes. This improves their inhibitory potential against α -amylase and α -glucosidase, thereby contributing to more effective regulation of post-meal blood glucose levels.

Protection of Bioactive Compounds

Nanosuspension systems offer a stabilizing matrix for sensitive phytochemicals, protecting them from degradation caused by oxidation, fluctuations in gastrointestinal pH, and enzymatic activity. This protective effect helps preserve the integrity of the active constituents, allowing a higher fraction to reach the intended site of action in an active form.

Sustained Release and Pharmacokinetic Improvement

An additional key benefit of nanosuspension systems is their capability to provide controlled or prolonged drug release. This results in more consistent plasma drug levels over an extended period, thereby decreasing dosing frequency and enhancing patient adherence to therapy.

Methods of Preparation of Baobab Nanosuspension

Nanosuspensions can generally be produced using two main strategies: top-down and bottom-up approaches. In top-down techniques, larger particles are mechanically reduced to the nanoscale, while in bottom-up methods, nanoparticles are formed through controlled assembly from molecular or solution-based precursors.

For herbal substances such as *Adansonia digitata*, these approaches are especially valuable, as many of their active phytochemicals exhibit low solubility and limited stability in traditional formulation systems.

Ultrasonication

The ultrasonication method for preparing a baobab nanosuspension begins with accurately weighing the *Adansonia digitata* extract. This extract is then dispersed in an appropriate aqueous phase, followed by the incorporation of stabilizers such as Tween 80, Poloxamer 188, polyvinylpyrrolidone, or hydroxypropyl methylcellulose to maintain system stability. The mixture is initially homogenized using magnetic stirring to ensure uniform particle distribution.(Figure 2) . Thereafter, the dispersion is exposed to ultrasonic energy using either a probe or bath sonicator. High-frequency sound waves generate acoustic cavitation, in which microscopic bubbles form and collapse, producing strong shear forces that progressively reduce particle size. The process is carried out for a defined time under controlled temperature conditions to avoid thermal degradation of heat-sensitive phytoconstituents. With continued sonication, particles are reduced to the nanometer scale, yielding a stable nanosuspension. The final formulation is then cooled if required and stored under suitable conditions for further analysis.[15](Table 2)

High-Speed Homogenization

In the high-speed homogenization technique, the baobab extract is first dispersed in a suitable liquid medium containing stabilizing agents to prevent particle aggregation. This initial mixture is gently stirred to obtain a coarse, uniformly distributed suspension. The pre-suspension is then processed in a high-speed homogenizer equipped with a rotor–stator system. The equipment operates at high speeds, typically between 10,000 and 25,000 rpm, generating intense shear forces, turbulence, and pressure gradients. These mechanical effects progressively break down particles into finer sizes. The homogenization is generally performed over several cycles to achieve a uniform nanoscale distribution. Throughout the process, temperature is carefully monitored to prevent degradation of thermolabile bioactive compounds present in baobab. After completion, the resulting nanosuspension is collected and allowed to stabilize, forming a homogeneous and finely dispersed system suitable for further evaluation.[16]





Asiya et al.,

Anti-Solvent Precipitation

The anti-solvent precipitation method begins with dissolving the baobab extract in a suitable organic solvent that ensures complete solubilization of its active constituents, forming a clear solution. Separately, an aqueous anti-solvent phase containing stabilizers is prepared. The organic phase is then introduced gradually or rapidly into the anti-solvent under constant stirring. This sudden change in solvent environment causes a sharp reduction in solubility, resulting in a supersaturated state. This condition initiates rapid nucleation, where fine particles are formed almost instantaneously. As mixing continues, these nuclei grow into nanoparticles, while stabilizers adsorb onto their surfaces to prevent aggregation and control particle growth. Critical process parameters such as addition rate, stirring speed, and temperature are optimized to ensure uniform particle formation. After precipitation, the organic solvent is removed using evaporation or other suitable techniques, yielding a stable nanosuspension. The final product is then collected and stored appropriately for subsequent characterization and applications.[16]

Role of Stabilizers

Irrespective of the preparation technique employed, stabilizers play a crucial role in ensuring the physical stability of nanosuspensions. They prevent particle aggregation and maintain a uniform dispersion over time.

Frequently used stabilizing agents include:

- Tween 80, a surfactant that reduces surface tension
- Poloxamer 188, a non-ionic stabilizer that enhances dispersion stability
- Polyvinylpyrrolidone (PVP), which provides steric stabilization
- Hydroxypropyl methylcellulose (HPMC), used to improve viscosity and prevent particle coalescence

Evaluation tests

Particle Size and Polydispersity Index (PDI)

Particle size is a fundamental parameter in nanosuspension systems as it directly affects solubility, dissolution behavior, bioavailability, and overall therapeutic efficiency. For measurement, the nanosuspension is first suitably diluted to prevent multiple scattering effects, and then analyzed using techniques such as dynamic light scattering (photon correlation spectroscopy), laser diffraction, or Coulter counter methods. Among these, dynamic light scattering is most commonly used because it evaluates Brownian motion and translates it into particle size distribution. (Table 1). Along with particle size, the polydispersity index (PDI) indicates the uniformity of particles within the formulation. A PDI value in the range of 0.1–0.25 reflects a narrow and homogeneous size distribution, suggesting uniform particles. Higher PDI values indicate greater variability, which may contribute to instability through aggregation or uneven sedimentation. For herbal nanosuspensions like those derived from *Adansonia digitata*, achieving uniform particle distribution is particularly important due to the complex nature of plant-derived constituents. Together, particle size and PDI provide a reliable assessment of formulation quality and consistency.[17]

Zeta Potential

Zeta potential represents the surface charge of nanoparticles and is a key factor in predicting nanosuspension stability. It is determined using electrophoretic light scattering, where particle movement under an electric field is measured to calculate surface charge. In general, zeta potential reflects the degree of repulsion between particles. Higher absolute values (usually above ± 30 mV) indicate strong electrostatic repulsion, which helps prevent aggregation and improves stability. In formulations stabilized with both polymers and surfactants, stability may arise from combined electrostatic and steric effects, known as electrosteric stabilization; in such systems, even values around ± 20 mV may be sufficient. In baobab-based nanosuspensions, polymers not only impart surface charge but also create a protective coating around particles, enhancing long-term stability. Low zeta potential values suggest a higher tendency for aggregation, leading to sedimentation and reduced nanosuspension efficiency. Thus, zeta potential serves as both a stability indicator and a predictor of formulation shelf life.[13]





Saturation Solubility and Dissolution Rate

Enhancement of solubility and dissolution rate is one of the main objectives of nanosuspension development. Saturation solubility is assessed by adding excess nanosuspension to a solvent, followed by continuous shaking until equilibrium is achieved. The mixture is then filtered, and the dissolved drug concentration is measured using techniques such as UV–visible spectrophotometry. The improvement in dissolution can be explained by the Noyes–Whitney equation, which relates increased surface area to faster dissolution rates. Due to their nanoscale size, nanosuspensions provide a much larger surface area compared to coarse particles, leading to significantly improved dissolution. Dissolution studies are typically performed using standard apparatus under controlled conditions, with samples withdrawn at specific time intervals to determine release profiles. Enhanced dissolution improves absorption and in vivo performance, particularly for poorly water-soluble compounds. In baobab systems, this is especially important for polyphenols, which otherwise show limited bioavailability.

Stability Studies

Stability studies are conducted to evaluate the long-term behavior of nanosuspensions under different storage conditions. Formulations are stored at varying temperatures such as 15°C, 25°C, and 45°C, and monitored for changes in particle size, zeta potential, and physical appearance over time. In addition, thermal cycling tests are performed by alternating between high and low temperatures to simulate real-life storage conditions. Mechanical stress tests, including shaking and centrifugation, are also used to assess formulation robustness. A key instability phenomenon observed is Ostwald ripening, where smaller particles dissolve and redeposit onto larger ones, leading to gradual particle growth. This can compromise stability and performance. For herbal nanosuspensions, stability assessment is particularly important because natural phytochemicals may degrade or interact over time. A stable formulation should maintain consistent particle size, show no visible aggregation, and retain uniform drug content throughout the study period.

pH and Drug Content

pH measurement is an important quality parameter as it influences both stability and solubility of the formulation. It is determined using a calibrated digital pH meter to ensure accuracy. For oral applications, the pH should ideally be compatible with physiological conditions to avoid irritation and maintain stability. Drug content analysis is carried out to quantify the amount of active ingredient present in the nanosuspension. This typically involves extracting the drug using a suitable solvent followed by analysis using UV–visible spectrophotometry or other validated methods. This ensures uniform drug distribution and confirms that the formulation process has not led to significant loss of active compounds. In baobab-based nanosuspensions, maintaining consistent drug content can be challenging due to the complex nature of plant extracts. Therefore, this parameter is essential for ensuring dosing accuracy, reproducibility, and therapeutic effectiveness.[15]

RESULTS

Baobab fruit pulp exhibits notable antioxidant properties, contributes to glycemic control, and supports prebiotic activity through the generation of short-chain fatty acids. However, the therapeutic potential of its bioactive compounds can be limited by poor solubility. Nanosuspension technology addresses this limitation by reducing particle size to the nanometre scale, which significantly increases surface area, dissolution rate, and saturation solubility. As a result, the intestinal absorption and systemic bioavailability of poorly soluble phytoconstituents are markedly improved, leading to enhanced therapeutic effectiveness of herbal extracts. Key advantages of this approach include a faster dissolution rate, improved saturation solubility, increased bioavailability, high drug-loading capacity, and a reduced risk of adverse effects associated with excipients. Overall, nanosuspension-based delivery offers an efficient strategy to optimize the performance of bioactive compounds from baobab fruit pulp.





DISCUSSION

Baobab nanosuspensions have shown promising therapeutic potential across several biomedical and nutraceutical applications, including oral nutraceutical formulations, antioxidant therapy, immune-support supplements, functional beverages, and cosmeceutical products. In the context of diabetes management, nanosuspension-based delivery significantly enhances the bioavailability of baobab-derived polyphenols and vitamin C, leading to improved inhibition of carbohydrate-hydrolyzing enzymes such as α -amylase and α -glucosidase. This contributes to reduced postprandial hyperglycemia, improved insulin sensitivity, and protection of pancreatic β -cells against oxidative stress-induced damage. The enhanced antioxidant delivery further helps in neutralizing reactive oxygen species, thereby supporting better glycemic control. In addition, baobab nanosuspensions demonstrate notable cardiovascular protective effects, particularly relevant in diabetes-associated complications. Increased systemic availability of its phytoconstituents helps reduce lipid peroxidation, suppress pro-inflammatory cytokine production, and improve endothelial function. They also contribute to favorable modulation of lipid profiles, thereby addressing key risk factors associated with cardiovascular disease. Overall, the combined metabolic and cardioprotective actions highlight baobab nanosuspensions as a promising and multifunctional strategy for the management of metabolic syndrome and its associated complications.

Baobab fruit pulp-based nanosuspension offers a novel and scientifically promising strategy to overcome solubility and bioavailability limitations of conventional formulations. Nanosizing enhances dissolution rate, antioxidant activity, and therapeutic performance of its bioactive constituents. Although current evidence supports its nutraceutical and pharmaceutical potential, comprehensive *in vivo* studies, toxicity assessments, and scale-up investigations are essential prior to clinical translation.

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Asiya et al.,

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Table 1: Evaluation parameters of baobab nanosuspension

Parameter	Method of Evaluation	Significance
Mean Particle Size	Photon Correlation Spectroscopy (PCS), Laser Diffraction (LD), Coulter Counter	Influences dissolution rate and bioavailability
Polydispersity Index (PDI)	PCS analysis	Indicates uniformity of particle size distribution (Ideal: 0.1– 0.25)
Zeta Potential	Electrophoretic mobility analysis	Determines physical stability (± 30 mV ideal for electrostatic stabilization)
Saturation Solubility	Shake flask method in different pH media	Reflects improved dissolution characteristics
Dissolution Rate	USP dissolution apparatus	Predicts in vivo drug release behaviour
Stability Studies	Storage at 15°C–45°C, thermal cycling, mechanical shaking	Evaluates aggregation and Ostwald ripening
pH	Digital pH meter	Ensures formulation compatibility
Drug Content	Solvent extraction + UV spectrophotometry	Confirms uniform drug distribution





Asiya et al.,

Table 2: Comparison of Methods

Method	Type	Energy Requirement	Particle Size	Scalability
Ultrasonication	Top-down	Moderate	Small	Limited
High-speed homogenization	Top-down	High	Moderate-small	High
Anti-solvent precipitation	Bottom-up	Low	Very small	High

**Figure 1: Baobab fruit powder****Figure 2:Probe Ultrasonicator**

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RESEARCH ARTICLE

AI-Based Proactive Vehicle Threat Detection Using YOLO for Smart Surveillance Systems

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ABSTRACT

The drive to develop surveillance infrastructure in both public and commercial spheres has led to more sophistication in the requirement to develop smart systems with the capacity to detect multi-faceted, context-oriented security threats. Traditional CCTV based surveillance systems are very dependent on human personnel and they are not usually good in identifying such subtle but dangerous activities like people staying in their cars longer than expected. This paper proposes a smart video surveillance system that is based on deep learning and temporal analysis to identify long human occupancy within immobile vehicles. The suggested scheme combines vehicle and person detection by YOLO with occupancy checking and time-loitering evaluation in order to differentiate between normal and suspicious vehicle occupancies. On identifying abnormal conditions, the system sends real time alerts in various communication channels such as visual overlays, SMS, email messages, and logging on a database. This proposed system is edge-friendly, can safely be deployed in areas of practical surveillance, including occlusion, lighting conditions, and poor connectivity. The practicality of the method to improve the situational awareness and active security observation within the parking areas is proven by experimental observations.

Keywords: Intelligent surveillance, Vehicle loitering detection, YOLO, Human occupancy detection, Deep learning, Edge computing.

INTRODUCTION

The high population density and the increased urbanization have intensified the fears in terms of road security, malpractice of vehicles by criminals, and security of important structures, which have burdened the traditional



**Thamarai Selvi**

CCTV security system. Conventional surveillance is highly reliant on human operators whose operational performance is compromised in large volume video feeds and leads to sluggish response and high-risk activities in a complicated traffic scenario as observed by Llorca et al. [10]. Deep-learning object detectors, specifically the You Only Look Once (YOLO) family, have transformed single-stage computer vision that is jointly optimized both in speed and accuracy as described by Terven et al. [1], Sapkota et al. [2], and Wang and Liao [5]. Moreover, Wang and Liao [5] and Hussain [8] show that the consecutive steps of the YOLO, the YOLOv1, to YOLOv10 allow considering the significant increase in the accuracy of detection, the decrease in the latency, and the increase in the efficiency of the deployment. Due to these developments, these models can be best utilized with safety-critical systems, including autonomous driving, and traffic monitoring, which has been supported by Sapkota et al. [2], Wang and Liao [5], and Hussain [8]. The superiority of YOLO in different areas of use is also illustrated by the extensive deliberations presented by Terven et al. [1] and Ali and Zhang [3], which highlight that it is stronger to work under dynamic environments and that it could relate more to the edge devices to deliver low-latency inferences.

In the specific field of automobile environment, according to Kosasi et al. [6], YOLO variants (e.g., YOLOv4, YOLOv8) are routinely able to achieve vehicle detection rates of 90 percent or above in mixed weather and lighting. Nevertheless, it is still hard to cope with mini-sized objects and extreme light. Llorca et al. [10] also provide information about the increased use of YOLO in vision-based vehicle speed estimation and speed enforcement systems, pointing out that in order to take proactive safety-based actions, real-time detection is needed. The concomitant advancements in the AI-based threat detection show that machine learning can evolve into the vigilant prevention of risk instead of passive monitoring. The article presented by Kheddar [9] proves that the sophisticated, dynamic patterns of threats in the cyber-physical systems can be identified with the help of advanced deep models, including transformers and hybrid architectures. These models emphasize the importance of explainability, robustness and real-time response in deployments of security related nature. However, most of the existing implementations of YOLO-based vehicle use are focused on generic detection or post-detection analysis rather than proactive vehicle threat detection. In proactive detection, the challenge is to detect possible dangerous vehicles or actions, such as abnormal paths, excessive speed, or hanging around the sensitive zones, before things get out of control. This gap is explicitly stated in surveys of the history of YOLO and its use by Terven et al. [1], Sapkota et al. [2], Ali and Zhang [3] and Gallagher and Oughton [4], which require context-sensitive, multimodal and security-enhanced real-time detectors. According to this gap, this paper proposes an AI-based active vehicle threat detection system based on a current YOLO framework to run smart surveillance systems. The suggested architecture is an expansion of the works of Terven et al. [1], Sapkota et al. [2], and Wang and Liao [5], seeking to give rise to a scalable, robust deployment. This system complements the real-time vehicle detection based on the YOLO algorithm with risk-aware driving behavior analysis and automatic notification.

LITERATURE REVIEW

Object detection in deep learning has become part of intelligent transportation systems (ITS) and smart surveillance and has since replaced traditional image-processing pipelines due to its much better resistance to occlusion, changing lighting, and heavy traffic, as demonstrated by Farid et al. [14], Liang et al. [17], and Shokri et al. [18]. It is on this framework that the YOLO (You Only Look Once) family of one-stage detectors is appealing since they cast detection to a one-regression problem enabling real-time inference at scalable accuracies, which is a crucial quality to proactive monitoring and early response in safety-critical settings, as discussed by Farid et al. [14] and Liang et al. [17]. The relative studies of YOLO with other more recent multi-stage algorithms such as Faster R-CNN and SSD show evidence that later versions of YOLO have a better speed-accuracy trade-off in car-centric tasks in complex urban environments as observed by V. Sisaudia et al. [13] and Shokri et al. [18]. The literature on the use of YOLO based vehicle detection and counting has been abundant in investigating transportation analysis, tolling, and urban planning. Farid et al. [14] approach the YOLOv5 in transfer learning and custom and highly congested traffic datasets and demonstrate higher performance as compared to the traditional algorithms in multiple weather and density settings. Comparing SSD, R-CNN, and several versions of YOLO (v5-v8), Shokri et al. [18] report that the YOLO



**Thamarai Selvi**

architectures achieve detection and over 90% overall classification accuracy on cars, trucks, and buses, which prove the success of YOLO in ITS applications. In the other studies, the logic of tracking and counting vehicles based on the detection of vehicles via YOLO can be applied and visualized when using V. Sisaudia et al. [13] and R. Srimathi et al. [15] logic, to obtain statistical data of vehicles flow and support the analysis of a smartcity. Other more recent studies are also doing environmental and operational constraints such as low-light, fog and resource-based edge platforms. Deng et al. [21], propose O-YOLOv2, which contains extra feature extraction and residual modules, to enable it to scan small vehicles in KITTI with 94% precision and without the necessity to decelerate on speed, which is crucial when handling long-range or tiny objects in video surveillance. Liang et al. [17] suggest Edge YOLO, a pruned and compressed version of YOLOv4 to the edge to detect on the autonomous car; it can produce 47.3% mAP using 25.67 MB parameters and 26.6 frames per second, offering a solution to the latency and energy constraints of on-vehicle and roadside devices.

The aerial and adverse-condition surveillance have become the focus of studies as more recent generations such as YOLOv8 have been introduced. Bakirci et al [11] works with YOLOv8n/x on traffic images collected by UAVs and determines that the former is more appropriate to real-time ITS due to lower latency, whereas the latter is more accurate and has higher recall in case of the need to achieve high accuracy. N. Raza et al. [20] apply lightweight YOLOv8n/s models to detect and avoid collisions in low-visibility fog to illustrate that YOLOv8n can reach mAP50 scores of up to 85 per cent as well as run at over 100 FPS on multiple data sets; more significantly, on-device real-time warnings can be executed with the models of TensorFlow Lite on Android. Parallel work concentrates on compression of models as well as edge deployment with references to vehicle detecting. Liu et al. [19] introduce SMA-YOLO, a model built on iOS V7 and MobileNetV3 as the backbone, SimAM attention, and SIoU loss. This architecture is far smaller and cheaper in regard to parameters and computation but is highly accurate when applied to UA-DETRAC data. Other similar experiments using YOLO based detectors, multi-object tracking, multi-object hybrid ML/DL by Singh et al. [12] and Chaman et al. [16] are also pointing towards detecting, classifying and tracking of objects in real time and the viability of end-to-end smart surveillance systems. In spite of such developments, most of the ITS applications implemented with the YOLO technique are generic vehicle detection, counting and incident recognition. It is the relative emphasis on presence of the proactive vehicle threat detection where the models should not only identify vehicles but also determine the risk associated behaviour to give early warnings in intelligent surveillance networks. This deficiency has been the catalyst that has led to the development of AI based solutions that scale the high performance YOLO detection to the threat aware detection in intelligent surveillance and security sensitive environment.

METHODOLOGY

System Architecture

It works on a modular architecture that consists of four major stages Data Acquisition and Preprocessing, Core Detection Engine, Threat Logic Analysis and Response and Visualization. The video stream is fed in at a rate of 1920x1080, and scaled down to a processing size of 960px as a trade

Object Detection and Tracking

The perception layer is made up of the core that uses YOLOv8s (You Only Look Once, version 8 Small) to detect the object. This model has been chosen as a trade-off of the best between the inference speed (needed to operate in real-time) and the mean Average Precision (mAP). Class Filtering: This model is limited to the identification of only the relevant classes: Person (Class 0) and Vehicle (Classes 2 and 7). Tracking: To measure identity between frames we use a tracking algorithm which matches detections by both Euclidean distance and Intersection over Union (IoU) by which each vehicle and person is assigned a unique ID. This enables the system to trace the time stability of interactions.





Thamarai Selvi

Threat Detection Algorithm (Geometric Validation)

An original algorithm of geometric validation is proposed to differentiate between the legitimate parking behavior and the suspicious lingering. The system provides a definition of a threat based not on the presence of a person, but an individual in relation to a parked car over time. `check_Overlap(Person_Box, Car_Box)` is an algorithm, which finds the spatial interaction with three criteria Centroid Containment: This is required so that the center of mass of the person is within the horizontal limits of the vehicle bounding box. Vertical Constraint: This is used to distinguish between somebody passing a car and somebody interacting with the car (e.g. bending over, trying handles) checking the feet of the person (the bottom bounding box coordinate) against the tire line of the car.

Automatic License Plate Recognition (ALPR).

This is a specialized YOLOv8n model that is trained on license plates and is used to localize the plate region of the vehicle crop. The system of Optical Character Recognition (OCR) is based on multi-pass plate enhancement pipeline that includes 2x image upscaling to improve clarity, plate inversion to fit different plate color schemes and alpha numeric recognition with EasyOCR architecture based on ResNet, LSTM, and CRNN. An OCR output is verified by a heuristic filter (e.g. verification of certain country code or alpha numeric structure) to filter out noise.

Database and Data Persistence

MongoDB is a NoSQL database that deals with data persistence and is selected because it is flexible in the storage of unstructured data (images) and structured metadata.

Event Schema: Each threat event is stored as a document that has the `car_id`, `time_stamp_utc`, `time_stamp_ist` and binary encoded images of the `evidence_snapshot` and `license_plate`.

Status Tracking: The database has state (PARKED vs THREAT) to avoid recording the same ongoing incident more than once.

Alert and Response Mechanism

This system has a multi-channel alert mechanism to assist in responding to threats quickly. The email is formatted in HTML and sent via SMTP to the security personnel with the vehicle ID, the time spent loitering and a high-resolution snapshot of the vehicle. At the same time, a Telegram bot will be used to send live image-based notifications to a specified security group, and a local audio alarm activated via a pygame library will serve as the instant scarecrow to the possible intruders.

Evaluation Metrics

Precision

Precision indicates the proportion of correct detections among all predicted objects

$$Precision = \frac{TP}{TP + FP}$$

Where TP-True Positives, TN-True Negatives, FP-False Positives, FN-False Negatives

Recall

Recall measures the system's ability to identify actual objects present

$$Recall = \frac{TP}{TP + FN}$$

F1-Score

F1-score providing an overall measure of detection performance.

$$F1 = 2 \times \frac{Precision \times Recall}{Precision + Recall}$$

Accuracy

Accuracy is used to determine the general accuracy of correctly classified tokens:

$$Accuracy = \frac{TP + TN}{TP + TN + FP + FN}$$





Thamarai Selvi

RESULT AND ANALYSIS

The proposed system was strictly tested on the basis of the real-life surveillance footage, in which a benchmark model (yolov8x.pt) collected ground truth annotations on the key frames sampled at 1 FPS to obtain a non-biased test against the deployed lightweight model (yolov8s.pt). As determined in Table 1, the system proved to be highly effective, classifying vehicles at the accuracy of 76.2% compared to the benchmark and at the impressive 94.7% accuracy of person detection, and hence it proved to be efficient in classifying human silhouettes at difficult settings. Further performance evaluation as in Table 2 shows that the system scored a Precision and Recall of 98.4% and 76.2% accuracy on vehicles respectively, producing an F1-Score of 85.9%, and on persons, its F1-Score was 93.9%, which is a good balance between false alarms and false missed detections. Furthermore, the real time applicability of the system was confirmed with an average alert latency of 3.71 seconds and processing throughput of 15.0 FPS which is very appropriate in edge computing applications where TP represents true positives, FP false positives, and FN false negatives. Vehicle detection was very accurate (98.4) which means that there were few false alarms, but there are chances that vehicles were overlooked according to the recall. Pedestrian detection also had a balanced performance with an F1-score of 93.9, which validates the strength of the system in detecting the presence of human beings in the surveillance zone.

System Latency and Throughput

Intelligent surveillance systems need to be responsive in real-time. The average alert latency of the proposed framework was 3.71 seconds, as a time range between threat detection and the delivery of Telegram notification. The system was also capable of processing a throughput of about 15 FPS, which made the system very real-time with minimal computation delay

DISCUSSION

The results of the experiment confirm the efficiency of the suggested AI-based surveillance system to automate parking security processes. The efficiency of the system in the real-life conditions is proven by high pedestrian detection accuracy and high precision in both classes. The limitation was relatively less with vehicle recall although it depends mainly on environmental aspects like occlusion and space congestion and not algorithmic instabilities. The proposed system, in comparison to current methods, which tend to be less accurate than new YOLO versions, more focused on classification instead of behavior detection, or limited to general object detection without prioritizing the detection of dangerous objects in the environment, promotes surveillance with built-in threat analytics. Nevertheless, the framework demands annotation of threats, and has increased complexity of the system since it is multi-functional. Future development could involve better tracking systems, use of multi-cameras, and more resolution inputs to increase the area of detection. In general, the suggested system is a scalable and efficient way of providing intelligent parking surveillance because it involves less use of manual monitoring and provides a quicker response to any possible threat.

CONCLUSION

This paper proposed a smart video surveillance system of monitoring a long stay of humans within parked vehicles by means of deep learning and temporal analysis. The proposed solution incorporates the YOLO-based object detection with the context-sensitive occupancy verification and real-time warning, to bridge a critical gap between the current surveillance solutions. The system is efficient in providing situational awareness and proactive security surveillance in parking environments as well as is edge deployable. Further development will be done to enhance the robustness in the extreme lighting conditions and expand the framework to a multi-camera tracking and predictive threat assessment.





Thamarai Selvi

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Thamarai Selvi

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Table 1: Performance Metrics

Object	Precision	Recall	F1-Score
Vehicle	98.4%	76.2%	85.9%
Person	93.1%	94.7%	93.9%

Table 2: Comparative analysis

Study	Model Focus	Accuracy / mAP (%)
Edge YOLO (Liang et al., 2022)	Pruned YOLOv4 with edge–cloud cooperative computing	mAP $\approx$ 47.3% at IoU 0.5
Shokri et al., 2023	Comparative YOLO-based multi-label classifiers	Detection accuracy >95%; Classification >90%
Bakirci, 2024 (YOLOv8 ITS)	Comparative study of YOLOv8n and YOLOv8x	YOLOv8n AP $\approx$ 83%, YOLOv8x AP $\approx$ 96%
Proposed System	YOLO-based detector integrated with behavior analysis and automated alerting	Person: 94.7%; Vehicle: 76.2%





Thamarai Selvi

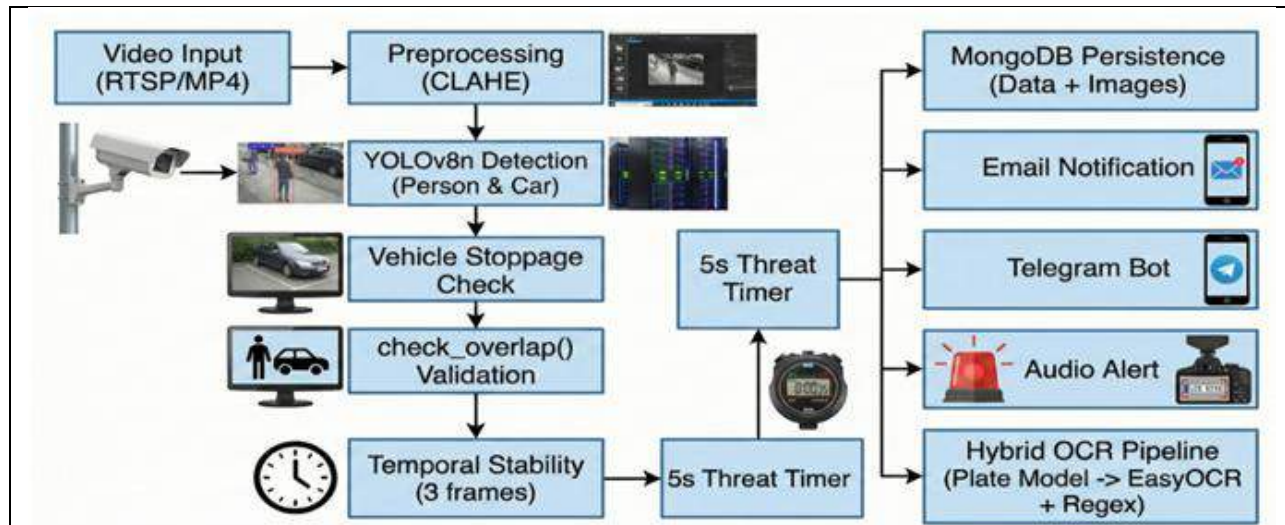


Figure 1: AI Vehicle Loitering Detector (AVLD)

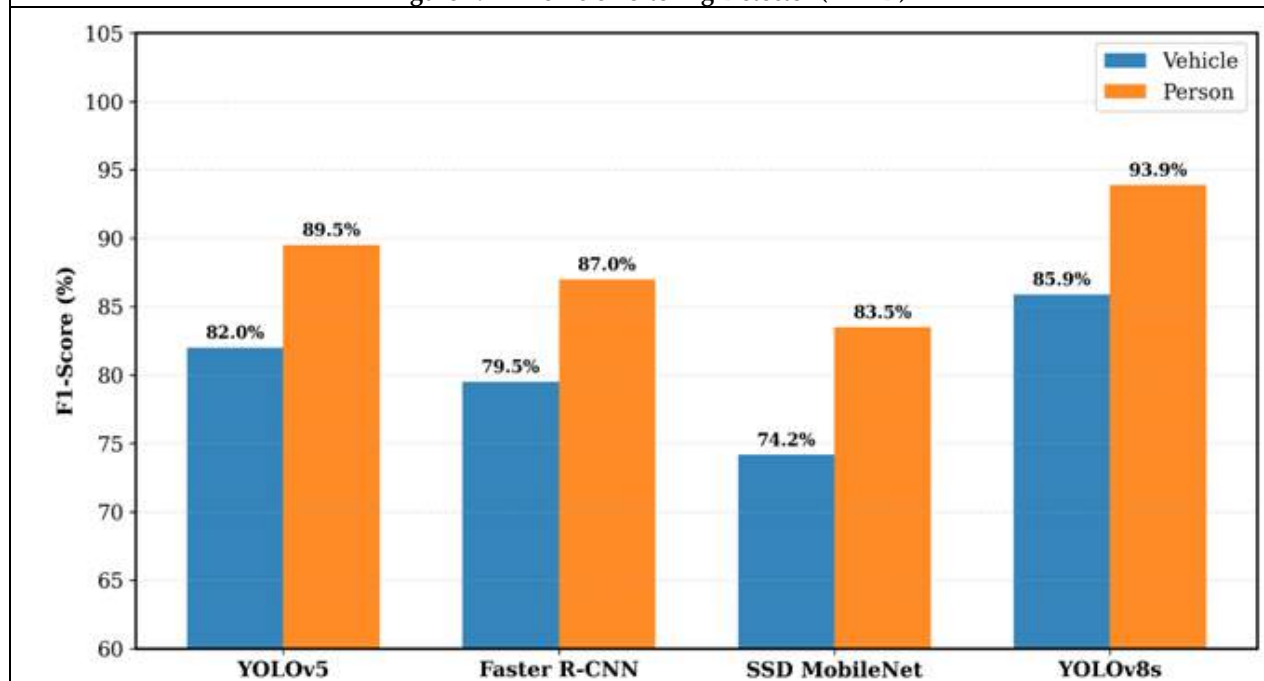


Figure 2: F1-score Comparison

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