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The full manuscript should contain first page with full author names, affiliation, ORCID No and the corresponding author email / ORCID details, followed by full text of the MS file in word format. All data/tables/figures/Images (images must be submitted with the MS in high print-reproducible resolution).

Introduction: This section must provide a brief review of literature, purpose of the study, objectives and the rationale of the research undertaken should be given with proper clarity.

Material and Methods: This section of material and methods /procedures should be concise but detailed enough to enable the reader to reproduce the experiments / methodology. Commonly used procedures and methods in detail need not be described, but require a reference to the original source.

Results (Including Tables/Fig/Images): Give only brief findings, presented in the form of tables or figures, should be included without duplication of presentation and no discussion of the significance of the data, either tables or figures be given, avoid duplication of data.

Discussion should present the significance of the present data under the prevalent understanding and interpretation of the phenomenon. Speculative discussion is allowed but it should be concise and corroborated by the presented data.

Conclusion: summarizes the study and is drawn from the results and discussion, should not be more than 100 words.

Acknowledgements/ Financial Acknowledgements if any, should be placed at the end of Conclusion before References.

References (In Vancouver Style ie in numbers serially arranged as per standard format).

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1. Lindley, S. T., Estimation of population growth and extinction parameters from noisy data. Ecol. Appl., 2003, 13, 806-813.
2. Martin, H., The Archaean grey gnesisses and the genesis of continental crust. In Archaean Crustal Evolution (ed. Condie, K. C.), Elsevier, Amsterdam, 1994, pp. 205-259.

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Systematic Reviews or Meta-Analysis should be systematic, critical assessments of most recently updated literature and data sources pertaining to basic biological or bio-medical science topics that include a statistical technique for quantitatively combining the results of multiple studies that measure the same outcome into a single pooled investigation. Data must be searched for and selected systematically for inclusion and critically evaluated, and the search and selection process for compiling the review must be mentioned. The text should NOT exceed 5000 words excluding abstract, references, tables and figures.

Each of the sections of the Systematic Review or Meta Analysis articles should include specific sub-sections as follows:

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Screening And Isolation of Lactic Acid Bacteria from the Gut of African Palm Weevil *(Orycete Rhinoceros)* Larvae as Starter Culture in Yoghurt Production

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ABSTRACT:
African palm weevil larvae *(Oryctes rhinoceros)* are widely consumed in the South-South region of Nigeria and valued as a nutritious traditional food. The larvae, naturally distributed across tropical regions, are mostly harvested from the wild. This study investigated the presence and viability of lactic acid bacteria (LAB) in the gut microbiota of the larvae as a potential alternative starter culture for yoghurt production. Larvae were aseptically collected from Odi community, dissected, and cultured using the pour plate technique on de Man Rogosa Sharpe (MRS) agar. Incubated cultures were purified by streaking, and isolates were identified microscopically based on cell morphology, catalase reaction, and Analytical Bacterial Identification System (ABIS) Map. The isolate was used to ferment milk, and key indicators such as pH, titratable acidity, and organoleptic properties were assessed. The LAB isolate from the larvae showed no effective fermentation activity, maintaining a near-neutral pH (6.8), low titratable acidity (72%), fresh-milk odor, and absence of curd formation. In contrast, the standard yoghurt starter culture produced the expected uniform gel structure, characteristic yoghurt aroma, higher titratable acidity (96–120%), and pH 4.5, confirming its functional suitability for yoghurt production. The findings demonstrate that the LAB isolate from *Orycete rhinoceros* larvae is not appropriate as a yoghurt starter culture due to its inability to meet essential fermentation, safety, and physicochemical requirements. Further research should include molecular identification and screening for functional LAB to determine whether safe, technologically relevant strains exist within the larvae microbiome.

KEY WORDS: Yoghurt, Lactic acid bacteria, Starter culture, Palm weevil larvae, *Orycete rhinoceros*, Food safety.

INTRODUCTION

Yoghurt is a very nutritious food and its continued consumption in the Western World owes much to the development of its health food image (Early, 1998).The methods of production of yoghurt have, in essence, changed little over the years and although there have been some refinements, especially in relation to lactic acid bacteria, that bring about the fermentation. Yoghurt is produced in the form of a highly viscous liquid. Yoghurt is also produced in a drinking form and can be frozen or blended with other ingredients to create, for example, mousse type products, sorbet, yoghurt ice-cream or other forms of dairy dessert (Early, 1998). The initial popularity of yoghurt in Western Europe owed much to the work of the Russian and Metchnikoff, 1908 he attributed the good health and longevity of Balkan peasants to the effects of certain bacteria in the yoghurt they consumed. He postulated the theory that prolongation of life would follow ingestion of a lactic acid bacterium named as

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A Review on Polymers Used in Pharmaceutical Formulations

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ABSTRACT:
Pharmaceutical formulations and are therefore an essential part of a pharmaceutical formulation. Their flexibility, having so many chemical structures, physical characteristics, and behaviors, permits tight control of drug release profiles, greater stability, and even better bioavailability. The review has successfully presented the centrality of pharmaceutical science to governments, mainly because of the use of polymers. We consider how polymers are classified on the basis of their source (natural, synthetic, or semisynthetic) and then the role that they play in a formulation, either as binders, disintegrants, coating agents, or matrix formers. We explore the use of polymers in the development of advanced delivery systems such as controlled-release matrices, nanoparticles, micelles, and hydrogels (purposefully designed to avoid obstacles posed by the physiology and deliver targets). Particular focus is on so-called smart or stimuli-sensitive polymers, which can undergo a property change in response to an environmental stimulus such as pH, temperature, or a particular enzyme, resulting in site-specific release of a drug. This abstract relies on the critical analysis of the underlying postulates and modern contributions to polymer-based drug delivery and thus emphasizes the revolution brought to the development of more effective, safer, and patient-friendly therapeutic alternatives through polymer science.

KEY WORDS: Polymers, Pharmaceutical Formulations, Synthetic & Natural Polymers.

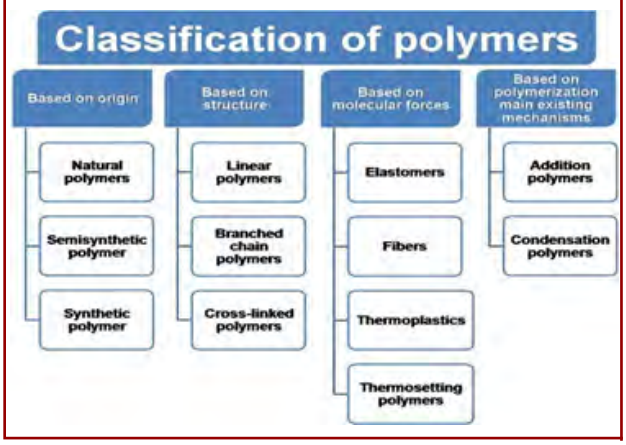
INTRODUCTION

In contemporary pharmaceutical science, the polymers are fundamental ingredients that are indispensible, extending far beyond the more mundane use as inert excipients to form the core of sophisticated drug delivery systems [1,2]. The functionality of such big molecules is carefully designed into formulations so as to regulate release characteristics, stabilize the formulation and curtail the bioavailability of active pharmaceutical ingredients (APIs) [2,3]. Polymers can also be used to modulate the pathway of a drug through the human body to produce a controlled-release dosage form that maintains steady therapeutic levels and thus has reduced the number of doses required and the dose-limiting side effects [3-5]. The use of polymers can be attributed to the capacity to regulate drug release through possible processes such as diffusion, erosion and swelling [5]. Not only does the category of natural polymers such as cellulose and starch adapted as a binder and disintegrant, semisynthetic polymers such as hydroxypropyl methylcellulose (HPMC) in sustained-release matrices and advanced synthetic polymers such as poly (lactic-co-glycolic acid) (PLGA) in biodegradable implants determine

the release pattern of the drug and the therapeutic performance of the drug, but also forms the basis of a huge industry in the pharmaceutical industry [6-10]. New evolutions in polymeric materials, such as the creation of smart polymers that react to certain physiological stimuli, are bringing a new generation of highly-targeted and personalized therapies, and polymers are at the core of the future of pharmaceutical formulation [10].

Classification of Polymers: Polymers [11-13] can have different chemical structures, physical properties, mechanical behavior, thermal characteristics and can be classified in different ways by following below are.

Figure 1: Classification of Polymers



Based on Origin

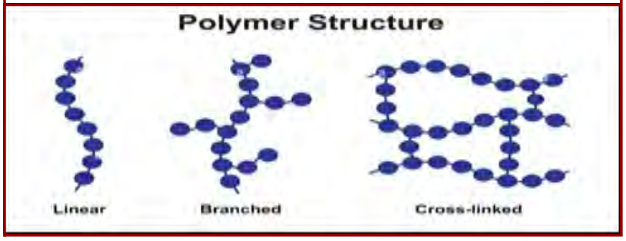
1. Natural Polymers: Natural polymers are those which are derived from either plants or animals. We refer to them as animal and plant polymer.
2. Examples : Cellulose, Jute, Silk, Wool, RNA, DNA, Natural rubber
3. Semisynthetic Polymers: Semisynthetic fibres are created from natural fibres that have been chemically treated to improve specific physical traits, such as tensile strength and lustre.
4. Examples include Cupra, ammonium silk, and viscose rayon.
5. Synthetic Polymers: Synthetic fibres are manufactured in laboratories through the polymerisation of fundamental chemical components [13 & 14].

Examples include nylon, Orlon, polystyrene, PVC, and Teflon.

Based on structure

1. Linear polymers: In these polymer, monomers are interconnected to form an elongated, linear structure. These chains do not possess any additional branches or side chains. Examples: Polyester, Polyethene 2. Branched Polymers: The straight long chain of molecules is accompanied by various side chain. Due to there irregular packing these molecules exhibit low density, tensile strength and melting point [14].
2. Examples: Polypropylene, Amylopectin, Glycogen
3. Crosslinked Polymers: The monomeric units are interconnected to form a three-dimensional framework in which cross link play a crucial role. These cross link contribute to the hardness, rigidity and brittleness of the network structure. Examples: Bakelite, Formaldehyde resin, Vulcanized rubber.

Figure 2: Structure of Polymers



Based on molecular forces

1. Elastomer

These polymers are characterized by polymer chains being held together by the weakest attractive force. They consist of randomly coiled molecular chains with minimal cross links. When a strain is applied, the polymer stretches and upon release of the force, it return to its original position. Such polymers exhibit elasticity and are commonly referred to as elastomers [15]. Examples : Silicone, Natural rubber

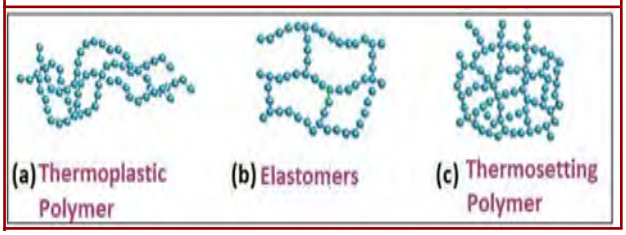
2. Fibers They possess a strong intermolecular attractive force similar to hydrogen bonding. Additionally, they exhibitremarkable tensile strength making them highly valuable in the textile industries.
Examples: Nylon-66, Terlyene

3. Thermoplastic Polymers

Polymers with intermolecular force between elastomer and fibers can be easily shaped by heating and then cooling at room temperature. These polymers may have a linear or branched chain structure. Examples : Acrylic, Polypropylene

4. Thermosetting Polymer This polymer exhibit high hardness and remain non melting when exposed to heat. They do not soften when subjected to pressure and cannot be reshaped. Due to their cross-linked structure these polymers are not recyclable.
Examples:Melamine,Silicone,Polyurea.

Figure 3: Structure of Polymers based on molecular forces



Based on Polymerization Addition Polymer

1. Addition Polymer: Addition polymers are formed by repeatedly adding monomers without removing any byproducts. As a result these polymers consist of all the atom from the monomers making

them a multiple of the monomer unit [15-19]. Examples: Orion, Teflon There are two types of addition polymer:

1. Homopolymer The formation of addition polymers due to the polymerization of single polymeric species is called homopolymer.
2. Copolymer The formation of addition polymer which occur due to addition polymerization from two different monomer is called a copolymer.
3. Condensation Polymer: The formation of these compounds occur through the combination of two monomers resulting in the elimination of small molecules such as water, alcohol, or ammonia. Ester and amide linkage are present in their molecular mass does not correspond to an integral multiple of monomer units [9]. Examples: Polyamide, Polyurethane

Characteristics of an ideal polymer:

1. It should be inert and compatible with the terrain.
2. It should be non- poisonous and physiologically inert.
3. It should be fluently administrable.
4. It should be easy to fabricate and must be affordable.
5. It should have good mechanical strength.
6. It must have comity with utmost of the medicines.
7. It mustn't negatively affect the rate of release of the medicine.
8. It mustn't have tendency to retain in towel and must be a good biodegradable material.
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Mechanism of drug release:

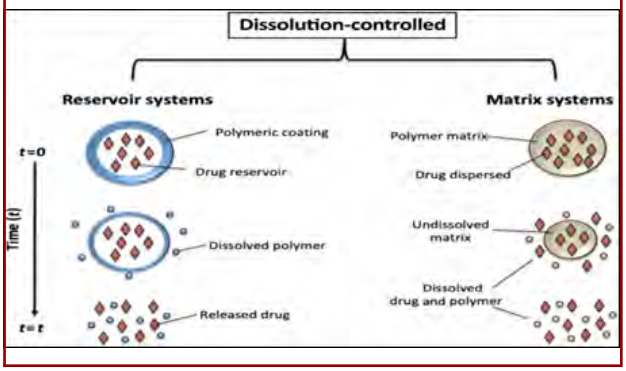
1. Dissolution controlled release.
2. A Matrix dissolution control
3. B. Encapsulation dissolution control
4. 2.Diffusion controlled release.
5. A. Reservoir device
6. B. Matrix device
7. 3.Combination of dissolution and Diffusion release systems
8. 4.Osmotic controlled release
9. 5.Ion exchange system

Dissolution controlled release: Controlled-release drug formulations can be created by reducing their dissolution rate. Strategies to achieve this include the development of suitable salts or derivatives, coating the drug with a slowly dissolving substance, or embedding it within a tablet that contains a slowly dissolving carrier. Various methods can be utilized to create dissolution-controlled systems: One approach involves layering the drug with rate-controlling coatings, allowing for pulsed delivery. If the outermost layer is a rapidly dissolving bolus of the drug, it can quickly establish initial drug levels in the body, followed by subsequent pulsed intervals. Another alternative is to deliver the drug in the form of beads, each featuring coatings of varying thicknesses. Due to the different coating thicknesses, the release of the drug will occur progressively. Beads with thinner coatings will supply the initial dose, while those with thicker coatings will maintain drug levels later on. This principle underpins spansule technology or microencapsulation. The dissolution rate at a steady state is represented by the Noyes-Whitney equation: dC/DA (C-C) dt h, where dC/dt indicates the rate of dissolution, D represents the drug's diffusion coefficient through the pores, h denotes the thickness of the diffusion layer, A is the surface area of the exposed solid, C is the saturated solubility of the drug. Depending on their technical complexity, these systems can be classified into two categories: A. Matrix type B. Encapsulation type [13-18].

A.Matrix dissolution: Matrix dissolution devices are created by compressing the medication with a slowly dissolving carrier to form a tablet. The controlled release is achieved by: 1. Modifying the tablet's porosity 2. Reducing its wettability 3. Allowing it to dissolve at a slower pace. The rate of drug release depends on the dissolution of the polymer. Examples include Dimetane Extencaps and Dimetapp Extentabs.

B. Encapsulation dissolution/reservoir dissolution-controlled system: The microencapsulation method involves coating or enclosing drug particles. These pellets are placed inside hard gelatin capsules, commonly referred to as 'spansules'. Once the coating material dissolves, the entire drug contained within the microcapsule becomes readily available for dissolution and absorption. In this case, the drug release is influenced by both the dissolution rate and the thickness of the polymer membrane, which can vary from 1 to 200 µ. The rate at which the coat dissolves is affected by the stability and thickness of that coating. Examples include: 1. Ornade spansules. 2. Chlorpheniramine repetabs [11-15].

Figure 4: Dissolution model for the Reservoir & Matrix systems for Encapsulation



2. Diffusion controlled release: Diffusion systems are defined by the fact that the rate at which a drug is released depends on its movement through an inert membrane barrier, typically made of an insoluble polymer. Generally, two categories of diffusional systems are identified: reservoir devices and matrix devices. In reservoir devices, the drug release adheres to Fick's first law

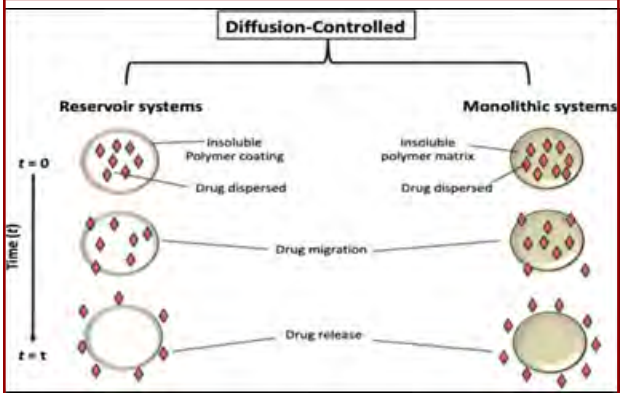
of diffusion. Here, D represents the diffusion coefficient of the drug within the polymer, J denotes the flux (amount per area per time), and dc/dx indicates the change in concentration concerning polymer distance [10-13].

These systems can be classified into two categories: A.Reservior Devices , B.Matrix Devices

A. Reservoir Device: Reservoir devices consist of a drug core, known as the reservoir, which is encased in a polymeric membrane. The characteristics of the membrane influence how quickly the drug is released from the system. One of the benefits of reservoir diffusional systems is that they can achieve zero-order delivery, and the release rate can be adjusted depending on the type of polymer used. However, reservoir diffusional systems have drawbacks, including the need for the system to be physically implanted at the site, challenges in delivering high-molecular-weight substances, and the risk of dangerous dose dumping if the system ruptures [7-9].

B. Matrix Devices/ Monolithic system: A matrix device is made up of a drug that is uniformly distributed within a polymer matrix. In this model, the drug located in the outer layer, which is in contact with the bathing solution, dissolves first and then diffuses out of the matrix. This process continues as the boundary between the bathing solution and the solid drug shifts inward. Clearly, for this system to be governed by diffusion, the dissolution rate of the drug particles within the matrix must significantly exceed the rate at which the dissolved drug diffuses out of the matrix.

Figure 5: Dissolution model for the Reservoir & Matrix systems for Monolithic system



3. Combination of Dissolution and Diffusion release systems: These systems can integrate both the diffusion and dissolution of the drug and the matrix material. The drug can diffuse from the dosage form similar to some previously described matrix systems, while the matrix simultaneously undergoes a dissolution process. The complexity arises from the fact that as the polymer dissolves, the path length for the drug's diffusion may change. This typically results in a moving boundary diffusion scenario. Zero-order release can occur only when surface erosion takes place and the surface area remains constant over time. A key benefit of such a system is that the bioerodible nature of the matrix prevents the formation of a ghost matrix, thus eliminating the need for removal from implant sites. However, the drawbacks of this system include the challenges in controlling release kinetics due to multiple release processes and the potential toxicity of the degraded polymer must be taken into account. Another approach to creating a bioerodible system is by chemically bonding the drug directly to the polymer. Typically, the drug is released from the polymer through hydrolysis or enzymatic reactions. A third type, which employs a combination of diffusion and dissolution, is the swelling-controlled matrix. In this case, the drug is dissolved within the polymer; however, instead of an insoluble or eroding polymer as seen in prior systems, the polymer swells. This swelling allows water to penetrate, leading to drug dissolution and diffusion from the swollen matrix. In these systems, the rate of release is strongly influenced by the polymer's swelling rate, the drug's solubility, and the proportion of the soluble fraction within the matrix. This system often minimizes burst effects since polymer swelling must first occur before drug release [11-18].

4.Osmotic controlled release: In these systems, osmotic pressure acts as the driving force for the controlled release of medication. Imagine a semi-permeable membrane that allows water to pass through but blocks drug molecules. A tablet with a drug core encased by such a membrane, when immersed in water or any bodily fluid, will draw water into the tablet due to the difference in osmotic pressure. These systems usually come in two distinct configurations. The first type features the drug in a solid core along with an electrolyte, which dissolves upon water interaction. The electrolyte creates a significant osmotic pressure difference. The second configuration contains the drug in a solution inside an impermeable membrane within the device, while the electrolyte is situated around the bag. Both types have one or more holes drilled through the membrane that permit drug release. In the first scenario, the high osmotic pressure is alleviated by forcing a solution containing the drug out through the opening. Likewise, in the second scenario, the elevated osmotic pressure compresses the inner membrane, causing the drug to be expelled through the hole. The benefits of osmotically controlled devices include the ability to achieve zero-order release. There is no need for reformulation for various drugs, and drug release is not affected by the surrounding environment of the system. However, the drawbacks of these systems are that they can be significantly more costly than traditional alternatives, and the quality control measures required are more extensive compared to standard tablets [5-15].

5.Ion exchange system: Ion-exchange systems typically utilize resins made of cross-linked, water-insoluble polymers. These polymers feature functional groups that form salts, positioned at regular intervals along the polymer chain. The drug is attached to the resin and is released through an exchange with ions in the vicinity of the ion-exchange sites. The process can be represented as Resin Drug + X → Resin-X + Drug, and conversely, Resin Drug + Y → Resin-Y + Drug, where X and Y are ions found in the gastrointestinal (GI) tract. The released drug then diffuses out from the resin. The drug-resin complex is formed by combining the resin with the drug solution, either through repeated exposure of the resin to the drug in a chromatography column or through

extended contact in a solution. The timing of drug release from the resin is determined by factors such as the diffusion area, length of the diffusion path, and the rigidity of the resin, which relates to the amount of cross-linking agent used during resin production. This system is particularly beneficial for drugs that are prone to degradation due to enzymes, as it provides a protective mechanism by temporarily altering the substrate. However, this controlled release method has the drawback that the release rate depends on the concentration of ions in the administration area. While the ionic concentration in the GI tract generally remains fairly stable, variations in diet, fluid intake, and individual intestinal content can influence the rate at which the drug is released. One enhancement to this system is the application of a hydrophobic rate-limiting polymer, like ethyl cellulose or waxes, as a coating for the ion-exchange resin. These systems depend on the polymer coating to regulate the rate of drug release [3-14].

Application of polymers used in pharmaceutical formulation: Polymers are play a vital role in pharmaceutical formulations given as follows:

Tablets: Tablets are the most prevalent form of dosage for medications intended for oral administration. The drug's release from the tablet can be regulated by modifying the formulation's design and components. In tablet formulations, polymers serve as disintegrants and binders. For example, disintegrants include starch, cellulose, alginates, polyvinylpyrrolidine, and sodium CMC. Binders made from polymers consist of glucose, starch, HPMC, gelatin, alginic acid, polyvinylpyrrolidine, sucrose, and ethyl cellulose. Additionally, polymers can be used to conceal a drug's unpleasant taste and to provide enteric coating for tablets, such as shellac and zein. Microcrystalline cellulose (MCC) improves the compressibility of tablets [2-10].

Capsules: Capsules are typically made from gelatin. There are two types of gelatin: hard gelatin and soft gelatin, each with a different composition. Fillers like microcrystalline cellulose (MCC) and starches are utilized to occupy space within the capsule. To address the issue of aggregation, various polymers such as starch and sodium starch glycolate are combined with the capsule material [2-13].

Polymers in gels: Gel systems are comprised of either physical or chemical cross-links that limit the movement of interconnected polymer chains. Gels exhibit unique rheological characteristics. Cross-linked gels are often referred to as hydrogels. These materials are also classified as smart polymers because they exhibit varying gelling behaviors depending on the water environment. The most frequently utilized hydrogels include poly(hydroxyethyl methacrylate), poly(methacrylic acid), and poly(acrylamide). In the pharmaceutical sector, cross-linked gels are mainly employed for localized drug delivery to the skin, oral cavity, vagina, and rectum [7-19].

Swelling controlled release systems: In numerous drug delivery systems, the size of the dosage form can change during the drug release process due to the swelling of the polymer matrix. While the primary mechanism for drug release is diffusion, examples of systems that demonstrate swelling-controlled release include physically and chemically crosslinked gels. For controlled drug release, chemically crosslinked hydrogels, such as poly(hydroxyethylmethacrylate), have been utilized to facilitate controlled drug release from medical devices, whereas physically crosslinked hydrogels that rely on swelling can be easily produced by directly compressing a drug with a hydrophilic polymer, such as HPMC [8-16].

Temperature-responsive drug release: Numerous studies have been conducted on the design and use of controlled systems for drug delivery that utilize temperature as an external trigger. The polymers employed to achieve such release characteristics are known as thermoresponsive polymeric systems. Typically, homopolymers and copolymers of N-substituted acrylic and methacrylate amides (for example, poly(isopropyl acrylamide)) are used for these applications. More specifically, there are two categories of thermoresponsive polymer systems: those that demonstrate a positive temperature response and those that exhibit a negative temperature response. Polymers in the first category show an upper critical solution temperature, below which the polymer contracts as the temperature decreases. In contrast, negative temperature-dependent polymers possess a lower critical solution temperature and will shrink when the temperature rises above this threshold [6-14].

Polymers in parenteral: We have various kinds of polymers, such as methacrylic acid and its alkyl amide derivative. Methacrylic acid functions as an interferon inducer, which is effective in treating cancer-related illnesses, while its alkyl amide variant serves as a plasma expander, increasing plasma levels in the human body. For instance, insulin injections are utilized in diabetes management, and multiple polymer types are employed in their formulation, acting as reservoirs that bond with insulin and release it at the intended site [4-11].

Ocular drug delivery systems: Enhancing the ocular contact duration of solutions involves adding polymers to an aqueous medium such as polyvinyl alcohol (PVA), polyvinylpyrrolidone (PVP), methylcellulose, carboxymethylcellulose (CMC), and hydroxypropyl cellulose (HPC). The increased viscosity of the solution helps to minimize its drainage. By elevating the viscosity of a pilocarpine solution from 1 to 100 cps through the addition of methylcellulose, the rate constant for solution drainage was decreased by a factor of ten, while the concentration of pilocarpine in the aqueous humor only increased twofold. Ocusert consists of a drug reservoir, which is a thin disk made of a pilocarpine-alginate complex, situated between two clear discs of microporous membrane created from ethylene-vinyl acetate copolymer. These microporous membranes allow tear fluid to flow into the drug reservoir, facilitating the dissolution of pilocarpine molecules, which are then released at a steady rate of 20 or 40 mg/hr for a duration ranging from one hour to seven days [2-9].

Application in cosmetics: The treatments for hair and skin that are revealed include a novel quaternary chitosan derivative. These

derivatives serve as effective agents, especially for keratin in hair, as well as for enhancing and conditioning hair. Several products are available on the market, such as oxidation hair coloring formulas, hair toning products, skin creams, hair setting lotions, hair treatment solutions, and gels.

Nanoparticles and microparticles: Polymeric nanoparticles and microparticles are utilized for drug encapsulation, offering protection against degradation, regulated release, and targeted delivery. These particles can be engineered to respond to specific physiological conditions, such as changes in pH or temperature, releasing the drug only under predefined circumstances. PLGA nanoparticles are widely researched for delivering anticancer drugs and vaccines, safeguarding the encapsulated drug from degradation and facilitating controlled release. They can also be modified with targeting ligands for precise drug delivery. Like nanoparticles, polymeric microparticles are employed for drug encapsulation and regulated release and are frequently utilized in depot formulations to ensure sustained drug release over longer durations.

The creation of advanced medication delivery systems that enhance patient adherence and treatment effectiveness is significantly reliant on polymers. Targeted delivery systems direct medications to specific tissues or cells, while controlled-release formulations extend the therapeutic effect's duration. Polymeric microparticles and nanoparticles provide protection, regulated release, and targeted administration while adapting to physiological conditions to achieve optimal therapeutic outcomes. Their functionality and versatility make them a vital component of modern medication delivery systems [3-15].

Water-soluble polymers: New applications for water-soluble synthetic polymers span a wide array, including scientific initiatives like drug delivery systems and tissue engineering scaffolds, as well as environmental efforts such as removing heavy metals. Fields focused on information generation also explore fresh opportunities for these materials, such as in electrically responsive or optical films. Water-soluble synthetic polymers have been engineered with properties previously only seen in natural polymers to meet the demands of these innovative applications. The introduction of reactive functional groups is not the only method to address specific challenges. This structural versatility places water-soluble polymers in a pivotal role within the realms of nanotechnology and smart materials.

This brief overview highlights the most recent advancements in the applications of water-soluble synthetic polymers, particularly concentrating on polyethylene glycol, polyvinyl alcohol, polyacrylamide, polyvinylpyrrolidone, and poly (N-isopropyl acrylamide). Through this summary, the intelligent features and sensitive structural control accessible to this class of materials via manipulation of strong hydrophilic interactions will be clarified. Polyethylene glycol, commonly referred to as PEG or polyethylene oxide (PEO) based on its molecular weight, is a typical water-soluble polymer. It has been utilized in a variety of applications, including lubricating coatings, osmotic pressure agents, electrolyte solvents, cosmetic ingredients, and medical laxatives. Recent advancements in nanoscience and technology, as well as in environmental engineering, have opened new opportunities for these polymers and are propelling the development of innovative properties [2-8].

Types of Polymers in Pharmaceutical Drug Delivery

1.Polymers in floating drug delivery system: Polymers play a vital role in floating drug delivery systems, helping ensure that medications reach specific areas of the gastrointestinal tract, particularly the stomach. Researchers have been exploring natural polymers for their effectiveness in targeting drug delivery to the stomach. Some notable examples include chitosan, pectin, xanthan gum, guar gum, gellan gum, karaya gum, psyllium husk, starch, and alginates [3-9].

2.Polymers in Mucoadhesive Drug Delivery Systems: The latest advancements in mucoadhesive polymers are making waves in drug delivery via the buccal route. These innovative polymers provide several advantages, such as adhering longer to the mucosal surface, enhancing penetration through the mucus layer, targeting specific areas, and minimizing enzyme activity. These characteristics make them incredibly valuable for delivering a range of therapeutic agents through the buccal mucosa. Current studies are delving into the use of lectins and "lectinomimetics" to ensure safe and effective drug delivery through this pathway.
3.Polymers for Colon Targeted Drug Delivery: Polymers are essential in colon-targeted drug delivery systems. They safeguard drugs from being degraded or released prematurely in the stomach and small intestine, ensuring that the medication is released in the proximal colon.

For instance, Wong et al. investigated the release of dexamethasone and budesonide from formulations containing guar gum and discovered that drug release significantly increased in simulated colonic fluid when higher concentrations of galactomannanase were present. A new colon-targeted tablet formulation was developed using pectin as a carrier, with diltiazem hydrochloride and indomethacin as model drugs. In vitro tests indicated that these dosage forms released minimal amounts of the drug in the stomach and small intestine, while most of the drug was released in the colon. Additionally, McLeod et al. synthesized glucocorticoid-dextran conjugates, incorporating dexamethasone and methylprednisolone for enhanced delivery.

Advantages of polymer used in pharmaceutical formulation

1. Colloidal drug carrier systems that utilize polymers made of small particles offer significant benefits in drug delivery due to their enhanced drug loading and release capabilities.
2. In controlled drug delivery, a polymer (either natural or synthetic) is combined with a drug, facilitating an effective and regulated dosage while preventing overdose.
3. Degradable polymers break down into biologically compatible molecules that can be absorbed and eliminated by the body through normal pathways.
4. Reservoir-based polymers provide multiple advantages,

such as improving the solubility of poorly soluble drugs and minimizing adverse side effects.

5. Magneto-optical nanoparticles that are polymer-coated and targeted are detectable by both optical and MRI methods, whereas quantum dots are only detectable optically.
6. Some quantum dots contain calcium, which is recognized as toxic to humans. In contrast, polymer-coated or targeted magneto/optical nanoparticles consist of iron oxides/ polymers that are considered safe, indicating a promising future.
7. Dextran, a common polymer used for coating iron oxide, has been utilized as a plasma expander and has affinity for iron, and it has been employed in treating iron-deficiency anemia since the 1960s, still continuing today.
8. In controlled release applications, certain polymers, such as polyurethanes known for elasticity and polysiloxanes valued for insulating properties, are chosen for their specific non-biological physical attributes.
9. Modern polymers like poly 2-hydroxyethyl methacrylate, polyvinyl alcohol, and polyethylene glycol are preferred due to their inert qualities and the absence of leachable impurities.
10. Biodegradable polymers are biocompatible, ensuring that no residual dosage remains at any time, while maintaining their properties until the drug is fully depleted.
11. In hydrogels used for drug delivery, the characteristics of polymer materials like PEG (a commonly utilized polymer in designing hydrogels) can be adjusted to optimize features like pore size, thereby controlling the diffusion rate of the delivered drugs. PEGylation has been found beneficial for various conditions, including hepatitis B and C, cancer-related neutropenia (PEG-GCSF), and several cancer types [PEG]. Glutaminase was combined with the glutamine anti-metabolite 6-diazo-5-oxo-norleucine (DON).
12. Polymers serve a variety of roles, from acting as films or binders in tablet coatings to flow-managing agents in liquids or emulsions, enhancing drug safety and modifying delivery properties. Micelles, due to their smaller size, have a brief circulation time in the body, granting them an advantage in easily penetrating tumor cells through the EPR effect.

Disadvantages of polymer used in pharmaceutical formulation

1. It cannot handle very high temperatures because all plastics start melting quickly compared to essence.
2. The strength-to-size ratio of polymer is lower, whereas for essence it is higher.
3. It is difficult to machine smoothly, and the machining speed is limited.
4. The heat capacity of polymer is much lower, making it unsuitable for heat-related applications.
5. It is not possible to create heavy structures with polymer because its structural strength is much lower.
6. Disposal becomes a problem because some polymers cannot be recycled, while all essence can be reclaimed.
7. After being delivered, polymers have a propensity to release a significant amount of medication rapidly.
8. From the beginning, polymers exhibit a high degree of drug release.

CONCLUSION

To sum up, the use of polymers has made a big difference in the pharmaceutical industry, starting from ancient times. Polymers have special qualities that make them perfect for many uses in medicine. They are commonly used in everyday medicine forms like tablets and capsules as binders, fillers, dissolving agents, and coatings. These help keep the medicine strong, uniform, and make sure the active ingredients are released at the right time. In special medicine forms that control how fast a drug is released, both types of polymers—those that don't break down and those that do—are important. They help manage how much drug is released and when, which makes treatment more effective. Polymers are also widely used in medicine packaging because they are flexible, strong, and protect against chemicals and bad weather. Using polymers in the pharmaceutical industry helps make medicines work better, last longer, and be safer. New discoveries in polymer science could lead to even more useful ideas and uses in medicine.

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Effects of Exposure to Magnetic Fields in MRI: A Review of Current Evidence and Future Directions

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ABSTRACT:
This review aims to examine the biological effects of Magnetic Resonance Imaging (MRI) exposure in healthcare professionals working with high-field MRI systems (1.5 Tesla and above). By synthesizing current evidence, it seeks to inform future safety guidelines and highlight potential risks associated with cumulative, long-term exposure among MRI technicians and radiologists.A systematic literature search was conducted on studies published from 2000 to 2024. Databases (e.g., PubMed, Scopus) were queried using key terms such as “MRI exposure,” “occupational health,” and “high-field MRI.” Studies were evaluated based on methodological rigor, relevance to occupational settings, and reported biological effects. The search aimed to identify both short-term and long-term physiological impacts of repeated MRI exposure. Although short-term MRI exposure is generally safe, healthcare workers consistently operating high-field MRI systems may experience transient sensory disturbances (e.g., vertigo, nausea) and possible long-term physiological effects. Findings suggest an elevated concern for cumulative exposure, yet conclusive evidence on the magnitude and nature of these risks remains limited due to variability in study designs and outcomes. While MRI technology plays a pivotal role in diagnostic medicine, ongoing research is needed to clarify the occupational hazards for healthcare professionals exposed to high-field environments. Improved monitoring, standardized safety protocols, and comprehensive longitudinal studies are recommended to ensure the continued safe use of MRI in clinical settings.

KEY WORDS: electromagnetic fields; Magnetic Resonance Imaging; MRI safety.

INTRODUCTION

Magnetic Resonance Imaging (MRI) is a cornerstone of modern diagnostic medicine, providing high-resolution images of internal body structures using non-ionizing electromagnetic fields [1-3]. Since its introduction in the 1980s [4], MRI has revolutionized the way medical professionals diagnose and monitor a wide range of conditions, from neurological disorders to cardiovascular diseases. The widespread use of MRI is due in large part to its ability to offer detailed anatomical and functional information without the risks associated with ionizing radiation, such as that used in X-rays and CT scans. This has established MRI as a preferred modality in many clinical and research settings [5-8].

However, as MRI technology has advanced, so too have concerns regarding its long-term safety, particularly for healthcare workers who are regularly exposed to the powerful magnetic fields generated by MRI machines. While MRI is

generally regarded as safe for patients due to the relatively short duration of exposure, the same may not hold true for medical staff, who may be exposed to these fields for hours each day over the course of their careers. These concerns have been amplified with the increasing use of high-field MRI systems, such as those operating at 3 Tesla (T) or higher, which offer greater image resolution and contrast but also produce stronger magnetic fields that could pose a greater risk of biological effects [9,10].

The potential biological effects of MRI exposure are associated with three primary sources: static magnetic fields, time-varying gradient magnetic fields, and RF fields [11]. Static magnetic fields, which are a constant feature of MRI, can interact with biological tissues, leading to effects such as magneto hydrodynamic phenomena, which can cause dizziness and other sensory disturbances [12,13]. Time-varying gradient magnetic fields are used to spatially encode the MRI signal and can induce electrical currents in the body, potentially leading to peripheral nerve stimulation. RF fields are responsible for heating tissues, and while regulations limit the specific absorption rate (SAR) to prevent excessive heating, higher field strengths could pose a greater risk of localized tissue damage [14].

A key concern for healthcare workers is the cumulative effect of prolonged exposure to these fields over time. While individual MRI examinations may not pose a significant risk, repeated exposure over months or years could potentially lead to adverse health outcomes. For example, studies have reported transient effects such as dizziness, vertigo, metallic taste, and concentration difficulties among MRI technicians and radiologists, suggesting that there may be physiological consequences to long-term occupational exposure to MRI environments [15-17].

To date, research on the long-term effects of MRI exposure has been inconclusive. Several studies have examined the acute effects of MRI exposure, often focusing on short-term symptoms such as dizziness or nausea. However, fewer studies have investigated the long-term health consequences for healthcare workers who are exposed to MRI environments on a regular basis. Existing studies are often limited by small sample sizes, short follow-up periods, or a lack of consistent exposure measurement. Additionally, there is significant variability in the levels of exposure experienced by MRI staff, depending on factors such as their proximity to the MRI machine, the duration of exposure, and the strength of the magnetic field. [18-23].

Despite the growing body of research, many questions remain unanswered. For instance, it is unclear whether chronic exposure to high-field MRI could lead to more serious health conditions, such as cognitive impairment, cardiovascular issues, or even cancer. Additionally, the variability in exposure levels among MRI staff highlights the need for more precise monitoring and risk assessment protocols. Understanding the mechanisms by which MRI exposure might affect biological tissues is crucial for developing effective safety guidelines and minimizing risks for healthcare workers.

Given the increasing reliance on high-field MRI in both clinical and research settings, it is critical to assess the safety of long-term exposure to MRI environments, particularly for healthcare professionals who may be at higher risk. This study aims to provide a comprehensive review of the current literature on the biological effects of MRI exposure, with a particular focus on healthcare workers who are regularly exposed to high-field MRI. By synthesizing existing research, this review will identify both the potential benefits and risks associated with long-term MRI exposure, and will highlight areas where further research is needed to fill existing knowledge gaps. The outome of this review will contribute to the development of more robust safety guidelines for healthcare workers, ensuring that the benefits of MRI technology can be fully realized without compromising the health and safety of those who operate these systems daily. Furthermore, by identifying gaps in the current literature, this study will help to inform future research priorities and guide the development of new safety protocols for high-field MRI environments.

MATERIAL AND METHODS

Figure 1 illustrates a flow chart describing that A comprehensive review was conducted of the biological effects of MRI exposure by systematically searching relevant literature across multiple databases, including PubMed, Google Scholar, Scopus, and Science Direct, ensuring broad coverage of scientific publications and clinical studies .The search was performed using specific keywords and Boolean operators, such as “MRI exposure,” “biological effects of MRI,” “occupational health MRI staff,” “high-field MRI safety,” and “MRI-induced cognitive effects.” Boolean operators (e.g., AND, OR) were applied to refine and combine search terms where necessary. The complete list of all search terms and combinations used in this review is provided in Appendix A to facilitate reproducibility and to support future related research. The search spanned studies published between 2000 and 2024, focusing on research related to MRI machines operating at field strengths of 1.5 Tesla and above.

This threshold was chosen because these field strengths are the most widely used in clinical environments and generate stronger magnetic fields, making them more relevant for evaluating occupational safety. Figure 2 illustrates the study review criteria applied in this review, including both inclusion and exclusion parameters. Studies investigating biological effects on healthcare staff—such as MRI technicians, radiologists, and other medical professionals frequently exposed to MRI environments—were included.

In addition to the database search, we conducted a manual review, which involved screening the reference lists of all included studies to identify additional relevant articles

not captured during the initial search. These additional articles were retrieved, read in full, and assessed against the same inclusion and exclusion criteria to ensure eligibility. physiological changes, and potential long-term risks such as DNA damage. Studies that did not meet these criteria were excluded from the review.

Figure 1: Methodology Flow Chart

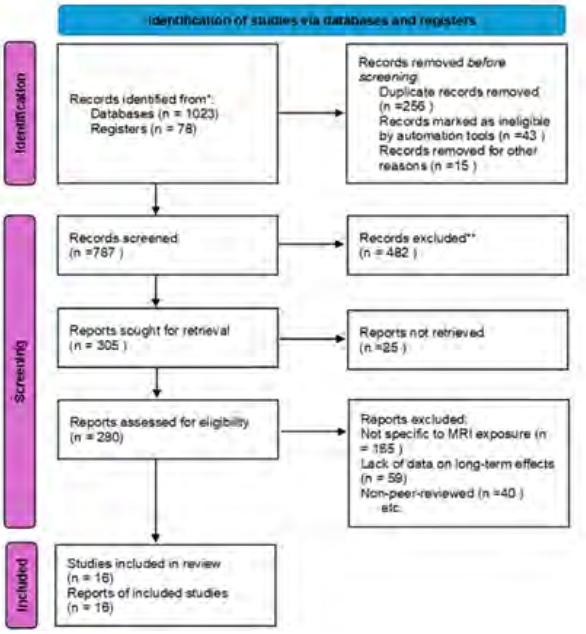

Figure 2. Study Review Criteria

RESULTS

The analysis of studies [24–39] on magnetic resonance imaging (MRI) exposure reveals both immediate and potential long-term health effects for individuals in MRI environments. Studies such as [24] identified short-term subjective symptoms (e.g., drowsiness, concentration issues) among healthcare professionals exposed to MRI. However, symptoms typically resolved within weeks, suggesting possible adaptation. Studies [25–28] explored the physiological impacts of ultra-high-field (UHF) MRI systems (≥7T), noting transient effects like dizziness and vertigo but insufficient evidence for lasting biological damage, underscoring the need for regular monitoring and strict adherence to occupational safety protocols.

For MRI technicians and workers in industries with static magnetic fields, studies [29,30] noted sleep disturbances and inconclusive cancer risks, emphasizing the need for improved exposure assessment and ongoing health monitoring. In particular, the lack of conclusive epidemiological studies limits understanding of MRI's cumulative effects over extended periods, especially at high field strengths. Studies focusing on childhood leukemia and environmental EMF exposure, such as studies [31–39], present conflicting findings. While some meta-analyses [35,39] indicate a potential link between extremely low-frequency (ELF) magnetic fields (≥0.4 μT) and childhood leukemia, others report no definitive associations, suggesting that results may vary by exposure intensity and environmental factors. These discrepancies highlight the necessity for further large-scale, longitudinal studies to clarify long-term EMF impacts, particularly among children and other vulnerable groups.

DISCUSSION

This review highlights the current understanding and gaps in knowledge regarding the health impacts of MRI and EMF exposure. Short-term symptoms like dizziness, concentration issues, and drowsiness are frequently reported by healthcare workers in MRI environments, with most symptoms resolving over time. However, there remains limited and inconclusive data on the long-term effects of high-field MRI and EMF exposure, particularly concerning cumulative health risks such as cancer, reproductive effects, and neurological impacts. Notably, studies focusing on extremely low-frequency (ELF) magnetic fields in residential settings indicate a potential association with an increased risk of childhood leukemia, though findings vary across studies and depend on factors such as exposure intensity and environmental context. The lack of definitive evidence on long-term effects points to an urgent need for more rigorous epidemiological studies, particularly longitudinal research that examines cumulative exposure in both occupational and residential contexts.

For healthcare professionals, especially MRI technicians and radiologists, the growing use of ultra-high-field (≥7T) MRI technology warrants strict adherence to occupational safety protocols, including regular monitoring and exposure assessments. Furthermore, the potential vulnerability of children and other high-risk groups to prolonged EMF exposure emphasizes the need for precautionary measures in both clinical and residential environments. Considering these findings, developing robust, evidence-based guidelines for MRI and EMF safety is essential to balance the benefits of MRI technology with the health and safety of both patients and healthcare workers. Future research should prioritize understanding the biological mechanisms behind EMF-

related health effects and evaluating the long-term impacts on diverse populations to establish clear and actionable safety standards. This study underscores the necessity of proactive safety protocols and continued research to support the safe and effective use of MRI in healthcare settings.

Summary table MRI exposure

Author/Year	Population	MRI Field Strength	Exposure Duration/Type	Reported Symptoms/Effects	Short/Long Term	Severity/Frequency
Zanotti et al., 2015 [24]	MRI technicians	1.5T–3T	Routine occupational work	Drowsiness, concentration issues	Short	Moderate, resolved in weeks
Ladd et al., 2018 [25]	Healthy volunteers	≥7T	Single and repeated scans	Dizziness, vertigo	Short	Mild, transient
Schenck, 2000 [26]	General MRI population	≥1.5T	Routine scanning	Safety and static field interaction effects	Short/Long	Varied, inconclusive for long-term
Huss et al., 2021 [29]	MRI technicians	1.5T–3T	Occupational, routine work	Sleep disturbances	Long	Persistent in subset
Feychting, 2005 [30]	MRI workers	Mixed	Occupational	Inconclusive cancer risk	Long	Not quantified
Schüz, 2011 [35]	Children (environmental EMF)	Residential ≥0.4 μT	Residential exposure	Potential link to childhood leukemia	Long	Risk varies by exposure level
Ghahremani et al., 2020 [39]	Children (environmental EMF)	Residential ≥0.4 μT	Residential exposure	Association with childhood leukemia (meta-analysis)	Long	Risk varies by exposure level

CONCLUSION

This review highlights the current understanding and remaining gaps regarding the health impacts of MRI and EMF exposure in occupational settings. Short-term effects such as dizziness, vertigo, and concentration difficulties are commonly reported but generally resolved over time, while evidence for long-term risks remains limited and inconclusive. The growing use of ultra-high-field MRI underscores the need for rigorous exposure monitoring, standardized safety protocols, and targeted research particularly large-scale longitudinal studies to clarify potential cumulative effects. For now, strict adherence to occupational safety measures remains the most effective strategy to protect MRI staff and patients while enabling the continued safe use of MRI technology.

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Effect of *Mussaenda glabrata* Leaf Extract on Cisplatin Induced Hematotoxicity, Nephrotoxicity And Neurotoxicity in Albino Wistar Rats

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ABSTRACT:
This research was aimed to explore effect of *Mussaenda glabrata* extract of leaves against cisplatin induced hematotoxicity, neurotoxicity and nephrotoxicity in experimental rats. Toxicities were induced in healthy adult wistar rats by administering cisplatin, single dose of 8mg/kg, on 5th day through intraperitoneal route. After completion of nine days dosing with the test drug, on 10th day, various hematological, biomarkers, antioxidants and histopathological studies were performed. *Mussaenda glabrata* leaf extract had a significant beneficial role in cisplatin induced hematotoxicity by increasing the hematological parameters compared to cisplatin treated group. It also exhibited a significant protection against cisplatin induced nephrotoxicity and neurotoxicity by suppressing the biomarkers and by elevating the antioxidant parameters at different doses compared to cisplatin treated group. According to research findings, *Mussaenda glabrata* extract of leaves may be protective against the neurotoxicity, nephrotoxicity and hematotoxicity caused by the anti-cancer medication cisplatin. MGLE's antioxidant action may thus be directly linked to its protection. These results support the theory that antioxidant-rich medicinal herbs may have protective properties.

KEY WORDS: Cisplatin, *Mussaenda glabrata* leaf extract, Hematoprotective, Neuroprotective, Nephroprotective.

INTRODUCTION

Cisplatin is considered one of the key chemotherapy agents for cancer treatment that may be used to treat a variety of human cancers in various organs [1].

Nephrotoxicity can develop from cisplatin buildup in the tubules that are proximal inside the kidney once it is excreted by the kidneys. Subjects having a creatinine clearance more than 60 milliliters per minute are often the only ones who can use it; nonetheless, cisplatin-induced nephrotoxicity is frequent and can restrict dosage and/or dose intensity. When patients who have received a complete course of chemotherapy develop peripheral neuropathy, cisplatin neurotoxicity is medically obvious and may impact the treatment plan and the patient's quality of life [2].

One of the main causes of neurotoxicity is believed to be the build-up of cisplatin within dorsal root ganglia neurons as platinum-DNA adducts. There are currently no medications that can stop the development of cisplatin-induced neurotoxicity and the high frequency of neurotoxicity restricts the chemotherapeutic efficacy of cisplatin [3].

The development of synthetic medications has led to the necessity for alternative therapies and medical care due to their negative effects. From ancient times to the present, herbs and herbal treatments have been used extensively to heal illnesses [4].

Cisplatin's anticancer properties have been attributed to its capacity to bind DNA and generate covalent cross-links, which in turn block transcription and DNA replication. However, the host's development of numerous adverse effects and/or the cancer cells' acquisition of drug resistance limit the complete therapeutic efficacy of cisplatin [5].

Mussaenda glabrata syn. Mussaenda frondosa, also called "Vellaiilai" in Tamil, is a member of the Rubiaceae family and one of the medicinally significant plants. Jaundice, hyperacidity, ulcers, diuretic, wound, leprosy, swelling, asthma, antibacterial, hypolipidemic effect, hepatoprotective action, fever and cough are among the traditional ailments that leaves are used to cure [6].

Till now no study has been reported, regarding, protective effect of *Mussaenda glabrata* leaf extract [MGLE] against cisplatin induced hematotoxicity, nephrotoxicity and neurotoxicity. Hence the current study was designed to demonstrate the impact of extract of leaves from *Mussaenda glabrata* in different doses against cisplatin induced hematotoxicity, nephrotoxicity and neurotoxicity.

MATERIAL AND METHODS

Chemicals: Cisplatin was obtained from Cipla, Mumbai, India. Biochemical estimation kits were obtained from Precision Biomed Pvt. Ltd., Mumbai, India. All chemicals, solvents used for this study were of the analytical grade obtained from reputed suppliers from India.

Animals: Both sexes of experimental rats weighing between 175 and 250 grams were kept in an adequately conditioned animal housing with a 12:12 light-dark cycle at 25 ° ± 5 °C and 50± 5% relative humidity. Every rat was given a commercially accessible standard pellet diet and unlimited access to water. In accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals' (CPCSEA) recommendations, the animals were kept and research was carried out. Institutional Animal Ethics Committee [IAEC] approved the study bearing Reference No.: SDCP/IAEC/13/2020 dated 15/02/2020.

Preparation of *Mussaenda Glabrata* leaf extract [MGLE] [7] Kerala was the source of M.glabrata. The plant material was identified by the Department of Botany, University College, Mangalore, 575001. Leaves that had been shade-dried were ground into a reasonably coarse powder. The Soxhlet extractor was used to obtain petroleum ether, chloroform, alcohol, and aqueous extracts of leaves. Till the solvent in the thimble was clear, the extractions were carried out 12 times. The deep brown semisolid substance was stored for later use in an airtight container once the solvent had evaporated.

Phytochemical estimation of the extract [8]: The presence of several phytochemical elements, including alkaloids, proteins, carbohydrates, glycosides, phytosteroles, saponins, tannins and flavonoids were examined by qualitative analysis of the MGLE extract.

Experimental Protocol

Dose selection [9,10]: Cisplatin dosages for rats have been determined to be 200 mg/kg and 400 mg/kg by oral route based on earlier studies of the literature.

Cisplatin induced toxicity model [9,10]: The current study has nine-day duration. On the fifth day, a single intraperitoneal (i.p.) dosage of 8 mg per kg of cisplatin was given to healthy adult Wistar rats in order to cause hematotoxicity, nephrotoxicity, and neurotoxicity. The MGLE was given orally to each group for nine days.

Following nine days of test medication dosage, the rats being studied was put to sleep by ether inhalation on the tenth day (six days following cisplatin injection). From each rat, blood was taken and split into two samples. Also kidneys and brain were removed and one sample was used to estimate hematological parameters while the other was utilized to estimate biomarkers. The kidney's two sections were employed for histological research and antioxidant assessment, respectively. The animal's brain was removed, cleaned in cold saline, blotted and prepared for biochemical along with histological analysis in order to assess neurotoxicity.

Groupings: Good health mature wistar rats were split into four distinct groups of six rats each. Group I: Normal (saline 10 ml/kg oral) for 9 days. Group II: Cisplatin (8mg/kg intraperitoneal) on the 5th day of treatment. Group III: Low dose MGLE (200 mg/kg intraperitoneal) + CIS on the 5th day of treatment. Group IV: High dose MGLE (500 mg/kg intraperitoneal) + CIS on the 5th day of treatment.

RESULTS

Isolation of *Mussaenda Glabrata* Leaf Extract: The practical yield of MGLE from 100g of dried plant powder of M. glabrata by maceration process was found to be 35 g. The evidence of carbohydrates, steroids, triterpenoids, glycosides and flavonoids were confirmed by preliminary qualitative analysis of the MGLE extract.

Effect of MGLE on CIS induced hematotoxicity: MGLE showed high efficacy against CIS induced hematotoxicity. CIS treated rats explored extremely significant ($P<0.001$) decrease in RBC (red blood corpuscles) compared to normal group. The rats pretreated with MGLE (200 mg/kg) and MGLE (400 mg/kg) showed extremely significant ($P<0.001$) increase in RBC compared to CIS group. CIS treated rats showed

extremely significant (P<0.001) increase in WBC (white blood corpuscles) compared to normal group. The rats pretreated with MGLE (200 mg/kg) and MGLE (400 mg/kg) showed extremely significant (P<0.001) decrease in WBC compared to CIS group.CIS treated rats showed most significant (P < 0.01) decrease in Hb (haemoglobin) compared to normal group. The rats pretreated with MGLE (200 mg/kg) and MGLE (400 mg/kg) showed most significant (P<0.001) increase in Hb compared to CIS group.CIS treated rats showed extremely significant (P<0.001) decrease in platelets compared to normal group. The rats pretreated with MGLE (200 mg/kg) and MGLE (400 mg/kg) showed extremely significant (P<0.001) increase in platelets compared to CIS group. See table 1.

Table 1. Effect of MGLE on CIS induced hematotoxicity

TREATMENT	RBC (106 cell/µL)	WBC (103 cell/µL)	Hb (g/dL)	Platelets (10^5 cell/µL)
NORMAL	4.69±0.101	4.656±0.003	13.825±0.006	6.256±0.009
CIS	2.548±0.073###	7.305±0.067###	10.345±0.007###	2.915±0.001###
MGLE200+CIS	2.913±0.005**###	6.631±0.192**###	10.685±0.087**###	3.391±0.151*###
MGLE400+CIS	3.31±0.026***###	6.039±0.166***###	10.896±0.077***###	3.626±0.171**###

n=6; data are presented as MEAN ± SEM. Statistical significance was determined using one-way ANOVA then Tukey-Kramer multiple comparison test. **P<0.01 and ***P<0.001 indicate significant differences compared to the control group, while ##P<0.01 and ###P<0.001 denote significant differences compared to the normal group.

Effect of MGLE on CIS induced nephrotoxicity serum biomarkers: In this model of experimentation, the CIS induced rats showed an extremely significant (P<0.001) increase in creatinine level when compared with normal control. Prior treatment of MGLE (200 mg/kg) and MGLE (400 mg/kg) showed highly significant (P<0.001) decrease in serum creatinine level respectively when compared with the control group.In this experimental model, the CIS induced rats control showed an extremely significant (P<0.001) increase in urea level when compared with normal control. Prior treatment of MGLE (200 mg/kg) and MGLE (400 mg/kg) showed extremely significant (P<0.001) decrease in urea level respectively when compared with the control group. In this model of experimentation, the CIS induced rats revealed an extremely significant (P<0.001) increase in uric acid level when compared with normal control. Prior treatment of MGLE (200 mg/kg) and MGLE (400 mg/kg) showed more significant (P<0.001) decrease in uric acid level respectively when compared with the control group. See table 2.

Table 2. Effect of MGLE on CIS induced nephrotoxicity serum biomarkers

TREATMENT	CREATININE (mg/dl)	UREA (mg/ml)	URICACID (mg/ml)
NORMAL	0.963±0.009	37.858±0.032	1.587±0.021
CIS	4.457±0.019###	70.808±0.048###	6.312±0.029###
MGLE200+CIS	3.510±0.225**###	59.352±4.008*###	5.065±0.269*###
MGLE400+CIS	3.203±0.297***###	57.809±3.788*###	4.732±0.494**###

n=6; data are presented as MEAN ± SEM. Statistical significance was determined using one-way ANOVA then Tukey-Kramer multiple comparison test. **P<0.01 and ***P<0.001 indicate significant differences compared to the control group, while ##P<0.01 and ###P<0.001 denote significant differences compared to the normal group.

Effect of MGLE on CIS induced nephrotoxicity antioxidants: Investigation of antioxidants in homogenate kidney tissue showed an extremely significant (P<0.001) decrease in GSH compared to normal group. The rats pretreated with MGLE (200 mg/kg) and MGLE (400 mg/kg) showed more significant (P<0.001) increase in GSH compared to CIS group. Investigation of antioxidants in homogenate kidney tissue showed more significant (P<0.001) decrease in catalase compared to normal group. The rats pretreated with MGLE (200 mg/kg) and MGLE (400 mg/kg) showed extremely significant (P<0.001) increase in catalase compared to CIS group. Investigation of antioxidants in homogenate kidney

tissue showed more significant (P<0.001) decrease in SOD (super oxide dismutase) compared to normal group. The rats pretreated with MGLE (200 mg/kg) and MGLE (400 mg/kg) showed extremely significant (P<0.001) increase in SOD compared to CIS group. See Table 3.

Table 3. Effect of MGLE on antioxidants in CIS induced nephrotoxicity antioxidants

TREATMENT	GSH (ηM/g wet gland)	SOD (U/mg wet gland)	Catalase (U/mg wet gland)
NORMAL	17.509±0.058	96.262±0.007	22.75±0.012
CIS	8.737±0.006###	49.566±0.007###	12.08±0.017###
MGLE200+CIS	10.36±0.530*###	66.977±4.388*###	15.729±0.660**###
MGLE400+CIS	10.867±0.548**###	75.715±6.885**##	17.488±1.003***###

n=6; data are presented as MEAN ± SEM. Statistical significance was determined using one-way ANOVA then Tukey-Kramer multiple comparison test. **P<0.01 and ***P<0.001 indicate significant differences compared to the control group, while ##P<0.01 and ###P<0.001 denote significant differences compared to the normal group.

Effect of MGLE on CIS induce neurotoxicity antioxidants: Investigation of antioxidants in homogenate brain tissue showed an extremely significant (P<0.001) decrease in GSH of CIS treated group compared to normal. The rats pretreated with MGLE (200mg/kg) and MGLE (400mg/kg) showed extremely significant (P<0.001) increase in GSH compared to cisplatin control. Investigation of antioxidants in homogenate brain tissue showed an extremely significant (P<0.001) decrease in catalase of CIS treated group compared to normal. The rats pretreated with MGLE (200mg/kg) and MGLE (400mg/kg) showed extremely significant (P<0.001) increase in Catalase compared to cisplatin control. Investigation of antioxidants in homogenate brain tissue showed an extremely significant (P<0.001) decrease in SOD of CIS treated group compared to normal control. The rats pretreated with MGLE (200 mg/kg) and MGLE (400 mg/kg) showed extremely significant (P<0.001) increase in SOD compared to Cisplatin control. See Table 4.

Table 4. Effect of MGLE on CIS induced neurotoxicity antioxidants

TREATMENT	GSH (mmol/g tissue)	SOD (U/g tissue)	CAT (U/g tissue)
NORMAL	17.781±0.024	15.86±0.025	17.735±0.007
CIS	8.715±0.024###	8.558±0.019###	10.556±0.014###
MGLE200+CIS	9.853±0.270**###	10.655±0.489**###	11.648±0.286**###
MGLE400+CIS	10.253±0.360***###	11.490±0.508***###	12.597±0.318***###

n=6; data are presented as MEAN ± SEM. Statistical significance was determined using one-way ANOVA then Tukey-Kramer multiple comparison test. **P<0.01 and ***P<0.001 indicate significant differences compared to the control group, while ##P<0.01 and ###P<0.001 denote significant differences compared to the normal group.

DISCUSSION

This study aimed to determine if *Mussaenda glabrata* extract of leaves could prevent cisplatin-induced hematoxicity, nephrotoxicity, and neurotoxicity in albino wistar rats. The study found that *Mussaenda glabrata* leaf extract significantly reduced the effects of cisplatin. The Rubiaceae family includes the flowering plant species M. glabrata. The entire wild Mussaenda plant has anti-inflammatory properties and is used to treat pruritis, bronchitis, fever, wounds, ulcers, cough and jaundice [11].

One well-known chemotherapeutic medication is CIS. Numerous human malignancies, including as those of thelung, ovaries, bladder, head and testicles have been treated with it. Nephrotoxicity is the most prevalent of the roughly 40 distinct toxicities of cisplatin. Ototoxicity, haematological neurotoxicity, hepatotoxicity, gastrointestinal and cardiotoxicity are additional frequent adverse effects [12,13]. One of the most critical metrics for determining the toxicity of the anti-cancer medication cisplatin in both people and animals is the haematopoietic system. According to the current study, rats treated with CIS for five days experienced considerable toxic effects in their haematological

parameters, while animals pretreated with MGLE shown a strong defence against hematotoxicity. The substantial reduction in the number of erythrocytes, hemoglobin, and platelets validated the harmful effects of CIS on haematological parameters [14].

The CIS demonstrated a significant decrease in the RBC level in the current investigation, confirming the hematotoxicity. When compared to a normal control group, the observed decline was highly significant, and the magnitude of toxicity was quite high. Several factors could be responsible, including bone marrow cell deterioration or an increase in the osmotic brittleness of red blood cells. Therefore, CIS poisoning may result in a reduction of red blood cells due to either haematopoietic tissue activity suppression, erythropoiesis impairment, or both. Because of the increased permeability of the RBC membrane, cisplatin treatment accelerated the destruction of RBCs and decreased erythropoietin, which is a haemopoietic growth factor. This, in turn, caused changes in haematological parameters [14].

In platelets and lymphocytes, CIS induces oxidative stress that may impact their lifespan, trigger apoptosis, and ultimately decrease the quantity of these cells in the circulation. In addition to a drop in RBC count, a decrease in platelet count may result from cisplatin's inhibition of bone marrow function, reduced platelet generation or consumption, or enhanced platelet aggregation. Also results suggests that thrombocytopenia with leukopenia in the cisplatin-treated group may have been caused by the apoptotic impact of cisplatin on platelets and lymphocytes, which in turn decreased the quantity of these cells in the blood. Additionally, decreased haemoglobin and erythrocyte counts may be caused by bleeding from intestinal affections caused by cisplatin and red cell destruction caused by free radicals [14].

Animals treated with CIS in the current study had higher WBC levels. This may be the result of an inflammatory response or the infection brought on by the injection of CIS. Additionally, CIS may cause oxidative stress in lymphocytes and thrombocytes of human being, resulting in their necrosis 14. Due to haemoglobin's sensitivity to oxidative stress, we observed a decrease in platelets and haemoglobin levels following CIS administration. Extracellular signal-regulated protein kinase (ERK) in platelets is activated in a dose-dependent manner by cisplatin, resulting in platelet death and decreased platelet function, both of which are signs of haematological toxicity. RBC, Hb, and platelet counts are beneficially increased in response to MGLE dose-dependent prophylactic therapy against CIS-induced hematotoxicity indicators. An increase in these erythrocytes may be linked to either preventing bone marrow suppression or promoting erythropoiesis. However, the inhibition of ERK in platelets is the cause of the increase in platelet counts. A lower WBC count may be associated with reduced inflammation across the haemopoietic system [14].

The CIS-treated group exhibits a substantial increase in urea, creatinine and uric acid in nephrotoxicity. The two primary indicators of nephrotoxicity and hepatorenal development are serum creatinine and urea. The natural product of muscle digestion, serum creatinine is removed unaltered by renal system. An anomaly in the kidney's glomerular filtration mechanism might be the cause of the rise in blood creatinine levels. The waste product produced by the liver's urea cycle during protein metabolism is called urea. Serum urea levels rise as a result of GFR deficit and decreased blood volume. The enzyme uridase produces uric acid as a byproduct of purine biotransformation [15,16].

The elevated levels of creatinine, urea, and uric acid brought on by CIS are effectively corrected by the MGLE pretreatment. The pathophysiology of CIS and other cytotoxic drug-induced nephrotoxicity has been proposed to include oxidative stress damage and generation of reactive oxygen species (ROS). The disturbance of the dynamic balance between pro-oxidants and free radicals, which antioxidants scavenge, results in cellular damage [17]. The current investigation shows that CIS administration significantly lowers GSH levels. The poisonous molecule acrolein is the cause of the decrease in GSH content in renal tissues following CIS treatment. Acrolein causes the kidney's tubular cells to necrotise by binding GSH in the plasma membrane, interfering with the antioxidant defence mechanism, and raising ROS. Following CIS administration, a significant decrease in SOD and catalase levels was noted in the current study.

The essential antioxidant enzymes SOD and catalase transform molecules of oxygen into non-toxic byproducts. When ROS levels rise, SOD levels fall [18]. The inhibition of catalase function is the cause of the increase in H2O2 levels. Consequently, the SOD activity is likewise inhibited by catalase deficiency. Increased ROS and lipid peroxidation are the primary causes of the decrease in these antioxidant enzymes. Following CIS therapy, the MGLE pretreatment dramatically raises the levels of GSH, SOD, and catalase [18]. Histopathological analysis and the results of serum markers and antioxidant parameters were connected. Bowman's capsule shrinkage, congestion, blood vessel dilatation, inflammation, and infiltration are all symptoms of CIS treatment. By examining positive effects such no alteration in Bowman's capsule size, mild inflammation, and no blood capillary congestion, the MGLE pretreatment effectively increased the toxic consequences brought on by the CIS renal system [18].

Numerous research in the field of neuroprotection have shown that oxidative stress, LPO, and mitochondrial dysfunction are all involved in the neurotoxicity caused by CIS. CIS causes cytotoxicity by producing reactive oxygen species (ROS). In brain tissues, injection of CIS resulted in reduction in activity of antioxidant defense enzymes, increase in levels of LPO, NO and decrease in concentrations of non-enzymatic components of GSH that prevent/defend against LPO. It is acknowledged that both are associated with oxidative stress and lead to andiscrepancy between the antioxidant capacity of the body and the production of radicals obtained from oxygen [19-22].

The study's findings demonstrate that, in comparison to the normal group, the CIS-treated animals' neural SOD and GSH levels dramatically dropped. The reduction of brain antioxidants

is prevented by simultaneously administering MGLE (400 mg/kg, orally) and CIS therapy. The loss of both zinc and copper, which are necessary for enzyme function, may be the cause of the drop in SOD activity following CIS injection. The superoxide anion generated during the regular metabolic process cannot be scavenged by the reduced SOD activity. LPO can be initiated and progressed by the superoxide anion. Following CIS injection, there is also a reduction in GSH activity. As a result, the brain's capacity to scavenge harmful H2O2 and lipid peroxides was diminished [23-25].

The fact that MGLE restored neuronal SOD and GSH activity indicates that the extract can shield the enzymes. The toxicity of CIS can be significantly increased by GSH deficiency. The neurotoxicity caused by CIS is known to be significantly influenced by free radicals and MGLE clearly shows that the elevated GSH levels provide protection. Oxidative stress is brought on in the brain by ROS and free radicals. CIS becomes more harmful when GSH is depleted. GSH depletion, which seems to be the primary mechanism for LPO and reduced antioxidant enzyme activity, is another cause of LPO. These findings provide credence to the idea that antioxidant system depletion is a contributing factor in the neurotoxicity process observed in rats treated with CIS [23-25]. MGLE's antioxidant action may thus be directly linked to neuroprotection. These results support the theory that antioxidant-rich medicinal herbs may have neuroprotective properties.

CONCLUSION

The current study showed that MGLE (200 mg/kg and 400 mg/kg orally) exhibited hematoprotective, nephroprotective, and neuroprotective properties against cisplatin. Cisplatin caused experimental rats to become nephrotoxic, neurotoxic, and hematotoxic. The herb's possible antioxidant properties, free radical-fighting activity, regulation of serum indicators and safeguarding of histopathological characteristics may all contribute to its effectiveness as a preventative therapy. To prove the reality clinically, more study is needed.

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Iron Homeostasis and Insulin Resistance in Type 2 Diabetes: A Cross-Sectional Study

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ABSTRACT:

Type 2 diabetes mellitus (T2DM) is closely associated with iron biology and oxidative stress. Indian data are, however, sparse regarding the association of abnormal iron indices, particularly hyperferritinemia, with insulin resistance and cardiometabolic risk. Iron status, glycaemia, dyslipidaemia, and oxidative stress in adults with T2DM receiving routine care were investigated in this thesis. (1) compared markers of iron homeostasis (ferritin, transferrin and total iron binding capacity [TIBC]) in adults with T2DM versus age-matched healthy controls; (2) examined the relationship of ferritin with insulin resistance (HOMA IR score) among subjects with well-controlled glycaemia and finally (3) profiled glycaemic indicators, apolipoproteins, inflammatory markers and oxidative stress biomarkers by disease status. A comparative cross-sectional study recruited 100 adults with T2DM and 100 age and sex matched controls (35–65 years). Anthropometry, blood pressure, fasting/post prandial glucose, HbA1c, lipid profile, ApoB/ApoA1, insulin (with HOMA IR), complete blood count, CRP, ESR, vitamins C and E, oxidative stress (MDA, SOD, GST), and iron indices (serum iron, ferritin, transferrin, TIBC) were obtained using standard biochemical and immunoturbidimetric methods; statistics used SPSS 20 with t tests and Pearson correlations (α=0.05).

Compared with controls, the T2DM group had higher BMI (24.83 ± 1.99 vs 23.54 ± 2.54 kg/m²; $p<0.01$) and blood pressure (SBP 134.05 ± 14.25 vs 123.69 ± 8.4 mmHg; DBP 83.12 ± 4.16 vs 80.1 ± 1 mmHg; both $p<0.01$). Fasting glucose (167.3 ± 51.56 vs 85.05 ± 8.54 mg/dL) and PPBS (216.06 ± 69.13 vs 114.15 ± 12.31 mg/dL) were higher (both $p<0.01$). Insulin (15.0 ± 10.21 vs 8.72 ± 11.29 µIU/mL) and HOMA IR (6.11 ± 4.70 vs 1.84 ± 2.40) were elevated ($p<0.01$). Ferritin was markedly higher (155.41 ± 31.91 vs 39.12 ± 21.45 ng/mL; $p<0.01$), while transferrin (244.31 ± 40.35 vs 280.94 ± 60.71 mg/dL; $p<0.01$) and TIBC (251.38 ± 67.72 vs 313.97 ± 64.79 µg/dL; $p<0.01$) were lower. Oxidative stress was higher (MDA 25.64 ± 12.81 vs 5.44 ± 6.04 nmol/mL; $p<0.01$) with lower SOD and GST and reduced vitamins C and E (all $p<0.01$). Ferritin correlated with HOMA IR ($r=0.424$; 95% CI 0.304–0.532; $p<0.001$) among those with satisfactory glycaemic control. Adults with T2DM showed a distinct iron inflammation–oxidative stress phenotype: higher ferritin (with lower transferrin/TIBC), stronger insulin resistance, and heightened oxidative stress. Ferritin's positive association with HOMA IR supports a role for iron dysregulation in insulin resistance in this Indian cohort.

KEY WORDS: Type 2 Diabetes Mellitus; Insulin Resistance; Serum Ferritin; Transferrin; TIBC; Oxidative Stress; Malondialdehyde; Apolipoprotein B.

INTRODUCTION

The prevalence of type 2 diabetes mellitus (T2DM) is increasing around the world, including India, with significant microvascular and macrovascular complications [1]. Indian patients generally exhibit a clustering of dyslipidaemia, hypertension, and inflammation, which enhances vascular risk [2]. In addition to glucose, iron has been identified as an essential mediator of metabolic risk [3]. Elevated ferritin has been linked to insulin resistance, β-cell stress, and unfavourable cardiometabolic phenotypes: in particular, the pathogenetic role of excessive labile iron-mediated production of reactive oxygen species (ROS) and protein glycation mechanisms that lead to impaired activity of the insulin receptor [4]. Data from cohort and mechanistic studies indicate that ferritin and transferrin kinetics (including reduction of Fe^{3+} binding to transferrin by glycation3) are impacted in diabetes, which can further create a vicious circle of oxidative stress and inflammation [5].

Oxidative stress is integral to diabetic vascular pathology: lipid peroxidation by products such as malondialdehyde (MDA) are elevated, while enzymatic antioxidants (e.g., SOD, GST) and antioxidant vitamins (C, E) may be depleted [6]. Dyslipidaemia (notably ApoB-rich lipoproteins) further compounds risk. Despite this, Indian data simultaneously profiling iron indices, insulin resistance, oxidative stress, and emerging lipid markers in a single study remain scarce [7].

Knowledge gap and rationale: While prior work has linked iron status to glucose dysregulation and cardiometabolic risk, there is limited integrative evidence from Indian outpatient populations receiving routine care—especially examining correlations between ferritin and insulin resistance under satisfactory glycaemic control. This study addresses that gap.

Objectives: We sought to (i) compare iron homeostasis markers between T2DM cases and matched controls; (ii) quantify the relationship between ferritin and HOMA IR; and (iii) characterise oxidative stress, antioxidant status, inflammatory markers, and apolipoproteins in the same cohort.

MATERIAL AND METHODS

Study design and setting: This was a comparative cross-sectional study conducted at a diabetic clinic, Kozhikode, Kerala, India, from December 2020 to May 2022.

Participants. Adults aged 35–65 years: 100 with previously diagnosed T2DM on treatment and 100 apparently healthy controls, matched by age and sex. Inclusion required willingness to participate; individuals with life-threatening comorbidities were excluded.

Data collection and assays: Anthropometry and blood pressure were recorded; fasting/post-prandial blood glucose (oxidase–peroxidase method) and lipid profile were measured. ApoB/ApoA1 were quantified by immunoturbidimetry. Oxidative stress was assessed via MDA (thiobarbituric acid reactive substances method) and antioxidant enzymes (SOD, GST); vitamins C and E were measured with standard spectrophotometric protocols. Insulin and HOMA IR were obtained; complete blood count, CRP, ESR, iron, ferritin, transferrin, and TIBC were analysed using standard laboratory methods.

Outcomes: Primary: between-group differences in ferritin, transferrin, and TIBC; association between ferritin and HOMA IR under satisfactory glycaemic control. Secondary: between-group differences in glycaemic, lipid, inflammatory, haematological, and oxidative stress indices.

Table 1. Baseline characteristics of participants (Control vs Test)

Parameter	Control (n=100)	Test (n=100)	t test	p value
Age (years)	47.5 ± 10.56	51.5 ± 10.32	−2.71	<0.01**
Height (cm)	161.94 ± 6.46	162.06 ± 7.58	−0.12	0.904
Weight (kg)	61.96 ± 7.82	64.55 ± 8.07	−2.30	<0.05*
BMI (kg/m²)	23.54 ± 2.54	24.83 ± 1.99	−4.01	<0.01**

Statistical analysis: Data are mean ± SD. Group comparisons used independent samples t tests; Pearson correlations examined relationships among ferritin, insulin, and HOMA IR. Significance was set at $p \leq 0.05$ (SPSS v20).

RESULTS

Participant characteristics: The T2DM group had higher BMI (24.83 ± 1.99 vs 23.54 ± 2.54 kg/m²; p<0.01) and blood pressure (SBP 134.05 ± 14.25 vs 123.69 ± 8.4 mmHg; DBP 83.12 ± 4.16 vs 80.1 ± 1 mmHg; both p<0.01).

Glycaemia and insulin resistance: FBS and PPBS were higher in T2DM (FBS 167.3 ± 51.56 vs 85.05 ± 8.54 mg/dL; PPBS 216.06 ± 69.13 vs 114.15 ± 12.31 mg/dL; both p<0.01). Insulin (15.0 ± 10.21 vs 8.72 ± 11.29 µIU/mL; p<0.01) and HOMA IR (6.11 ± 4.70 vs 1.84 ± 2.40; p<0.01) were elevated.

Lipid and apolipoprotein profile: Traditional lipids were higher in T2DM; ApoB was significantly higher (1.55 ± 0.19 vs 1.46 ± 0.17 g/L; p<0.01), while ApoA1 was modestly higher but not statistically significant (1.29 ± 0.33 vs 1.23 ± 0.13 g/L; p = 0.077).

Oxidative stress and vitamins: MDA was markedly higher (25.64 ± 12.81 vs 5.44 ± 6.04 nmol/mL; p<0.01). Antioxidant enzymes SOD (55.15 ± 37.90 vs 498.73 ± 671.12 U/L; p<0.01) and GST (21.31 ± 18.13 vs 74.89 ± 32.15 ng/mL; p<0.01) were lower. Vitamins C and E were significantly reduced (both p<0.01).

Table 2. Glycaemic parameters in Control vs Test

Parameter	Control (n=100)	Test (n=100)	t test	p value
FBS (mg/dL)	85.05 ± 8.54	167.30 ± 51.56	−15.74	<0.01**
PPBS (mg/dL)	114.15 ± 12.31	216.06 ± 69.13	−14.51	<0.01**
HbA1c (%)	4.87 ± 0.38	5.82 ± 1.16	−7.75	<0.01**

Table 3. Lipid profile in Control vs Test

Parameter	Control (n=100)	Test (n=100)	t test	p value
Total Cholesterol (mg/dL)	178.39 ± 13.57	231.60 ± 27.88	−17.16	<0.01**
HDL C (mg/dL)	47.22 ± 4.48	36.50 ± 3.03	19.80	<0.01**
LDL C (mg/dL)	111.92 ± 10.20	157.96 ± 26.70	−16.11	<0.01**
VLDL C (mg/dL)	20.75 ± 5.06	33.96 ± 11.15	−10.79	<0.01**
Triglycerides (mg/dL)	122.93 ± 18.84	177.46 ± 30.85	−15.08	<0.01**

Inflammation and haematology: CRP and ESR were higher in T2DM; total WBCs, neutrophils, lymphocytes, platelets, RBCs, haematocrit, and MCV were elevated, while MCH/MCHC/RDW indices showed no significant rise. Hb did not differ significantly.

Table 4. Oxidative stress and antioxidant markers

Parameter	Control (n=100)	Test (n=100)	t test	p value
MDA (nmol/mL)	5.44 ± 6.04	25.64 ± 12.81	−14.26	<0.01**
SOD (U/L)	498.73 ± 671.12	55.15 ± 37.90	6.60	<0.01**
GST (ng/mL)	74.89 ± 32.15	21.31 ± 18.13	14.52	<0.01**
Vitamin C (ng/mL)	93.08 ± 40.11	11.07 ± 7.71	20.08	<0.01**
Vitamin E (nmol/mL)	64.94 ± 21.45	7.80 ± 5.42	25.83	<0.01**

Iron homeostasis: Ferritin was substantially higher in T2DM (155.41 ± 31.91 vs 39.12 ± 21.45 ng/mL; p<0.01), whereas transferrin (244.31 ± 40.35 vs 280.94 ± 60.71 mg/dL; p<0.01) and TIBC (251.38 ± 67.72 vs 313.97 ± 64.79 µg/dL; p<0.01) were lower. Among participants with satisfactory glycaemic control, ferritin correlated with HOMA IR (r=0.424; p<0.001) and with insulin (r=0.214; p=0.002). Insulin correlated strongly with HOMA IR (r=0.848; p<0.001).

DISCUSSION

In this clinic-based Indian cohort, T2DM was characterised by higher adiposity and blood pressure; marked hyperglycaemia with elevated insulin/HOMA IR; an atherogenic lipid pattern with higher ApoB; pronounced oxidative stress (high MDA; low SOD/GST and vitamins C/E); systemic inflammation (CRP/ESR); and a distinct iron signature—high ferritin with lower transferrin/TIBC. Significantly, ferritin correlated positively with HOMA IR under satisfactory glycaemic control, strengthening the link between iron stores and insulin resistance. Our results are consistent with previous evidence that has associated ferritin with insulin resistance and T2DM risk [8]. Harrison et al. emphasized the involvement of iron in glucose toxicity and insulin action [9]. A meta-analysis by Yang et al. supported the relationship between abnormal iron homeostasis and T2DM [10].

Conway et al. In population studies, body iron was independently associated with serum insulin and glucose [11]. While mechanistically hepcidin is known to modulate iron flux, transferrin glycation may reduce the binding capacity of iron and increase labile iron, leading to ROS and inflammation – consistent with our high ferritin and oxidative stress [12].

The documented lower transferrin/TIBC in T2DM likely mirrors inflammation-induced negative acute phase-reactants and glycation-mediated function impairment of transferrin, as evidenced by in vitro and clinical studies. In concert, they may attenuate iron binding and exaggerate oxidative damage in line with our increased MDA and depleted antioxidant levels. Our pattern of oxidative stress—increased MDA, decreased SOD/GST, and lower vitamins C/E—is consistent with traditional diabetes reports and newer endothelial/metabolic dysregulation studies that emphasize ROS damage.

On lipids, ApoB no question complements the idea that higher ApoB is better than LDL C as a risk marker, significant in DM, where dense LDL rules the roost. Discrepancies with some literature (for example, the lack of a substantial increase in ApoA1 in our study) may result from the status of treatment, diet, or background inflammation in our cohort.

Strengths are age/sex matching, a large biomarker panel (glycaemic, lipid, inflammatory-oxidative-iron indices), and formal correlation of ferritin against insulin resistance over an interval clinically pertinent. The thesis addressed these limitations: cross-sectional nature (i.e., no causality), single-centre recruitment, incomplete formal risk assessment for diabetic complications, and potential residual confounding (dietary iron; hepcidin status; medication effects). Limitations Analytic choices (e.g., use of HOMA IR rather than clamp studies; lack of measurement of hepcidin levels) limit mechanistic inference.

CONCLUSION

This study demonstrates that, in Indian adults with T2DM, iron dysregulation (high ferritin with lower transferrin/TIBC) coexists with insulin resistance and heightened oxidative stress. Ferritin relates positively to HOMA IR under satisfactory glycaemic control. These data support integrating iron indices into cardiometabolic risk appraisal and motivate prospective/interventional work (e.g., defining optimal ferritin targets, clarifying hepcidin dynamics) in Indian settings.

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Conflict of interest: The authors declare no competing interests.

Data availability: The identified data underlying this study's findings (biomarker matrices and statistical code) are available from the corresponding author upon reasonable request, subject to ethical approvals and institutional policies.

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Knowledge, Attitude, and Practice of Digital Dentures Among Practising Dentists in the Mumbai Metropolitan Region: A Cross-Sectional Questionnaire Study

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ABSTRACT:

Digital dentures are increasingly being introduced into prosthodontic practice because of their potential to improve workflow standardization, denture accuracy, and record preservation. However, their successful clinical adoption depends on the knowledge, attitude, and practical exposure of dentists. To assess the knowledge, attitude, and practice regarding digital dentures among practising dentists in the Mumbai Metropolitan Region. A questionnaire-based observational cross-sectional study was conducted among practising dentists in the Mumbai Metropolitan Region using a web-based survey. A structured close-ended questionnaire assessed demographic details and responses related to knowledge, attitude, practice, perceived barriers, and future prospects of digital dentures. Data were analyzed using descriptive statistics, and qualification-wise comparisons were performed using tests for categorical variables. A p value of less than 0.05 was considered statistically significant. A total of 111 responses were analyzed. Most respondents had heard of digital dentures (93.7%), and 93.7% considered them a valuable innovation in prosthodontics. However, only 24.3% had tried fabricating digital dentures in clinical practice, and 24.3% reported hands-on experience. Formal curricular learning was reported by 30.6% of respondents. Most participants felt that affordability depended on locality (68.5%), and 50.5% reported multiple concurrent barriers to learning or practice. MDS respondents showed significantly greater clinical exposure, curricular learning, and academic exposure than BDS respondents. Practising dentists showed high awareness and a favorable attitude toward digital dentures, but practical exposure and routine use remained limited. The findings indicate a gap between awareness and implementation and support the need for stronger educational and hands-on training opportunities.

KEY WORDS: DDigital dentures; CAD-CAM dentures; Digital dentistry; Prosthodontics; Questionnaire surveyQuestionnaire survey

INTRODUCTION

Edentulism remains an important prosthodontic problem, particularly in older adults, and complete dentures continue to play a major role in restoring mastication, speech, esthetics, and quality of life. Conventional complete denture fabrication has been used successfully for many years, but it involves multiple clinical and laboratory steps and is often time-consuming and technique-sensitive. In recent years, digital workflows have gained increasing attention because they offer a more standardized approach to denture fabrication and may reduce chairside and laboratory time while preserving digital records for future remakes ([1,2]

Digital complete dentures are fabricated using computer-aided design and computer-aided manufacturing technologies, usually through subtractive milling or additive three-dimensional printing. These methods have expanded the scope of removable prosthodontics by introducing new options for denture design, manufacturing precision, and workflow simplification. Reviews of currently available CAD-CAM complete denture systems have noted that digital workflows are becoming more clinically relevant as materials, software, and manufacturing systems continue to improve [3].

Accuracy of the denture base is one of the most important factors influencing retention, stability, and adaptation of complete dentures. Experimental studies have shown that digitally fabricated denture bases can achieve clinically acceptable adaptation. [4] reported that both milled and digital light processing-generated mandibular denture bases demonstrated intaglio surface adaptation within a clinically acceptable range. Similarly, [5] found that digitally fabricated maxillary denture bases showed favorable trueness and tissue surface adaptation when compared with conventional pack-and-press methods [6]. further observed that CAD-CAM milled and rapid prototyping methods showed better overall accuracy than conventional injection molding in their comparative evaluation of denture base fabrication techniques [4,5 & 6].

Despite these advancements, successful incorporation of digital dentures into routine practice depends not only on the technology itself but also on the clinician's knowledge, acceptance, and practical exposure. In a metropolitan region such as Mumbai, where digital dentistry is gradually becoming more visible in academic and private practice settings, it is important to understand how practising dentists perceive and use digital denture technology. Therefore, the present study was undertaken to assess the knowledge, attitude, and practice of digital dentures among practising dentists in the Mumbai Metropolitan Region.

MATERIALS AND METHODS

Study design and setting: This questionnaire-based observational cross-sectional study was conducted to assess the knowledge, attitude, and practice regarding digital dentures among practising dentists in the Mumbai Metropolitan Region (MMR). The study was carried out as a web-based survey, which allowed inclusion of respondents from different clinical and academic practice settings within the region. The study was conducted according to the approved study protocol and in accordance with accepted ethical principles for questionnaire-based research. The respondents were informed about the objectives of the study before participation, and confidentiality of their responses was maintained throughout the study. As the study was non-interventional and based solely on an anonymous web-based questionnaire, no clinical procedure was involved.

Study population and eligibility criteria: The study population comprised practising dentists working in the Mumbai Metropolitan Region. Both Bachelor of Dental Surgery (BDS) graduates and Master of Dental Surgery (MDS) practitioners were considered eligible for participation. Dentists practising outside the Mumbai Metropolitan Region and undergraduate dental students were excluded from the study. Since the study was based on voluntary participation in an online questionnaire, withdrawal criteria were not applicable.

Sample size and survey approach: A target sample of 200 practising dentists was planned for the survey. The questionnaire was circulated electronically among eligible participants, and responses received from those who met the inclusion criteria were considered for analysis. Only completed responses were included in the final dataset. The web-based approach was chosen to improve accessibility and facilitate participation from dentists across different areas of the metropolitan region.

Study instrument: Data were collected using a structured, close-ended questionnaire designed for this study. The questionnaire included items related to demographic details such as qualification and specialty, followed by questions assessing knowledge, attitude, and practice regarding digital dentures. The knowledge component assessed awareness of the term digital dentures, prior exposure to the subject in the academic curriculum, and understanding of the digital denture workflow. The attitude component included questions related to the perceived value of digital dentures, their future role in prosthodontic practice, affordability, the need for additional undergraduate training, and confidence in using the technology in future. The practice component assessed previous fabrication of digital dentures, hands-on experience, and extent of academic exposure. Additional questions were included to identify perceived barriers to learning or practising digital denture fabrication and the expected impact of digital dentures on patient care.

Data collection procedure: The questionnaire was distributed through online platforms to practising dentists in the Mumbai Metropolitan Region. Before participation, the respondents were informed about the purpose and nature of the study. They were assured that the information provided by them would be kept confidential and used only for academic and research purposes. No open-ended questions were included in the survey.

Study variables and outcome measures: The primary outcome of the study was the assessment of knowledge, attitude, and practice related to digital dentures among practising dentists in the Mumbai Metropolitan Region. The main study variables included awareness of digital dentures, curricular learning, perception of their value in prosthodontics, opinion regarding their future use, confidence in adopting the

technology, actual clinical exposure, and hands-on experience. Secondary observations included perceived barriers to learning or implementation, opinion regarding affordability, expected impact on patient care, and views on the integration of digital denture training into the undergraduate curriculum.

Data management and statistical analysis: The collected responses were compiled in a master chart and reviewed for completeness before analysis. Categorical variables were summarized using frequencies and percentages. The findings were presented in tabular form for clarity. For subgroup comparisons, qualification-based differences between BDS and MDS respondents were assessed using appropriate tests for categorical data, such as the chi-square test or Fisher's exact test wherever required. A p value of less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

A total of 111 completed questionnaire responses were included in the analysis. Of these, 69 respondents (62.2%) were MDS practitioners and 42 (37.8%) were BDS practitioners. With respect to specialty distribution, 34 respondents (30.6%) belonged to Prosthodontics, Crown and Bridge, followed by Pediatric and Preventive Dentistry and Periodontology with 14 respondents (12.6%) each. Conservative Dentistry and Endodontics and Oral Medicine and Radiology each contributed 7 respondents (6.3%). In 35 responses (31.5%), the branch was not applicable or not specified, corresponding largely to BDS participants. The distribution of participant characteristics is shown in Table 1.

Table 1. Participant characteristics

Characteristic	Category	n (%)
Qualification	BDS	42 (37.8)
	MDS	69 (62.2)
Specialty/branch	Not applicable/not specified	35 (31.5)
	Prosthodontics, Crown & Bridge	34 (30.6)
	Pediatric and Preventive Dentistry	14 (12.6)
	Periodontology	14 (12.6)
	Conservative Dentistry and Endodontics	7 (6.3)
	Oral Medicine and Radiology	7 (6.3)

Knowledge and practice related to digital dentures: Awareness regarding digital dentures was high, with 104 respondents (93.7%) reporting that they had heard of the term. However, only 27 respondents (24.3%) had tried to fabricate a digital denture in their clinic, and an identical proportion reported hands-on experience with digital denture fabrication. Learning about digital dentures through the formal curriculum was reported by 34 respondents (30.6%), while 77 (69.4%) stated that they had not learned about the topic in their curriculum. Regarding academic exposure, the most common response was "rarely" in 56 respondents (50.5%), followed by "never" in 21 (18.9%), "frequently" in 20 (18.0%), and "occasionally" in 14 (12.6%). These findings are presented in Table 2.

Table 2. Knowledge and practice related to digital dentures

Variable	Response	n (%)
Heard of the term digital dentures	Yes	104 (93.7)
	No	7 (6.3)
Tried to fabricate digital dentures in clinic	Yes	27 (24.3)
	No	84 (75.7)
Learned about digital dentures in curriculum	Yes	34 (30.6)
	No	77 (69.4)
Hands-on experience with digital denture fabrication	Yes	27 (24.3)
	No	84 (75.7)
Exposure to digital denture content in academic curriculum	Frequently	20 (18.0)
	Occasionally	14 (12.6)
	Rarely	56 (50.5)
	Never	21 (18.9)

Attitude, perceived barriers, and future prospects: Most respondents expressed a favorable attitude toward digital dentures. A total of 62 respondents (55.9%) strongly agreed and 42 (37.8%) agreed that digital dentures are a valuable innovation in prosthodontics. Regarding future replacement of conventional dentures, 63 respondents (56.8%) believed that digital dentures would completely replace conventional dentures, whereas 48 (43.2%) felt that a combination of both approaches would be ideal. With respect to affordability, 76 respondents (68.5%) felt that affordability depended on locality, 28 (25.2%) considered digital dentures affordable, and 7 (6.3%) considered them non-affordable.

For undergraduate training, 62 respondents (55.9%) strongly agreed and 28 (25.2%) agreed that students should receive more training on digital denture fabrication. Confidence in using digital denture technology in the future was reported as very confident by 48 respondents (43.2%), somewhat confident by 28 (25.2%), and requiring further learning and training by another 28 (25.2%), while 7 respondents (6.3%) reported that they were not confident. The most common perceived barrier was the presence of multiple simultaneous barriers, reported by 56 respondents (50.5%). With regard to future prospects, 76 respondents (68.5%) expected a revolutionary improvement in patient care, and 55 (49.5%) strongly agreed while 35 (31.5%) agreed that digital denture training should be integrated more strongly into the undergraduate curriculum. These data are summarized in Table 3.

Table 3. Attitude toward digital dentures, perceived barriers, and future prospects

Variable	Response	n (%)
Digital dentures are a valuable innovation in prosthodontics	Strongly agree	62 (55.9)
	Agree	42 (37.8)
	Neutral	7 (6.3)
Belief that digital dentures will replace conventional dentures in future	Yes, completely	63 (56.8)
	A combination of both is ideal	48 (43.2)
Affordability of digital dentures for patients	Affordable	28 (25.2)
	Depends on locality	76 (68.5)
	Non-affordable	7 (6.3)
Need for more undergraduate training	Strongly agree	62 (55.9)
	Agree	28 (25.2)
	Neutral	14 (12.6)
	Disagree	7 (6.3)
Confidence in ability to understand and use digital denture technology in future	Very confident	48 (43.2)
	Somewhat confident	28 (25.2)
	Need some learning and training sessions	28 (25.2)
	Not confident	7 (6.3)
Perceived barriers to learning or practicing digital denture fabrication	All above	56 (50.5)
	Limited resources	14 (12.6)
	Lack of teaching in curriculum	14 (12.6)
	Lack of hands-on training	14 (12.6)
	High cost of equipment	7 (6.3)
	None	6 (5.4)
Perceived impact on patient care	Revolutionary improvement	76 (68.5)
	Moderate improvement	35 (31.5)
Recommendation for greater integration into undergraduate curriculum	Strongly agree	55 (49.5)
	Agree	35 (31.5)
	Neutral	7 (6.3)
	Disagree	14 (12.6)

Comparison according to qualification: Qualification-wise comparison demonstrated statistically significant differences for several variables. Previous clinical fabrication of digital dentures was reported by 27 of 69 MDS respondents (39.1%), whereas none of the 42 BDS respondents reported such experience ($p<0.001$). Similarly, learning about digital dentures in the curriculum was more frequent among MDS respondents than BDS respondents (39.1% vs 16.7%; $p=0.023$). A significant difference was also observed in perception of digital dentures as a valuable innovation: 48 MDS respondents (69.6%) strongly agreed compared with 14 BDS respondents (33.3%), while neutrality was reported only among BDS respondents (16.7%) ($p<0.001$).

Exposure to digital denture-related content also differed significantly between the two qualification groups ($p<0.001$). Frequent exposure was reported only among MDS respondents (29.0%), whereas "never" was more common among BDS respondents (33.3% vs 10.1%). Confidence regarding future use showed a significant association with qualification ($p=0.034$), with "very confident" being reported by 50.0% of BDS respondents and 39.1% of MDS respondents, while lack of confidence was reported only among MDS respondents (10.1%). Affordability perception also differed significantly between groups ($p=0.015$). In contrast, no statistically significant association with qualification was observed for awareness of the term digital dentures ($p=0.084$), belief regarding complete future replacement of conventional dentures ($p=0.148$), perceived impact on patient care ($p=0.914$), or recommendation for curricular integration ($p=0.157$). The detailed comparison is presented in Table 4.

The present study showed that awareness of digital dentures among practising dentists in the Mumbai Metropolitan Region was high, but this did not translate into equivalent levels of clinical use or hands-on experience. This gap between

awareness and implementation is clinically important because adoption of digital dentures depends not only on knowing the term, but also on exposure to scanning, digital design, manufacturing workflow, and post-processing steps. A similar pattern was reported by [7], who found high awareness of digital dentures among dentists, while actual practice and confidence remained lower. Likewise, [8] reported that perception toward digital dentistry was generally favorable, but emphasized that stronger educational exposure and practical training were still needed for meaningful clinical integration.

Table 4. Qualification-wise comparison of selected variables

Variable	Response	BDS n (%)	MDS n (%)	p value
Heard of the term digital dentures	Yes	42 (100.0)	62 (89.9)	0.084
	No	0 (0.0)	7 (10.1)	
Tried to fabricate digital dentures in clinic	Yes	0 (0.0)	27 (39.1)	<0.001
	No	42 (100.0)	42 (60.9)	
Learned about digital dentures in curriculum	Yes	7 (16.7)	27 (39.1)	0.023
	No	35 (83.3)	42 (60.9)	
Digital dentures are a valuable innovation in prosthodontics	Strongly agree	14 (33.3)	48 (69.6)	<0.001
	Agree	21 (50.0)	21 (30.4)	
	Neutral	7 (16.7)	0 (0.0)	
Affordability of digital dentures for patients	Affordable	7 (16.7)	21 (30.4)	0.015
	Depends on locality	35 (83.3)	41 (59.4)	
	Non-affordable	0 (0.0)	7 (10.1)	
Confidence in ability to understand and use digital denture technology in future	Very confident	21 (50.0)	27 (39.1)	0.034
	Somewhat confident	7 (16.7)	21 (30.4)	
	Need some learning and training sessions	14 (33.3)	14 (20.3)	
	Not confident	0 (0.0)	7 (10.1)	
Exposure to digital denture content in academic curriculum	Frequently	0 (0.0)	20 (29.0)	<0.001
	Occasionally	7 (16.7)	7 (10.1)	
	Rarely	21 (50.0)	35 (50.7)	
	Never	14 (33.3)	7 (10.1)	
Perceived impact on patient care	Revolutionary improvement	28 (66.7)	48 (69.6)	0.914
	Moderate improvement	14 (33.3)	21 (30.4)	
Recommendation for greater integration into undergraduate curriculum	Strongly agree	21 (50.0)	34 (49.3)	0.157
	Agree	14 (33.3)	21 (30.4)	
	Neutral	0 (0.0)	7 (10.1)	
	Disagree	7 (16.7)	7 (10.1)	

The positive attitude observed in the present study is understandable in light of the growing evidence supporting digital complete denture workflows. Systematic review evidence suggests that CAD-CAM complete dentures are not inferior to conventional dentures and may offer additional advantages such as better retention, improved mechanical properties, reduced chairside time, and preservation of digital records for future remakes [10]. Experimental studies have also shown that CAD-CAM dentures, particularly milled dentures, demonstrate superior fit compared with conventionally fabricated dentures, which may explain why many clinicians increasingly regard digital dentures as a valuable innovation in prosthodontics [11]. Similarly, [12] found that milled PMMA denture bases showed better adaptation than printed and conventionally fabricated denture bases, supporting the perception that digital methods may improve prosthesis accuracy.

In the present study, MDS respondents showed greater curricular exposure, greater clinical experience, and significantly more prior fabrication of digital dentures than BDS respondents. This finding is logical because postgraduate training provides more specialty-based exposure, especially in prosthodontics and related digital workflows. The importance of such training has been highlighted in earlier literature [13]. demonstrated that a complete denture workflow based on intraoral scans is feasible, but such techniques require familiarity with digital records, maxillomandibular relationship registration, and appropriate case selection [14] also noted that although digital workflows may improve clinician-reported

and patient-related outcomes in several aspects, adoption still depends on practical understanding, operator learning curve, and clinical infrastructure. Therefore, the present finding that many respondents supported stronger undergraduate integration of digital denture training appears justified and reflects a real educational need.

Another notable finding was that affordability was considered dependent on locality by most respondents, and the most frequently reported barriers were multiple concurrent obstacles, limited resources, lack of curricular teaching, lack of hands-on training, and high equipment cost. These responses reflect the current practical situation in removable digital prosthodontics. Although digital denture systems may reduce appointments and improve workflow efficiency, the required infrastructure remains expensive and is not uniformly available across all practice settings. Earlier survey-based work has also identified cost, equipment access, and insufficient practical training as major barriers to adoption [7,8]. Clinically, this suggests that digital dentures are presently being viewed more as a promising adjunct or evolving alternative rather than a universal replacement for conventional dentures in all settings.

This study has certain limitations. It was a cross-sectional, questionnaire-based survey and therefore depended on self-reported responses, which may be influenced by recall bias or response bias. The sample was restricted to practising dentists in one metropolitan region, so the findings may not be generalizable to other regions or institutional settings. In addition, the questionnaire assessed perceived knowledge and practice rather than objectively measured competence. Future studies should include multicentric samples, validated scoring systems for knowledge and attitude, and subgroup analysis based on specialty, years of experience, and type of practice. Longitudinal or interventional studies evaluating the effect of structured teaching modules, hands-on workshops, and continuing dental education programs on actual adoption of digital denture workflows would provide stronger evidence for curriculum planning and clinical implementation.

CONCLUSION

Digital dentures were widely recognized by practising dentists in the Mumbai Metropolitan Region and were generally viewed positively as an emerging advancement in prosthodontics. However, actual clinical use, hands-on experience, and curricular exposure remained limited, indicating a gap between awareness and implementation. Within the limits of this study, the findings suggest a need for stronger educational exposure and practical training to support more confident and effective adoption of digital denture technology.

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In vitro Clonal Propagation of Medicinally Valuable sp. *Centella asiatica* (L.) for Conservation and Sustainable Utilization

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ABSTRACT:
In Vitro clonal propagation of medicinally important plant species: Ensuring High Alkaloid Production and Genetic Stability in *Centella asiatica* (L.). This clearly conveys both the purpose conservation and maintaining biochemical traits. The plant is believed to be native to tropical and subtropical regions, particularly Sri Lanka, India, Madagascar, South Africa, and Malaysia. It grows abundantly in moist, swampy, and shaded environments such as riverbanks, paddy fields, and wetlands, where it forms a dense green cover. *Centella asiatica* (L.) belong to the Apiaceae family important valuable alkaloid medicinal herb and It helps in curing many diseases like Memory-Enhancing [7], Anti-ulcer, Antitumor, Antitumor, Cardio-protective, Antiviral Activity etc. because of this plant immediate conservation and develop cost effective micropropagation technique with help of suitable combination and concentration with MS media reduce and minimize contamination percentage. MS basal medium was supplemented with 3% sucrose, seven different concentrations of BAP with 0.5 mg/l NAA, separately. It was noted that MS + 3% sucrose, gelled with 0.8% agar + The best response in the present study was observed at 3.0 mg/L BAP + 3.0 mg/L IAA, which showed maximum initiation (85%), shoot proliferation (95%), and elongation (90%) along with excellent rooting. Bavistin and $HgCl_2$ treatments significantly reduced contamination rates, with maximum effectiveness observed at higher concentrations and optimal exposure times. High contamination rates during initial culture establishment have also been reported in *Centella asiatica*, emphasizing the importance of effective sterilization protocols.

KEY WORDS: *Centella asiatica*, Micropropagation, conservation, Medicinal, MS media, PGR and Contamination rate.

INTRODUCTION

Medicinal plants have been an essential component of human healthcare systems since ancient times, serving as a primary source of therapeutic agents for the treatment of various diseases. Traditional systems such as Ayurveda, Unani, and Traditional Chinese Medicine have extensively relied on plant-based remedies long before the development of modern pharmaceuticals [3]. Even today, a significant proportion of the global population, particularly in developing countries, depends on herbal medicine due to its affordability, accessibility, and relatively fewer side effects.

The medicinal value of plants is attributed to the presence of diverse bioactive compounds, including alkaloids, flavonoids, tannins, and phenolic substances. These phytochemicals exhibit a wide range of pharmacological activities such as antioxidant, anti-inflammatory, antimicrobial, and anticancer effects. Their combined action enhances the therapeutic

potential of medicinal plants, making them highly valuable in both traditional and modern healthcare systems [10].

Among the various medicinal plants, *Centella asiatica* (L.), belonging to the family Apiaceae, is one of the most important herbs due to its extensive pharmacological properties. Commonly known as Gotu Kola or Mandukaparni, it is widely distributed in tropical and subtropical regions, including India, Sri Lanka, China, and Africa. The plant thrives in moist and shaded environments and is characterized by its creeping growth habit and kidney-shaped leaves. Traditionally, C. asiatica has been used for enhancing memory and cognitive function, promoting wound healing, reducing inflammation, and managing stress and anxiety.

The therapeutic efficacy of C. asiatica is primarily due to its rich phytochemical composition, particularly triterpenoids such as asiaticoside and madecassoside, along with flavonoids and phenolic compounds. These constituents contribute to its antioxidant, neuroprotective, antimicrobial, and anti-inflammatory activities [9]. Owing to these properties, the plant has gained considerable importance in pharmaceutical, cosmetic, and nutraceutical industries. (South African Journal of Botany 2021).

Despite its immense medicinal value, C. asiatica faces several challenges related to natural propagation. Poor seed viability, slow growth rate, and increasing commercial demand have led to overexploitation of natural populations, resulting in a decline in its availability and genetic diversity. Conventional propagation methods are insufficient to meet the growing demand and ensure sustainable utilization [12].

In this context, plant tissue culture techniques, particularly *in vitro* propagation, offer a promising alternative for the rapid multiplication and conservation of C. asiatica. Micropropagation enables the production of large numbers of disease-free and genetically uniform plantlets under controlled conditions, independent of seasonal variations. Therefore, the present study focuses on developing an efficient *in vitro* propagation protocol for *Centella asiatica* to support its large-scale production, conservation, and sustainable utilization.

The study demonstrates that *in vitro* propagation is an efficient and reliable method for the mass multiplication of *Centella asiatica*. Rapid shoot initiation and successful culture establishment confirmed the suitability of tissue culture techniques for this medicinal plant [2]. Murashige and Skoog (MS) medium proved effective in supporting optimal explant growth. Among plant growth regulators, BAP significantly enhanced multiple shoot induction, while auxins such as NAA and IBA promoted efficient root formation. The combined application of cytokinins and auxins resulted in well-developed plantlets with balanced shoot and root systems. A high survival rate during acclimatization indicated the physiological stability and adaptability of regenerated plants. The findings support previous studies and highlight the potential of this protocol for large-scale production, conservation, and sustainable utilization of *Centella asiatica* in pharmaceutical and cosmetic applications.

MATERIALS AND METHODS

Collection of Plant Material: Healthy plants of *Centella asiatica* were collected from the medicinal nursery established under the Biodiversity Division of the State Forest Research Institute, Jabalpur, Madhya Pradesh, India. The collected plants were maintained under suitable conditions prior to experimentation.

Selection of Explants: Different explants such as nodal segments, root segments, and leaf tissues were considered for *in vitro* culture. Among these, nodal segments were found to be most suitable for shoot induction due to the presence of axillary buds. Leaf explants were mainly used for callus formation, while root explants exhibited a comparatively low response in shoot regeneration. Therefore, nodal and root explants were selected for the present study, with nodal segments showing the highest regeneration efficiency.

Sterilization Procedure: Sterilization is a critical step in plant tissue culture to eliminate microbial contamination. Since the culture medium is nutrient-rich, it favors the growth of microorganisms; hence, strict aseptic conditions were maintained.

Physical Sterilization Methods

Dry Heat Sterilization

- Glassware such as Petri dishes, flasks, and pipettes were sterilized using a hot air oven or microwave at 160–180°C for 2–3 hours.

Moist Heat Sterilization

- Culture media and instruments were sterilized in an autoclave at 121°C and 15 psi pressure for 15–20 minutes.
- **Filtration:**
- Heat-sensitive substances such as plant growth regulators (BAP, NAA) and vitamins were sterilized using membrane filters (0.22 μm).

Chemical Sterilization: Surface sterilization of explants was carried out using the following chemicals:

- Ethanol (70%) for 30–60 seconds for initial disinfection
- Mercuric chloride ($HgCl_2$) at varying concentrations (0.01–0.10%) for 2–5 minutes
- Bavistin (1–4%) as an antifungal treatment for 5–25 minutes

After chemical treatment, explants were thoroughly rinsed with sterile distilled water to remove traces of sterilants.

Culture Media: Murashige and Skoog (MS) medium (1962) was used as the basal medium for *in vitro* culture. It contains

essential macro- and micronutrients, vitamins, and a carbon source necessary for plant growth and development.

Preparation of MS Medium: The MS medium was prepared using standard stock solutions:

- Macronutrients (Stock I, 20×)
- Micronutrients (Stock II, 200×)
- Iron source (Stock III)
- Vitamins (Stock IV, 200×)

To the medium, 3% (30 g/L) sucrose was added as a carbon source. The pH was adjusted to 5.7–5.8 using 1N HCl or 1N NaOH before adding 0.8% agar (8 g/L) for solidification. The medium was then sterilized by autoclaving. Plant growth regulators (PGRs) such as BAP, NAA, and IBA were added as required depending on the experimental objectives.

Surface Sterilization Protocol: Explants were first treated with Bavistin solution (1–4%) for 5–25 minutes to remove fungal contamination. This was followed by treatment with mercuric chloride (0.01–0.10%) for 2–5 minutes under aseptic conditions. Finally, explants were rinsed multiple times with sterile distilled water before inoculation.

Preparation of MS Medium (1 Litre): Murashige and Skoog (MS) medium were prepared following standard procedures. Initially, 500 ml of double distilled water (DDW) was taken in a conical flask, and 30 g of sucrose was added and dissolved completely. Thereafter, 50 ml of Stock I (macronutrients), 5 ml each of Stock II (micronutrients), Stock III (iron source), and Stock IV (vitamins) were added sequentially with continuous stirring [12].

The required concentration of plant growth regulators (PGRs) was then added. The volume was made up to 1000 ml using DDW. The pH of the medium was adjusted to 5.7–5.8 using 1N HCl or 1N NaOH. Subsequently, 8 g/L agar was added as a solidifying agent, and the medium was heated in a microwave to dissolve the agar completely. The prepared medium was poured into culture tubes or bottles and allowed to solidify. Finally, the media were sterilized in an autoclave at 121°C and 15 psi pressure for 30 minutes.

Inoculation Procedure: All inoculation procedures were carried out under aseptic conditions. Sterilized explants were transferred into pre-sterilized culture vessels using sterile forceps. The explants were trimmed using a scalpel to remove damaged portions and then inoculated onto the culture medium under flame or within a laminar airflow cabinet. The culture vessels were sealed properly with caps or closures and further secured with parafilm or tape. Each culture was labeled with the name of the explant and date of inoculation before being transferred to the culture room.

Fresh Culture Establishment: Healthy and phenotypically superior plants were selected as the source of explants. The collected explants were washed thoroughly under running tap water to remove surface contaminants. This was followed by washing with detergent (Extran) to reduce microbial load and rinsing with double distilled water (3–4 times).

Explants were then treated with Bavistin solution to eliminate fungal contamination, followed by repeated washing with DDW. Surface sterilization was carried out using 0.1% mercuric chloride ($HgCl_2$) for 5 minutes under aseptic conditions. After sterilization, explants were rinsed 4–5 times with sterile double distilled water to remove any traces of $HgCl_2$. The sterilized explants were aseptically inoculated onto culture media using sterile forceps and scissors under a laminar airflow chamber. Cultures were maintained in a culture room at 25 ± 2°C.

Sub-culturing: Sub-culturing was performed to maintain and multiply the cultures. It involves transferring explants from an old medium to a fresh nutrient medium to ensure continuous growth and development. Before sub-culturing, the laminar airflow chamber was sterilized using UV light for 20 minutes. Explants were carefully removed from the culture vessels using sterile forceps and transferred to a sterile Petri plate. The ends of the explants were trimmed using a sterile scalpel and then inoculated into fresh media under aseptic conditions. The culture vessels were sealed, labeled, and transferred back to the culture room for further growth.

Culture Room Conditions

The culture room was maintained under controlled environmental conditions to ensure optimal growth of cultures. The temperature was maintained at 25 ± 2°C using air conditioners. A photoperiod of 16 hours light and 8 hours dark was provided, with light intensity ranging between 2000–3000 lux. Relative humidity was maintained at 60–70%. Special racks or shelves made of glass or plywood were used for placing culture vessels. Proper precautions were taken to prevent disturbance and contamination.

Hardening of Plantlets: Regenerated plantlets were transferred to a mist chamber for acclimatization. The process of hardening helps the plantlets to gradually adapt from *in vitro* to ex vitro conditions, ensuring higher survival rates under natural environmental conditions.

Growth Regulators: Different concentrations of plant growth regulators were used to study their effect on shoot initiation, proliferation, elongation, and rooting.

- BAP (6-Benzylaminopurine): 0.0, 1.0, 2.0, 3.0, 4.0 mg/L
- IAA (Indole-3-acetic acid): 0.0, 1.0, 2.0, 3.0, 4.0 mg/L

The combination of BAP and IAA at 3.0 mg/L each showed the best response, with 85% shoot initiation and 95% shoot proliferation along with healthy rooting. Higher concentrations (4.0 mg/L) resulted in excessive callus formation and reduced rooting efficiency. These conditions were found to be optimal

for the *in vitro* growth and development of *Centella asiatica* [14].

RESULTS AND DISCUSSION

The present study was conducted to evaluate the effect of different concentrations of BAP and IAA on the *in vitro* growth and development of *Centella asiatica* at different culture periods (7, 14, 21 and 28 days).The results clearly indicated that growth response varied significantly with different concentrations of plant growth regulators. Explants cultured on hormone-free medium (0.0 mg/L BAP + 0.0 mg/L IAA) showed no response, confirming that exogenous supply of plant growth regulators is essential for *in vitro* morphogenesis. Similar observations have been reported in *Centella asiatica*, where growth regulator-free media failed to induce significant shoot or root development. [10].

Among different explants types, nodal explants showed the highest response, followed by leaf and root explants. This is consistent with earlier findings that nodal segments possess pre-existing meristems, making them more responsive for shoot induction in tissue culture.

With the application of growth regulators, a gradual improvement in growth response was observed. The treatment containing 1.0 mg/L BAP + 1.0 mg/L IAA showed moderate response, with 45% initiation, 55% shoot proliferation and 60% elongation, but poor rooting. Similar moderate responses at lower hormone concentrations have been reported, indicating that suboptimal levels of cytokinin and auxin limit morphogenetic efficiency [15].

The best response in the present study was observed at 3.0 mg/L BAP + 3.0 mg/L IAA, which showed maximum initiation (85%), shoot proliferation (95%), and elongation (90%) along with excellent rooting. These findings are in agreement with previous studies reporting that balanced combinations of cytokinins (BAP/BA) and auxins (IAA/NAA) significantly enhance shoot multiplication and plant regeneration in *Centella asiatica* [8].

A slightly lower but still effective response was observed at 2.0 mg/L BAP + 2.0 mg/L IAA, indicating that optimal hormonal balance is critical for coordinated shoot and root development. The synergistic interaction between cytokinin and auxin has been widely reported to regulate organogenesis, where cytokinin promotes shoot induction and auxin supports root formation. [15].

However, further increase in hormone concentration (4.0 mg/L BAP + 4.0 mg/L IAA) resulted in reduced growth performance, including decreased elongation and callus formation [3]. This observation supports earlier reports that excessive concentrations of growth regulators can disrupt endogenous hormonal balance and lead to abnormal growth or callogenesis instead of organized plant development.

Surface sterilization treatments also played a crucial role in successful culture establishment. Bavistin and $HgCl_2$ treatments significantly reduced contamination rates, with maximum effectiveness observed at higher concentrations and optimal exposure times. High contamination rates during initial culture establishment have also been reported in *Centella asiatica*, emphasizing the importance of effective sterilization protocols.

Table 1. Optimization of bavistin concentration and exposure duration for effective surface sterilization of Centella asiatica explants

Experiment No	Concentration of Bavistin in %	Time Duration (min)	Contamination rate in (%)
T 0	Control	-	90%
T 1	0.2	2min	70%
T 2	0.4	4min	50%
T 3	0.6	5min	30%
T 4	1.0	10min	3%
T 5	0.8	15min	20%

Table 2. Effect of varying HgCl2 concentrations on microbial contamination control in Centella asiatica tissue culture

Experiment No	Concentration of Hgcl2	Time Duration (min)	Contamination rate in (%)
T 0	Control	-	90%
T 1	0.2	2min	70%
T 2	0.4	3min	50%
T 3	0.6	4min	30%
T 4	1.0	5min	2%
T 5	0.8	6min	10%

Table 3. Comparative regeneration potential of Nodal, Leaf and Root explants of Centella asiatica under In Vitro conditions

S.No	Explants type	Response Observation
1.	Nodal explants	High response
2.	Leaf explants	Moderate response
3.	Root explants	Low response

The use of Murashige and Skoog (MS) medium provided essential nutrients for optimal growth and development of explants. Previous studies have also confirmed that MS

medium supplemented with appropriate growth regulators is highly effective for micropropagation of *Centella asiatica*.

The regenerated plantlets showed good rooting and high survival rates during acclimatization, indicating their physiological stability. Similar results have been reported where *in vitro* raised plantlets of *Centella asiatica* exhibited genetic and biochemical stability after acc limatization.

Overall, the study confirms that *in vitro* propagation is an efficient and reliable method for mass multiplication of *Centella asiatica*. The optimal treatment identified was 3.0 mg/L BAP + 3.0 mg/L IAA, which produced maximum shoot proliferation, elongation, and rooting. This is consistent with previous research highlighting the importance of balanced growth regulator combinations for large-scale propagation and conservation of medicinal plants.

Table 4. Effect of different concentrations of BAP and IAA on In Vitro shoot initiation, proliferation, elongation and rooting of Centella asiatica.

S.No.	BAP Concentration (mg/L)	IAA Concentration (Mg/L)	7Day Initiation	14 day Shoot Proliferation	21 day Elongation	28day Rooting Status
1.	0.0	0.0	0%	0%	0%	No response
2.	1.0	1.0	45%	55%	60 %	Very poor rooting weak
3.	2.0	2.0	65%	75%	90%	Moderate rooting
4.	3.0	3.0	85%	95%	90%	Excellent rooting. Healthy plant
5.	4.0	4.0	50%	60%	55%	Callus formation, poor Rooting

Figure 1: In Vitro regeneration of Centella asiatica: shoot proliferation and root induction under optimized culture conditions.

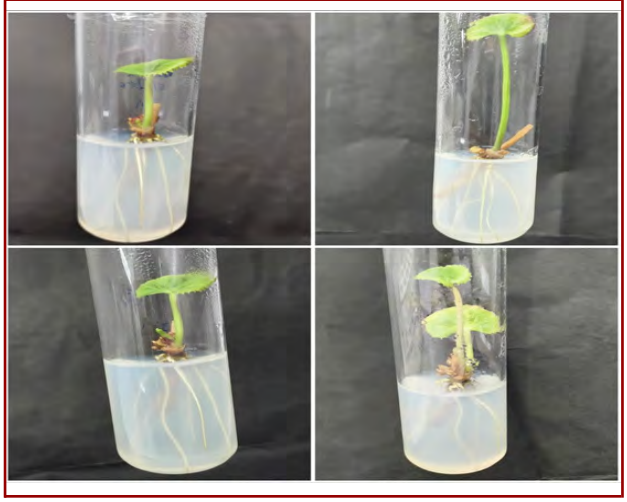

Figure 2: Ex Vitro acclimatization of micropropagated Centella asiatica plantlets under greenhouse conditions

CONCLUSION

The present study successfully demonstrates that *in vitro* propagation of *Centella asiatica* is highly influenced by the concentration and combination of plant growth regulators (PGRs), specifically BAP (Benzylaminopurine) and IAA (Indole-3-acetic acid). The results clearly show that an exogenous supply of PGRs is essential for promoting growth and development, as the hormone-free medium failed to induce any significant morphological changes. Among the different explant types, nodal explants proved to be the most responsive, likely due to the presence of pre-existing meristems.

The optimal combination of 3.0 mg/L BAP and 3.0 mg/L IAA yielded the best results, with the highest percentages of initiation, shoot proliferation, elongation, and excellent rooting. This aligns with earlier studies that emphasized the role of balanced cytokinin and auxin levels for enhancing shoot multiplication and plant regeneration in *Centella asiatica*. Lower concentrations of 2.0 mg/L BAP and 2.0 mg/L IAA also provided satisfactory results, confirming that hormonal balance is crucial for coordinating both shoot and root development. However, higher concentrations of PGRs (4.0 mg/L BAP + 4.0 mg/L IAA) resulted in a decline in growth performance, with decreased elongation and callus formation, supporting the concept that excessive hormone levels can disrupt plant development.

Surface sterilization, utilizing Bavistin and $HgCl_2$, effectively reduced contamination rates, further highlighting the importance of proper sterilization protocols in the success of *in vitro* cultures. The use of Murashige and Skoog (MS) medium provided essential nutrients, contributing to the successful

growth and development of explants, and subsequent acclimatization of regenerated plantlets showed high survival rates and physiological stability.

In conclusion, the study affirms that *in vitro* propagation of *Centella asiatica* is a reliable method for large-scale multiplication, and the optimal PGR combination of 3.0 mg/L BAP + 3.0 mg/L IAA is recommended for efficient propagation and conservation of this medicinal plant species.

Conflict of interest: There is no conflict of interest.

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