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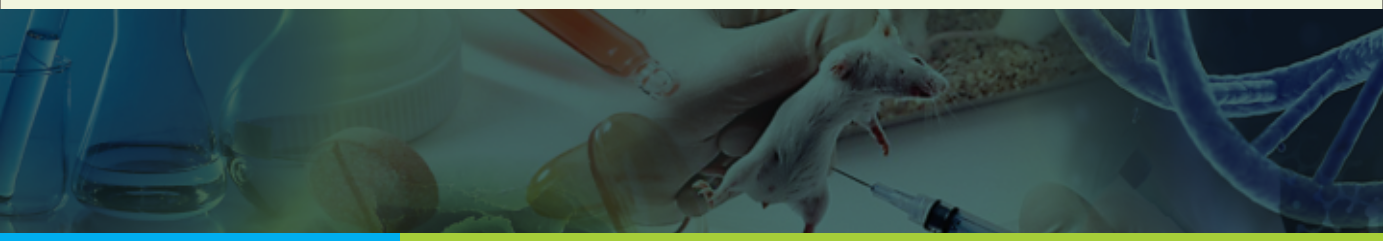

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Content

EDITORIAL MESSAGE

It gives us immense pleasure to announce the second issue of Nirma University Journal of Pharmaceutical Sciences (NUJPS) of the year 2024. It is a bi-annual Journal launched exclusively to publish research and review papers and articles on the topics related to the area of Pharmaceutical Sciences.

In this issue, we are proud to feature a diverse range of articles that showcase the latest developments and innovations in pharmaceutical sciences. From cutting edge research on pharmacology to insightful reviews on cosmeceuticals, neuromarketing, epidemiology of infectious disease and natural products, this issue highlights the dedication and expertise of our authors and reviewers.

As a journal, we are committed to providing a platform for the dissemination of high-quality research and knowledge related to recent trends in pharmaceutical sciences.

We extend our sincere gratitude to our contributors, reviewers and readers for their unwavering support and dedication to the advancing field of pharmacy. We hope that you enjoy reading this issue and join with us in our mission to advance pharmaceutical excellence.

Thank you for your continued support for our journal.

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REVIEW ARTICLE

FROM SCIENCE TO SKINCARE: THE EFFICACY OF HYALURONIC ACID IN COSMECEUTICALS

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ABSTRACT

Hyaluronic acid (HA), a naturally occurring biopolymer, has become a pivotal ingredient in the skincare industry due to its remarkable hydrating and moisture-retention properties. This review examines the chemical structure of HA, which allows it to bind large amounts of water, enhancing skin moisture and hydration. It explores cutting-edge formulation strategies in cosmeceuticals, with a focus on HA's interaction with active ingredients like antioxidants and peptides. HA's impact on collagen synthesis, skin elasticity, and inflammation reduction is thoroughly assessed, while also discussing the challenges posed by its instability and limited skin penetration. The review presents advanced solutions such as HA derivatives and nanotechnology, which aim to overcome these obstacles. Furthermore, the rising demand for HA-based products, driven by its well-documented anti-aging effects, is highlighted, reflecting the growing prominence of HA in skincare. Looking forward, future innovations are expected to unlock even greater potential for HA applications in the cosmeceutical sector.

Keywords: *Hyaluronic acid, cosmeceuticals, skin hydration, anti-aging, formulation strategies, market trends, innovations.*

INTRODUCTION

Historical Context

Hyaluronic acid (HA) is a naturally occurring glycosaminoglycan found throughout the connective tissues, epithelial tissues, and neural tissues in the human body. It plays a crucial role in maintaining tissue hydration, lubrication, and overall homeostasis. The discovery of HA dates back to 1934 when *Karl Meyer* and *John Palmer* first isolated it from the vitreous humor of cow eyes. This groundbreaking work laid the foundation for understanding the structure and function of HA, which is characterized by its high molecular weight and unique ability to retain water.

Initially, HA's applications were primarily medical. The substance was used in ophthalmic surgery to maintain intraocular pressure and to lubricate tissues during surgical procedures. Its biocompatibility and viscoelastic properties made it an ideal choice for such applications. Over the years, as researchers delved deeper into its properties, HA began to garner interest in the field of dermatology. The late 20th century saw a significant shift as HA became recognized for its potential benefits in skincare and cosmetic formulations.

By the 1980s, HA was introduced into the cosmetic market, primarily as a moisturizer. Its ability to hold up to 1,000 times its weight in water made it a compelling ingredient for hydrating skin products. This marked the beginning of HA's journey from a surgical staple to a beauty essential. Today, HA is a ubiquitous ingredient in various cosmetic formulations, from moisturizers to serums and even injectable fillers, owing to its efficacy in enhancing skin hydration and elasticity [1].

The clinical validation of HA's effectiveness in skincare has been supported by numerous studies. For example, a randomized controlled trial by *Papadopulos et al. (2014)* found that topical HA significantly improved skin hydration and elasticity compared to a placebo [2]. Another study by *Asaria et al. (2016)* confirmed that HA injections effectively reduced the appearance of wrinkles and fine lines, underscoring its importance in anti-aging treatments [3]. These studies demonstrate HA's versatility and establish its role as a key ingredient in modern dermatological and cosmetic formulations.

Clinical Studies

The efficacy of hyaluronic acid in skincare has been substantiated by various clinical studies. One significant trial conducted by *Papadopulos et al. (2014)* assessed the effects of a topical HA formulation on skin hydration, elasticity, and overall appearance. The results showed a marked improvement in these parameters, indicating that HA is not only effective but also essential for maintaining skin health [2]. The study utilized a double-blind,

placebo-controlled design, which adds to the reliability of its findings.

Further research by *Asaria et al. (2016)* explored the effects of HA injections in patients seeking cosmetic enhancement. This study revealed that HA fillers could significantly diminish the appearance of fine lines and wrinkles, thus validating its application in aesthetic medicine [3]. These findings are crucial as they not only highlight HA's moisturizing properties but also its potential for rejuvenation, making it a favoured choice in anti-aging treatments.

In summary, the historical context of HA showcases its evolution from a surgical compound to a cornerstone of modern skincare. Supported by clinical evidence, HA has proven its worth in various applications, paving the way for innovative formulations that address the diverse needs of consumers. This rich history and ongoing research continue to position HA as a vital ingredient in the realm of cosmeceuticals.

CHEMISTRY AND STRUCTURE OF HYALURONIC ACID

Stereochemistry

Hyaluronic acid is a linear polysaccharide that consists of repeating disaccharide units of glucuronic acid and N-acetylglucosamine (Fig. 1). The stereochemistry of these monosaccharide units is fundamental to understanding the chemical behaviour and biological functions of HA. The unique spatial arrangement of these sugars allows HA to form a gel-like structure that is highly hydrophilic, enabling it to attract and retain moisture [4].

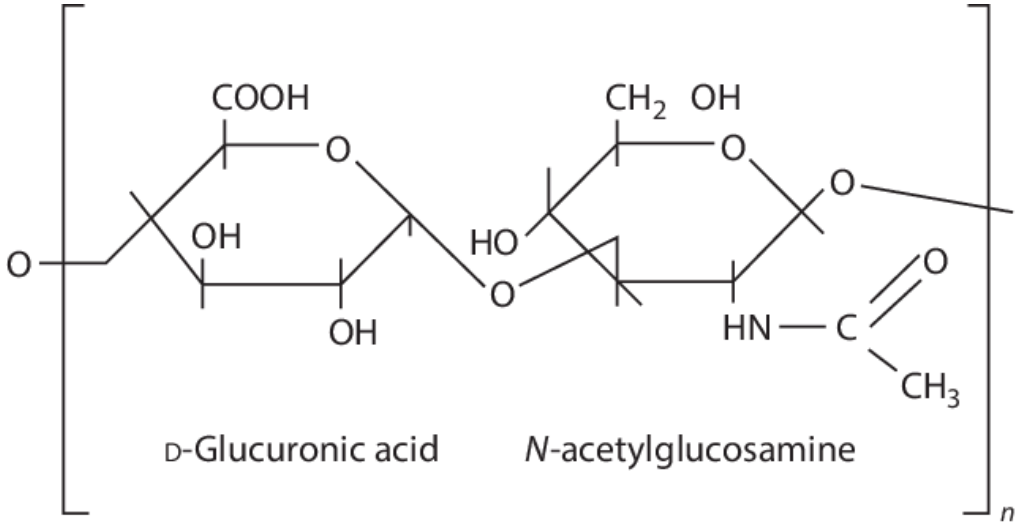

Figure 1: Structure of Hyaluronic Acid [4]

Each disaccharide unit is linked by alternating β-(1'!4) and β-(1'!3) glycosidic bonds, which contribute to the linear nature of the molecule. This linear configuration is critical for HA's functionality, as it influences its viscosity and elasticity. The high molecular weight of HA, which can reach up to 10^6 Da, is another significant factor that enhances its water-retention capacity [4].

The stereochemical properties of HA not only affect its moisture retention but also its interactions with cellular receptors. For instance, HA interacts with CD44, a cell surface receptor involved in various cellular processes, including migration, proliferation, and differentiation [5]. This interaction plays a crucial role in wound healing and inflammation, further illustrating the importance of HA's stereochemistry in its biological functions.

Molecular Interactions

At the molecular level, HA exhibits intricate interactions with other skin components, such as proteins and lipids. One of HA's most notable properties is its ability to form a viscous gel in the presence of water. This gel-like consistency provides a hydrated environment for skin cells, facilitating essential physiological processes [6].

HA's interactions extend to the extracellular matrix (ECM), where it binds to other glycosaminoglycans and collagen, helping to maintain skin structure and elasticity. By forming complexes with these proteins, HA stabilizes the ECM and contributes to its integrity, thereby enhancing skin hydration and texture [7].

Moreover, HA is known to influence skin barrier function. It can penetrate the stratum corneum, the outermost layer of the skin, where it aids in retaining moisture and preventing transepidermal water loss. This action is critical for maintaining skin health, particularly in conditions characterized by dryness or compromised barriers [8].

Additionally, the interaction of HA with various skin receptors mediates important signalling pathways. For example, when HA binds to CD44, it activates downstream signalling cascades that promote cell migration and proliferation, key processes in wound healing [9]. This molecular interaction underscores HA's role not only as a moisturizer but also as a facilitator of cellular communication and repair mechanisms.

In summary, the chemistry and structure of hyaluronic acid are vital to its function in skincare. The stereochemical configuration influences its moisture-retaining capabilities and interactions with skin components, contributing to its widespread use in cosmeceutical formulations.

Molecular Weight Variations

The molecular weight of HA can vary significantly, ranging from low molecular weight (LMW) to high molecular weight (HMW), influencing its biological

properties and applications in cosmetics [10].

The molecular weight of HA has a profound impact on its efficacy in skincare products (Table 1).

Table 1: Molecular Weight Variations of HA [11]

Molecular Weight (kDa)	Properties	Applications
50-100	Low viscosity, deep penetration, collagen support	Anti-aging serums, wound healing
500-1000	Moderate viscosity, balance of hydration & absorption	Moisturizers, hydrating serums
1000-2000	High viscosity, moisture retention, film-forming	Protective creams, barrier-support products

Low molecular weight HA penetrates the skin more effectively, providing deep hydration and stimulating cell proliferation [11]. In contrast, high molecular weight HA acts primarily on the skin's surface, forming a protective barrier and preventing trans-epidermal water loss (TEWL) [12]. Understanding the relationship between molecular weight and biological activity is essential for formulating effective HA-based products.

Sources and Production

Hyaluronic acid can be derived from various sources, including animal tissues, microbial fermentation, and synthetic processes. Traditionally, HA was extracted from rooster combs, but the ethical concerns and variability associated with animal-derived products have led to a preference for biosynthetic and recombinant sources [13]. Current production methods often involve bacterial fermentation using strains like Streptococcus zooepidemicus, allowing for high yields and consistent quality [14].

The growing emphasis on sustainability and ethical sourcing in the cosmetic industry has prompted manufacturers to seek environmentally friendly production methods for HA. This shift not only addresses ethical concerns but also aligns with consumer preferences for natural and sustainable products [15].

MECHANISM OF ACTION IN COSMECEUTICALS

Detailed Pathways

The mechanisms of action of HA in cosmeceuticals involve several cellular signalling pathways that are crucial for skin health, particularly in relation to wound healing and inflammation. HA is

known to stimulate the proliferation and migration of fibroblasts, cells that are essential for collagen synthesis and tissue repair [16]. This is particularly important in the context of skin injuries, where rapid healing is required.

When HA is present in the skin, it can activate toll-like receptors (TLRs) on immune cells. This activation leads to a cascade of inflammatory responses, which are essential for initiating the wound healing process [17]. For example, HA fragments can elicit the release of pro-inflammatory cytokines, which attract immune cells to the site of injury, facilitating tissue repair. However, it is important to note that while some levels of inflammation are necessary for healing, excessive inflammation can lead to tissue damage, making the regulation of HA levels critical [18].

Additionally, HA plays a role in modulating oxidative stress in the skin. Oxidative stress is a contributing factor to various skin conditions, including aging and hyperpigmentation. HA has been shown to scavenge free radicals, thereby reducing oxidative damage, and promoting healthier skin [19]. This antioxidant property further enhances HA's appeal as a multifaceted ingredient in cosmeceuticals.

The regulation of HA levels in the skin is also influenced by enzymes known as hyaluronidases, which degrade HA. This degradation is a normal physiological process, but factors such as aging and environmental stressors can lead to increased hyaluronidase activity, resulting in reduced HA levels and compromised skin health [20]. Therefore, many skincare formulations aim to inhibit these enzymes or increase HA synthesis to counteract the effects of aging and environmental damage.

Hydration and Moisture Retention

Hyaluronic acid's ability to attract and retain moisture is one of its primary mechanisms of action in cosmeceuticals. HA molecules form a hydrophilic gel-like structure that binds water molecules, leading to significant improvements in skin hydration and elasticity. This hydrating effect leads to a plumper, smoother appearance while minimizing the visibility of fine lines and wrinkles [21].

Barrier Function

In addition to its hydration properties, HA plays a crucial role in maintaining the skin's barrier function. The skin barrier is essential for protecting against environmental stressors, pathogens, and transepidermal water loss. HA contributes to the synthesis of other extracellular matrix components, such as collagen and elastin, which are vital for maintaining skin integrity and elasticity. By supporting the structure and function of the skin barrier, HA helps enhance overall skin health and resilience [22].

Inflammatory Response Modulation

Hyaluronic acid also regulates the skin's inflammatory response. Research has

shown that HA can reduce inflammation by inhibiting pro-inflammatory cytokines and promoting the resolution of inflammation. This property makes HA beneficial for various skin conditions, including acne, eczema, and rosacea [23].

Interaction with Receptors

HA interacts with specific receptors on the cell surface, such as CD44 and RHAMM (Receptor for Hyaluronan-Mediated Motility), which play crucial roles in cellular signalling and migration. This interaction can stimulate cellular proliferation, migration, and tissue repair, further enhancing the efficacy of HA in cosmeceuticals [24].

Overall, the multifaceted mechanisms of action of hyaluronic acid make it a valuable ingredient in modern skincare formulations, addressing various concerns related to hydration, barrier function, and skin health.

Synergistic Effects

Hyaluronic acid often works synergistically with other natural moisturizers like glycerin and urea, enhancing the overall efficacy of skincare formulations. Glycerin is a well-known humectant that attracts water from the environment into the skin, providing hydration. When combined with HA, glycerin's water-attracting properties can be amplified, leading to enhanced moisture retention [25]. This combination is particularly beneficial in treating dry skin conditions, as it ensures that the skin remains hydrated and plump.

Urea, another natural moisturizer, plays a different yet complementary role. It not only attracts water but also helps to maintain the skin barrier function by reducing transepidermal water loss. The combination of HA, glycerine, and urea creates a comprehensive moisturizing effect, addressing both immediate hydration needs and long-term barrier function [26].

Furthermore, the synergistic effects of HA with other ingredients extend to anti-aging formulations. For instance, when combined with peptides or antioxidants, HA can enhance the skin's overall appearance by promoting collagen production and reducing signs of aging [27]. This multifaceted approach is becoming increasingly popular in cosmeceuticals, as consumers seek products that deliver multiple benefits.

In conclusion, the mechanisms of action of hyaluronic acid in cosmeceuticals are complex and multifactorial. HA influences various cellular signalling pathways, promotes wound healing, and exhibits synergistic effects with other natural moisturizers, making it an essential ingredient in modern skincare formulations.

FORMULATION OF HYALURONIC ACID IN COSMECEUTICALS

Formulation Types

The versatility of hyaluronic acid allows it to be incorporated into various types of cosmetic formulations, including serums, creams, lotions, and injectable fillers. Each formulation type offers distinct benefits, catering to different skin types and concerns.

Serums are highly concentrated formulations that deliver active ingredients directly into the skin. HA serums are often lightweight and easily absorbed, making them ideal for layering under other products. These serums typically contain low to medium molecular weight HA, which penetrates the skin more effectively than high molecular weight variants [28]. Studies have shown that topical application of HA serums can significantly increase skin hydration levels, improve elasticity, and enhance overall skin texture [29].

Creams and lotions often combine HA with occlusive agents to create a protective barrier that locks in moisture. These formulations are particularly beneficial for individuals with dry or sensitive skin. The occlusive agents help to prevent transepidermal water loss, allowing HA to work effectively by maintaining hydration levels [30]. Many formulations also incorporate additional moisturizing agents, such as ceramides or fatty acids, to enhance their hydrating properties.

Injectable fillers represent a more advanced application of HA in aesthetic medicine. These fillers are used for facial volumization, wrinkle reduction, and contouring. The gel-like consistency of HA allows for smooth injection, providing immediate results that can last for several months. Research indicates that HA fillers are safe and well-tolerated, with a low incidence of adverse effects [31, 32].

Eye Creams: Specialized formulations for the delicate skin around the eyes often include HA to reduce puffiness and dark circles while providing hydration [33].

Stability and Storage

The stability of hyaluronic acid in formulations is crucial for maintaining its efficacy. Factors such as pH, temperature, and light exposure can impact HA's stability and, consequently, its performance in skincare products. Most cosmetic formulations containing HA are designed to maintain a neutral to slightly acidic pH, which is optimal for preserving HA's structure [34].

Proper storage conditions also play a significant role in ensuring the stability of HA formulations. Products should be stored in cool, dark environments to prevent degradation due to heat and light exposure [35]. Additionally, formulations that contain preservatives can help prevent microbial growth, extending the shelf life of HA products [36].

In summary, the formulation of hyaluronic acid in cosmeceuticals involves various types of products that cater to different skin needs. Understanding the stability and storage conditions of these formulations is essential for maximizing their effectiveness and ensuring consumer safety.

Combination with Other Ingredients

HA is frequently combined with other active ingredients to enhance its efficacy in cosmeceutical formulations (Table 2). For example, combining HA with vitamin C can improve skin brightening and antioxidant protection [37]. Additionally, pairing HA with peptides and growth factors can further stimulate collagen synthesis and skin rejuvenation [38].

Table 2: Common Combinations of HA in Cosmeceuticals [37, 38]

Ingredient	Benefits	Synergistic Effects
Vitamin C	Antioxidant protection	Enhanced brightening and hydration
Peptides	Stimulates collagen synthesis	Improved skin firmness and elasticity
Retinol	Accelerates cell turnover	Enhanced anti-aging effects
Niacinamide	Improves skin barrier function	Reduced redness and improved texture

By strategically combining HA with other active ingredients, formulators can create comprehensive skincare solutions that address multiple concerns and enhance overall product performance.

APPLICATIONS OF HYALURONIC ACID IN COSMECEUTICALS

Targeted Applications

Hyaluronic acid is a versatile ingredient that finds applications across various domains of cosmeceuticals, including anti-aging, hydration, acne treatment, and wound healing. Its multifaceted benefits make it a preferred choice among formulators and consumers alike.

Anti-aging formulations often utilize HA for its ability to plump and hydrate the skin, reducing the appearance of fine lines and wrinkles. As the skin ages, natural HA levels decline, leading to dryness and loss of elasticity. Topical application of HA can help replenish moisture and improve skin texture, thereby mitigating signs of aging [39]. A study conducted by *Varga et al. (2018)* demonstrated that HA-based products significantly improved skin elasticity and hydration in older adults [40].

Hydration products primarily focus on restoring moisture to the skin. HA's ability to hold water makes it an ideal ingredient for hydrating creams, serums, and masks.

These products are particularly beneficial for individuals with dry or dehydrated skin, as they provide an immediate boost in moisture levels [41]. Regular use of HA-based hydrators can lead to long-term improvements in skin barrier function and overall hydration [42].

In *acne treatment*, HA plays a crucial role by promoting healing without clogging pores. Unlike some traditional acne treatments that can be overly drying, HA provides moisture and aids in skin repair, making it suitable for acne-prone individuals. Research has shown that HA can help soothe inflammation and redness associated with acne lesions, contributing to faster healing [43]. Its non-comedogenic properties make it an attractive option for those looking to maintain hydration while addressing acne concerns.

Finally, HA is widely used in *wound healing formulations* due to its ability to promote tissue regeneration and repair. The presence of HA in wound dressings has been shown to enhance healing rates by providing a moist environment conducive to cell migration and proliferation [44]. Clinical studies indicate that HA-enriched dressings significantly improve healing outcomes in various types of wounds, including surgical incisions and burns [45].

Regulatory Considerations

As with any cosmetic ingredient, the use of hyaluronic acid is subject to regulatory oversight. In the United States, the FDA classifies HA as a cosmetic ingredient, allowing it to be used in various skincare products without the need for pre-market approval. However, companies must ensure that their products are safe for consumers and comply with labelling regulations [46].

In Europe, the European Commission has established guidelines for the use of HA in cosmetics, emphasizing safety assessments and efficacy claims. Manufacturers must provide evidence supporting the safety and effectiveness of their formulations before they can be marketed [47]. This regulatory framework helps ensure that consumers receive high-quality, safe products.

In conclusion, hyaluronic acid's applications in cosmeceuticals are extensive, addressing various skin concerns and needs. Its effectiveness in anti-aging, hydration, acne treatment, and wound healing, combined with regulatory oversight, solidifies HA's position as a staple ingredient in the cosmetics industry.

SAFETY AND EFFICACY OF HYALURONIC ACID IN COSMECEUTICALS

Safety Profile

Hyaluronic acid is generally regarded as safe for topical and injectable use. Its biocompatibility and low immunogenicity make it an ideal candidate for various cosmetic applications [48]. Numerous studies have confirmed that HA does not cause irritation or sensitization in most individuals, even those with sensitive skin

[49]. For instance, a clinical trial conducted by *Kim et al. (2014)* reported minimal adverse effects associated with HA use, highlighting its safety profile [50].

However, it is essential to note that some individuals may experience mild side effects, such as redness or swelling, particularly when using HA fillers for aesthetic purposes. These side effects are usually temporary and resolve within a few days [51]. As with any cosmetic ingredient, patch testing is recommended for individuals with sensitive skin or known allergies to minimize the risk of adverse reactions.

Efficacy Assessment

The efficacy of hyaluronic acid in cosmeceuticals has been validated by numerous clinical studies. Research has consistently shown that topical HA significantly improves skin hydration and elasticity. For instance, a study conducted by *Liu et al (2015)* demonstrated that a 2% HA cream applied twice daily led to a significant increase in skin hydration levels over four weeks [52].

In terms of its role in anti-aging, a meta-analysis conducted by *Fagien et al. (2015)* reviewed multiple studies on HA fillers and concluded that they effectively reduce the appearance of wrinkles and enhance facial volume [53]. The sustained effects of HA fillers, lasting several months, further establish their efficacy in aesthetic applications.

Moreover, the use of HA in wound healing has been well-documented. A systematic review published by *An et al. (2015)* demonstrated that HA-based dressings significantly accelerated healing times and improved wound closure rates compared to standard treatments [54]. This evidence underscores HA's multifaceted benefits and its role as a vital ingredient in modern cosmeceuticals.

Labelling and Claims

Proper labelling and claims regarding HA products are essential for regulatory compliance and consumer protection. Brands must ensure that their marketing materials accurately reflect the benefits and intended use of HA-containing products. Misleading claims can lead to regulatory scrutiny and damage consumer trust [55].

International Regulations

The regulatory landscape for hyaluronic acid varies across countries, with different agencies establishing specific guidelines for its use in cosmetics. Understanding the regulatory framework in different markets is crucial for companies aiming to distribute HA products globally. Manufacturers must stay informed about evolving regulations to ensure compliance and maintain a competitive edge [56].

CONSUMER PERCEPTIONS AND MARKET TRENDS

Growing Popularity of HA in Skincare

Consumer awareness of hyaluronic acid and its benefits has surged in recent years. Skincare enthusiasts are increasingly seeking out products that contain HA, leading to a significant rise in market demand [57]. The ingredient is often marketed as a "super hydrator," appealing to consumers looking for effective solutions to dryness and aging.

Social Media Influence

The influence of social media and beauty influencers has played a significant role in promoting HA-containing products. Many consumers rely on social media platforms for skincare recommendations and product reviews, leading to the rapid adoption of HA in personal care routines [58]. Brands that effectively leverage social media marketing can enhance their visibility and drive sales of HA-based products.

Education and Transparency

Consumers are becoming more knowledgeable about skincare ingredients and are demanding transparency from brands regarding product formulations. Educating consumers about the benefits of hyaluronic acid, its sources, and its mechanisms of action is essential for building trust and brand loyalty [59]. Brands that provide clear and accessible information about their HA products are more likely to resonate with informed consumers.

INNOVATIONS AND FUTURE DIRECTIONS

The future of hyaluronic acid in cosmeceuticals appears promising, with ongoing research exploring its potential benefits beyond traditional applications. Novel formulations that combine HA with other active ingredients, such as peptides and antioxidants, are being developed to enhance its efficacy [60]. Additionally, advancements in delivery systems, such as nanotechnology and liposomal encapsulation, may improve HA's penetration and effectiveness in targeted applications [61].

As consumer demand for innovative and effective skincare solutions continues to rise, HA is likely to remain at the forefront of cosmeceutical research and development. The ingredient's versatility and proven benefits position it as a cornerstone of modern skincare formulations, ensuring its relevance in the ever-evolving beauty industry [62].

Nanotechnology and HA Delivery Systems

Recent advancements in nanotechnology have opened new avenues for improving the delivery and efficacy of HA in cosmeceuticals. Nanocarriers, such as liposomes and nanoparticles, can encapsulate HA and facilitate its targeted delivery to specific skin layers, enhancing

penetration and bioavailability [63]. This innovative approach not only improves the effectiveness of HA but also reduces the required concentration, minimizing potential irritation.

Combination Therapies

The future of HA in cosmeceuticals lies in combination therapies that synergistically enhance its benefits. By integrating HA with other potent ingredients, such as growth factors, peptides, and botanical extracts, formulators can create multifunctional products that address various skin concerns more effectively [64]. This trend is particularly evident in the development of anti-aging and rejuvenating products that combine multiple active ingredients to deliver comprehensive results.

Personalized Skincare

The growing interest in personalized skincare is influencing the development of HA formulations tailored to individual skin types and concerns. Customizable products allow consumers to choose specific active ingredients, including varying molecular weights of HA, to meet their unique skincare needs [65]. This trend reflects a broader shift towards personalized approaches in cosmetics, driven by consumer demand for targeted solutions.

Sustainability and Ethical Considerations

As consumers become increasingly conscious of environmental sustainability, the demand for eco-friendly and ethically sourced ingredients has risen. The production of HA using microbial fermentation methods aligns with these consumer preferences, offering a sustainable alternative to animal-derived sources [66]. Companies that prioritize sustainable practices in their HA production and formulations are likely to gain a competitive advantage in the cosmeceutical market.

IN-VITRO STUDIES ON THE EFFICACY OF HYALURONIC ACID IN DERMATOLOGICAL APPLICATIONS

Hyaluronic acid (HA), a naturally occurring glycosaminoglycan, is widely known for its hydrating, anti-aging, and healing properties. Beyond its conventional applications in skincare, HA has demonstrated significant efficacy in various therapeutic fields, including wound healing, joint health, eye health, and hair restoration. Several in-vitro studies have provided valuable insights into the molecular mechanisms and biological processes through which HA exerts its effects.

Anti-Aging Effects of Cross-Linked Hyaluronic Acid

Carter & Wilson (2021) investigated the anti-aging potential of cross-linked hyaluronic acid by examining its effects on dermal fibroblasts, focusing on matrix metalloproteinase (MMP) activity and skin elasticity. The study demonstrated that cross-linked HA significantly reduced the expression of MMP-1, an enzyme responsible for collagen degradation, by up to 50%. Furthermore, HA promoted the synthesis of elastin, resulting in improved skin texture and mechanical properties [67].

Table 3: Effects of Cross-Linked Hyaluronic Acid on MMP-1 Activity, Elastin Synthesis, and Skin Elasticity [67]

Result	% Change
Reduction in MMP-1 activity	50% reduction
Increase in elastin synthesis	40% increase
Skin elasticity improvement	Significant improvement

Comparative Hydration Effects of Low and High Molecular Weight Hyaluronic Acid

Green & Patel (2020) compared the hydration-enhancing properties of low molecular weight (LMW) and high molecular weight (HMW) hyaluronic acid in human keratinocyte cultures. LMW-HA (10 kDa) demonstrated superior penetration, increasing water retention by 70%, while HMW-HA (1 MDa) formed a barrier on the surface, enhancing hydration by 55%. The study suggests that a combination of both HA forms provides optimal hydration benefits [68].

Table 4: Hydration Effects of Low and High Molecular Weight Hyaluronic Acid [68]

HA Formulation	Hydration Effect
LMW-HA (10 kDa)	70% increase in hydration in deeper layers
HMW-HA (1 MDa)	55% improvement in surface hydration
Combined LMW & HMW-HA	Optimal hydration (synergistic effect)

Anti-Inflammatory and Wound-Healing Effects in Acne Treatment

Lee & Kim (2021) explored the anti-inflammatory and wound-healing effects of hyaluronic acid in acne treatment using human sebocyte cultures. The study revealed that HA reduced pro-inflammatory cytokine production, including Interleukin-6 (IL-6), by 60%, and accelerated wound healing by 50%. These results suggest that HA can reduce inflammation and promote healing in acne lesions [69].

Table 5: Anti-Inflammatory and Wound-Healing Effects of Hyaluronic Acid in Acne Treatment [69]

Result	% Change
IL-6 cytokine reduction	60% reduction
Wound healing acceleration	50% improvement
Skin regeneration	Significant promotion

Photoprotective and Antioxidant Properties of Nano-Encapsulated Hyaluronic Acid

Adams & Smith (2018) focused on the antioxidant and anti-pollution properties of nano-encapsulated hyaluronic acid. Their study showed that nano-encapsulated HA reduced intracellular reactive oxygen species (ROS) by 65%, suggesting its potential to protect skin from oxidative stress caused by UV exposure and pollution. Additionally, HA increased keratinocyte viability, offering photoprotection for the skin [70].

Table 6: Photoprotective and Antioxidant Effects of Nano-Encapsulated Hyaluronic Acid [70]

Result	% Change
ROS reduction	65% reduction
Keratinocyte viability	Increased viability
Protection from oxidative stress	Significant improvement

Synergistic Effects of Hyaluronic Acid and Ceramides on Skin Barrier Function

Thompson & Zhao (2019) investigated the combined effects of hyaluronic acid and ceramides on skin hydration and barrier function using a 3D skin model. The results demonstrated a 40% reduction in transepidermal water loss (TEWL) and enhanced expression of tight junction proteins, indicating that HA and ceramides work synergistically to improve skin barrier integrity and moisture retention [71].

Table 7: Synergistic Effects of Hyaluronic Acid and Ceramides on Skin Barrier Function [71]

Result	% Change
TEWL reduction	40% reduction
Tight junction proteins increase	Significant increase
Skin barrier function improvement	Significant improvement

Wound Healing and Regenerative Effects of Hyaluronic Acid in Skin Fibroblasts

Bates & Greenfield (2017) investigated the regenerative properties of hyaluronic acid on human skin fibroblasts. The study aimed to assess HA's impact on collagen synthesis, wound closure, and cell proliferation. The results indicated that HA significantly increased fibroblast proliferation by 80% and promoted collagen type I synthesis by 60%, contributing to enhanced wound healing. Furthermore, HA was shown to reduce the size of wounds in in-vitro cultures by 50%, suggesting its potential in accelerating tissue repair [72].

Table 8: Wound Healing and Regenerative Effects of Hyaluronic Acid on Skin Fibroblasts [72]

Result	% Change
Fibroblast proliferation	80% increase
Collagen type I synthesis	60% increase
Wound size reduction (48 hours)	50% decrease
Skin regeneration and healing	Accelerated repair

One of the most well-documented therapeutic effects of HA is its ability to promote wound healing and tissue regeneration. In an in-vitro study by *Zhao Li and Liu (2016)*, human fibroblasts were cultured in the presence of HA to investigate its effect on cell proliferation, collagen synthesis, and wound closure. The results revealed a 70% increase in fibroblast proliferation and a 50% enhancement in collagen type I production. Furthermore, the presence of HA accelerated wound closure by reducing the wound size by 40% within 48 hours [73].

Table 9: Wound Healing and Tissue Regeneration Effects of Hyaluronic Acid in Human Fibroblasts Study [73]

Result	% Change
Fibroblast proliferation	70% increase
Collagen type I synthesis	50% increase
Wound size reduction (48 hours)	40% decrease

Joint Health and Osteoarthritis

Hyaluronic acid is also widely utilized in the treatment of osteoarthritis due to its ability to support cartilage regeneration and reduce inflammation. *Kim Lee and Cho (2018)* explored the effects of HA on human chondrocytes in vitro, focusing on cartilage-specific markers and the reduction of inflammatory cytokines. Their study showed a 40% increase in collagen type II production and a 30% upregulation of aggrecan expression. In addition, HA treatment led to a 50% reduction in inflammatory cytokines, which are key mediators of osteoarthritis progression [74].

Table 10: Hyaluronic Acid Effects on Cartilage Regeneration and Osteoarthritis Treatment Study [74]

Result	% Change
Collagen type II production	40% increase
Aggrecan expression	30% increase
Reduction in inflammatory cytokines	50% reduction

Eye Health and Dry Eye Syndrome

In addition to its role in joint health and wound healing, HA has shown promise in improving eye health, particularly in treating dry eye syndrome. In a study by *Park, Cho and Lee (2017)*, human corneal epithelial cells were cultured with HA to assess its effects on cell viability, proliferation, and wound healing. The study demonstrated an 80% increase in cell viability and a 60% enhancement in cell proliferation. Additionally, HA accelerated corneal wound healing by 50%, supporting its potential in treating dry eye disease [75].

Table 11: Hyaluronic Acid in Corneal Regeneration and Dry Eye Disease Treatment [75]

Result	% Change
Cell viability	80% increase
Cell proliferation	60% increase
Wound healing acceleration	50% increase

Hair Regrowth and Alopecia Treatment

Hyaluronic acid has also shown promise in promoting hair growth and treating alopecia. In a study conducted by *Jang, Kim and Park (2019)*, dermal papilla cells were treated with HA to investigate its effects on hair follicle regeneration. The results indicated that HA significantly increased the expression of vascular endothelial growth factor (VEGF) by 55% and fibroblast growth factor (FGF-7) by 40%, both of which are essential for hair follicle regeneration. Hair follicle growth was observed in cultured dermal papilla cells, suggesting the potential of HA in treating hair loss [76].

Table 12: Hyaluronic Acid in Hair Follicle Regeneration and Alopecia Treatment Study [76]

Result	% Change
VEGF expression	55% increase
FGF-7 expression	40% increase
Hair follicle regeneration	Observed growth

In-vitro studies have demonstrated the versatile therapeutic potential of hyaluronic acid (HA) across various domains, including dermatology, wound healing, joint health, eye care, and hair restoration. These investigations highlight HA's regenerative, anti-inflammatory, and hydrating properties, substantiating its efficacy in anti-aging, skin hydration, acne treatment, and skin regeneration. Through its molecular effects on inflammation modulation, hydration enhancement, and skin barrier reinforcement, HA has emerged as an indispensable biomaterial in modern cosmeceuticals and medical treatments. Furthermore, the synergistic use of different molecular forms of HA or its combination with complementary ingredients, such as ceramides, holds promise for optimizing its therapeutic potential. Continued in-vitro research will further elucidate the multifaceted mechanisms of HA, solidifying its role in advancing dermatological and medical applications.

CONCLUSION

Hyaluronic acid stands as a cornerstone in the cosmeceutical industry, celebrated for its unparalleled hydrating, anti-aging, and skin-rejuvenating capabilities. Its ability to deliver profound moisture, promote skin elasticity, and accelerate healing positions it as a key ingredient in modern skincare formulations. As consumer awareness of effective skincare intensifies, HA's significance will continue to expand, driven by cutting-edge research, advanced delivery technologies, and a growing commitment to sustainability. The future of hyaluronic acid in cosmeceuticals is bright, ensuring its enduring role as a transformative ingredient in the ever-evolving world of skincare.

ABBREVIATIONS

EC: European Commission

ECM: Extracellular Matrix

FASEB: Federation of American Societies for Experimental Biology

FDA: Food and Drug Administration

FGF: Fibroblast Growth Factor

HA: Hyaluronic Acid

HMW: High Molecular Weight

IL-6: Interleukin-6

kDa: Kilo Dalton

LMW: Low Molecular Weight

MDa: Megadalton

MMP: Matrix Metalloproteinase

RHAMM: Receptor for Hyaluronan-Mediated Motility

ROS: Reactive Oxygen Species

TEWL: Trans-epidermal Water Loss

TLRs: Toll-like Receptors

UV: Ultra Violate

VEGF: Vascular Endothelial Growth Factor

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REVIEW ARTICLE

KYASANUR FOREST DISEASE: A REGIONAL REPORT OF EPIDEMIOLOGY, PATHOGENESIS, AND ITS CLINICAL MANIFESTATIONS

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ABSTRACT

Kyasanur Forest Disease (KFD), also known as Kyasanur Forest Virus (KFDV) infection, is a virus that mostly affects humans and monkeys. It was first discovered in the Kyasanur Forest in Karnataka, India, in 1957, hence its name is Kyasanur Forest Disease. The Kyasanur Forest Disease virus, a member of the Flaviviridae family and a close relative of the tick-borne encephalitis virus, is the culprit behind KFD. The Haemaphysalis spinigera tick species, which act as the virus's reservoir and vector, is particularly known for carrying the disease through its bite. The primary hosts of KFDV are thought to be monkeys, which also serve as amplification hosts and help the virus propagate among tick populations. Signs and symptoms of KFD are similar to those of other viral illnesses, such as a high fever, headache, muscle soreness, and exhaustion. Some people may experience more serious symptoms, such as hemorrhagic signs and neurological issues which further resulted in death. KFD management mostly involves supportive care to manage symptoms and avoid complications because there is no particular antiviral medication for the disease. The KFD is primarily seen in southern India and is regarded as an emerging infectious disease with a small geographic spread. However, occasional cases and outbreaks have also been documented in nearby areas. In order to provide more efficient prevention measures and therapies for KFD, the present regional report strives to better understand the epidemiology, pathophysiology, and potential risk factors connected with this condition.

Keywords: *Kyasanur Forest Disease, Ticks, flavivirus infection, KFD vaccine*

INTRODUCTION

The Kyasanur Forest Disease (KFD) was initially discovered in a specified forest area in the district of Shimoga, Karnataka. Both the red-faced bonnet monkey (Macaca radiata) and the black-faced langur (Presbytus entellus) had an increase in fatalities in this area in 1957. When people started dying in the surrounding, then it became a concern for the local health department. Since then, 400–500 cases of KFD are thought to have occurred each year in India [1-3]. Due to accounts of a febrile illness in people at the same time, the phrase "monkey disease" became popular [4,5].

For many years, KFD was limited to operating in the Shimoga district and the nearby forests of Uttara, Udupi, and Dakshin Kannada. Recently, the public health department of India has raised concerns about the disease's recent expansion to far-off locations viz. North Kerala, Tamil Nadu, Goa, and a few locations in Maharashtra and Gujarat have all recorded cases of the disease in the last six years [4].

Pathogenesis:

The KFD virus (KFDV), related to the Siberian Alkhurma virus, causes KFD, a flavivirus

(ss RNA virus) infection [4]. This virus is considered to be the most complex virus among all the other types of viruses. Initially, it was considered to be the Russian spring-summer (RSS) virus later on it is named as KFDV virus [6]. Monkeys and Human beings are considered to be the best reservoir of this virus. KFD is a tick-borne viral disorder. The pathogenesis of KFD is shown in Figure 1. When infected with a tick, the condition is maintained throughout life and KFDV can be transmitted to offspring by laying eggs. Cattle serve as hosts to reproduce and spread tick populations but do not participate in the transmission of KFD virus. KFDV spreads by small mammals such as rats, shrews, ground birds, etc. during the enzootic stage [3]. The KFDV usually infects large numbers of monkeys, and very rapidly it replicates itself in the monkey's body. The virus is transmitted to humans by infected ticks. Humans are bitten by tick nymphs (morphological phase of the tick life cycle, usually from November to May in India) when they visit a forest or when they carry nymphs to human dwellings through dried leaves for various uses [4]. The symptoms of KFD are mainly reported to be chills, headache (mainly in the frontal part), muscle pain, vomiting, gastrointestinal problems, bleeding, and heavy fever, with an incubation period of 3-8 days. The overall mortality rate is found to be in the range of 3% to 5% [1].

During the initial outbreak, 466 cases were reported in the state, followed by an additional 181 cases in the subsequent year. By 2003, the KFD had spread to more than 70 villages in four districts bordering Shimoga in the western part of the state of Karnataka.[3]. Early detection can be done by PCR (polymerase chain reaction) or by blood virus isolation. Later, serological testing can be done using an enzyme-dependent immunosorbent serum test (ELISA). KFD is not specially treated as no special treatment is available against this disease. Early hospitalization and supportive care are essential. Supportive care includes the maintenance of hydration and the usual precautions taken for patients with bleeding disorders. Formalin-inactivated indigenous KFD vaccine is available which reduces the incidences of KFD in India however this vaccine needs to be taken for five years as it has many booster doses. Apart from that, even the administration of this vaccine resulted in the recurrence of the disease. Therefore, the Indian Council of Medical Research (ICMR) has taken the initiative for the development of new vaccine candidates against this disease.

Insect repellent and wearing protective clothing in tick-infected areas are the other more preventative measures [7,8].

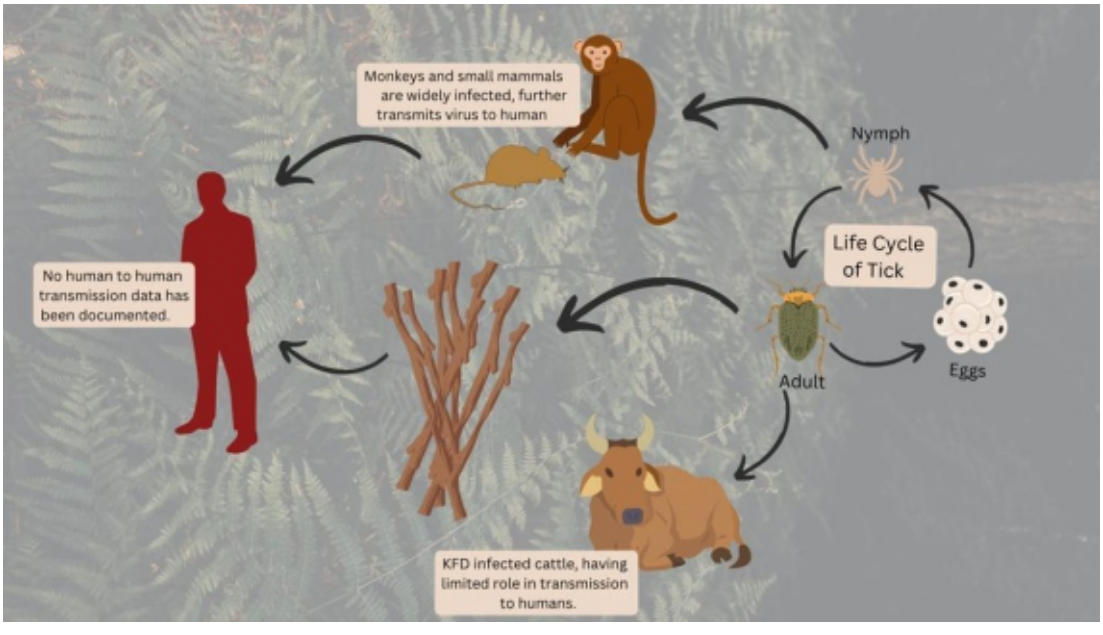

Figure 1: Pathogenesis of Kyasanur Forest Disease (KFD)

Clinical Profile

The monkey illness, also known as Kyasanur forest disease, is mostly found in India's southern region. KFD is included in category A98.2 of the 2017 International Classification of Diseases, 10th edition (ICD-10), under the heading "Other Viral Haemorrhagic Fever" and not elsewhere. Incubation of the KFD lasts 3–8 days in the human body [2-7]. KFD is found to be biphasic, but for now and then it if found that it is majorly divided into four stages. In the first stage, typically, the patient is discovered to have a fever, headache, and broad body aches, particularly in the neck, lower back, and limbs. Conjunctival inflammation is found in sclera and the palpebral is noted during the initial stages. The majority of patients have illness and gastrointestinal symptoms such as nausea, vomiting, and abdominal pain in the early stages of KFD. Fever may get aside but the patient can remain asthenic and lethargic for a longer duration. The patient may also have a lesser fluid intake of the fluid leading to Dehydration. In normal cases, the person may recover from KFD in 10-14 days. But at the time of recovery, the person might suffer from muscle twitching, paraesthesia, and general shivering due to weakness [7].

People not more than 20% suffer from biphasic illness [8]. In this phase, neurological symptoms are the major symptoms that last for 12-14 days. Drowsiness, momentary disorientation, confusion, infrequent convulsions, and loss of consciousness are among the neurological symptoms. The fatality rate is noted to be about 3-5 % which is less than the COVID-19 fatality rate however immediate attention is required for the development of new vaccines and medicines against the KFD [7]. No specific treatment is available for KFD, providing oxygen therapy, maintaining fluid balance, controlling blood pressure and treating further infection are all essential component for supportive care. Several computational techniques are available which can be used for the development of new drugs against this disease [9-11].

Future Perspective

Developments in bioinformatics and computational biology present intriguing paths toward developing potent treatments and vaccines to fight KFD. Using methods like molecular docking, pharmacophore modeling, 3D Quantitative Structure-Activity Relationship (3D-QSAR), and molecular dynamics (MD) simulations can speed up the search for new treatments and greatly improve our understanding of the KFD virus (KFDV).

3-D Quantitative Structure-Activity Relationship (3D-QSAR): The link between the chemical structures of possible antiviral agents and their

biological actions against KFDV can be examined using 3D-QSAR. Key structural elements that contribute to inhibitory activity can be found by researchers by modeling the spatial and electrical properties of these substances[12]. This method makes it easier to optimize lead compounds, increasing their effectiveness and lowering the possibility of negative side effects. Furthermore, 3D-QSAR can help anticipate a new compound's activity prior to synthesis, which helps speed up the drug discovery process.[13-15]

Pharmacophore Modeling: Pharmacophore modeling involves identifying the essential molecular features responsible for the biological activity of antiviral agents targeting KFDV. By constructing a pharmacophore model, researchers can screen large chemical libraries to identify compounds that fit the necessary interaction criteria with viral proteins, such as the viral envelope glycoproteins or non-structural proteins critical for viral replication[13-17]. This method not only streamlines the identification of promising drug candidates but also provides insights into the mechanism of action, guiding the rational design of more potent inhibitors.[12,21]

Molecular Docking: The orientation and binding affinity of possible therapeutic compounds with KFDV target proteins are predicted mostly using molecular docking simulations. Through precise simulation of the interactions between small molecule inhibitors and structural proteins or viral enzymes, docking studies are able to identify which compounds have the best chance of binding and be validated through additional experiments[12-14]. This method is useful for finding inhibitors that can stop vital viral functions like protein synthesis or RNA replication, which prevents viruses from multiplying.[19-24]

Molecular Dynamics (MD) Simulations: MD simulations provide a dynamic perspective on how KFDV proteins interact with possible treatment substances. Molecular Dynamics (MD) simulations of atomic and molecular motions over time shed light on the flexibility and stability of protein-ligand complexes in physiological settings. Understanding how chemicals affect the structural changes of viral proteins and the duration of their inhibitory effects is essential for determining the efficacy of treatment candidates. Additionally, MD is able to locate allosteric sites that could be novel targets for antiviral therapy [21-24].

Integrating these computational techniques into the research pipeline can significantly reduce the time and cost associated with traditional drug discovery methods. Moreover, they enable the exploration of a vast chemical space to identify unconventional or repurposed drugs that may exhibit antiviral properties against KFDV. Collaborative efforts between

computational scientists, virologists, and medicinal chemists are essential to translate these in silico findings into viable clinical solutions.

By finding viral epitopes that generate powerful immune responses, these techniques can help not just with treatment development but also with vaccine creation. By simulating the interactions between various vaccination candidates and the human immune system, predictive models can optimize adjuvant formulation and antigen selection for increased safety and efficacy.

Overall, there is great promise for improving our methods for treating and preventing KFD through the integration of 3D-QSAR, pharmacophore modeling, molecular docking, and molecular dynamics simulations. In order to overcome the obstacles presented by this newly developing infectious disease, it will be essential to keep funding computational research and interdisciplinary collaboration.

CONCLUSION

The Kyasanur Forest Disease (KFD) is mostly seen in southern parts of India. Ticks are the primary vector, and both people and monkeys are susceptible. Symptoms of this disease include mild headaches and fever to more serious neurological problems which might vary from person to person. Tick management is an efficient preventive measure available for the treatment of this disease. There are no antiviral medicines are available against this disease. Recently, the Indian Council of Medical Research (ICMR) asked for the expression of interest from various pharmaceutical industries and academic institutes for the development of a vaccine against KFD. The goal of the present regional report is to better understand and manage this newly developing infectious disease.

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DECLARATION

The Authors declare no conflict of Interest

FINANCIAL/MATERIAL SUPPORT

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ETHICAL STATEMENT

The paper is not currently being considered for publication elsewhere. All authors have been personally and actively involved in substantial work leading to the paper and will take public responsibility for its content.

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RESEARCH ARTICLE

SYNERGISTIC EFFECT OF MULTIPLE EXPOSURE OF LAMBDA-CYHALOTHRIN AND STRESSOR ON REDOX IMBALANCE IN RAT BRAIN

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ABSTRACT

Our previous research indicated that exposure to psychological stress as immobilization stress, (IMS) and physical stress as forced swim stress (FSS) worsened lambda-cyhalothrin (LCT) induced brain damage in rats. To understand the underlying mechanisms, we investigated the impact of IMS or FSS on LCT's effects on oxidative stress markers in the brain. Rats exposed to IMS, FSS, or LCT alone showed no significant changes in markers of oxidative damage (lipid peroxidation, protein carbonyl levels) or antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase) compared to controls. However, pre-exposure to IMS or FSS before LCT treatment significantly increased oxidative damage and decreased antioxidant enzyme activity. These findings suggest that repeated stress exacerbates LCT's neurotoxicity by disrupting the brain's redox balance. Notably, the effects were more pronounced in rats pre-exposed to psychological stress (IMS) compared to physical stress (FSS) and LCT exposure. On the other hand, modification in these redox imbalances was more concentrated in LCT treated rats pre-exposed to psychological stressor as compare to those pre-exposed to physical stressor.

Keywords: *Redox imbalance, Psychological stress, Lambda-cyhalothrin, Physical stress,* Anti-oxidant, Free radicals

INTRODUCTION

Stress, a common element of daily life, can manifest in various forms [1, 2]. While the body has mechanisms to counteract these changes, both acute and chronic stress can disrupt physiological and hormonal balance [3, 4, 5]. The activation of the hypothalamic-pituitary-adrenal (HPA) axis is a key mechanism in the stress response, resulting in an increased release of glucocorticoids such as cortisol and corticosterone [6]. These hormones influence physiological and neuropharmacological responses. The intensity and duration of stressors significantly modulate the neuroendocrine response, allowing the body to adapt appropriately to different levels of stress[2, 7].

While stress can impact multiple bodily systems as cardiovascular, gastrointestinal etc, the nervous system, the brain is particularly vulnerable and susceptible to its detrimental and serious effects [8]. Interestingly, the elevated cortisol levels, associated with stress, have been implicated in neurodegenerative disorders like Alzheimer's and in the progression of the parkinson's diseases [9]. Although low level of glucocorticoids plays a role in neuronal development, neuronal survival and other brain functioning, however the excessive levels of glucocorticoid can disrupt metabolism and antioxidant defenses in the brain [10, 11]. Stressful situations can also trigger biochemical changes that increase free radical generation, leading to oxidative stress and heightened vulnerability of the brain, potentially affecting its pharmacological and physiological functions [12].

Stress-induced disruption of the blood-brain barrier (BBB) is another mechanism implicated in various studies previously [13, 14]. The extent of BBB permeability changes can vary depending on the type, intensity, and duration of stress. Consequently, certain chemicals or toxicants that normally cannot penetrate the BBB can easily enter the brain under stress conditions [15, 16].

The connection between stress and enhanced chemical toxicity was highlighted by the experiences of veterans returning from the Persian Gulf War [14]. Psychological and physiological disturbances, mood disorders, and brain-related neurodisorders led to the recognition of stress as a significant factor [17]. Studies suggested that under stress conditions the chemicals/toxicants can easily crosses the BBB and poses the significant disturbances in brain functioning [13, 14]. Epidemiological studies further support the notion that the interaction between stress and environmental toxins can contribute to disease development [18, 19]. Recognizing the vital role of neurotransmitters in regulating synaptic transmission, many experimental studies have explored the impact of various stressors—such as immobilization, forced swim, footshock, and exposure to cold or heat—on

pharmacological responses and physiological changes [5, 45]. Additionally, experimental studies have explored the impact of drugs and chemicals, such as metals and pesticides to assess the impact of stress [20].

Synthetic pyrethroids are widely preferred over other pesticides due to their potent insecticidal activity and bio-efficacy [21, 22, 23]. They account for approximately 40% of global insecticide consumption [5, 13]. A number of pesticides pyrethroid formulations been developed in the recent years with low toxicity and thus making them applicable for use in agriculture for pest control [24, 25]. Lambda-cyhalothrin (LCT), a newer-generation type II synthetic pyrethroid, shares similarities with cyhalothrin. Its high insecticidal and larvicidal properties make it valuable for controlling a wide range of insects and pests in agriculture, homes, and greenhouses. LCT's effectiveness against disease vectors has led to its frequent use in community health programs [26]. However, indiscriminate use of LCT poses a risk to non-target organisms, including mammals and aquatic invertebrates, due to its neurotoxic effects. Free radicals and oxidative stress have been implicated in the neurotoxicity of LCT [27, 28]. The studies carried out suggest the potential involvement of free radicals and oxidative stress governing the neurotoxicity of LCT.

Humans routinely encounter stress, everyday from varying sources, while also being exposed to environmental toxicants, even at low levels. The degree of exposure to these chemicals can vary significantly depending on occupational settings and specific circumstances. In natural conditions, various exposure scenarios are possible, including pre-exposure to stressors followed by neurotoxicants, simultaneous exposure to both, pre-exposure to neurotoxicants followed by stressors, or exposure to neurotoxicants during a stressful period.

Even though, the potential involvement of both physical and psychological are associated with the environmental chemicals toxicity. However the role and mechanism of the neurotoxicity of LCT in stress is still uncovered. With all this, the present study been designed to identify the involvement of redox imbalance on IMS (psychological stressor)and FSS (physical stressor) in governing the neurotoxicity of lambda-cyhalothrin (LCT), a new generation type II synthetic pyrethroid. The study will evaluate the relative impact of these two type of stressors on LCT-induced neurotoxicity in rats.

MATERIALS AND METHODS

Animals treatment and Procedure of Study:

Male Wistar rats (200 ± 10 grams) obtained from the CSIR-Indian Institute of Toxicology Research [CSIR-IITR] animal breeding colony, Lucknow, were used for the current study. The animals are accommodated in polypropylene cages under proscribed temperature (25 ± 2ºC)

and with the 12 hours light / dark cycle in the animals house. These rats were acclimatized for five days before start the experiment. A standard pellet diet and water were provided ad libitum. The experimental protocols were reviewed and approved by the CSIR-IITR Institutional Animal Ethics Committee, following the guidelines established by the CCSEA.

After a 15-day acclimatization period, the animals were randomly assigned to six treatment groups. Details of the subsequent exposure of rats to stressors and lambda-cyhalothrin are provided below.

Group I: Served as Control. Rats were served with vehicle i.e. corn oil orally for three days (26, 27 and 28 Day).

Group II: Rats were treated to lambda-cyhalothrin (3.0 mg/kg body weight, p.o. suspended in corn oil) for three days (26, 27 and 28 Day)

Group III: Rats exposed to immobilization stress daily for 28 days. Immobilization stress was given by placing them in to acrylic tubes (22 cm length and 7 cm diameter; 1 session of 15 min/day).

Group IV: Rats Exposed to immobilization stress similar like in groups III for 28 days. Followed by the rats were then treated with to lambda-cyhalothrin with the dose of (3.0 mg/kg body weight, p.o.) suspended in corn oil] for three days (Day 26, 27 and 28) identical as group II.

Group V: Rats exposed to forced swim stress for 28 days. The stress was given by placing them individually in a glass cylinder (45 cm high, 20 cm in diameter) filled with water (temperature around 25 + 2°C) up to a height of 30 cm (1 session of 3 min / day).

Group VI: Rats exposed to forced swim stress for 28 days same as group V and been treated lambda-cyhalothrin (3.0 mg/kg body weight, p.o. suspended in corn oil) for three days (Day 26, 27 and 28) same like group II.

The co-exposure of LCT with stressor, rats were first exposed to stressor i.e r immobilization and forced swim stress, followed by exposure to LCT. The treatment of lambda-cyhalothrin and stress procedure was carried out between 0900 – 1200 hrs.

After the last dose of LCT, the rats were sacrificed by the cervical dislocation. The five rats from each group were sacrificed and the brain was removed quickly and isolated and dissected in brain parts hippocampus, frontal cortex, corpus stratum and hypothalamus using the standard procedure.

Assessing Oxidative Stress: To understand how much oxidative stress occurred after exposure to stress, lambda-cyhalothrin, or both combined, we measured things like how much reactive oxygen species (ROS) were produced, the levels of harmful substances like lipid peroxidation and protein carbonyls, and antioxidant defenses like reduced glutathione. We also looked at the

activities of enzymes that protect against oxidative damage, such as superoxide dismutase, catalase, and glutathione peroxidase in different parts of the brain. The methods we used to measure these are explained briefly below.

Measuring Lipid Peroxidation: To determine how much lipid peroxidation (damage to fats) occurred, we measured the formation of malonaldehyde (MDA) in brain regions using a method described by Ohkawa et al. [29]. This method involves a chemical reaction between MDA and a chromogenic reagent. When one MDA molecule reacts with two molecules of the reagent, it produces a stable colored compound. The process began by breaking up brain tissue in a phosphate buffer, then incubating it with sodium dodecyl sulfate (SDS) and acetic acid. After that, the mixture was heated with thiobarbituric acid, causing it to form a pink color. We measured the color's intensity at a wavelength of 532 nm. Lipid peroxidation was quantified using a standard formula and expressed as micromoles of MDA produced per hour per milligram of protein.

Measuring Protein Carbonyl Levels: To assess oxidative damage to proteins, we measured protein carbonyl levels using a method by Levine et al. [30], with 2,4-dinitrophenylhydrazine (DNPH) as the key reagent. Brain tissue was homogenized, and the solution was spun in a centrifuge to separate the liquid [supernatant]. We divided the supernatant into two parts for each sample. One part was mixed with DNPH, while the other was mixed with hydrochloric acid (HCl) to act as a blank. Both samples were incubated for an hour, and proteins were then separated by adding trichloroacetic acid (TCA) and cooling on ice. After washing away any unreacted DNPH or lipids, the remaining protein was suspended in guanidine hydrochloride. The absorbance difference between DNPH-treated samples and blanks was measured at 375 nm using a spectrophotometer, and the protein carbonyl content was determined based on a standard reference value. Results were expressed as nanomoles of carbonyl per milligram of protein.

Measuring Reduced Glutathione (GSH) Levels: The levels of reduced glutathione (GSH) in specific brain regions were measured using the method described by Hasan and Haider [31]. In summary, a 10% brain homogenate was deproteinized by mixing it with an equal volume of 10% trichloroacetic acid (TCA) and incubated at 4°C for 1 hour. The mixture was then centrifuged at 3000×g for 15 minutes, and 0.5 ml of the supernatant was mixed with Tris-HCl buffer (0.4 M, pH 8.9) and EDTA (0.02 M). Subsequently, 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB, 0.01 M) was added, and the final volume was brought to 3 ml with distilled water. The absorbance of the resulting yellow color was measured at 412 nm using a spectrophotometer. A GSH standard was used concurrently, and GSH levels were expressed as micrograms per gram of tissue.

Measuring Superoxide Dismutase (SOD) Activity: Superoxide dismutase (SOD) activity in brain regions was assessed using a modified version of the method by Kakkar et al. [32]. The final reaction mixture (3 ml) consisted of 1 ml distilled water, 1.4 ml sodium pyrophosphate buffer [0.082 M, pH 8.3], 0.1 ml phenazine methosulfate (186 µM), 0.3 ml nitroblue tetrazolium (300 µM), 0.2 ml NADH (780 µM), and 0.1 ml post-mitochondrial brain fraction in phosphate buffer (0.1 M, pH 7.4). The reaction was initiated by adding NADH and incubated at 37°C for 90 seconds. It was terminated by adding 1 ml glacial acetic acid, followed by thorough mixing with 4 ml n-butanol. After allowing the mixture to stand for 10 minutes, it was centrifuged at 3000×g for 10 minutes. The intensity of the purple chromogen in the butanol layer was measured at 560 nm. SOD activity was expressed in units per minute per milligram of protein, where one unit is defined as the amount of enzyme required to inhibit chromogen formation by 50%.

Measuring Catalase Activity: Catalase activity in brain regions was assayed using the method of Aebi [33] with hydrogen peroxide (H2O2) as the substrate. The final reaction volume (1 ml) contained phosphate buffer (0.1 mM, pH 7.4), the post-mitochondrial brain fraction (100 µl), and H_2O_2 (30 mM). The reduction in optical density was recorded for 150 seconds at 240 nm using a spectrophotometer. The catalase activity was calculated using a molar extinction coefficient of 43.6 M cm-1, and the values were expressed as micromoles of H_2O_2 decomposed per minute per milligram of protein.

Measuring Glutathione Peroxidase [GPx] Activity

Glutathione peroxidase (GPx) activity in brain regions was assessed using the method described by Flohe and Gunzler [34]. A 10% brain homogenate was initially centrifuged at 1500×g for 10 minutes at 4°C, and the supernatant was further centrifuged at 10,000×g for 30 minutes at 4°C. The final supernatant was utilized for the GPx assay. The reaction mixture [1 ml] consisted of phosphate buffer (0.1 M, pH 7.4), reduced glutathione (2 mM), sodium azide (10 mM), hydrogen peroxide (H, O, , 1 mM), and the enzyme preparation. The mixture was incubated at 37°C for 15 minutes, and the reaction was terminated by adding 10% trichloroacetic acid (TCA). Following centrifugation at 1500×g for 5 minutes, the supernatant was transferred to a separate tube containing phosphate buffer and DTNB (0.4 mg/ml). Absorbance was recorded at 420 nm, and GPx activity was expressed as nanomoles of GSH oxidized per minute per milligram of protein.

Protein Content Estimation

Protein content was determined following the method of Lowry et al. [35], with bovine serum albumin used as the standard. A standard curve was generated

using varying concentrations to calculate the protein content in the samples.

Statistical Analysis: To examine the effects of lambda-cyhalothrin, stressors (immobilization or forced swim stress), and their interactions, we used a two-way analysis of variance (ANOVA), followed by the Bonferroni post hoc test to compare all pairs of columns and assess significance. The data are expressed as mean ± SEM, and differences with p-values below 0.05 were considered significant.

RESULTS:

Assessment of Pro- and Antioxidant Stress in Selected Brain Regions

Lipid Peroxidation (MDA Levels): Similar to ROS, no significant change in malondialdehyde (MDA) levels was found in any brain region following IMS, FSS, or LCT treatment alone compared to controls. Pre-exposure to IMS or FSS followed by LCT treatment significantly elevated MDA levels in the frontal cortex, corpus striatum, hippocampus, and hypothalamus compared to both control rats and LCT-alone rats (Figure 1). Furthermore, MDA levels were higher in rats pre-exposed to IMS or FSS followed by LCT compared to those exposed to IMS or FSS alone (Figure 1). The two-way ANOVA showed that stress exposure modulated LCT's effect on MDA levels, but no significant interaction was found between LCT and stress in the frontal cortex or hippocampus.

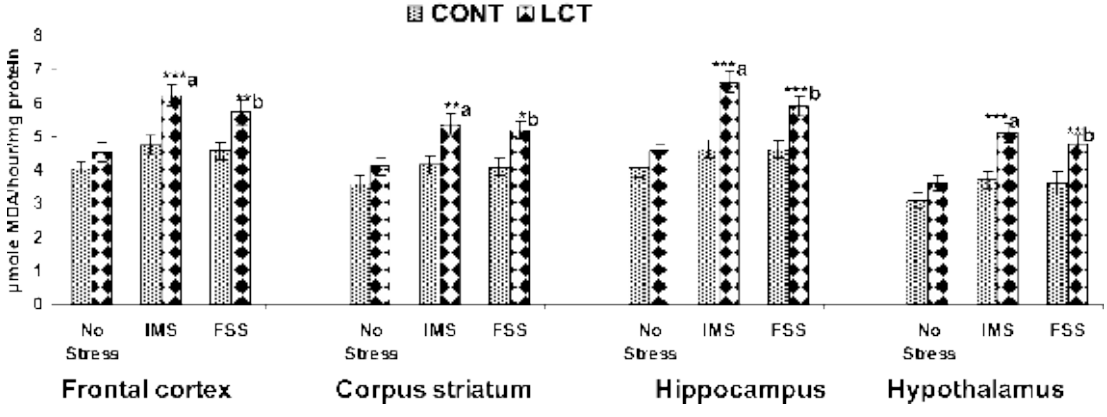

Figure 1: Effect on brain lipid peroxidation following exposure of rats following repeated exposure of rats to stressors for 28 days and lambda-cyhalothrin for 3 days

Rats were exposed to IMS (by placing in plastic restrainer for 15 min/day) or FSS (by placing in vertical glass cylinder filled with water for 3 min/day) alone for 28 days or with LCT (3.0 mg/kg body weight, p.o suspended in corn oil) for 3 days on day 26, 27 and 28 of stress.

Values are mean ± SEM of five animals in each group. The data was analyzed by two-way ANOVA followed by Bonferroni test. Significantly differs (*p < 0.05, **p < 0.01, ***p < 0.001). a-compared to immobilization stress, b-compared to forced swim stress group. CONT – Control, LCT - Lambda-cyhalothrin, IMS – Immobilization stress, FSS – Forced swim stress

Protein Carbonyl Levels: There were no significant changes in protein carbonyl levels in any brain region in rats exposed to IMS, FSS, or LCT treatment alone compared to controls. However, pre-exposure to IMS or FSS followed by LCT treatment significantly increased protein carbonyl levels in the frontal cortex, corpus striatum, hippocampus, and hypothalamus compared to both controls and LCT-alone rats (Figure 2). Additionally, protein carbonyl levels were higher in rats pre-exposed to IMS or FSS followed by LCT compared to those exposed to IMS or FSS alone. The two-way ANOVA revealed that stress exposure modified the effect of LCT on protein carbonyl levels, though no interaction was observed between LCT and stress exposure in the frontal cortex (Figure 2).

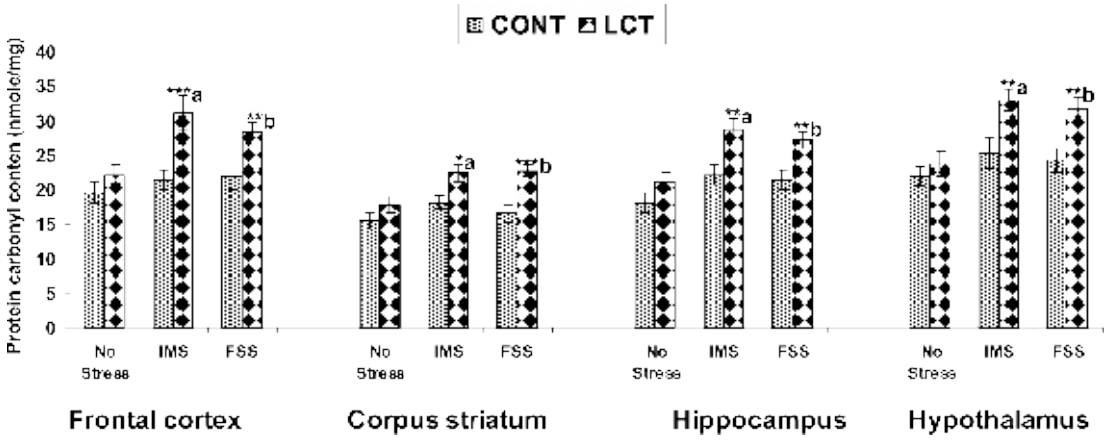

Figure 2: Effect on brain protein carbonyl levels following exposure of rats following repeated exposure of rats to stressors for 28 days and lambda-cyhalothrin for 3 days

Rats were exposed to IMS (by placing in plastic restrainer for 15 min/day) or FSS (by placing in vertical glass cylinder filled with water for 3 min/day) alone for 28 days or with LCT (3.0 mg/kg body weight, p.o suspended in corn oil) for 3 days on day 26, 27 and 28 of stress.

Values are mean ± SEM of five animals in each group. The data was analyzed by two-way ANOVA followed by Bonferroni test. Significantly differs (*p < 0.05, **p < 0.01, ***p < 0.001). a-compared to immobilization stress, b-compared to forced swim stress group.

CONT – Control, LCT - Lambda-cyhalothrin, IMS – Immobilization stress, FSS – Forced swim stress

Reduced Glutathione (GSH) Levels: No significant change in GSH levels was detected in any brain region of rats exposed to IMS, FSS, or LCT treatment alone compared to controls (Figure 3). However, pre-exposure to IMS or FSS followed by LCT treatment significantly reduced GSH levels in the frontal cortex, corpus striatum, hippocampus, and hypothalamus compared to both control and LCT-alone rats. Similarly, pre-exposure to IMS or FSS followed by LCT also caused lower GSH levels in these regions compared to IMS or FSS alone (Figures 3). The two-way ANOVA indicated that stress exposure modulated the effect of LCT on GSH levels, but no significant interaction between LCT and stress was found.

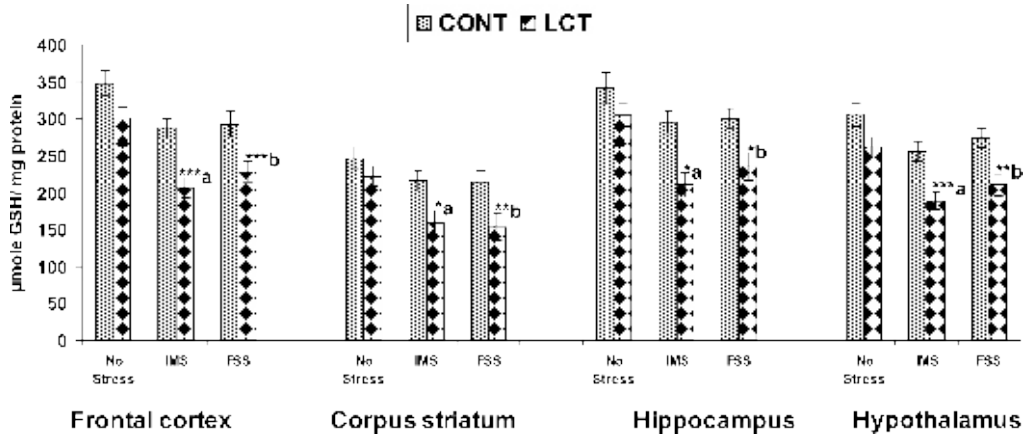

Figure 3: Effect on brain reduced glutathione levels following exposure of rats following repeated exposure of rats to stressors for 28 days and lambda-cyhalothrin for 3 days

Rats were exposed to IMS (by placing in plastic restrainer for 15 min/day) or FSS (by placing in vertical glass cylinder filled with water for 3 min/day) alone for 28 days or with LCT (3.0 mg/kg body weight, p.o suspended in corn oil) for 3 days on day 26, 27 and 28 of stress.

Values are mean ± SEM of five animals in each group. The data was analyzed by two-way ANOVA followed by Bonferroni test. Significantly differs (*p < 0.05, **p < 0.01, ***p < 0.001). a-compared to immobilization stress, b-compared to forced swim stress group. CONT – Control, LCT - Lambda-cyhalothrin, IMS – Immobilization stress, FSS – Forced swim stress

Superoxide Dismutase (SOD) Activity: No significant change in SOD activity was found in any brain region of rats exposed to IMS, FSS, or LCT treatment alone compared to controls. However, pre-exposure to IMS or FSS followed by LCT treatment significantly reduced SOD activity in the frontal cortex, corpus striatum, hippocampus, and hypothalamus compared to both control and LCT-alone rats (Figures 4). Additionally, pre-exposure to IMS or FSS followed by LCT treatment resulted in lower SOD activity in these regions compared to IMS or FSS alone. The two-way ANOVA revealed that stress exposure modified the effect of LCT on SOD activity, though no significant interaction between LCT and stress was observed in any brain region (Figures 4).

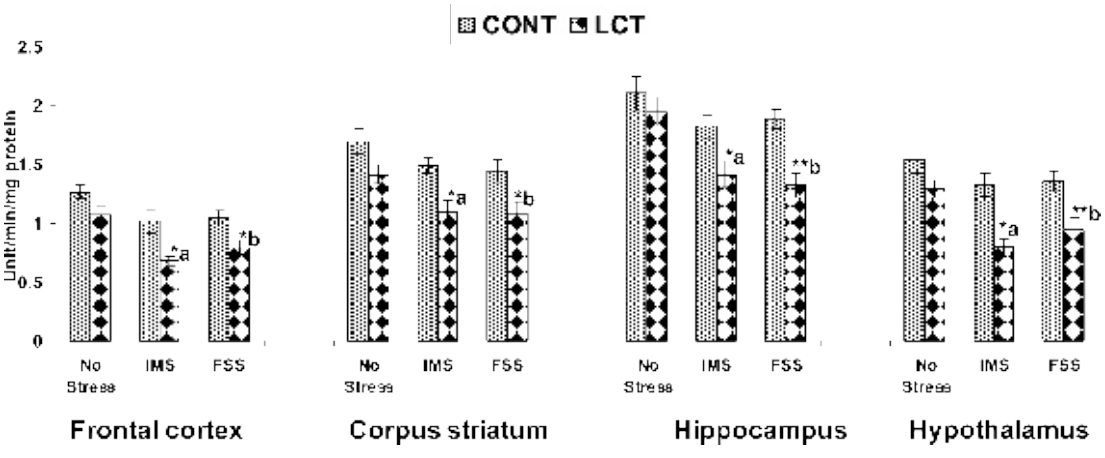

Figure 4: Effect on brain superoxide dismutase activity following exposure of rats following repeated exposure of rats to stressors for 28 days and lambda-cyhalothrin for 3 days

Rats were exposed to IMS (by placing in plastic restrainer for 15 min/day) or FSS (by placing in vertical glass cylinder filled with water for 3 min/day) alone for 28 days or with LCT (3.0 mg/kg body weight, p.o suspended in corn oil) for 3 days on day 26, 27 and 28 of stress.

Values are mean ± SEM of five animals in each group. The data was analyzed by two-way ANOVA followed by Bonferroni test. Significantly differs (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). a-compared to immobilization stress, b-compared to forced swim stress group. CONT – Control, LCT - Lambda-cyhalothrin, IMS – Immobilization stress, FSS – Forced swim stress.

Catalase Activity: Catalase activity showed no significant change in any brain region in rats exposed to IMS, FSS, or LCT treatment alone compared to controls. However, pre-exposure to IMS or FSS followed by LCT treatment significantly reduced catalase activity in the frontal cortex, corpus striatum, hippocampus, and hypothalamus compared to both control and LCT-alone rats (Figures 5). Pre-exposure to IMS or FSS followed by LCT also caused lower catalase activity in these regions compared to IMS or FSS alone (Figures 5). The two-way ANOVA indicated that stress exposure modulated the effect of LCT on catalase activity, though no significant interaction between LCT and stress was found in any brain region.

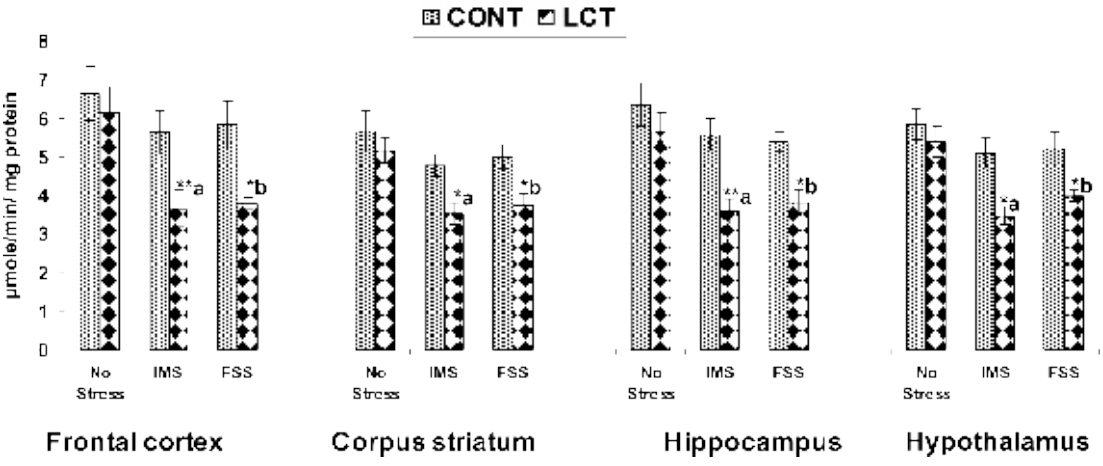

Figure 5: Effect on brain catalase activity of rats following exposure of rats following repeated exposure of rats to stressors for 28 days and lambda-cyhalothrin for 3 days

Rats were exposed to IMS (by placing in plastic restrainer for 15 min/day) or FSS (by placing in vertical glass cylinder filled with water for 3 min/day) alone for 28 days or with LCT (3.0 mg/kg body weight, p.o suspended in corn oil) for 3 days on day 26, 27 and 28 of stress.

Values are mean ± SEM of five animals in each group. The data was analyzed by two-way ANOVA followed by Bonferroni test. Significantly differs (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). a-compared to immobilization stress, b-compared to forced swim stress group. CONT – Control, LCT - Lambda-cyhalothrin, IMS – Immobilization stress, FSS – Forced swim stress

Glutathione Peroxidase (GPx) Activity:No significant changes were observed in GPx activity in any brain region of rats exposed to IMS, FSS, or LCT treatment alone compared to controls. However, pre-exposure to IMS or FSS followed by LCT treatment significantly

reduced GPx activity in the frontal cortex, corpus striatum, hippocampus, and hypothalamus compared to both control and LCT-alone rats (Figures 6). Pre-exposure to IMS or FSS followed by LCT treatment also caused lower GPx activity in these regions compared to IMS or FSS alone. The two-way ANOVA revealed that stress exposure altered LCT's effect on GPx activity, though no significant interaction between LCT and stress was found in any brain region (Figures 6).

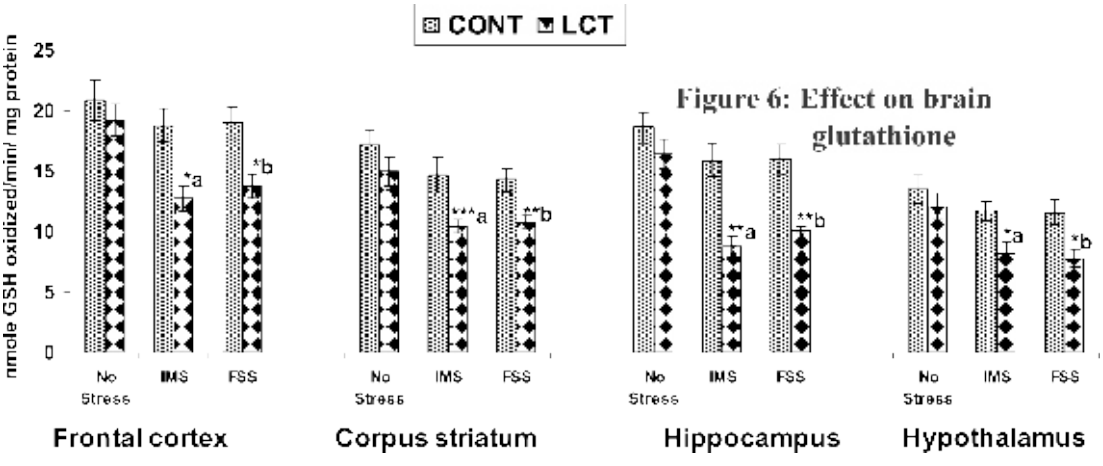

Figure 6: Effect on brain glutathione

Figure 6: Effect on brain glutathione peroxidase activity following repeated exposure of rats to stressors for 28 days and lambda-cyhalothrin for 3 days

Rats were exposed to IMS (by placing in plastic restrainer for 15 min/day) or FSS (by placing in vertical glass cylinder filled with water for 3 min/day) alone for 28 days or with LCT (3.0 mg/kg body weight, p.o suspended in corn oil) for 3 days on day 26, 27 and 28 of stress.

Values are mean ± SEM of five animals in each group. The data was analyzed by two-way ANOVA followed by Bonferroni test. Significantly differs (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). a-compared to immobilization stress, b-compared to forced swim stress group. CONT – Control, LCT - Lambda-cyhalothrin, IMS – Immobilization stress, FSS – Forced swim stress

DISCUSSION:

Elevated glucocorticoid levels in plasma/serum, often indicative of stress and its severity, have been widely documented [1, 36]. Research suggests that exposure to environmental neurotoxicants can also trigger this increase [19, 27, 28]. For instance, Soderlund et al. [46] observed heightened plasma corticosterone in rats exposed to low-dose deltamethrin, a synthetic pyrethroid, implying potential impacts on neuroendocrine and autonomic systems. Similarly, Righi and Palermo-

Neto [36] noted elevated serum corticosterone in rats exposed to higher doses of LCT (3.0 mg/kg body weight/day) for 7 days, but not at lower doses (1mg/kg body weight/day), while examining its behavioral effects. These findings underscore the complex relationship between environmental chemical exposure, stress response, and potential neuroendocrine disruption. Initial studies on rats, exploring the combined impact of restraint stress (lasting 5 to 60 minutes) and low-dose pyridostigmine bromide, revealed a significant cortisol spike at the 15–20-minute stress mark [13, 15]. Mirroring this, our current study showed no significant plasma corticosterone change in rats exposed to LCT (3.0 mg/kg/day) for 3 days. Similarly, isolated exposure to either IMS (10 minutes/day) or FSS (3 minutes/day) for 28 days didn't alter corticosterone levels. However, previous work by Shukla et al. [37, 38] demonstrated that pre-exposure to IMS or FSS for 28 days, followed by 3 days of LCT, heightened both plasma corticosterone and blood-brain barrier permeability. This suggests a potential synergistic effect between prior stress and LCT exposure, leading to a pronounced HPA axis response and redox imbalance.

A landmark study by Friedman et al. [14] revealed that stress could compromise the blood-brain barrier (BBB) in mice. This vulnerability was further exacerbated when combined with exposure to chemicals like pyridostigmine bromide, DEET, and permethrin [13, 15, 23]. Our current findings align with this, demonstrating that while neither stress nor LCT alone significantly impacted BBB permeability, the sequential exposure to stress (IMS or FSS) followed by LCT treatment did. This suggests a heightened susceptibility of the BBB to chemical insult under prior stress conditions. However, the precise mechanisms underlying this interaction remain to be elucidated [37, 38].

Experimental studies have shown varying degrees of oxidative stress in the brain following exposure to stress, likely due to differences in the types of stressors, experimental models, methodologies, and durations of stress procedures [36, 39]. Additionally, stress-induced changes in neurotransmitter and metabolite levels have been observed, often suggesting an adaptive response [19]. In our study, rats exposed to LCT (3 mg/kg body weight) for 3 days showed no significant changes in brain pro- and antioxidant levels, suggesting the dose was well-tolerated and non-neurotoxic. However, when rats were pre-exposed to IMS or FSS before LCT treatment, we observed decreased antioxidant levels and increased pro-oxidant levels in the hypothalamus, frontal cortex, corpus striatum, and hippocampus.

The brain is highly susceptible to various stressors, including physical, psychological, social, and environmental factors. Research has shown that stress can increase the production of reactive oxygen species (ROS) in the brain by altering biochemical pathways [22]. This

heightened ROS production, coupled with the brain's limited antioxidant capacity and high levels of polyunsaturated fatty acids (PUFAs), makes it particularly vulnerable to oxidative damage [40]. While ROS play essential roles in biological processes, excessive ROS generation can lead to lipid peroxidation, compromising cellular structure and function [41]. Studies have demonstrated that even short-term psychological stress can increase lipid peroxidation in the brain without affecting other organs like the liver. Chronic stress has also been linked to elevated lipid peroxidation and nitrite levels in the hippocampus, accompanied by cognitive impairments. Our study found that rats exposed to prolonged immobilization stress (IMS) or forced swimming stress (FSS) exhibited increased lipid peroxidation, protein carbonyl levels, and decreased antioxidant defenses in various brain regions [3]. However, these effects were not observed in rats subjected to shorter durations of stress, highlighting the importance of stress duration in causing these changes. Overall, exposure to acute or chronic stress can disrupt biochemical pathways, generate free radicals, and damage biomembranes, ultimately affecting cellular integrity and function in the brain [40].

Research suggests a strong link between stress and neuropsychiatric disorders. Stress-induced oxidative stress can trigger inflammation and disrupt the immune system, contributing to neurodegenerative diseases [42]. Observations from veterans after the Gulf War highlighted how stress can heighten the toxic effects of drugs on the central nervous system, even those considered safe under normal conditions [13]. Experimental studies have confirmed that stress can significantly increase the neurotoxicity of various drugs, environmental chemicals, metals, and pesticides. Furthermore, stress has been shown to worsen the neurotoxic effects of these substances [16]. The blood-brain barrier (BBB) plays a critical role in defensive the brain from injurious chemicals. However, stress can compromise the integrity of the BBB, allowing substances that would normally be blocked to enter the brain and cause damage [13, 15]. This study observed that pre-exposure to stress followed by LCT treatment, or simultaneous exposure to both, increased lipid peroxidation and decreased reduced glutathione (GSH) in rat brains [45]. The GSH depletion likely stems from its increased use in conjugation and/or antioxidant activities to neutralize reactive free radicals [44]. LCT toxicity may also be attributed to cyanohydrins, unstable free radical species under physiological conditions [46]. The simultaneous stress and LCT exposure impaired the antioxidant defense in rat brains, evidenced by decreased superoxide dismutase, catalase, and glutathione peroxidase activity, likely due to excessive free radical generation. Reduced catalase and Gpx activity, key enzymes in hydrogen peroxide degradation, may elevate brain-toxic hydrogen peroxide levels. Increased free radicals could disrupt the blood-brain

barrier, impacting brain homeostasis and metabolic functions.

This study's findings suggest that stress intensity and duration significantly impact neurobehavioral changes. Rats exposed to IMS for 6 hours/day or FSS for 15 minutes/day over 28 days exhibited these changes, whereas those exposed to IMS for 15 minutes/day or FSS for 3 minutes/day did not. The LCT doses used were well-tolerated and didn't independently affect neurobehavioral endpoints. However, concurrent exposure to IMS (exposure time 6 hours/day per session) or FSS (exposure time 15 minutes/day per session) and LCT worsened certain neurobehavioral parameters related to brain cholinergic and dopaminergic systems [37, 38]. Notably, pre-exposure to IMS (15 minutes/day) or FSS (3 minutes/day) combined with LCT led to brain cholinergic alterations, impacting learning and memory, and brain dopaminergic alterations, causing motor deficits. These changes appear linked to increased corticosterone levels, disrupted blood-brain barrier permeability, mitochondrial dysfunction, heightened oxidative stress, and apoptosis [22, 44].

While the study shows that psychological stress (IMS) may cause more intense oxidative stress changes in rats exposed to LCT compared to physical stress (FSS), it is evident that both types of stress significantly increase LCT's neurotoxic effects. This finding is particularly important considering a large portion of the population experiences stress regularly.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REVIEW ARTICLE

A SYSTEMATIC REVIEW ON NEUROMARKETING IN PHARMACEUTICAL INDUSTRIES

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ABSTRACT

Neuromarketing has garnered significant academic and commercial interest due to advancements in neural recording techniques and interpretation algorithms, making it an effective tool for deciphering consumers' implicit responses to marketing stimuli. This article presents a pioneering systematic review of neuromarketing within the pharmaceutical advertising domain. The study commences with an in-depth exploration of the background and rationale for conducting this review, followed by clearly articulated objectives and research questions. A thorough literature review provides insights into neuroscientific perspectives relevant to consumer behavior and decision-making, laying the foundation for the methodology section. Here, the search strategy, inclusion and exclusion criteria, study selection process, and data synthesis methods utilized in this systematic review are delineated. The systematic review section is structured around key themes, including an analysis of neuromarketing techniques applied in advertising within the pharmaceutical sector and specific types of pharmaceutical advertisements.

Additionally, the review investigates the methodologies employed in neuromarketing practices, offering an overview of commonly utilized techniques. Furthermore, the review discusses the implications of neuromarketing approaches in pharmaceutical advertising, highlighting practical applications and emerging trends. The discussion section critically examines key findings concerning the effectiveness of

neuromarketing in pharmaceutical advertising, outlining both the strengths and limitations of these approaches. Ethical considerations inherent in neuromarketing techniques are also explored, alongside a gap analysis identifying areas for future research and development. In conclusion, this systematic review provides valuable insights into the applications of neuromarketing in pharmaceutical advertising, offering recommendations for future directions and enhancements in this dynamic field.

***Keywords**: Neuromarketing, Pharmaceutical drugs advertising, Neuromarketing techniques, Pharmaceutical Advertising, Consumer behaviour*

INTRODUCTION

Marketing involves creating, communicating, and delivering value to satisfy customer needs, with two main types: B2B (business-to-business) and B2C (business-to-consumer). B2B marketing focuses on businesses and uses the 7 P's (product, price, place, promotion, people, process, and physical evidence), while B2C targets individual consumers. Emerging trends include C2B (consumer-to-business) and C2C (consumer-to-consumer) marketing, emphasizing flexibility and the sharing economy through platforms like e-commerce[1]. Traditional market research relies on self-report methods like questionnaires and focus groups but can be biased and unreliable.

Neuromarketing, a field introduced by Ale Smidts in 2002, offers deeper insights by using neuroscience and biometric technologies to assess consumers' subconscious responses to stimuli. Key aspects include emotion, attention, and memory, which help marketers create more effective strategies by understanding how the brain reacts to advertising [2,3]. This interdisciplinary field bridges marketing and neuroscience, aiding in predicting consumer behavior in a rapidly changing marketplace [4].

A systematic review is vital to consolidate knowledge in neuromarketing, identify research gaps, and assess study quality. This helps guide marketers in choosing effective techniques, supports evidence-based decision-making, and contributes to theory development and future research directions.

The review focuses on:

1. Common neuromarketing techniques used in pharmaceutical advertising.

2. The link between brain activity and consumer decision-making in this context.
3. The effectiveness of neuromarketing in influencing consumer behavior.
4. Strengths and limitations of neuromarketing in pharmaceutical advertising.
5. Gaps in current research and future research directions.

Neuromarketing is an emerging field that combines marketing strategies with insights from neuroscience to understand consumer decision-making[5]. The Foundation of neuromarketing is built on the "three-brain" model, as outlined by Renvoisé and Morin, which divides the brain into:

1. Neocortex (Thinking Brain): Responsible for logical analysis and rational decision-making.
2. Limbic System (Emotional Brain): Influences preferences subconsciously through emotions and feelings.
3. Reptilian Brain (Old Brain): Governs survival instincts and basic functions, playing a key role in evaluating risks and needs.

Companies leverage neuromarketing to design advertisements that target these areas, particularly the reptilian brain[6]. Strategies include focusing on self-preservation, using contrasts to capture attention, emphasizing beginnings and endings, and relying on emotional and visual cues to make advertisements more memorable and impactful[5].

Emotional advertising has been shown to outperform rational advertising, particularly in memory retention. Techniques such as fMRI and EEG analyses reveal brain regions involved in decision-making, like the striatum and ventromedial prefrontal cortex, providing deeper insights for companies to enhance consumer engagement.

In the context of social media, neuromarketing shows that ads with minimal editorial content and featuring celebrities gain more attention. Neuromarketing is becoming increasingly sophisticated with technology, using neural networks and neuroscience-based metrics to predict ad effectiveness[7-9].

This approach helps companies understand consumer behaviour and create stronger emotional connections through targeted advertising strategies. Table 1 shows various examples of neuromarketing strategies, modes of action, and their prospective effect on customers.

Table 1: Various examples of neuromarketing strategies, mode of action, and their prospective effect on customers

Sr. No.	Strategy	Mode of action	Effect on customers
1.	Utilising visual and audio to promote items	Direct effect on the brain of an individual	- Strong bass draws people's attention to dark items unconsciously. - More high -frequency music draws the listener's attention to bright items. - When it comes to attraction, white is more appealing than black.
2.	Advertising that emphasizes scarcity is the most effective.	Persuading individuals through advertising campaigns.	- Gain frames are statements like "Grab the latest edition today," while loss frames are phrases like "Don't miss out on the new edition." - People who seek uniqueness respond better to messages about what they'll miss if they don't bu y, while those less focused on uniqueness prefer hearing about the benefits of purchasing.
3.	Using gentle incentives to guide consumer behaviour on the internet.	Offering rewards to customers is an effective method to encourage repeat business.	- Stores of ten use delayed rewards, such as awarding points for each purchase, which can later be redeemed for store credit, to encourage customers to return. - Short-term rewards can motivate people to stay focused while they work toward their long-term goals.
4.	Developing a streamlined approach to product design.	Providing top -notch and distinctive packaging choices.	- An analysis of various packaging choices for products. - Volvo and Hyundai have employed analogous methodologies to identify the components of new car mo dels that resonated with consumers.
5.	Generating a multi - sensory incongruity.	Products and packaging that mimic the appearance of other materials.	- Leading brands involve consumers in multi-sensory experiences. - This indicates that they deliver a brand exp erience beyond visual elements, incorporating senses like smell and taste. When two sensory signals don't align, it's termed a mismatch.

6.	Forecasting future achievements using neuroscience.	Shaping the commercial success of a product.	- Impacts investment decisions, time allocation, and human and financial resource management.

MATERIALS AND METHODS

Search Strategy and Terms

A systematic literature review was conducted to evaluate neuromarketing's role in pharmaceutical advertising. A search on PubMed, Scopus, and ScienceDirect identified 19,497 articles, which were narrowed to 1,000 based on relevance. 110 were selected for detailed analysis. The review examined the significance, techniques, advantages, disadvantages, ethical considerations, and future prospects of neuromarketing in pharmaceutical advertising, ensuring data reliability through quality assessment tools. This provided insights into neuromarketing's impact on marketing strategies and consumer behaviour in the pharmaceutical industry.

Inclusion Criteria

- Primary research articles published in peer-reviewed journals between 2004 and April 2024.
- Studies focused on marketing aspects involving brain or physiological mechanisms, consumer behaviour, psychology, or neurology.
- Articles using neuroimaging techniques (EEG, fMRI, PET) or physiological measures (ET, GSR) for marketing insights.
- Only English-language articles.
- Studies providing relevant insights into neuromarketing.

Exclusion Criteria

- Articles published before 2004 or after April 2024.
- Duplicates across databases.
- Studies lacking sufficient methodological details.
- Articles not offering clear insights on neuromarketing techniques or their impact on consumer behaviour.

Study Selection Process

1. Reviewed all titles, removing duplicates and irrelevant articles based on exclusion criteria.
2. Evaluated the abstracts of the remaining articles, eliminating those that did not meet the exclusion criteria.
3. We proceeded with full-text screening for the articles that remained after abstract evaluation.
4. For the qualified articles, checked their reference lists and searched for any subsequent publications that cited them.

5. Compiled this comprehensive list and conducted another round of title and abstract screening using the established criteria

6. Repeated this process iteratively until the compiled list from step 5 no longer contained articles that met the criteria for full-text screening.

Data Synthesis

In the data synthesis phase, key insights from 110 shortlisted articles on neuromarketing in the pharmaceutical industry were analyzed. Using an aggregative approach, the review evaluated the significance, advantages, disadvantages, and ethical considerations of neuromarketing techniques like EEG, fMRI, and PET. The synthesis provided a comprehensive overview of neuromarketing's impact on pharmaceutical advertising, ensuring reliability and validity through quality-assessed studies. The PRISMA flow diagram shown in Figure 1 shows the extensive literature selection process in the following sections.

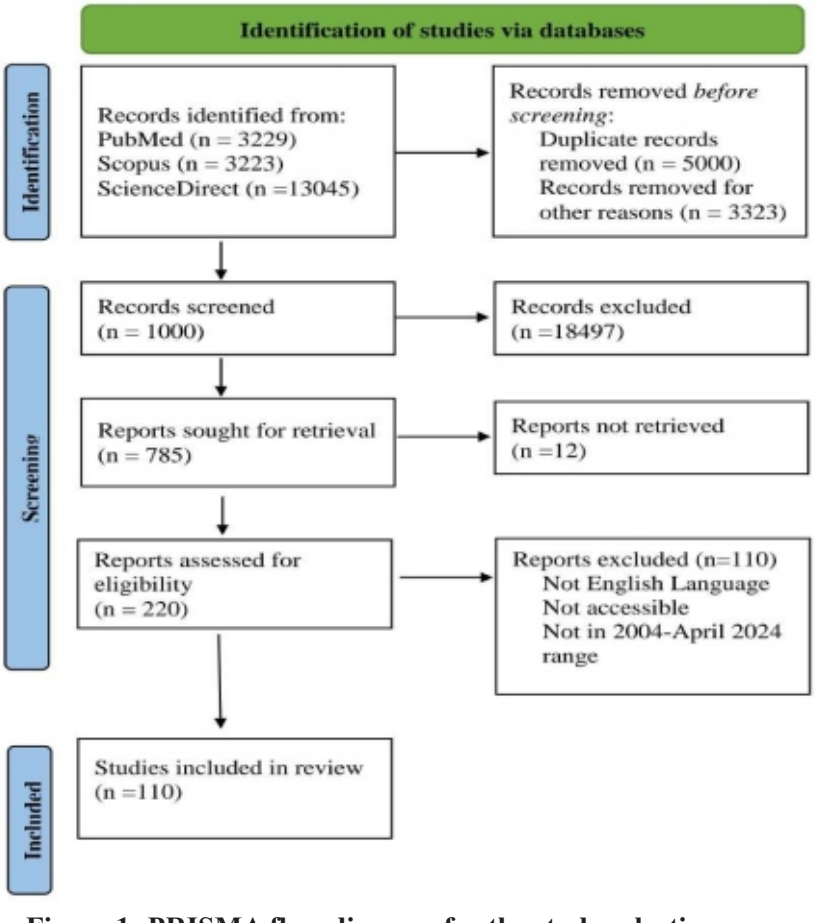


Figure 1: PRISMA flow diagram for the study selection process

SYSTEMATIC REVIEW

Advertising

Pharmaceutical drug advertising is controversial, balancing health promotion (e.g., vaccinations) with marketing products that may not address core health issues[10-12]. Authorities work to protect consumers, as advertisements focus on sales rather than education, raising concerns about potential drug misuse[13]. The pharmaceutical industry heavily invests in marketing new, high-priced drugs, which can harm consumers[6,14]. Neuromarketing techniques in ads, due to their persuasive nature, are debated and often considered for restriction. Drug advertising is regulated by international and local laws, ensuring that new drugs undergo a thorough review by regulatory bodies like the FDA, EMEA, and MHLW[6,15]. A SWOT analysis was conducted to assess the impact of neuromarketing in advertising new health technologies, which is shown in Figure 2.

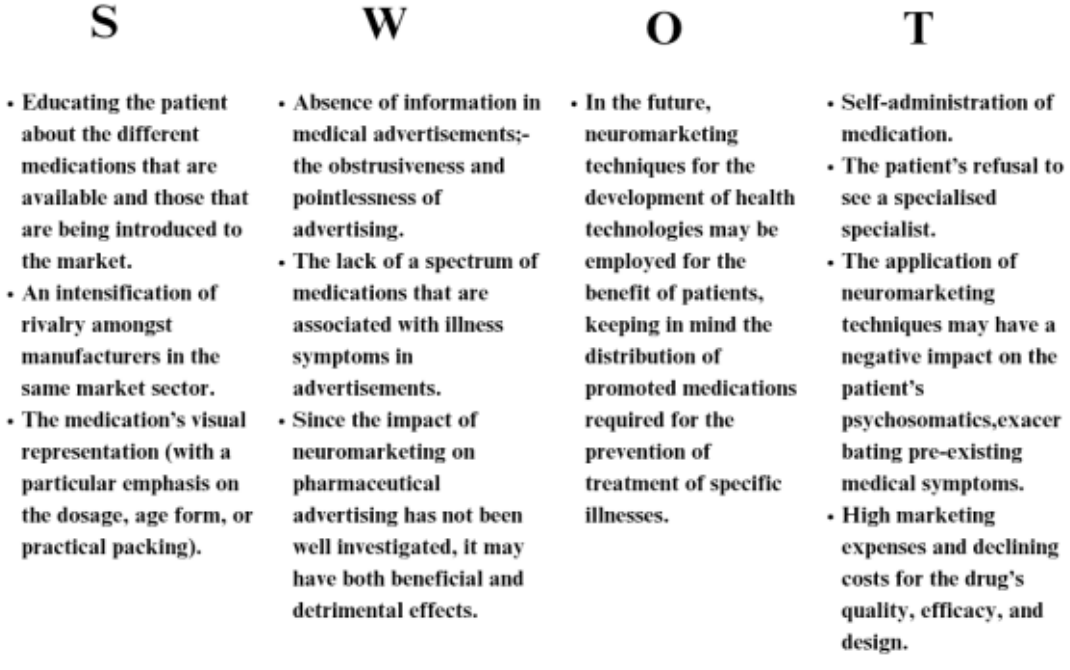


Figure 2: SWOT Analysis for neuromarketing in advertising

Types of Pharmaceutical Advertisements:

1. Direct-to-Consumer (DTC) Advertising: Includes help-seeking ads (informing about medical conditions without naming products), reminder ads (mentioning product name but not its use), and product claim ads (naming the product and making efficacy claims) [16]. Notable campaigns like "Lipitor"

and "Viagra" have demonstrated DTC's ability to educate and engage consumers[17-19].

2. Professional Advertising (DTP): Targets healthcare professionals, introducing new medications and providing details about the efficacy and side effects, as seen in Pfizer's "Lyrica" and Roche's "Tamiflu" campaigns[20-22].

3. Disease Awareness Campaigns: Focus on educating the public about conditions and treatment options, such as Janssen's schizophrenia awareness and Lilly's depression campaign[23,24].

4. Sponsorship and Event Advertising: Involves sponsoring relevant events, like Roche's support for the "American Society of Hematology" meeting, to engage specific audiences.

How is an applied neuromarketing study performed?

The process requires a series of specific steps to ensure the study is conducted accurately, which are shown in Figure 3[25]. These steps are as follows:

1. Client Briefing: Understand the client's goals.

2. Define Sample: Use a sample of at least 40 participants.

3. Choose Technologies: Select equipment based on desired data outcomes.

4. Develop Experimental Protocol: Design stimuli, control for bias, and consider neuroscience principles.

5. Organize Field Work: Conduct field activities, including a pilot test, in a well-equipped lab.

6. Data Collection: Use reliable technology for measurements.

7. Interpret Results: Analyse data to answer client questions and compile it into a final report.

Figure 3: All you need to know about neuromarketing

Overview of Neuromarketing Techniques

Neuromarketing strategies leverage neuroscientific methods to understand subconscious consumer behaviour, as 95% of decisions are made subconsciously. Techniques such as fMRI, EEG, galvanic skin response, and eye tracking are commonly used, each with benefits and drawbacks [26]. An overview of neuromarketing techniques is mentioned below in Table 2. These methods help reveal consumer emotions, expectations, and restrictions, making marketing more effective. Neuromarketing techniques are divided into two categories, which are shown in Figure 4:

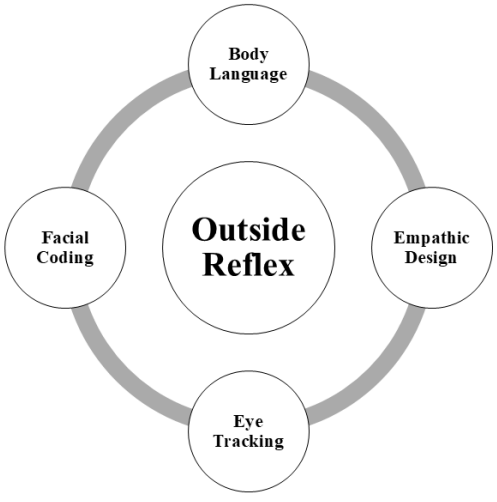
1. Outside Reflex Techniques: These include body language, empathic design, facial coding, and eye tracking. They measure external reactions like body movements, facial expressions, and eye gaze to understand consumer behaviour.

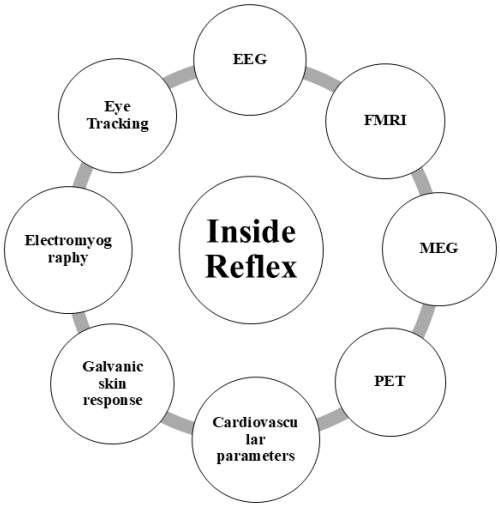
2. Inside Reflex Techniques: These focus on brain scanning (EEG, MEG, fMRI) and physiological measurements like heart rate and galvanic skin response. These methods monitor brain activity

and physiological reactions to marketing stimuli, providing deeper insights into emotional and cognitive responses.

Each method offers unique insights, and combining these techniques yields more comprehensive results for understanding consumer behaviour and improving marketing strategies[27]

Outside Reflex Neuromarketing Techniques





Inside Reflex Neuromarketing Techniques

Figure 4: Neuromarketing Techniques

Table 2: Overview of neuromarketing techniques

	Eye-tracking	EEG	Facial coding	fMRI	Physiological measures	Implicit Measures
How it works	Uses infrared light to detect a person's eye movements in real-time.	Detects the minuscule electrical charges produced by the activation of brain cells.	Identifies emotions from people's facial expressions and knows what they mean.	Detects the variations in blood flow brought on by changes in brain activity.	It covers a range of physiological measurement techniques.	Gather implicit preference information from non-conscious sources.
What it measures	Monitoring attention using pupil dilation, eye location, and eye movement	Cognitive functions, such as computations, to forecast a decision.	Human emotions as revealed via facial expression analysis.	Neural correlates of every consumer motivation.	Pupil dilation, heart rate, respiration, and galvanic skin reaction	Based on an examination of reaction times.
What it reveals about customers	Determines which products draw a customer's interest and focus.	Records the degree of stimulus, emotional arousal, and memory.	All-encompassing feelings such as joy, sorrow, shock, and terror.	Recall, emotional reactions, and degree of involvement.	Arousal is a term used to describe a range of emotions, including tension and enthusiasm.	The degree to which people are motivated, like, and associate a brand.

Implications of neuromarketing approaches in practice

Neuromarketing uses neuroscience techniques to evaluate the effectiveness of marketing materials, like ads and packaging, by analyzing brain responses. Studies show that consumer preferences, such as a liking for products with rounded edges, are linked to brain areas like the insula, which is involved in pain perception. This insight can guide product design.

Neuromarketing also reveals subconscious motivations that traditional methods may miss. For example, fMRI research by Martin Lindstrom found that emotional and personal associations with brands often differ from conscious preferences . Additionally, research on pricing strategies shows that consumers respond better to absolute savings (e.g., "$10 off") rather than percentage discounts, especially for tangible goods. These findings help optimize marketing and promotional

strategies . Some neuromarketing companies are listed in Table 3, which gives an idea of the data sources used by the companies for their products.

Table 3: List of neuromarketing companies and their product offerings

Company	Product Offering	Data Sources
Brain Intelligence	Measure the emotional reactions, moods, and perceptions triggered by various stimuli like media advertisements and product packaging.	Eye tracking, EEG, Galvanic skin Response; EMG; Implicit Association Test
Buyology	Collect quantitative data on emotions, moods, and perceptions in response to different types of stimuli, including media ads, produ ct packaging, and the shopper or user experience.	Go/No-Go Association T, implicit association test, EMG, fMRI, EEG, Eye Tracking.
FKF Applied Research	Monitors real -time emotional responses to stimuli, providing marketers with insights into customer attitudes.	fMRI
Forebrain	Examines the subconscious reactions consumers have to marketing cues and advises companies on leveraging this for better communication.	EEG; Eye Tracking
Innerscope Research	Examining both conscious and subconscious reactions to media and packaging.	Facial Coding, Eye Tracking, Biometrics, EEG, fMRI, Voice Analysis
Institute of Sensory Analysis	Identify emotions elicited by advertising through techniques like Eye Tracking, EEG, EMG, MRI, and GSR to improve user interfaces and the overall consumer experience.	Eye Tracking, EEG, EMG, fMRI, GSR
Keystone Network	Examines various elements of the consumer experience to gain insights into subconscious behavior.	Eye Tracking/Observation Cameras, EEG, GSR
Merchant Mechanics	Eye Tracking, EEG, EMG, and MRI are used to understand what customers think, feel, say, and do, exploring the scientific basis for the biometric variations in these behaviors.	Eye Tracking, EEG, EMG, fMRI, Biometrics

Mind Lab International	Investigate subconscious at titudes to uncover the underlying factors that drive consumer motivations and decision -making, revealing how they engage with marketing and its intended messages.	Implicit Association Test, EG, Eye Tracking Biometrics, EMG
MSW Research	Offers research and consulting grounded in neuroscience and other methodologies to craft compelling advertising messages and build more enduring, profitable brands.	Facial Coding, Eye tracking, GSR, EEG
Neurensics	Gaining insights into consumer behavior by tracking brain responses to various marketing stimuli involving all the senses.	fMRI, Eye Movements
Neuro-Insight	Focus on understanding how the brain reacts to messages delivered through branding and media channels.	EEG/SST
NeuroFocus	Analyse real-time subconscious and conscious reactions to grasp how consumers respond to marketing efforts.	EEG; Eye Tracking
Neurosense	Offer online consumer tests that enable the analysis of subconscious thought patterns, along with consulting services based on the results of these tests.	Implicit Reaction Speed Tests; fMRI
Neurospire	Studies the brain mechanisms underlying attention, memory, emotion, and decision-making.	EEG; Eye Tracking
Nielsen Neuro	Use neuroscience tools to reveal the subconscious elements of consumer decision-making.	EEG; Biometrics; Facial Coding; Implicit Association Testing; Eye Tracking; fMRI
One-to-One Insight	Employs EEG technology to understand how consumers react to media without prior interaction or experience.	EEG
Sales Brain	Centers on utilizing neuromarketing research to enhance the sales process.	EEG, Eye Tracking, Facial Imaging, Biometrics
Sands Research	Produces neurological data in a marketing setting to gauge levels of emotional engagement.	EEG, Eye Tracking, Biometrics

Applications of Neuromarketing

Neuromarketing offers valuable insights into consumer behavior and emotional responses across various marketing domains:

1. Branding: Techniques like fMRI and EEG help marketers understand subconscious consumer reactions to branding elements, fostering emotional connections and brand loyalty. Sensory branding, incorporating colors, sounds, and aromas, enhances the overall brand experience[32,33].

2. Advertising: Neuromarketing aids in developing effective ad campaigns by analyzing brain responses, allowing marketers to create targeted ads that resonate with consumers' desires, ultimately boosting brand loyalty and sales [11].

3. Digital Ecosystem: By examining brain activity in response to digital marketing, neuromarketing helps optimize campaigns for better engagement and conversions. It enables personalized marketing messages that enhance overall digital strategy effectiveness [34,35].

4. Packaging Design: Neuromarketing identifies emotional responses to packaging elements, guiding the creation of appealing designs that stand out and effectively communicate product information [36,37].

DISCUSSION

Key Findings on Neuromarketing in Pharmaceutical Advertising

Neuromarketing in pharmaceutical advertising offers significant advantages by measuring emotional responses, identifying attention-grabbing elements, and optimizing messages for better brand recall. It helps marketers understand subconscious decision-making, enhances brand differentiation, and supports personalized marketing strategies. However, ethical concerns related to privacy, consent, and potential manipulation must be addressed. Long-term studies are needed to assess neuromarketing's impact on consumer behavior, and cultural considerations are essential for global campaigns.

Limitations of Neuromarketing Approaches

Neuromarketing faces challenges, including a lack of reliability and validity in results, high costs of research, and potential manipulation of findings[4,38,39]. The field's popularity has not translated into sufficient academic literature, and the timing and context of marketing stimuli can affect individual responses[26].

Advantages of Neuromarketing Approaches

Neuromarketing integrates behavioural neuroscience to reveal consumer

behaviours that traditional methods might miss. It aids in advertising, brand development, and product design, enhancing the overall shopping experience[40]. Innovative applications, such as virtual stores, provide realistic marketing exposure to consumers[41,42].

Ethical Considerations

Ethical discussions around neuromarketing focus on human dignity, privacy, and the potential for manipulative advertising practices[43,44]. It is essential to ensure reliability and mitigate risks through ethical oversight. Although some argue neuromarketing aims to improve products rather than manipulate consumers, privacy concerns persist, leading to calls for stricter regulations[26,45].

Gap Analysis

Neuromarketing is still developing, with gaps in standard methodologies, ethical guidelines specific to healthcare, and the reliability of its techniques in predicting consumers. Privacy and data protection remain significant concerns, and there is a need for clearer regulatory compliance. Accessibility to neuromarketing technologies is limited, particularly for smaller companies, and the scarcity of comprehensive studies in the pharmaceutical sector poses challenges. Examples of successful neuromarketing applications in other industries include:

1. Coca-Cola: Utilizes neuromarketing for branding and product design, improving consumer appeal through extensive research[46-49].
2. Airbnb: Leverages user-generated content to enhance credibility and trust[50].
3. Walmart: Adjusts store layout based on consumer behaviour insights to optimize product sales[51].
4. IKEA: Employs psychological tactics in in-store design to influence purchasing decisions[52,53].
5. Nike: Uses emotional marketing strategies involving prominent figures to resonate with consumers[54-56].

Future Research Directions

Future directions in neuromarketing research are centered on leveraging advanced technologies, exploring cultural nuances, and addressing ethical concerns. One key area is the application of Artificial Intelligence (AI). AI and machine learning can analyze large datasets from social media, online browsing, and purchase histories to better understand customer behaviour and preferences. In the pharmaceutical industry, this could lead to personalized marketing messages and offers, improving customer engagement and conversion rates.

Another promising direction is the integration of multi-modal neuroimaging techniques. While functional magnetic resonance imaging (fMRI) and electroencephalography (EEG) are

commonly used, incorporating other methods like magnetoencephalography (MEG), near-infrared spectroscopy (NIRS), and transcranial magnetic stimulation (TMS) could offer a more nuanced understanding of how marketing stimuli affect brain responses. This integration can help pharmaceutical companies design more effective campaigns that resonate with consumers' neural responses. Cross-cultural studies in neuromarketing can provide insights into cultural differences in consumer behaviour, helping pharmaceutical companies create marketing strategies that are sensitive to these variations. This approach can guide more targeted campaigns in different regions, improving global marketing efforts.

Addressing ethical concerns is also crucial. Research focusing on the development of neuromarketing ethics can ensure that these techniques are used responsibly, with attention to privacy and autonomy. Lastly, the incorporation of behavioural economics into neuromarketing research can offer a deeper understanding of consumer decision-making. By combining insights from psychology, economics, and neuroscience, pharmaceutical companies can develop more effective and ethical marketing strategies that align with consumer needs and preferences. These future research directions offer a comprehensive approach to enhancing the impact of neuromarketing in the pharmaceutical industry while ensuring ethical practices and respect for consumer privacy.

CONCLUSION

Neuromarketing has attracted considerable attention from both academic researchers and the general public. Although there are relatively few scientific studies in this field, existing evidence indicates that neuroimaging could be advantageous in several marketing contexts. For marketing professionals, neuroimaging could be intriguing because it may offer faster and potentially less expensive insights compared to conventional marketing methods while also uncovering unique perspectives on consumer perceptions that might otherwise remain hidden. Among the various devices for recording brain signals, EEG is gaining popularity in neuromarketing studies, especially for analyzing television commercials (TVCs), owing to its high temporal resolution and cost-effectiveness.

However, the claim that neuroimaging is more cost-effective than traditional marketing approaches seems overstated. Nonetheless, ongoing progress in neuroimaging analysis, including techniques like multi-voxel pattern analysis (MVPA), could soon enable the extraction of subtle information about consumer preferences. This ability could improve sales strategies following product design, but the most profound impact might occur during the product development phase.

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REVIEW ARTICLE

RECENT ADVANCES IN PHARMACOLOGICAL PROPERTIES OF INULIN: A MINI REVIEW

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ABSTRACT

Inulin is a polymer primarily composed of fructose units, and glucose unit at the end. It is neither digested nor absorbed in the stomach, thus staying in the bowel. Although it gets metabolized by bacteria in the colon to a gel that helps certain beneficial gut microflora grow, it could be classified as a prebiotic. Multiple research studies have been conducted to uncover its pharmacological use in various chronic disorders, but compiled information is lacking in the current literature. The recent advances in its pharmacological effects such as neuroprotective effects, anti-diabetic, hepatotoxicity, anti-oxidant, and anti-inflammatory were discussed in this article. It showed promising pharmacological properties and can be explored further for developing formulations against the neuro-disorders, diabetes, liver and inflammatory bowel diseases.

INTRODUCTION

In 1804, German scientist Valentin Rose discovered inulin by extracting it from Inula helenium roots. Inulin is a polymer primarily composed of fructose units, and glucose unit at the end [1]. It is commonly found in herbs, fruits and vegetables, including garlic, bananas, onions, wheat, leeks, chicory, Jerusalem artichokes, asparagus, and artichokes [2]. It is utilized as an energy reserve and regulates cold resistance in these plants and found to be water-soluble therefore osmotically active [3]. Certain plants protect themselves from cold and drought during the winter period by changing their osmotic potential. This is achieved by altering the degree of polymerization of inulin molecules through hydrolysis without

changing the total carbohydrate content [4]. It is neither digested nor absorbed in the stomach, thus staying in the bowel. Although it gets metabolized by bacteria in the colon to a gel that helps certain beneficial gut microflora grow, it could be classified as a prebiotic [5]. A significant quantity of carbon dioxide, hydrogen, and/or methane is released, which could be responsible for bloating and flatulence. It is commonly taken by mouth for weight loss, constipation, diabetes, controlling hyperlipidemia, preventing traveller's diarrhoea, increasing calcium absorption in adolescents, and several different conditions, but there is a lack of research findings to aid most of these uses [6]. The clinical trials have revealed that it causes gastrointestinal adverse effects like flatulence and bloating, which advocates its use in moderate quantities [7]. Although, inulin is being used to find out the glomerular filtration rate, it is not being reabsorbed or secreted after introduction into tubules due to its resistance to enzymes and its high molecular weight [8].

Numerous research studies have been conducted to uncover its pharmacological use in various chronic disorders, but compiled information is lacking in the current literature. Hence, it was decided to compile the recent development in sources, process of extraction and pharmacological applications which will help researchers for further research. This review provides a deep insight about inulin's sources, chemistry & SAR, extraction process, role of inulin in various therapeutic application and its utilization as a functional ingredient in the development of novel products.

SOURCES

Inulin is found in over 36,000 plant species, such as chicory, agave, Jerusalem artichoke, wheat, asparagus, onion, garlic and banana, these plants have diverse therapeutic uses, inulin could be responsible for these actions [Table 1]. The dietary intake of the prehistoric hunter-gatherer in the Chihuahuan Desert is predicted to comprise 135 grams of inulin-type fructans daily [9].

Table 1: Sources of inulin

Sr No.	Source	Plant part	Inulin content [g/100g]	Therapeutic use
1.	*Cynara cardunculus* (Artichoke)	Leaves	85	Anti-rheumatic, diuretic, lithontripic, early stages of late-onset diabetes, chronic liver/gall bladder diseases, hepatitis, arteriosclerosis and the jaundice [10].
2.	*Articum lapp. L* (Burdock)	Fruits and Roots	50	Diabetes mellitus, skin inflammation and digestive tract diseases [11].
3.	*Allium sativum* (Garlic)	Rhizome	18-19	Diabetes and its complications [12].
4.	*Cichorium intybus* (Chicory)	Flowers	68	Diabetes mellitus, appetite stimulant, gallstones, gastroenteritis, sinus problems, cuts, and bruises [13].
5.	*Allium cepa* (Onion)	Bulb	25	Antidiabetic effects, bronchitis, pain and swelling after bee or wasp stings, inflammatory disorders, asthma, ulcer wounds dysentery, keloids and scars [14].
6.	*Asparagus officinalis* (Garden asparagus)	Root	2-3	Antidiabetic, remedy for schistosomiasis and tuberculosis [15].
7.	*Jerusalem artichoke* (Artichoke)	Root	53	Antidiabetic and increase the beneficial intestinal microbiota [16].

8.	*Allium porrum* (Leek)	Stem	16	Antidiabetic, gastric ulcer, tuberculosis, anti - hypertiensive and anti - helmenthic, blood clotting disease [17].
9.	*Triticum* (Wheat)	Seed	1-4	Diabetes, Anti -cancer, Strengthens the bones [18].
10.	*Musa* (Banana)	Leaves	0.3-0.7	Antidiabetic, ant -cancer, anti-ulcer. Anti-alzheimer's disease, anti -infection, anti-diarrhea, hemorrhoids, anti - diabetes, and anti - hypertension [19].
11.	*Taraxacum officinale* (Dandelions)	Leaves Roots Fruits	45	Anti-diabetes and anti - cancer, diuretic, choleretic, anti-inflammatory, anti - rheumatic, digestive - stimulant, alterative, and depurative properties. Anti-hypertension, dyspepsia, irritable bowel syndrome, and ovarian androgen excess [20].
12.	*Smallanthus sonchifolius* (Yacon)	Roots Leaves	9.25+/- 0.44	Anti-diabetic [21].
13.	*Hordeum vulgare* (Barley)	Fruit	0.5-1.5	Anti-diabetic [22].

CHEMISTRY

Inulin's structure primarily consists of β-D-fructosyl units connected by (2→1) glycosidic bonds, typically terminating with an α-D-glucosyl group linked via a (1↔2) bond. The fructose chain length varies, generally ranging from 2 to 60 monomers, though it can extend up to 100. Its physicochemical and functional characteristics are influenced by the degree of polymerization (DP) and structural branching. Higher DP inulin serves as a fiber-like prebiotic, offering potential health benefits. However, as DP increases, inulin’s solubility decreases.

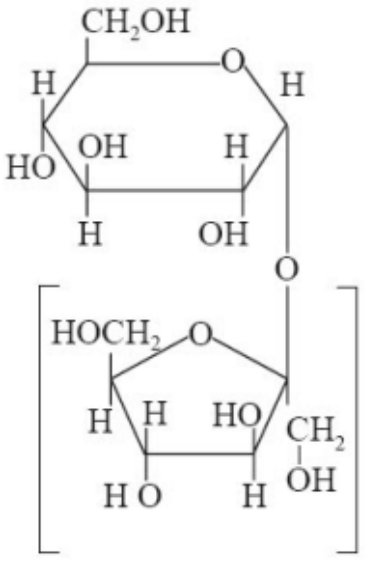

Figure 1: Structure of Inulin

Chemical formula:C12H22O11

Molar mass:342.3 g/mol

Boiling point =563.5°C

Melting point=176-181°C

Density 1.85 g/cm³

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RECENT ADVANCES IN THERAPEUTIC APPLICATIONS

Antidiabetic effect:

Diabetes is a medical disorder that affects the process of converting food into energy. Inulin has been screened for antidiabetic effect. Inulin and resveratrol together demonstrated renoprotective effects in diabetic rats by reducing oxidative stress and inflammation in kidney tissues, suggesting a low-cost treatment option for diabetic nephropathy [23]. In another study, inulin and Lycium barbarum polysaccharides improved the gut barrier and decreased hyperglycemia in rats by altering the gut microbiota and stimulating TLR2+ intraepithelial γδ T cells in the gut mucosa [24]. Furthermore, inulin-type fructan demonstrated a protective effect against gestational diabetes mice caused by a high-fat/sucrose diet [25]. In experimental animals, inulin fermentable fiber reduces type I diabetes via IL22 and short-chain fatty acids [26]. Recent research has indicated that inulin may prove to be a useful treatment for diabetes.

Antioxidant activity:

Inulin showed anti-oxidant activity in DPPH and ABTS radical scavenging assay [27]. The anti-oxidant activity of inulin is

correlated to its molecular characteristic [28]. Inulin intake results in increase in lactobacilli counts which increase lactic acid synthesis, lactic acid itself has antioxidant activity and thus inulin shows antioxidant activity [29]. In one of the studies, phosphorylated derivatives of long-chain inulin with different substitution degrees were prepared, the findings demonstrated that phosphorylation can improve its physicochemical characteristics and biological activity, including antioxidant effects, indicating its potential as a functional food ingredient and quality enhancer [28]. The effects of inulin supplementation on HFD-induced obesity with hepatic oxidative stress and anxiety-related defensive behavior were evaluated. Inulin supplementation restores the hepatic redox balance followed by a decrease in CAT activity and amounts of carbonylated protein [30].

Anti-inflammatory effect

Inulin is a prebiotic and polysaccharide has anti-inflammatory activity. A recent study highlighted that inulin may assist in managing recurrent inflammatory bowel disease symptoms by modulating gut microbiota, reducing inflammation, and alleviating endoplasmic reticulum (ER) stress [31]. Olsalazine-based MOF nanoneedle/inulin gel hybrid (Cu_2(Olsa)/Gel) reshaped intestinal homeostasis in inflammatory bowel disease. Cu_2(Olsa)/Gel displayed anti-oxidative and anti-inflammatory effect and enhanced bio-adhesion and colon retention [32]. Inulin administration in mice with type 2 diabetes (T2DM) Inulin administration reduced diabetes-induced chronic inflammation and mitigated renal damage [33].

Hepatoprotective property

Several research articles have proven inulin's hepatoprotective activity in their study. Catechin grafted inulin was investigated against carbon tetrachloride (CCl_4)-induced acute liver injury, it showed higher *in-vitro* antioxidant activity and stronger hepatoprotective effect *in-vivo* than inulin [34]. Inulin was found to be hepatoprotective against methotrexate induced hepatotoxicity might be mediated via the modulations of apoptotic and oxidative stress factors [35]. Inulin-type fructan demonstrated significant liver-protective effects in vivo, likely due to its antioxidant and immune-regulating properties [35]. Deoxynivalenol is a *Fusarium* mycotoxin which induced oxidative stress, cytotoxicity and genotoxicity. Inulin nanoparticles alleviated DON toxicity [36]. Inulin helps prevent non-alcoholic fatty liver disease by regulating gut microbiota and inhibiting the LPS-TLR4-Mψ-NF-κB-NLRP3 inflammatory pathway through the gut-liver axis [37].

Neuroprotective effect

The recent research showed promising neuroprotective effects of inulin, in one of the studies, evening administration of

inulin is more effective in reducing inflammation and enhancing amino acid metabolism and reduced CUMS-induced anxiety and depression. This study suggests a potential connection between the microbiota-gut-brain axis and chrono-nutrition, highlighting that optimal timing of administration enhances intervention effectiveness [38]. Inulin reduces blood-brain barrier permeability and mitigates behavioral disorders by regulating the TLR4/MYD88/NF-κB pathway in chronically stressed mice. Additionally, it modifies gut microbiota to relieve post-stroke depressive-like behavior through the IGF-1-mediated MAPK signaling pathway [40]. Prolonged preventive supplementation with inulin reduced anxiety, cognitive impairments, and dysbiosis in mice subjected to chronic unpredictable stress [41].

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