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Running title: Chitosan Nano-formulations

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ABSTRACT

Alopecia (hair loss) is a benign, treatable condition that affects a sizable portion of the population globally and develops into a chronic condition over time. It has an impact on the patient's quality of life and self-esteem. The current alopecia treatment plan is inadequate for drug delivery to hair follicles directly to produce continuous hair growth. Finasteride (FST), dutasteride (DST), and topical minoxidil (MNX) are examples of conventional therapies. These drugs currently have limited performance, lack of compliance and systemic side effects, including decreased libido, increased depression, and ejaculatory disorders. Given that the development of nanotechnology-based formulations as hair loss therapeutic strategies is clearly growing, topical FST, DST, and MNX delivery using these cutting-edge formulations is known to improve skin permeation and depot formation into hair follicles, increased skin bioavailability, and enhanced therapeutic efficacy with minimal side effects. Chitosan is a natural polymer that is frequently used to create drug delivery nano-systems. Because of its special qualities, including biocompatibility, biodegradability, and mucoadhesive properties, it enables targeted therapy without posing any hazardous risks. The potential of chitosan nanotechnology-based FST, DST, and MNX delivery formulations for better hair loss therapies is highlighted in this review, which also includes a full analysis of their in vitro and in vivo performances as well as regulatory and nano-safety considerations.

Keywords: Nanotechnology; Finasteride; Dutasteride; Minoxidil; Hair loss

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INTRODUCTION

Alopecia (hair loss) is among the most common and upsetting skin diseases, it advances from brief to a prolong condition over time [1]. It results from predetermined genetic background, unknown factors, and problems with the hormones [2] that have an impact on the patient's sense of self-worth, psychological health, and social standing [3]. During this time, other comorbidities such as vascular insufficiency and reactive oxygen species (ROS) cause the condition within hair follicles to worsen, which also causes the hair follicles to shrink. Alopecia's drugs comprise topical therapies (foam lotions and solutions) and prescription medications, depending on the severity of the condition [4]. There are numerous daily medication regimens that are often used. Regular use of these medications can cause a number of localized and systemic side effects, including tachycardia, hypertrichosis, and scalp irritation, which frequently start during the first few weeks of treatment [5]. These adverse effects suggest that it may be a potential clinical issue for biopharmaceutical development. Wigs, masks, and sunscreen are a few examples of physical interventions and Food and Drug Administration (FDA) endorsed creams to treat alopecia, and they show consumer non-compliance when used in conjunction with medications [6]. Topical corticosteroids and scalp micropigmentation are the most often utilized clinical therapy, and minoxidil and finasteride have also been shown to be successful adjuvant therapies [7].

The multifactorial character of alopecia has always been a problem for medication, regardless of age. It leads to the symptoms of systemic toxicity, like hypotension, and local pain [8]. As a result of the seriousness of these illnesses, new dosage schedules and medication adherence are advised but are ineffectual, which makes pharmacotherapy challenging. Physical methods and monotherapy applied externally and locally, which are currently used as non-unit dose treatment modalities, are insufficient in this circumstance to be completely effective. Therefore, improving drug therapy requires creating a unit dose approach to lower systemic and local toxicity, to provide comfort for the patients [9].

It may be beneficial to develop a programmable, reproducible nanocarrier depot for designed, controlled, targeted, and localized drug delivery [10] that delivers the desired drug release at a predetermined interval. In order to implement a variety of promising controlled release nanomedicines (CRNMs), significant advances are used in the formulation and production of therapies using controlled and targeted therapy [11].

Such a delivery system, when used in conjunction with combination therapy, offers a

wide range of possibilities for reducing dosage frequency while still maintaining clinical requirements for illness stability. A natural polymer called chitosan is frequently used to make drug delivery nano-systems. As a result of his biocompatibility, biodegradability, and mucoadhesive qualities, it improves targeted therapy without causing toxicity [12].[Ref]. This review focuses on alopecia, the most popular pharmacotherapies for it, their drawbacks, and the promise of FST, DST, and MNX delivery formulations based on chitosan nanotechnology for more effective hair loss treatments.

Skin Structure and Functions:

The human body's most versatile, largest, and heaviest organ is the skin. The body's temperature regulation, sensory awareness, and body's protection are the three most important functions of the skin. The skin shields the body from evaporation and the entry of potentially harmful substances, allergies, irritants, and microorganisms [13]. Healthy skin is necessary to guarantee that these many duties are carried out. The skin surface area of an adult is somewhere between 1.8 and 2.0 m^2 [14]. There are three main layers of the skin: the epidermis, dermis and hypodermis. The stratum spinosum, stratum corneum, stratum granulosum, stratum basal and stratum lucidum, make up the epidermis, which is the skin's outermost layer [15]. The stratum corneum is responsible for the skin's barrier qualities. Dermis is the second layer. The dermis is made up of elastic tissues and connective tissues like collagen fibrils, which primarily serve as the skin's support, mechanical strength, elasticity, and flexibility. An interconnection of lymphatic vessels, eccrine glands, sebaceous glands, nerve endings, eccrine glands and blood vessels supply the dermis [16]. Hypodermis also known as subcutaneous tissue is the innermost layer of skin and it is comprises of adipocytes inside the connective tissue. Insulation of the body is the main purpose of subcutaneous tissue [15].

Hair and Hair Growth Cycles:

The hair follicle is a skin component and an organ. Starting in the dermis, it goes to the epidermis layer through the skin's outer layer. The biggest appendages, which are made up of sebaceous glands and hair follicles, can offer "shortcut" routes for medications to overcome the stratum corneum barrier [15]. Proteins, lipids, water, trace elements, and colors make up hair [17]. Keratin is the name of the hair protein. Two categories of hair exist. The body's vellus hair, also known as hair which transforms into hair that appears at the surface of the body at puberty. The latter is referred to as terminal hair – (Ears, nose, and eyebrows) and long terminal hair (head hair, beard, underarm, pubic area). The hair shaft is the part of the hair that is visible above the skin. Cuticle, cortex, as well as medulla make up its structure. The term "hair root" refers to skin- or inconspicuous hair. Cell division and all other biological activities take

place in the hair bulb. The hair follicle is tube-like and hollow part in which the hair bulb is located. Among the organs which are impacted by androgens is the human hair follicle. Androgen encourages hair development but can also block it occasionally, leading to androgenetic alopecia (AGA) [18].

The hair follicle produces hair, which goes through a cyclic process having several stages. It begins in the anagen stage and transitions through the catagen to telogen stages. Every stage of body' hair growth happens simultaneously; some hairs may be in the anagen stage while others are in the telogen or catagen stage. The new follicle grows to make a new hair once the previous lower follicle is destroyed [19]. The hair growth cycle differs depending on the area. The anagen stage, which could also be referred to as the growth stage, is when hair is generated and during which hair achieves its maximum length. The anagen phases of scalp follicles can persist for several years [20]. The phase of regression also known as the catagen stage follows the anagen stage. During this phase, cell proliferation, differentiation, and pigmentation cease, decrease of dermal papilla and significant programmed cell death occur [20]. Typically, the catagen stage lasts 7-14 days. The club hair is created and moves upwards during the catagen stage, when the hair is fully keratinized [19]. Following the catagen stage is the telogen stage, and this could be for many weeks, the dermal papilla, which has a round form, is located very close to the secondary hair germ keratinocytes, that contain the stem cells for the hair follicles, during the telogen stage. A lower and new follicle forms during the early stages of the anagen phase, and the upper and old follicle is formed from the new hair, ejecting the current hair in the process [19].

Alopecia (hair loss):

According to Lin *et al*. [21], alopecia is a clinical illness that results in loss of hair from the scalp or other body's parts and is brought on by a genetic predisposition, hormone imbalance, infection, or idiopathic causes [22]. The majority of hair issues are caused by alopecia or hirsutism (excessive hair growth). The medical term for baldness or loss of hair is alopecia. AGA is the name for androgen-related baldness.

Etiology of Alopecia:

It is a broad category of illnesses with numerous and diverse etiologies. AGA is the most prevalent type of alopecia (female-pattern or male-pattern hair loss). Dihydrotestosterone is a key player in the androgen-dependent hereditary condition known as AGA. More than 70% of men (male-pattern hair loss) and 57% of women (female-pattern hair loss) who are above 80 years are affected by this kind of alopecia, whose prevalence rises with age [23]. Compared to Caucasians, the prevalence is lower among non-Caucasians [23]. [Ref]Drugs (including agents for chemotherapy), infections, systemic diseases (abnormalities that induce

high temperature, endocrine disorders), and trauma are additional major reasons of hair loss. Primary hair shaft anomalies, autoimmune illnesses, and uncommon dermatological problems are less frequent causes [24].

Mechanism of Hair Loss:

The hormones (androgens) that control hair growth are testosterone and dihydrotestosterone (DHT). The biologically active form of testosterone (male hormone) is DHT. In hair loss patients, the enzyme 5α-reductase converts testosterone, which is the primary androgen, to the more potent androgen, DHT which makes the hair follicle miniaturise and shed hair [25].

As a result of DHT, the normal hair begins as pigmented, long, and thick and later transforms to less pigmented, short and thin, at the end of the process. From one cycle to the next, the new hair color loses pigment, the hair shaft gets thinner, and bald spots start to form. 5α-reductase isozyme (Type I), which is found in the hair, skin, sebaceous glands, follicles and, prostate, kidney, and liver and 5α-reductase isozyme (Type II), which is found in prostate, male genitalia, and hair follicles, both play a role in androgen action [26]. Both of these isozymes interact with androgen receptors and are involved in the metabolism of steroids. The enzyme 5α-reductase serves as a catalyst in androgen production, transforming testosterone to the DHT, which is the biochemically active form of testosterone. A 5 α -reductase inhibitor can be added to prevent testosterone from being converted to DHT, which will stop hair loss.

Classification of Alopecia:

Alopecia, which is among the commonest skin diseases that affects the entire world's population [22] can be categorized into two: scarring and non-scarring [21]. The hair follicles are irreparably damaged in cases of scarring alopecia, resulting in permanent hair loss. According to Bolduc *et al*. [27], this kind of alopecia has lower prevalence and has a variety of etiologies, including lymphocytic, neutrophilic, and multifactorial origins [28, 29].

The capillary cycle is changed in non-scarring alopecia; however, the hair follicles are still present, causing hair development. In this group, there are a few disorders that can be distinguished, including trichotillomania, alopecia areata, and tinea capitis, which are marked by uneven loss of hair in a few particular areas. Telogen or anagen effluvium can also cause scattered hair loss. In turn, AGA can develop into total baldness and can either be diffuse or have a particular pattern [29]. The most prevalent kind of continuous loss of hair, AGA, affects roughly 30% of middle-aged women and 30–50% of males [30]. Its characteristics include shrinkage of the hair follicle as a result of a change in the hair development cycle brought on by high amounts of dihydrotestosterone (DHT), which is produced when the enzyme 5 α-reductase metabolizes the hormone testosterone [31].

Alopecia areata, which is connected to autoimmune conditions with a variety of causes [32], is the second most common form of non-scarring alopecia [33].

Alopecia Pharmacotherapy and their Inadequacies:

Mioxidil (MNX), finasteride (FST), and dutasteride (DST) are medications that are frequently prescribed to treat alopecia. Additionally, minoxidil (MNX) is frequently applied topically to treat alopecia. It works by opening potassium channels, causing nitric oxide release, enhancing the flow of blood to the hair follicles, and changing the E2 and D2 pathways of prostaglandins, which inhibits the pathway responsible for the development of alopecia [34]. As a result, it can lengthen the anagen period in hair follicles and effectively stop loss of hair [35]. It is just 30% to 40% of alopecia's patients who typically have noticeable hair growth, and MNX is typically ineffective or not recommended for other types of hair loss, with the possible exception of alopecia areata. It may take 8 to 12 months for hair to return. Because hair loss returns when medication is discontinued, treatment must be continued indefinitely. Mild scalp irritation, allergic contact dermatitis, and a rise in facial hair are the most frequent side effects. Sometimes used off-label, low-dose oral minoxidil is given once daily (0.25- 5 mg dosage), although its usage is restricted due to potential cardiovascular side effects [21, 34]. Increased adverse reactions cause patients to stop receiving treatment and make it more difficult for them to comply with it, which reduces the effectiveness of therapy [37]. Since the topical route is thought to increase MNX absorption, unique and effective formulations are required for MNX topical delivery [37].

In order to inhibit the conversion of testosterone into DHT, the FST, a synthetic azosteroid with powerful selective antagonistic effect on 5 α-reductase (type II) enzymes, binds permanently to the enzyme. Specifically, 5.0 mg/day and 1.0 mg/day for the treatment of prostate cancer and benign prostatic hyperplasia respectively, FST was initially licensed for the reduction of prostate size seen in patient with urinary blockage. Recently, it has been discovered that oral in-take of FST is effective for treating a variety of follicular and dermatological conditions, especially AGA [38].

Considering the manufacture of DHT, the usage of FST may be extremely beneficial as it will result in a reduction of the effects on the production of follicular cells' protein by inhibiting the conversion of testosterone to DHT. FST, however, has certain negative side effects, including reduced reproductive function, impotence, and erectile dysfunction, when taken orally. Hence, another method of delivering FST into the body free from those negative side effects is required. From another point of view, the majority of topical alopecia treatments in the market are made with the active ingredients dissolved in alcohol-water solutions.

Nevertheless, only a small portion of the administered dosage of the medication really gets to the target region, entering the hair follicles and the pores, as a result of the limited keratin layer's permeability. Because of this, those items fall short of consumer expectations, which can sometimes result in poor treatment compliance. Recent research has so supported the idea that nano-particles can successfully enter and stay in the pilous follicles for prolonged period of time [39]. Additionally, nano-particles could be able to solubilize hydrophobic medications, lower the dosage of a drug and, in some situations, avoid or eliminate negative effects [40]. It is occasionally used to treat AGA. Dutasteride (DST), a medication meant for the treatment of benign prostatic hyperplasia strongly inhibits 5 α-reductase than FST.

A promising treatment option for AGA is targeted hair follicle topical therapy with DST, which clinically appears to be more effective than FST [41, 42]. DST non-selectively inhibits 5 α-reductase types I and II. Patients using oral DST notice enhanced subjective appearance and higher hair density when their scalps are photographed [43]. Compared to the typical dose of 5.0mg of FST, a 2.5mg per day of DST promotes higher hair growth. However, because of DST's enhanced potency, more people are likely to experience adverse effects similar to FST, such as libido loss, erectile dysfunction, and gynecomastia [25]. DST has a low solubility in water and is very lipophilic. Due to these characteristics, it is challenging to include this molecule into standard topical method of drug delivery meant for the part of the head where hair grow, as a greasy or viscous residual may produce an unfavorable hair as well as being rejected by alopecia's patients.

Chitosan and Chitosan Nano-formulations in Alopecia's Drug Delivery:

When chitin is deacetylated under alkaline conditions in the solid state or when chitin deacetylase is hydrolyzed, chitosan, a linear polysaccharide, and a substance which occur naturally is created. In terms of use and distribution, it is regarded as the second-biggest renewable biomaterial after cellulose [44]. Due to their distinct biological characteristics, chitosan and biomaterials derived from it have been greatly considered recently in the biomedical arena. Chitosan's non-toxicity, biodegradability, biocompatibility, immuno-stimulating, anti-tumoral, antibacterial, as well as its ability to fight microbes are among its common medical attributes. Chitosan's ability to be broken down by biological activity was demonstrated in vivo as well as in vitro, where macromolecules were divided up into a number of smaller sections of monomers [45]. Living cells might interact with chitosan and the products broken down by it without any risk to their health.

According to Gallaher *et al*. [46], chitosan may reduce cholesterol absorption, stop oxidative stress by filtering reactive oxygen species, and function as an antibiotic and also shows ability

to fight bacteria [47]. It is proposed that a number of variables, including molar weight, the presence of metal cations, dissociation constant (pKa), the degree of deacetylation, species of microorganism, and pH affect the ability of chitosan to fight microbes [48].

Transdermal or topical medication delivery system offers an additional and efficient route of drug administration, because it eliminates first pass effect, poor patient compliance, and side effects produced by the system. Many formulations have been created in the modern period to offer local therapeutic effects [13, 49]. There are many techniques being researched and developed for maximizing drug absorption through skin, including micro-needling, iontophoresis, electroporation, microwave and ultrasounds therapy. Lately, nano-therapy carriers have sparked significant interest in the delivery of medication to the skin [14]. When compared to the regular dosage, nano-carriers such as nano-emulsions, vesicular drug carriers, and lipid nano-particles provide a number of advantages in the cosmetic and drug manufacturing companies [50].

Nano-technology and nano-carriers have been used in newer generations of drug application and targeting to enhance not only the effectiveness and use of pharmaceuticals but also their physical characteristics and delivery profiles [51, 52]. For the development of innovative nano-carriers and enhanced biomaterials, biomimetic methods are being investigated [53, 54]. With regulated drug delivery, nano-technology offers a wide range of topical treatments that will improve clinical results and deliver effective doses to specific areas. Older and generally used formulations like ointments, solutions, and many more are greatly reduced at the targeted site due to their passage through the gastrointestinal tract, and also have frequent administration schedules, uneven plasma concentration, and worse patient compliance.

By altering and changing those formulations to nano-particle carrier systems, researchers hope to lessen the negative effects of current traditional topical medicines for chronic conditions like alopecia. This will ensure effective delivery of medications and prevent adverse effects in the system [55]. Despite the fact that findings related to cutting-edge technologies in this area have been extensively utilized [56], demonstrating its potential for commercialization, nanomedicines for the treatment of hair loss are still in the development stage of successful marketable products. Numerous nanoparticles made from various substances, including lipids, phospholipids, biopolymers, degradable polymers, and even metals, have been suggested as potential treatments for alopecia. These nanoparticles can, in general, lessen the irritating potential of medicinal compounds and regulate their release.

Although this is a desired outcome given that the hair follicle is the drug target for hair loss, numerous findings have demonstrated that

topically administered nanoparticles prefer to collect there. The nanoparticles' interaction with the skin is essential for effective medication delivery in such nanostructured systems. A successful therapy in this situation depends on how well it's physical and chemical characteristics, such as surface charge, particle size, material composition, and application method are modulated.

Because they are biocompatible, not harmful, and can be decomposed by biological activity, natural polymers are frequently employed in therapeutic formulations.

Chitosan-based nanoparticles have a high permeability and retention impact and can also induce apoptosis, which inhibits the proliferation of tumor cells. Larger particles must be phagocytized in order to be taken up by receptor-mediated endocytosis, which can only take up particles between 100 and 200 nm in size [57].

Chitosan has been commonly utilized in many area of biomedical science, this include gene delivery [58], hemodialysis [59], to prevent microbes in wound [60] to deliver anti-cancer drugs to a targeted tissue without affecting other normal parts [61], and dentistry [62].

Particles of chitosan have been used to treat alopecia. To address alopecia issues, Matos *et al*. [35] created chitosan particles are loaded with minoxidil. They showed significant penetration and stability in the hair follicles and helped to create a medication release status that allowed for a therapeutically effective concentration to last for more than 12 hours.

The approach for the topical management of hair loss that has mostly been researched upon is polymeric nanoparticles loaded with minoxidil. It is always the goal to find a system that lessens the drawbacks of prolonged use of MNX conventional solutions, such as the skin's itches that these formulations and the medication cause, and to do away with the utilization of organic solvents in the formulation, which are frequently used to make the medication and cause a "dandruff" appearance on the scalp after evaporated. Evidently, the trials also focus on delivering the medicine to the hair follicles, where it is then released under controlled conditions. When applied to the epidermis, chitosan nanoparticles of about 235 nm revealed a two times rise in minoxidil in hair follicles post six hours compared to the control of the study [35]. Utilizing chitosan microparticles loaded with MNX sulfate, the combination of microencapsulation and iontophoresis was also investigated. The results demonstrated that both techniques work together to increase drug absorption into hair follicles. Microencapsulation of MNX led to a six times increase in drug accumulation of the follicles when compared to a free-drug control, while the use of iontophoresis increased drug absorption into hair follicles by up to 8-fold [63].

Chitosan-decorated Minoxidil for Treating Alopecia:

The creation of drug delivery systems that can improve MNX's stability [64], skin pharmacokinetic and pharmacodynamic profiles [22], permeation and formation of skin depots [9], therapeutic adherence [65], as well as the reduction of MNX's toxicity and treatment resistance has been made possible so far by nanotechnology [66].

As a result, formulations based on nanotechnology provide the opportunity for smart MNX delivery at lesser doses with enhanced healing effects as well as a greater advantageous release. Lipid [24], polymer [64], and formulations based on inorganic nanotechnology are examples of MNX-loaded nanotechnology [9].

Several research have looked into the delivery of MNX using chitosan-nanoparticle formulation so as to avoid the serious negative reactions including pain and inflammation, dryness of the scalp from the organic and water constituents in the present MNX drugs.

In comparison to pharmaceuticals in solutions, Matos *et al*. [35] developed MNX sulphate-loaded chitosan nanoparticles (MNX-CNP) that displayed prolonged release of of drug (five-times). The MNX-CNP formulation had a positive zeta potential and a mean diameter of 236 nm (chitosan/MNX; 1:1 w/w). They discovered that the application of MNX-CNP caused a two times increase in MNX transport in to the hair follicles post six hours compared to the solution used as control, according to drug permeation studies conducted in vitro through the skin. The differences in charge between the liposomes' positive ions and the surface of the skin caused MNX skin diffusion to rise when liposomes were coated with positive ions polymers like chitosan [67]. The disruption of the skin's tight connections may also result from the availability of the cationic polymers, which would enhance MNX skin permeability.

Due to a rise in the amount and length of hair follicles during the anagen stage, MNX nano-capsules promote growth and development of hair. Lipidic nanoparticles are less likely to form MNX depots, which emphasizes the nano-capsules' significance in the hair follicles' focused action improvement. Additionally, MNX-loaded chitosan polymeric nanoparticles had improved skin penetration and hair follicle accumulation as well as MNX sustained release qualities [35].

Chitosan-decorated Finasteride for Treating Alopecia:

As an oral medication, finasteride was created to treat androgen-dependent alopecia under the trade name Propecia [68]. It complexes in the hair follicles' protruding part, where the biomarkers of telogen control the androgen's concentration, that controls the cycle of hair development. The FDA has not cleared the oral medicine for use by women since it has been discovered to cause serious negative impacts including abnormalities of genitalia and

teratogenicity. More findings to develop novel therapeutic plan would likely be helpful in no distant time given the abundance of commercial and unique healing products that are available for treating loss of hair. An approachable way to achieve a regulated treatment regime with better patient compliance appears to be the manufacturing of controlled release profiles in nano-medicine. Finasteride-containing lipid nanoparticles with an average size of 200 nanometres that can last for about 4 weeks were created by Gomes *et al*. [69]. Only a minor amount of FST passed the skin during penetration tests on pig ear skin, indicating that this formulation is suitable for skin distribution of active ingredients used for treating alopecia. According to Caon *et al.* [70], the cation on the surface of the chitosan-coated polymersomes of FST caused them to engage with skin components more strongly than non-coated formulations. It was shown that the chitosan supplement increased the amount of FST that accumulated at the outer skin layer. The suggestion was that the materials, with an average diameter of 180 to 404 nanometres, selectively aggregated in the openings of follicles and also localized in the follicles and this was encouraged by the tiny size of the particle that will be readily conveyed down the channel of the hair follicles than the bigger one.

Even with droplet sizes of 800 nm, chitosan-decorated nano-emulsions with low molecular weight containing FST demonstrated enhanced skin retention and decreased drug penetration in comparison to non-decorated nano-emulsions. This may be because of their viscous nature and cations [71]. Chitosan-based FST for topical application enables continuous medication release and prolonged scalp contact [72]. By adopting nanoprecipitation and ionic gelation procedures, respectively, Fahad *et al*. [73] demonstrated the effective synthesis of FST- and MNX-loaded PLGA (Poly-lactic-co-glycolic acid) and chitosan-based nanomaterials. The effect of several formulation variables on the encapsulation effectiveness, particle size, % yield, as well as zeta potential of the functionalized nanoparticle formulations was effectively examined. Experiments involving ex-vivo permeability showed that FST and MNX released for up to 24 hours. This tactic can lower the dosage frequency while increasing patient adherence to medication.

A high-speed homogenization technique was used to successfully create FST-loaded chitosan-coated solid lipid nanoparticles (SLNs) from physiologically inert materials [74] In the formulations, the solid lipid used was stearic acid. The products could be applied to the skin based on the results considering the pH, zeta potential, as well as particle size. Without chitosan coating, in vitro release showed a burst release, but chitosan-coated SLNs showed a steady, sustained release pattern. In comparison to chitosan-coated solid lipid nanoparticles, more penetration was seen with chitosan-uncoated solid lipid nanoparticles. Furthermore, the retention of the medication

within the epidermal layers was greatly improved by the chitosan covering. These results led to the conclusion that chitosan-coated solid lipid nanoparticles could be employed instead of oral drug administration of FST in androgenic hair loss in order to surmount the negative side effects and improve patient compliance.

Chitosan-decorated Dutasteride for Treating Alopecia:

Olsen *et al*. [25] discovered that DST (2.5mg) was more effective than orally administered FST (5.0mg) at 3 and 6 months in increasing scalp hair growth in individuals with hair loss. DST was administered orally daily at a dose of 0.5 mg to treat male person suffering from hair loss, and Eun *et al.* [75] found that after six months, the DST group experienced significantly more hair growth than the placebo group. Another study [43] found evidence that DST dramatically slowed down the progression of masculine pattern of alopecia in seventeen sets of identical twin guys over the course of a year. In comparison to treatment with a placebo, they discovered that 0.5 mg per day of orally administered DST medication delayed the development of alopecia and improved the growth and development of hair. In 2014, Harcha *et al*. [76] conducted a randomised, active- and placebo-controlled research comparing DST to placebo as well as FST in the management of 917 participants (men) with AGA. In comparison to FST and placebo, they discovered that DST 0.5 mg significantly improved hair growth, width, and count at week 24. According to the oral DST (Avodart®) product features summary, taking oral DST may raise the chance of developing lower sexual urge, advanced prostate cancer, enlargement of breast and ejaculatory issues [77].

DST toxicity causes the skin to generate several red patches, indicating that it is a dermal irritant (in animals at 40 mg/kg) [77]. Medication delivery with continuous release should ideally target the hair follicle, in order to minimize severe negative effects. The oral route of DST delivery utilizing nanocarriers has received the most attention, and topical distribution has received less attention. DST nano-capsules have been shown by Camila *et al.* [78] to be able to target the casts of hair follicles. While a simple physical stimulus can increase the quantity of drug stored by around 5 times, coating with chitosan can also limits the release of drug, which may be helpful in lowering systemic side effects from drugs. In a study, chitosan-based DST for topical administration provided for continuous drug release and prolonged contact with the scalp [72].

Limitations and Challenges:

Due to the unique physicochemical properties of such formulations, the development of nanotechnology-based formulations for MNX, FST and DST topical distribution has resulted in significant advancements for the treatment of alopecia. However, such formulations should be

thoroughly evaluated with reference to their safety profile as nanoscale-tailored materials [79]. The action of the biological systems and the nanosized MNX carrier on each other is really amplified by the nanosized dimension, which causes a rise in surface area and, consequently, increases the surface contact area. Hence, as a result of increased contact or/and introduction of harmful and poisonous particles, there should be vigilance regarding the toxicity and reactivity when in touch with the body [80]. As a result, an analysis of their toxicological and pharmacological profiles is necessary, along with their characterization and analytical investigations, for the development and further application of MNX's, FST's and DST's nano-formulations for the treatment of hair loss. This is done so as to know better and to be able to say whether they would be suitable or not and also to identify their possible degradation product. Based on the anticipated harmful potential determined in cell cultures and animal models (in vivo research), the assessment of toxicity is based on the nano-bio interactions in detail [81]. Chitosan is frequently regarded as a non-harmful, biocompatible polymer that has received regulatory agency approval for a variety of uses. Chitosan, however, is not a substance that is generally acknowledged as safe (GRAS). This is because the safety profile of a particular polymer can be influenced by the particular properties and the chemical changes of the utilized chitosan. Therefore, each individual case should be evaluated to see whether using chitosan to treat alopecia is safe. For instance, the existence of charged particles in chitosan may change how it interacts with cells. Hence, the amount of cations present in the molecules of chitosan, which depends on the medium's pH, molecular weight, and degree of deacetylation, may change how chitosan interacts with cellular environment. As a result, controlling the aforementioned variables is crucial for modifying the probable harmfulness of chitosan formulations.

CONCLUSION

Since AGA is only found in pilosebaceous units, enhancing the distribution of medication at the target site in the hair follicles may be able to improve the result of alopecia treatment. Drug dose reduction and systemic drug absorption will both be minimized as a result of follicular drug targeting's benefits. The most auspicious method for localized delivery is chitosan-nanostructured drug delivery systems because they might enhance drug-selective tissue distribution in the hair follicle region, reducing adverse effects as well as lowering the needed dose. Additionally, by overcoming the barriers to skin penetration, the application of chitosan nanotechnologies could enhance medication solubility while concurrently increasing the concentration of the medication at the target region (stratum corneum and sebum). For effective penetration into the hair follicles as well as reaching the lower part of the hair bulb, the

ideal and favourable dimension range to address the target location inside the hair follicle needed to be sought. In order to extract the most pertinent information possible and raise the degree of general knowledge available, the data involving in vivo and in vitro assays should also receive a thorough study. In addition, the development of a chitosan nano-safety profile is crucial and necessitates international regulation and guidance, both of which are now lacking and impeding market endorsement. In order to address the above problems, international bodies must create guidelines that inform people about proper manufacturing procedures and underlying toxicology assessments, particularly with regard to long-term use of chitosan nano-formulations and, in particular, topical alopecia medications.

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CONFLICT OF INTEREST

The authors declared that they have no conflict of interest

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