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Potential biomedical and Pharmaceutical response of natural Chitosan

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ABSTRACT
Chitosan is a versatile natural polymer. Chitosan as one of the source of bioactive material stucturly it is liner amino-polysaccharide polymer consisting of two monomers 2-deoxy-Dglucosamine, 2-deoxy-N-acetyl-D-glucosamine units which bonded with glycosides' α(1-4) linkage. Both compounds are polysaccharides and they occurring naturally in exoskeleton on marine and insect, and they extracted from their sources by chemical and biological methods. Chitosan exhibited the importance due to its biological, pharmaceutical, and medical properties. This review emphasized that research on Chitosan based systems containing various therapeutic applications have increased in recent years.
Keywords: Chitosan, Pharmaceutical applications, Natural polymer

I. INTRODUCTION

Chitin is a biological substance that is found in almost every living thing on the planet. It is a linear homopolysaccharide with a high molecular weight, made up of repeated units of N-acetyl-D-glucosamine residues bound together by a -(1-4) linkage [1]. Invertebrates such as flies, shrimp, crabs, and many fungi have chitin. Chitosan is a chitin derivative that can be created through partial deacetylation. Chitosan is made up of a D-glucosamine and N-acetyl-D-glucosamine co-polymer. Depending on the degree of deacetylation, the quantity of D-glucosamine and N-acetyl-D-glucosamine residues in the co-polymer changes [2].

Aquaculture is one of the world's fastest-growing food industries. Shrimp sales have expanded considerably in recent years, particularly in Asia [3]. In the shrimp industry, however, the head and skin (exoskeleton) of the shrimp are separated from the flesh, leaving only the meat. Exoskeletons are discarded as bio-waste. During the processing procedures, 45-55 percent of the raw shrimp is thrown as a waste product [4,5]. Furthermore, crustaceans like shrimps may comprise a large amount of the entire weight of the shell or exoskeleton, according to a study. Every year, a large volume of shells and chitin compounds are thrown away for this purpose. It took 140 years for a book based on chitosan to be published, after it was first isolated and seen in mushrooms by French Professor Henri Braconnot in 1811.

Since then, numerous studies on chitosan technology have been undertaken [6]. Chitin may be converted swiftly into chitosan, a fiber-like material. Chitosan, on the other hand, is not like plant cellulose. Because chitosan has a positive ionic charge, it can bind to negatively charged molecules such as fats, ions, lipids, cholesterol, proteins, and so on. Furthermore, chitosan is non-toxic and has good bioavailability, biodegradability, and adsorption characteristics. These characteristics make it helpful in a variety of industries, including wound therapy, drug carrier, food packaging, dietary supplement, chelating agent, pharmaceutical and biomaterial uses, and so on [7,8].

Chemical and biological approaches can be used to remove chitin and chitosan from the exoskeleton [9,10]. The biological approach of chitin manufacturing is more ecologically friendly, cost-effective (depending on the microbe employed), and has a higher viscosity than the chemical method. The natural method also reduces the amount of protein left in the shrimp shell. Furthermore, investigations have shown that biological mechanisms create chitosan of greater quality than chemical processes [11]. Chitosan was approved as a feed additive by the Food and Drug Administration (FDA) in 1983. Chitosan is currently widely used in biotechnology, functional foods, and environmental protection [12]. Present reviews highlight the various methods of chitosan formation and the biomedical applications.

Chitosan Production by Chemical Method

The extraction and purification of chitin from the shrimp exoskeleton is the most important

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stage in the manufacturing of chitosan. Demineralization, deproteinization, and deacetylation are the three main processes of chitosan synthesis. To make chitosan from shrimp shells, the shrimp wastes are collected from the shrimp processing industries and washed and dried to obtain the shrimp waste powder. After that, a salicylic acid pre-treatment is performed for many hours. The demineralization stage comes after the pre-treatment step. HCl is used in the demineralization process.

However, the concentration will vary depending on the shrimp, as will the degree of demineralization. This process converts insoluble calcium carbonate to soluble calcium chloride, which can be easily removed by water in the subsequent stages. The deproteinization process follows the demineralization step, where is utilized. The NaOH concentrations may also differ by shrimp species. The protein component of the shrimp waste is removed in this process, and protein hydrolysate is formed, which is then separated by filtration. As a protein supplement, this protein hydrolysate can be utilized. Chitin and a little amount of proteins and other compounds remain after demineralization and deproteinization. The chitin is now available to be deacetylated and converted into chitosan. Extreme chemical conditions, such as 50 % NaOH, and extremely high temperatures, such as 70-90% or even higher, are necessary for the deacetylation phase. To make a reasonable amount of chitosan, 70-90 % of chitin deacetylation is required. However, the chemical technique is limited primarily by its harmful effects on the environment [13-15].

Biological Production of Chitosan

Because the natural process is eco-friendly, cost-effective, and appropriate for the chemical approach, the biological method of chitosan manufacturing is preferable to the chemical method. Two methods can be utilized in the biological approach. The enzymatic approach is one option, while the fermentation method is another. The enzymatic method is identical to the chemical method discussed previously. The sole difference is that in the deproteinization and demineralization phases, enzymes such as papain, trypsin, alcalase, and pepsin are utilized instead of different chemicals [16]. However, extra actions need be taken, such as enzyme inactivation followed by centrifugation. The protein in the supernatant and the chitin in the pellet will be separated by centrifugation. To obtain pure chitin, the pellet is washed with water, ethanol, and acetone in that order. The supernatant, on the other hand, is decolorized with charcoal before being filtered, neutralized (with NaOH), and lyophilized to produce protein hydrolysate. High-value peptides can be made from protein hydrolysate [17].

Various bacteria such as L. plantarum, P. aeruginosa, B. subtilis, and others are used in the fermentation of chitosan [18-19]. The shrimp waste is first collected and crushed in the fermentation procedure. The pulverized substance is then combined with distilled water and a suitable carbon source, then fermented at 370°C with the right microorganism. Filtration is performed to separate the filtrate after a 2-3 day incubation period. The proteins are found in the filtrate. The majority of the residue is chitin. Following that, in the fermentation phase, the chitin is deacetylated to generate chitosan; the chemicals produced by fermenting microbes are responsible for the shrimp waste's demineralization. In lactic acid fermentation, for example, the lactic acid produced by the bacteria combines with the calcium carbonate in the shrimp excrement to produce calcium lactate. The calcium lactate precipitates, which is easily eliminated by washing. However, in the fermentation approach, autolysis is used to deprotenized the chitin waste, which is a common phenomena in fish and shrimp waste [15, 17,20].

Biomedical Applications Of Chitosan
Antimicrobial Agent

Several investigations have found that chitosan has antibacterial action. Antibacterial activity against B. cereus, S. aureus, L. plantarum, B. megaterium, L. bulgaris, S. typhymurium, E. coli, P. fluorescens, and V. parahaemolyticus have been observed [21-23]. The bacteria' proteins and other cellular components leak as a result of this contact [24,25]. Because quaternized chitosan has a higher degree of substitution of the quaternary ammonium, it has shown to have more substantial antibacterial properties than chitosan in many investigations

As a result, it has a stronger interaction with the cell membrane and has more amazing antibacterial properties [26]. Chitosan has antifungal properties against B. cinerea, P. oryzae, F. oxysporum, T. equinum, and a variety of other fungi. Chitosan may demonstrate many ways for suppressing fungus. Chitosan, for example, may reduce S. cerevisiae protein synthesis while also affecting the

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intracellular ultra structure and membrane integrity of Candida albicans [27]. pH and molecular weight have an impact on the antibacterial agent. Chitosan's antibacterial activities are inversely proportional to its pH. Furthermore, investigations indicated that when the molecular weight of chitosan below 300 kDa was increased, the antibacterial effect on S. aureus was strengthened, while the impact on E. coli was diminished [28]. The mechanisms of chitosan and chitin's antibacterial properties are mostly unclear; nevertheless, various suggestions have provided light on a distinct mechanism. One of the most widely held beliefs is that a positively charged amino group is responsible for chitosan antibacterial properties. The negatively charged microbial cell membrane interacts with the positively charged amino group.

Drug Delivery

Chitosan's efficiency in the drug delivery system is one of its most noticeable and promising features. "Drug Delivery" refers to the method of safely transporting a pharmaceutical compound's desired therapeutic effect in the body via nanotechnology. Using nanoparticles or a combination of technologies, the approach primarily facilitates site-targeting in the body and systemic pharmacokinetics. Since the discovery of Chitosan's unique properties that are beneficial to pharmaceutical industries, scientists have begun to consider its usage in improving drug delivery processes. Chitosan is unique among all other biodegradable biopolymers claimed to be utilized in the field of pharmaceutics because it alone has cationic character. It can stick to negatively charged mucosal glycoproteins as a bioadhesive substance due to the presence of an amine group (positively charged) [29,30]. It's also a non-toxic amino polysaccharide that's biocompatible, biodegradable, and bioactive. It's also soluble in aqueous solutions with a pH of 6.5. As a result of its good superior mucoadhesive qualities, it can be considered a promising material for developing drug delivery techniques [31].

In addition to its mucoadhesive properties, chitosan possesses a number of other characteristics that make it ideal for drug delivery. It has an excellent regulated drug release capability. The drug-releasing procedure in the case of a sustained drug is predetermined and works for a set period of time. Furthermore, it occurs by a simple drug dissolving or diffusion process, or through the osmotic system, which is membrane regulated and frequently causes hurdles such as ionic contact. Cationic medicines can use anionic polymeric recipients like polyacrylates, sodium carboxymethylcellulose, or alginate to guarantee regulated release [32,33]. The mucoadhesive characteristics of chitosan, which are based on its cationic character, function in a unique way.

The anionic substructure is due to the presence of sialic acid and sulfonic acid in the mucus gel layer. The cationic primary amino groups of chitosan interact with the anionic gel layer, resulting in mucoadhesiveness. Drugs can be administered by the parenteral route and numerous non-invasive channels such as oral, nasal, and ocular mucosa routes, as well as intravesical mucosa using chitosan nanoparticles, using these varied outstanding features of chitosan. Several methods for producing chitosan nanoparticles exist, including ionotropic gelation, microemulsion, and polyelectrolyte complex approaches [34,35].

Due to its cationic nature, chitosan has additional good features such as in situ gelling and efflux pump inhibitory capabilities. This nanoparticle-based approach holds promise in a variety of pharmaceutical applications, including tissue engineering, controlled drug release carriers, and the oral drug delivery system. It improves absorption by opening the mucosal membrane's tight connections. Their positive charge makes them ideal for medication delivery in the lungs. These qualities are also exploited in nasal medication delivery systems, and chitosan can be used as an auxiliary agent [36]. In vaginal and buccal settings, methyl-pyrrolidinone chitosan had the most mucoadhesive and permeation-enhancing characteristics. The hydrogel nanoparticles of the chitosan system, based on non-toxic and permeation-enhancing effects, operate effectively in ocular drug administration [37]. Chitosan has been regarded a viable biomaterial in building drug delivery systems because of all of these remarkable ways to drug administration [38].

Gene Therapy with Chitosan

When it comes to using gene therapy to treat non-submissive disease, there is a lot of concern about immunogenicity and toxicity. Ensuring that these two requirements are met can lead to the proper application of this powerful viral system. However, this sector's development of non-viral vectors rather than viral vectors for precise gene delivery is clearly promising. Chitosan, a cationic polymer that may synthesize a complex form of DNA, is a safe and practical vector for nonviral gene therapy. Chitosan can bind

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negatively charged DNA and protect it from nuclease degradation because of its positive charge [39]. The conjugation of DNA with chitosan nanoparticles (electrostatic interaction) is clutter-free and remarkably stable during storage. The DNA-chitosan micro nanoparticle is the ideal size (20nm-500nm) for entering cells via endocytosis or pinocytosis and increasing transfection rates [40,41]. Both in vivo and in vitro transfection are possible, however the in vitro technique can provide successful transfection and high production [42,43]. Chitosan-based gene therapy underwent multiple trials and was refined based on transfection efficiency. The maximal modified chitosans discussed here have a lower transfection rate than regular DNA-Chitosan formulations and may damage DNA [44]. Small interfering RNA is delivered intravenously using chitosan-coated poly-isohexylcyanoacrylate nanoparticles. The strategy of using interfering short RNAs (siRNAs) to silence a specific gene and inhibit the corresponding protein has been widely employed in the treatment of a variety of disorders, including cancer [45]. Furthermore, when mannosylated Chitosan is combined with a plasmid, it can produce interleukin-12 (IL-12), which could be useful in cancer immunotherapy. Chitosan has been shown to suppress tumour cell proliferation as well as tumour growth by causing apoptosis and gradually lowering glycolysis [46].

Immunity with Chitosan

Chitosan can help to strengthen the immune system. The key to the highest development is to harness the immune systems' techniques on a daily basis using the most likely components. The immune response to vaccination is improved by suppressing secondary tumours or adjuvant. Working on this, it has been discovered that the vaccine adjuvant chitosan boosts cellular immunity by acting as a DNA sensor for type-I interferons, which could be the foundation for producing cell-mediated immunity vaccines. Chitosan has been shown to raise antigen-specific antibody titers by more than 5-fold and antigen-specific splenic $CD4^+$ proliferation by more than 6-fold. Chitosan produced both humoral and cell-mediated immune responses, as evidenced by antibody titers and robust delayed-type hypersensitivity reactions. Chitosan stimulates humoral immunity in serum and the interstitial fluid [47,48].

It also activates complement in the same way that anaphylatoxins like C3a and C5a do, with the exception of C4, which is activated by a different pathway. C3a triggers mast cells to produce histamine, whereas C5a is involved in the activation of phagocytic cells [49,50]. Chitosan can be utilized as an antigen store because of its higher viscosity than water. As a result, chitosan can be used in combination with vaccination to develop an adaptive immune response over time, proving that chitosan can boost the immune system as an adjuvant [51,52]. Furthermore, chitosan unique properties have allowed it to be employed in a variety of vaccine constructions and delivery methods, including influenza vaccine delivery, hepatitis B vaccine production, and polio vaccine preparation, among others [53,54].

Agriculture

The antimicrobial, against insecticidal, non-harmful, and biodegradability properties of chitosan can be utilized successfully for agrarian purposes. Chitosan-based nanoparticles have been created, which can be utilized to convey fundamental agrochemicals and hereditary materials to the plants. Chitosan nanoparticles can give pesticide and herbicide to trim insurance, convey manures, convey nanosensors for crop checking, and further develop soil wellbeing. Besides, studies have affirmed that utilizing chitosan nanoparticles can build the dirt's auxin and urea discharge. Chitosan additionally restrains the development of different plant pathogenic microbes and parasites like B. cinerea, F. oxysporum, M. nivalis, R. solani, E. carotovora, A. tumefaciens, and so on [55]. Chitosan biopolymers assist plants with guarding the microbe assaults by a few components. Chitosan can actuate a few siphons of the plant cells that might emit hostile to pathogenic mixtures. Chitosan can initiate the creation of key chemicals of the phenylpropanoid pathways. It can likewise animate the plant to create different sorts of resistant enhancers and elicitors and enact the Ca^{2+} subordinate cautious pathways like affidavit of Ca^{2+} subordinate callose synthase. These multitudes of reactions have antiviral, natural safe stimulatory impacts. Also, these reactions make the plants more impervious to optional contaminations and assist the plants with creating more auxiliary metabolites [56].

Chitosan additionally can further develop germination limit, root length, and movement, and seedling level. Studies have affirmed that chitosan can frame a semi-porous film on the film surface

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because of its film-shaping capacity, which keeps up with the plant seeds' dampness [57,58]. Besides, the chitosan covering likewise assists the plants with becoming lenient to the dry season pressure by fostering the underground root growth and expanding the plant roots' capacity to retain more water [59]. There is additionally proof that chitosan expands the creation of abscisic corrosive that diminishes the pace of happening and keeps up with the conclusion of stomata in low water conditions, for example, dry season [60,61]. In creature cultivation, chitosan can be utilized as protein supplementation of the feed. The protein buildup delivered as the result of chitosan can be utilized as a feed supplement, which can expand the invulnerability of creatures and satisfy the need for protein. Also, chitosan and its subsidiaries can be handled as creature feed [62].

Environmental Protection

Chitosan and its compounds can be used to clean up the environment and eliminate various pollutants. Both natural and inorganic contaminants can be present. For example, polyethylene glycol (PEG)-chitosan and polyvinylalcohol (PVA)-chitosan may remove nitrate impurities, phosphates can be removed by chitosan on which Cu(II) is immobilized, and so on [63]. Furthermore, chitosan can be used to remove pollution-causing dyes like as methylene blue and methylene violate. To use the polymeric combination, pesticides like glyphosate can be removed from the environment. Chitosan has been shown in experiments to be capable of removing heavy metal ions from the environment.

II. CONCLUSION

Chitin can be used to make chitosan, a biological molecule. It is a powerful molecule of interest since it is naturally biodegradable, eco-friendly, biocompatible, and non-toxic. Chitosan is made by deacetylating chitin to different degrees. Chitin can be produced using either chemical or biological techniques. Chitosan, on the other hand, is a versatile substance. Chitosan has a wide range of applications in biology. Chitosan is an antibacterial chemical with strong antimicrobial characteristics that can be used in food, agriculture, and medicinal therapies. Chitosan has a big impact on regeneration technology, namely corneal, skin, and heart regenerative technologies. Chitosan can also be utilized in immunological therapy, and a variety of other fields such as agriculture, environmental protection, and so forth. Although chitosan is not currently employed in all of the sectors mentioned, it is expected to have a substantial impact in these areas in the near future. More study is needed to make chitosan a chemical with a diverse range of products and opportunities.

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