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Original Research Article

Analysis of Chitosan in Terms of its Antimicrobial and Wound-Healing Properties

Jayantkumar Mafatlal Uperia[1], Navdeep Singh Osahan[2], Yash Solanki[3], Bikram Chandra Lenka[4]

[1]Assistant Professor, Dept. of General Surgery, Pacific Medical College and Hospital, Udaipur, Rajasthan

[2]PG Resident, Dept. of General Surgery, PMCH, Udaipur, Rajasthan

[3]PG Resident, Dept. of General Surgery, PMCH, Udaipur, Rajasthan

[4]PG Resident, Dept. of General Surgery, PMCH, Udaipur, Rajasthan

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Corresponding author: Dr. Bikram Chandra Lenka

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Abstract

Introduction: Chitosan is a biocompatible and biodegradable polymer derived from chitin, commonly found in the shells of insects and crabs. It has antimicrobial properties and has been shown to effectively kill bacteria, fungi, and viruses due to its cationic charge. Chitosan also has wound-healing properties and can stimulate cell proliferation and tissue remodelling, making it an effective agent in the healing process. Chitosan is a subject of significant research due to its non-toxicity and natural origin. This study focuses on chitosan's antibacterial and wound-healing properties.

Aims and objectives: To analyze the efficiency of chitosan as an anti-microbial and evaluate its effect on the growth kinetics of *S. aureus.*

Methods: This study involved 60 participants divided into two groups: S.aureus SG511 and SCV. They were exposed to chitosan at concentrations ranging from 10 to 60 g/ml. The experiment measured the effect of chitosan on S. aureus SG511 by analyzing the fluorescence emission magnitude of DiBAC4(3) and the amount of potassium produced. The analytical lipid II assay was performed in the same manner as described in previous studies. Results were compared between the two phenotypes. The cultures were maintained at 37 degrees Celsius, and the sample was discarded after 30-90 minutes.

Results: The study has shown that chitosan has a bacteriostatic effect on the bacteria, with its effectiveness increasing with concentration and exposure time. Figure 2 shows the results of an assay measuring the percentage of potassium release in the presence of chitosan and lantibiotic nisin over time, indicating membrane damage as a potential mechanism of action for antimicrobial agents. Both chitosan and nisin have a concentration-dependent membrane-damaging effect on bacterial cells. Further studies are needed to investigate their mechanism of action and potential toxicity in vivo.

Conclusion: The study has concluded that chitosan has a clinically efficient bacteriostatic effect and there is no significant difference in efficacy as compared to other antibiotics. Also, the study has concluded that chitosan has a dose-dependent bacteriostatic effect.

Keywords: Chitosan, Aureus, Staphylococcus, Bacteriostatic, Wound Healing.

Introduction

Chitosan is a polymer that is commonly arising and made from chitin. It is an essential ingredient that has been identified in the shells of insects and crabs [1]. Due to the fact that it is biocompatible, biodegradable, and safe. At the same time, Chitosan has been examined for a number of antimicrobial and wound-healing rates. This research paper has critically discussed chitosan's ability to destroy germs and aid wound healing [2]. Again, around 1811 Henri Braconnotwas found Chitosan by a French chemist and pharmacologist. Bracannotfound that a certain element (chitin) is open in mushrooms as well as it did not liquefy in sulfuric acid [3]. On the other hand, after the next century, chitin was found in crustaceans, as well as it is the indigestible outer structure of insects and the fabric from which the cell partitions of the mycelial fungi are made [4].

Antimicrobial Properties of Chitosan

In this research, it has been discussed that chitosan can be demonstrated to be adequate at killing bacteria, fungi, and viruses, which are a kind of microorganisms. Even chitosan is working against microorganisms due to it having a cationic charge, which mainly allows it to relate with the adversely indicated cell walls as well as membranes of microorganisms. On the other hand, positively charged chitosan molecules stick to the negatively affected cell wall and membrane of microorganisms [5]. Therefore, these breakups can lead to the extinction of the cell. Furthermore, research has demonstrated that chitosan would be able to kill bacteria as well as other germs. For example, Chauhan et al. (2021) found the impact of chitosan as an antibacterial representative on Gram-negative germs [6]. It has found Escherichia coli, Pseudomonas aeruginosa, as well as Klebsiella pneumonia which were destroyed by chitosan in an effective way. Furthermore, at the same time, Rajiv et al. (2020) declared that chitosan was an effective way to destroy the fungi such as Candida albicans as well as Aspergillus niger [7].

Wound Healing Properties of Chitosan

On the other hand, the term "wound-healing properties of chitosan" has a number of experimental results. Furthermore, it is a multi-step biological process such as hemostasis, inflammation, cell proliferation, and tissue remodelling [8]. Again, by raising the growth of fibroblasts, epithelial cells, as well as endothelial cells, stimulate angiogenesis. Even though it has been reducing inflammation of chitosan that has been illustrated to move quickly the comeback from wounds [9]. Again, chitosan can improve the healing process, that is the process of raising the synthesis of a combination of expansion factors, such as "vascular endothelial growth factor (VEGF)" as well as "fibroblast growth factor (FGF)" Again, the " exoskeletons of crustaceans" as well as "insects containing chitin", are part of the natural polymer in which chitosan is removed [10]. On the other hand, due to its biocompatibility, biodegradability, and non-toxicity, it has been found that chitosan is part of significant research into its antibacterial and wound-healing powers. Moreover, this study is mainly focused on wound healing as well as the antibacterial properties of chitosan.

Through this analysis, Chitosan has discussed different kinds of properties such as technological properties. During the deacetylation of chitin, a few N-acetylglucosamine units are transformed into glucosamine units, yielding chitosan. Again, the solubility of chitosan in acidic aqueous media is due to the existence of numerous protonated -NH2 groups in its design. Even, its pKa value is approximately 6.5. Approximately 50% of all amino groups must be protonated for chitosan to become soluble.

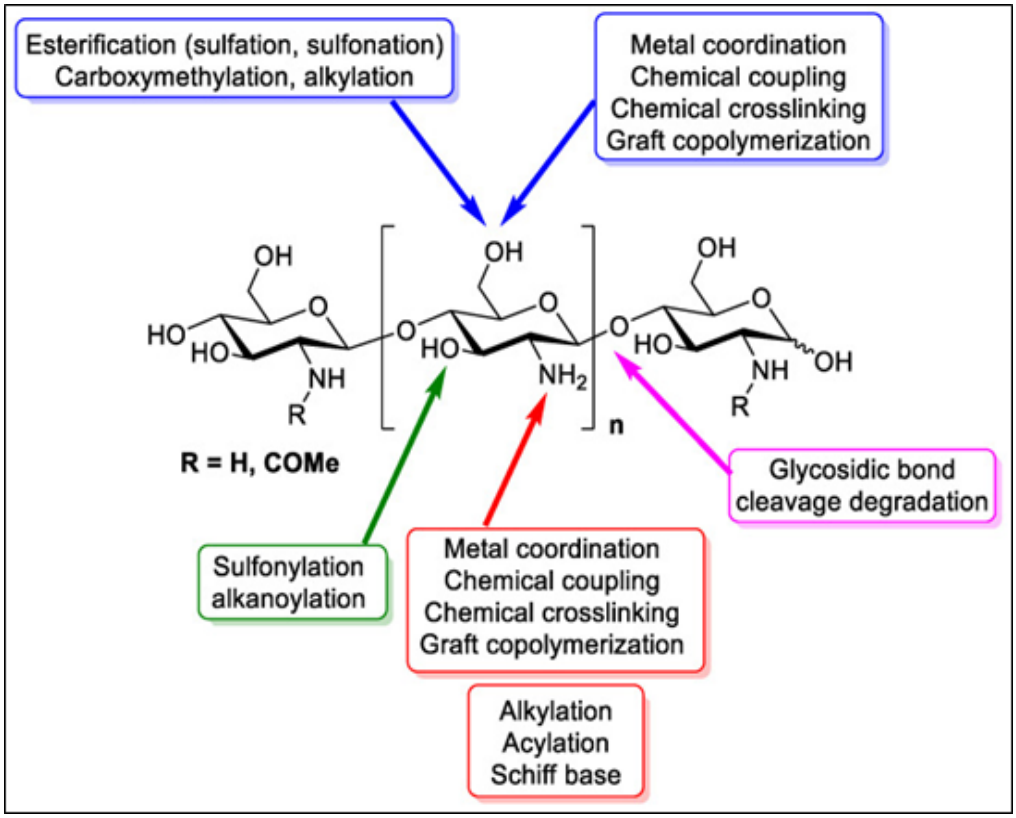

Figure 1: Structure of Chitosan showing chemically modifiable groups

Based on Figure 1, it has been observed that the reactive groups which are present in chitosan enclose a primary amino group (C2), primary and secondary hydroxyl groups (C6, C3), and a primary amine group (C2). Furthermore, it is also doable to consider glycosidic bonds and the acetamide group to be functional groups. Again, these functional groups are basically made it possible to complete a broad variety of alterations. As a result in the outcome of polymers with unique characteristics and behaviours. Chitin, chitosan, oligosaccharides, and derivatives all are exerted a variety of biological properties. For example anticancer, antibacterial, antioxidant, and anti-inflammatory effects. Furthermore, they have the possibility to be used as therapeutic polymers. Chitosan is derived from chitin. It is incredible that chitosan and chitosan hydrochloride are just carried out by regulatory tools for use in the production of excipients. It is not a medicine for the treatment of diseases. Inflammation is an automatic biological reaction of the body in its reaction to damaged tissue. The primary objective of the immune system's response to inflammation is to transport circulating leukocytes and plasma proteins to the site of the infection or tissue injury, to eliminate the causative agent if possible, and to initiate the process of wound healing. While inflammation is essential for survival, it can cause injury when it is excessive, unable to eradicate the factor responsible, or directed against the host. Free radical production is firmly linked to the inflammatory process. Again, this activity appears to be more pronounced when the molecular weight of the chitosan is diminished and when chitooligosaccharides exhibit greater activity.

Patients and Methods

Research design

This study was conducted from October 2021 to November 2022 on 60 participants who were divided into two groups, namely,

30 patients were randomized into the S.aureusSG511 group and 30 patients were randomized into the SCV group. The experiment that consisted of hemin auxotrophy was referred to as S.simulans22. It has also permitted growth in CAMHB at 37 degrees Celsius until it reached an OD600 value of 0.5. After being stored at room temperature, the cell suspension was incubated in the dark for twenty-five minutes with one milligram per millilitre of the membrane potential-sensitive fluorescent probe bis-trimethineoxonol. Following the application of the chitosan, the final concentrations of the substance ranged from 10 to 60 g/ml. In addition, we compiled data regarding the outcomes using the culture records of the strain S. aureus SG511. The analytical lipid II assay was performed in precisely the same manner as had been described in the previous explanation. After that, chitosan was added to the mixture of reactions in order to achieve the desired final levels, which were 67 and 267 g/ml respectively. The amount of potassium that was produced was measured at various concentrations of chitosan, ranging from 1 to 200 g/ml. After adding 46 litres of octyl glycoside with a concentration of 30% the liposomes were entirely dissolved, and leakage was evaluated about the total amount of potassium that was discovered. S.aureusSG511 which was grown in CAMHB had an optical density at 600 nanometers (OD600) of 0.8. Aliquots were taken during the first 20 minutes of treatment with chitosan at a concentration of 15 g/ml or baseline cultures that had not been treated and were then immediately stabilized using RNAprotect Bacteria Reagent. Following the application, the final concentrations of chitosan ranged anywhere from 10 to 60 g/ml. The variation in the fluorescence emission magnitude of DiBAC4(3) was measured using a Spectro fluoro-photometer from the RF-5301PC series that operated at wavelengths 492 and 515 nm, respectively, for the excitation and emission processes. This measurement was carried out over 15 minutes. In addition, the temperature that was maintained throughout the cultures of S. aureus SG511 in this investigation was 37 degrees Celsius. The sample was discarded somewhere between 30 and 90 minutes after exposure to the S. aureus strain SG511, and the MHA plates were used to determine the likely cell total resolution. Therefore, this conventional biochemical experiment was utilised by comparing both phenotypes.

Inclusion and exclusion criteria

The patients who visited our hospital were only included. The patients who were infected with S.aureusSG511 strain or SCV (Small Colony Variant), continued the treatment in our hospital and had no history of infection for the last 6 months, were included in our study. The patients who did not cooperate or followed up with our hospital, and had underlying chronic infection or other systemic diseases, were all excluded.

Statistical analysis

The study used SPSS 25 for effective statistical analysis. The continuous data has been written in mean ± standard deviation while the discrete data has been presented as frequency and its respective percentage. The study employed ANOVA as the statistical tool for its analysis. The level of significance was considered to be $P<0.05$. The growth kinetics and percentage of potassium release were made into graphs for schematic representation using MS Excel.

Ethical approval

Each patient explained the process of the study and consent was obtained from each of them. The study process has been approved by the Ethical Committee of the concerned hospital.

Results

Figure 2 displays the growth kinetics findings of S. aureus SG511 under the influence of chitosan at various time intervals (0h, 4h, 8h, 12h, 16h, 20h, and 24h) and different chitosan concentrations (0, 0.5, 1, 2, 5, and 10). The growth kinetics findings are measured by the optical density (OD) values. At 0h, the OD value of all chitosan concentrations (0 to 10) is the same, indicating that chitosan has not affected bacterial growth at this stage. However, at 4h, the OD values of the bacterial cultures treated with chitosan started to show differences compared to the untreated control. As the time of exposure to chitosan increased, the growth of S. aureus SG511 was progressively inhibited, and this effect was dose-dependent. At 24h, the highest chitosan concentration (10) completely inhibited bacterial growth. In contrast, the control without chitosan (0) showed the highest OD value at all time points, indicating unimpeded growth of the bacteria. In summary, the table indicates that chitosan has a bacteriostatic effect on S. aureus SG511 and its effectiveness increases with the concentration of chitosan and exposure time. These findings can be useful for developing antimicrobial strategies against S. aureus infections, particularly in combination with other compounds or treatments.

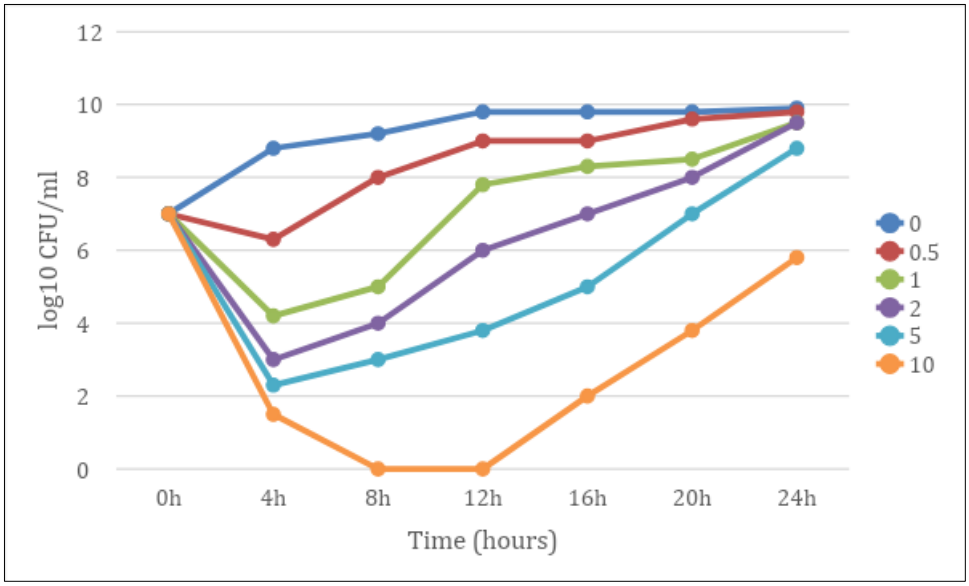

Figure 2: Growth Kinetic findings of S.aureusSG511 after adding chitosan

Figure 3 displays the results of an assay measuring the percentage of potassium release in the presence of different concentrations of chitosan and lantibiotic nisin over time. Potassium release is an indicator of bacterial membrane damage, which is a potential mechanism of action for antimicrobial agents. At the beginning of the assay (0s), all samples showed a low percentage of potassium release, indicating no or minimal bacterial membrane damage. As the assay progressed, the percentage of potassium release increased, suggesting increasing membrane damage caused by the treatment. At 180s, significant differences in the percentage of potassium release were observed between the control (without chitosan or nisin) and the samples treated with chitosan or nisin. At this time point, the samples treated with 60µg/ml chitosan or 1µM lantibiotic nisin showed the highest percentage of potassium release (40% and 100%, respectively), indicating a strong membrane-damaging effect. At the 240s and 300s, the membrane damage caused by a chitosan or nisin treatment continued to increase, with higher concentrations of chitosan or nisin leading to higher percentages of potassium release. The highest concentration of chitosan

(60µg/ml) and nisin (1µM) showed the highest percentage of potassium release throughout the assay. The study showed that both chitosan and lantibiotic nisin have a membrane-damaging effect on bacterial cells, and the effect is concentration-dependent. These findings can be useful for developing antimicrobial strategies that target the bacterial cell membrane. However, it is important to note that further studies are needed to investigate the mechanism of action and potential toxicity of these compounds in vivo.

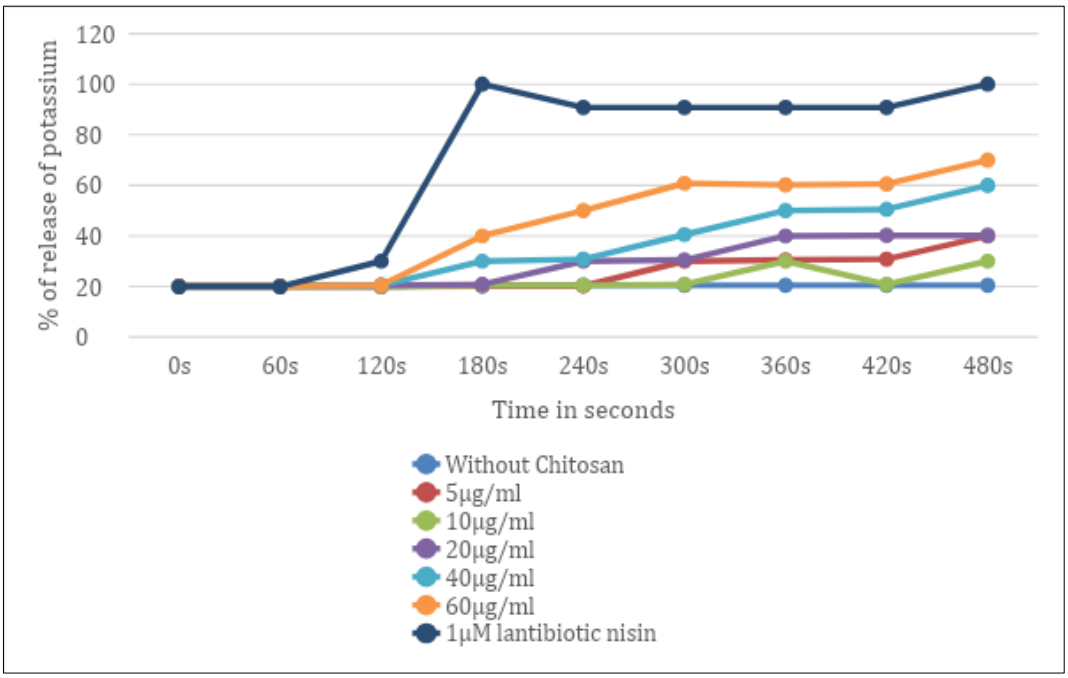

Figure 3: Assay result of the percentage of potassium release"

Discussion

Macrophages have a lot of contribution that is significant to this healing process of wounds. However, in the cases of chronic wounds, these cells would have a large contributing factor to the crisis [15]. In this chronic wounds would be unable to advance distance down the healing cascade because they stay attached in an inflammatory form. It is connected with the occurrence of "pro-inflammatory macrophages" as well as it is near the area of injury, which is the result of the increasing heights of cytokines, responsive oxygen types and apoptosis of "keratinocytes and fibroblasts" [16]. Furthermore, the form of inflammation that survives for a vast period is unwanted in wounds because it hinders the healing method. Again, the investigators experimented with the anti-inflammatory impacts of fiction formulations on murine macrophages in order to specify whether or not these formulations may have influenced the inflammatory reaction. Furthermore, there have been a number of research studies that identified the effectiveness of chitosan as a "wound-healing" accelerator, as well as there has been solid data that chitosan may positively impact wound healing at each stage of the process [17]. Chitosan and its related substances have the potential to expedite the process of wound healing by improving the actions of inflammatory cells. These cells include "polymorphonuclear leukocytes (PMN)" macrophages, as well as fibroblasts or osteoclasts. In addition to this, it has been suggested that the tensile strength of wounds might be improved by using chitosan [18]. The parameters of the

molecular weight, degree of degradation, as well as the condition of chitosan, could potentially have an impact on the wound-healing properties of chitosan. LPS is recognised by toll-like receptor 4 in macrophages, which leads to the invention of genes complicated by swelling when it binds to the receptor [19]. This causes overexpression of inducible nitric oxide synthase in mice, which then leads to high amounts, which may be used as a biomarker of anti-inflammatory reactions. Because this impact is far more pronounced in mice compared to humans, murine macrophages were used to evaluate the probable anti-inflammatory act. The findings of the inflammatory evaluations show that the formulations had a clear dose-dependent anti-inflammatory effect on the cells that were treated. When compared to LPS-induced macrophages that had not been treated with anything, the anti-inflammatory actions of the vesicles that comprised chitosan and CHX were much higher. However, it did not appear that the effects were synergistic; more specifically, the occurrence of both chitosan and CHX did not perform to augment the effects in a manner consistent with synergy [20]. The threshold was determined to be somewhere between a 55 and 65% decrease. It is interesting to note that the vacant and plain lipid transporters also produced a dose-dependent decrease in inflammatory reply; however, this result did not pointedly affect the outcome [21]. In addition, there have developed several study investigations found the achievement of chitosan as a wound-healing acceleration. In addition, there has been convincing proof chitosan could affect wound healing in a favourable method at each stage of the method. Chitosan has been demonstrated to be effective in accelerating the healing of wounds [22-24]. Chitosan and the combinations that are linked to it include the ability to quicken the method of wound healing by attracting the activities of inflammatory cells. This might be accomplished. "Polymorphonuclear leukocytes (PMN)", macrophages, fibroblasts, and osteoclasts are all samples of the cells that decline under this category. In addition to this, it has been hypothesised that the tensile strength of wounds could be increased by applying chitosan. Chitosan has been presented to have antimicrobial possessions. Chitosan's ability to promote wound healing could be affected by several factors, including its molecular weight, the degree of degradation it has undergone, and its current state [25].

Conclusion

The study has concluded that chitosan has a clinically efficient bacteriostatic effect and there is no significant difference in efficacy as compared to other antibiotics. Also, the study has concluded that chitosan has a dose-dependent bacteriostatic effect.

The compulsory of chitosan to cell barrier polymers is going to set off secondary cellular implications, such as destabilisation and afterwards the breakdown of the bacterial function of the membrane. That would compromise the cell membrane barrier function and result in the leaking of the cells without inflicting different pore development. Chitosan binding would have this effect, most likely because of an impairment in the appropriate functional organisation of the electron channel sequence. This disrupts the normal oxygen decrease process and compels the cells to switch to anaerobic drive production. It is possible that because of this, the cellular apparatus as a whole will become dysfunctional. It is also possible for us to the hypothesis, albeit only provisionally, that the growth of the polymer in the region of the membrane causes a variety of stress responses to be triggered as a result of a local low pH or other features that have yet to be found. Despite this, not all of the intricate mechanisms that are responsible for coupling or interrelating various processes have been identified. The authors suggest that more similar studies be conducted to

elucidate the molecular intricacies of the underlying mechanisms and the significance of those features to the antibacterial activity of chitosan. In addition, it is recommended that additional research be conducted in this field, specifically concerning the bacterial confrontation mechanisms that occur against this drug. Overall, this present research has shown to be clinically contributing to the management of wound healing and highlighting chitosan as an effective anti-microbial.

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