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ORIGINAL ARTICLE

Protective Effects of Chitosan Nanogels Loaded with Vancomycin against Oxidative Stress and Hepatotoxicity Induces by Methicillin-resistant *Staphylococcus aureus* (MRSA)

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KEYWORDS

MRSA;
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ABSTRACT: Methicillin-resistant *Staphylococcus aureus* (MRSA) has also become one of the significant clinical and epidemiological issues in hospital environments. *S. aureus* spreads the bacteria to liver tissue, brain tissue, heart tissue, lung tissue, spleen or kidney tissues. Oxidative stress markers including malondialdehyde (MDA), glutathione (GSH), catalase (CAT) and super-oxide dismutase (SOD) activity were studied from liver tissue. Besides, histopathological examination of liver tissues plasma levels of inflammatory mediators such as interleukin 6 (IL-6) and tumor necrosis factor-α (TNF-α) were measured, as well as ALT (alanine aminotransferase) and AST (aspartate aminotransferase). The results provided were that MRSA toxicity brings about dose-dependent cell death and inflammation in the liver. Oxidative stress markers also increased by MRSA-infectious treatment (10^6 CFU, 50 µl). Pro-inflammatory cytokines were also found that are implicated in inflammation and tissue damage. In addition, Chitosan nanogels encapsulating Vancomycin (100 mg kg^{-1}) treatment indicated reduced liver inflammation, plasma AST, ALT activity and oxidative damage compared to OTA group ($p<0.001$). On the whole, the findings of the study reveal that Chitosan nanogels encapsulating Vancomycin has strong antioxidant activity against inflammation and tissue damages exposed liver.

INTRODUCTION

Chromosome alterations and genetic mutations brought on by plasmids and transposons lead bacteria to develop resistance to antibiotics [1, 2]. *Staphylococcus aureus* bacteria are colonized in the form of normal flora on the skin and mucous membrane of humans and animals. Although *staphylococci* are part of the natural human flora, they are known as opportunistic pathogenic bacteria. Methicillin-resistant *Staphylococcus aureus* (MRSA) is the most important cause of hospital infections and community-acquired diseases, which causes a wide range of diseases with the help of a diverse set of pathogenic factors [3, 4]. And due to its potential pathogenic power and increasing resistance to antimicrobial drugs, it is considered one of the most important health problems in the world. Today, antibiotic resistance in bacteria is one of the important concerns of doctors, which is the main cause of failure. Treating patients and increasing mortality. As a result, creating

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innovative approaches to deal with this issue is imperative [1, 2].

Staphylococcus aureus strain resistant to methicillin has become one of the important clinical and epidemiological problems in hospitals. Resistance to methicillin is caused by a chromosomal segment called *SCCmec*, which contains the *mecA* gene. This gene encodes a protein called PBP2a (penicillin-binding protein), which has a lower affinity for methicillin. It is one of the other proteins that bind to penicillin in the bacterial wall. PBP proteins play a role in the construction of the bacterial cell wall, so the presence of such a new protein will not be affected by the antibiotic and the bacteria can easily survive [5, 6]. Today, with the advancement of nanotechnology, nanoparticles composed of various materials have been widely used in the field of medical and therapeutic applications [7, 8]. Nanoparticles not only protect nucleotides, but also attract target cells. Considering the practical advantages that they have compared to larger particles, nanoparticles have many capabilities in medical diagnosis and treatment approaches, especially in drug delivery and gene therapy systems. Molecules act on the surfaces of target cells. The connection between nanoparticles and target cells causes a special endocytosis that prevents the transformation of healthy cells into infected cells [7, 9]. The main obstacle restricting the utilization of this biopolymer is thought to be chitin's low solubility. Chitin and its derivatives have been claimed to have numerous applications thus far, notwithstanding this limitation. Consequently, chitosan has found a suitable place among polysaccharides because of the presence of free amine groups along the polymer chain and high solubility in mild acids like acetic acid [9, 10]. Chitin and chitosan fibers have enormous application potential in wound-healing products and biodegradable sutures. In this context, the present study aims to evaluate the antibacterial activity of vancomycin- and interferon-γ-loaded chitosan nanogels versus unloaded chitosan nanogels in a murine model of methicillin-resistant *Staphylococcus aureus* (MRSA) infection. Wound healing mechanisms in MRSA-infected mice were also explored [11, 12]. Since hepatocytes represent the major cell type of the liver and membrane integrity is a sine qua non of liver function, a secondary purpose of this research is to test the hepatoprotective effect of vancomycin-loaded chitosan nanogels against a model of MRSA-caused hepatic inflammation [13, 14].

To that end, the study is expected to critically examine the drug-loading chitosan nanogel's therapeutic efficiency against MRSA infections from wound healing at local sites to hepatic protection systemically [15].

MATERIALS AND METHODS

Synthesis of Chitosan Nanogels

In an aqueous solution of 1% (v/v) acetic acid, chitosan was prepared at a ratio of 0.3% (w/v) and continuously stirred. Using 10 N NaOH, the solution's pH was brought to 4.6. Furthermore, a 0.3% (w/v) concentration of Tripolyphosphate (TPP) was dissolved in pure water. [16]. The sonicated chitosan solution was then mixed with the Tripolyphosphate solution at a 5:1 ratio, drop by drop. The nanoparticles were separated from the suspension by centrifuging the resulting chitosan-TPP solution at 12,000 rpm for 15 minutes. After discarding the supernatant, the precipitate was stirred again to spread it evenly in water. After two more minutes of sonication, the solution was centrifuged once again. Ultimately, the silt was dried using a freeze drier and frozen at minus 80 degrees [17].

Animals and the Experimental Protocol

In this research 50 male albino mice (weighing 20 to 25 g) were purchased and randomly divided into 5 groups (N=10). Animals were housed in cages at 22 ± 1°C, relative humidity of 70-70%, exposure of 12:12 (light / dark), in additions food, water, and everything else they needed was available in sterile conditions. Mice were randomly divided into 5 groups (10 mice in each group): Group 1: Sham group, Group 2: Group of mice infected with MRSA (10^6 CFU, 50 µl) bacteria, Group 3: Group of mice infected with bacteria (10^6 CFU, 50 µl) and treated with *Chitosan nanogels loaded with Vancomycin*, Group 4: Group of mice infected with bacteria (10^6 CFU, 50 µl) and treated with drug nanogels, Group 5: Group of mice infected with bacteria (10^6 CFU, 50 µl) combined with INF-ɣ nanogels it was injected at 6, 12 and 24 hours.

Biochemical assessment

Animals were euthanized after treatment, and the liver tissues were removed and stored at -80 °C for biochemical analysis. We homogenized the samples in 9 g L-1 cold normal saline (1:9 w/v) volumes [18]. Following homogenates centrifugation at 4000 rpm min-1 for 10 min at 40° C, the supernatant was collected [19]. The activity of MDA, GSH, SOD, and CAT was determined using the liver supernatants. TNF-α (tumor necrosis factor-α) and interleukin 6 (IL-6) concentrations were determined according to the protocol included with the assay kits [18].

Liver enzymes activity

iver enzyme elevation could be a sign of inflammation or cellular injury in the liver. Damaged or inflamed liver cells release more amounts of some chemicals, i.e., liver enzymes, into the bloodstream, which give rise to elevated liver enzymes in blood samples [20]. Liver enzyme activities, e.g., Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST), were determined using automated analyzer after Kite's method (Auto Analyzer 7070, Hitachi, Japan) [21]. Use of the coefficients and chart reading was mentioned based on IU L^{-1}[22, 23].

Cytokines measurement

T cells or macrophages with endurated damaged tissues secrete two inflammatory cytokines called Tumor Necrosis Factor-α (TNF-α) and Interleukin-6 (IL-6). They were studied by this technique as specific inflammatory markers in inflammatory liver tissues. Immunological technique was used for measurement of both aforementioned tests and followed as per each kit's instruction [24].

Histopathological changes

After mice dissection, the liver of all the mice was selected, then fixed using 10% formalin. From the samples of the liver, by following the routine practices of tissue passage and pathological section preparation, 5 micron thickness sections were prepared using microtome and stored for hematoxylin-eosin (H&E) staining [25]. Blinded semi-quantitative analysis of the liver units was conducted. Liver injury was graded in terms of hyperemia, apoptosis and focal necro-inflammation, Leucocyte infiltration and other histopathological alteration parameters.

Statistical analysis

We have represented data as mean ± SEM. Graph Pad Prism 6.0 software (Inc., USA, version 6) has been used for calculating all the data and were compared by using one-way ANOVA followed by Tukey's post hoc comparison test. Differences were considered statistically significant at $P<0.05$.

RESULTS

Effect of Chitosan Nanogels on oxidative stress

As seen in Figure 1A, MRSA (10^6 CFU, 50 µl) treatment resulted in a significant rise in MDA level when compared to control group ($P<0.001$). Vancomycin-loaded Chitosan nanogels and Nanogels alone (100 mg kg^{-1}) treatment reduced the MDA content when compared to MRSA (106 CFU, 50 µl) ($P<0.001$), was administered at 6, 12 and 24 hours. Therefore, MRSA (106 CFU, 50 µl) treatment reduced the GSH level in mice liver (Figure 1B, $P<0.05$-$P<0.001$). In addition, exposure to MRSA (10^6 CFU, 50 µl) was significantly effective in changes the level of CAT Figure 1C and SOD Figure 1D contents. This results also indicated that, treatment with Chitosan nanogels loaded with Vancomycin (100 mg kg^{-1}) and Chitosan Nanogels alone (100 mg kg^{-1}) significantly increased GSH content, SOD and CAT activity compared to OTA treated rats ($P<0.001$). Changes in temperature and weight of mice show in Table 1.

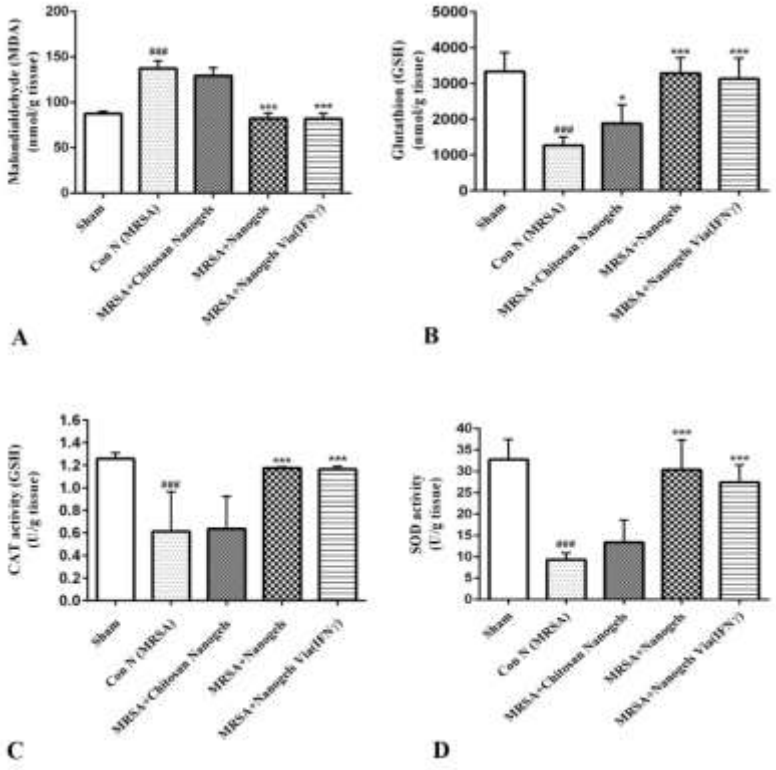

Figure 1: Effects of Chitosan nanogels loaded with Vancomycin on MDA concentrations (A), GSH (B), CAT activity (C), SOD activity (D), in the liver tissues mice. $^{###}P < 0.001$ vs. control, $^{***}P< 0.001$ and $^{*}P < 0.05$ compared to MRSA group.

Table 1. Changes in temperature and weight of mice

Groups	Treatment	Dosage	6 H		12 H		24 H	
			Temperature	Body Weight	Temperature	Body Weight	Temperature	Body Weight
1	Sham	Solvent	37.1±0.5	22±8	37.2±0.5	23±1	37.2±0.5	23±5
2	MRSA	0.5 µg kg^{-1}	38.1±0.4	20±1	38.2±0.7	17±5	38.2±0.5	17±2
3	Chitosan Nanogels loaded Vancomycin +MRSA	0.5 µg kg^{-1} +15 mg kg^{-1}	37.2±0.3	23±7	36.9±0.3	23±2	37.8±0.2	23±1
4	Nanogels +MRSA	0.5 µg kg^{-1} +20 mg kg^{-1}	37.5±0.6	22±9	36.7±0.5	23±7	37.2±0.8	23±2
5	Nanogels via INF-ɣ+MRSA	20 mg kg^{-1}	37.2±0.2	22±9	37.4±0.4	22±9	37.1±0.1	22±9

MRSA and Chitosan Nanogels effect on the liver enzymes activity

The results of this research demonstrated that serum levels of liver enzymes including ALT (Figure 2A) and AST (Figure 2B) significantly increased 14 days after MRSA administration (was injected at 6, 12 and 24 hours). It has also been shown that serum levels of these enzymes in *Chitosan Nanogels* receiver mice significantly decreased in comparing to MRSA groups (P<0.01-P<0.001). There were also significant changes in the activity of AST and ALT in mice administered only *Chitosan Nanogels* compared to MRSA group (P<0.001).

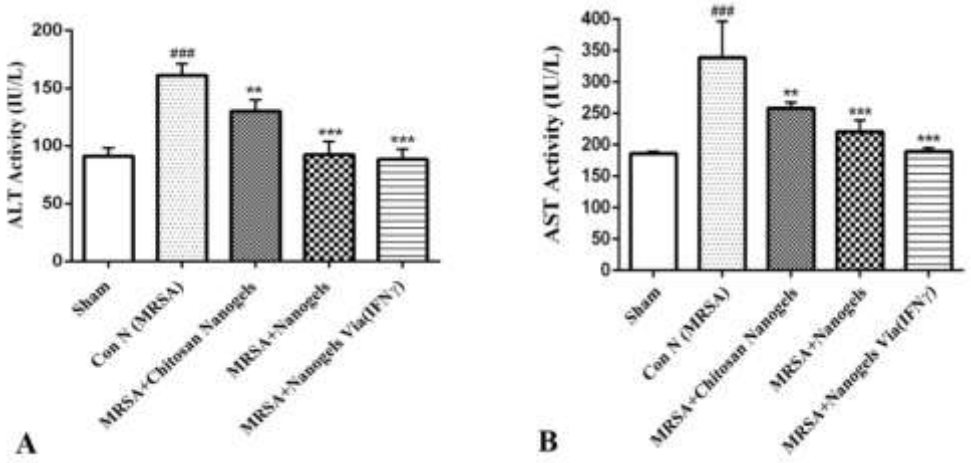

Figure 2. Effect of Chitosan nanogels on AST (A) and ALT (B) activity in the serum of MRSA-treated mice. $^{###}P < 0.001$ vs. control, $^{***}P < 0.001$ and $^{**}P < 0.01$ compared to MRSA group. ALT: Alanine aminotransferase, AST: Aspartate aminotransferase.

Effect of Chitosan Nanogels on IL-6 and TNF-α

Our finding revealed that there is a remarkable increase in IL-6 level and also TNF-α of the MRSA treated mice's (P<0.001). As shown in Figure 3A, administration of *Chitosan Nanogels* (100 mg kg^{-1}) significantly decreased the level of IL-6 as compared to the MRSA group (P<0.001). Furthermore, the results show that TNF-α amounts in *Chitosan Nanogels* (100 mg kg^{-1}) group were basically decreased in compared with control groups (P<0.001) Figure3B.

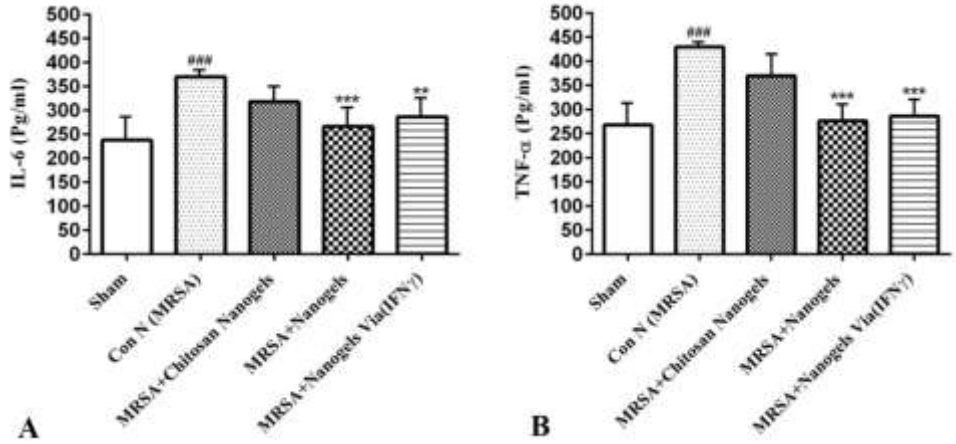

Figure 3. Levels of proteins related to Levels of inflammatory markers IL-6 (A) and TNF-α (B) in MRSA-administered mice treated with Chitosan nanogels and Chitosan nanogels and combined with INF-ɣ (100 mg kg^{-1}). $^{###}P < 0.001$ vs. control, $^{***}P < 0.001$ compared to OTA-administered mice.

Histopathological results

In the control group, no pathologic changes were observed. However, moderate hyperemia of central venous, Leucocyte infiltration in liver sinusoids, Sinusoidal inflammatory cells were detected in the liver of the mice in the MRSA group (P<0.001) Figure 4. The experimental group that received the *Chitosan nanogels loaded with Vancomycin at* a concentration of 100 mg kg^{-1} represented moderate to negative central venous dilatation, hyperemia or focal necroinflammation which show in Table 2 in details.

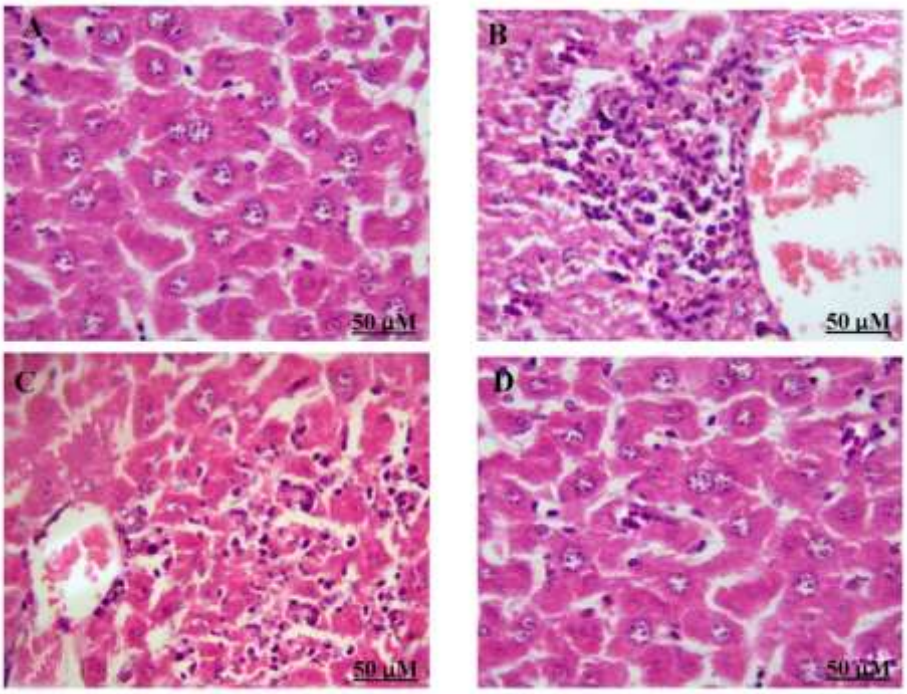

Figure 4. The histopathological changes of mice liver from various treatment groups stained with hematoxylin and eosin (H&E) by Light microscopy (× 400 magnification). (A): control group. (B): MRSA treated group. (C): MRSA+ Chitosan nanogels 100 mg kg^{-1}. (D): MRSA+ Chitosan nanogels 100 mg kg^{-1}.

Table 2. Liver histopathological changes of MRSA administration.

Groups / Histopathological findings	Control	OTA	OTA+ZM 15 mg kg^{-1}	OTA+ZM 20 mg kg^{-1}	ZM 20 mg kg^{-1}
Hyperemia	Negative	Moderate	Mild to moderate	Mild	Negative
Cholestasis	Negative	Moderate	Mild	Mild	Negative
Apoptosis and focal necroinflammation	Negative	Severe	Mild to moderate	Negative	Negative
Portal inflammation	Negative	Sever	Mild	Negative	Negative
Feathery change	Negative	Severe	Mild to moderate	Negative	Negative
Sinusoidal inflammatory cells	Negative	Severe	Mild	Negative	Negative
Leucocyte infiltration	Negative	Mild to moderate	Negative	Negative	Negative

DISCUSSION

Multidrug resistance is a growing problem worldwide. Indiscriminate and irresponsible antibiotic use leads to resistance to the easily available antimicrobial agents in most bacterial pathogens and also causes a significant health threat. For example, in some countries, 25% of pathogenic *staphylococcal* isolates are methicillin-resistant *Staphylococcus aureus* (MRSA). Vancomycin is still widely used in case of MRSA infection. Nevertheless, cytokine use is also a valuable and potent therapeutic strategy to stimulate the immune system and generate protective responses. Cytokine use has become increasingly popular in cancer immunotherapy, but its use is limited by its side effects since systemic administration leads to undesired effects. However, in some patients, this treatment has brought many advantages and they have been provided with a better prognosis for some diseases. But encapsulation of therapeutic molecules within polymer nanospheres can reduce their side effects and improve their therapeutic effects and chitosan as a biodegradable nanopolymer due to improved encapsulation, controlled release and low toxicity in this regard is very interesting. It is thought

that positively charged chitosan can bind to the negatively charged cell membrane and disrupt the tight junctions between epithelial cells and promote the transport of macromolecules in epithelial tissue. Therefore, it can improve the absorption of hydrophilic macromolecule drugs. In earlier studies, it has already been determined that chitosan nanoparticles by themselves are toxic to the majority of microorganisms and are capable of inhibiting a broad spectrum of bacteria. In addition, studies have determined that these nanoparticles are antibacterial in character as an anti-*staphylococcal* agent, i.e., methicillin-resistant *Staphylococcus aureus*[26, 27]. The results of the present study also showed that single treatment with chitosan nanogel was able to successfully reduce the gene expression of TNF-α when compared with the MRSA group. However, it is proven that peritoneal macrophages induced by *Staphylococcus aureus* produce reactive oxygen species (e.g., superoxide, nitric oxide, and hydrogen peroxide) and some cytokines like TNF-α and IL-1β [28]. And these cytokines also become bound to their respective receptors, cell surface TNFR1 and IL-1R, and result in the transmission of the inflammatory message and regulation of cytokine and other inflammatory gene expression [29].

The TNF-α gene expression is thereby somehow directly connected with decrease or increase with *staphylococcal* infection. Here in the current work, IFN-γ has also been employed for the treatment of the MRSA-infected mice and shown by the groups treated with the interferon gamma nanogel that the aforementioned cytokine can be an advancement and a drawback regardless of the TNF gene expression. Furthermore, the role of TNF-α also encompasses the role of macrophages which one of the main sources of the production of TNF-α [30]. Nevertheless, the bactericidal role of macrophages in fighting *Staphylococcus aureus*, because of *Staphylococcus aureus* capacity to survive inside endocytic compartments of macrophages, has been well defined [31]. A number of studies have confirmed that macrophage bactericidal activity against *Staphylococcus* in vitro is enhanced by IFN-γ signaling. In addition, IFN-γ and IL-17 signaling and pathways associated with *Staphylococcal* infections in human beings have also been reported. Host susceptibility to *Staphylococcal* infection has been among the critical factors reported in patients with inflammatory diseases and immune deficiency [32, 33]. In this study, it was also determined that with time, the effectiveness of IFN-γ increases significantly in the interferon-gamma nanogel than in the MRSA. On the other hand, in vitro investigations in this study also demonstrated that the MIC of vancomycin-loaded nanogels decreases when pH is 6.5 compared to pH 7.4. There have been various other studies in this field, which report that Kalhapure et al developed pH-sensitive chitosan nanoparticles with vancomycin loading (CSSNPs) from AGS. They found higher release of vancomycin from CSSNPs at pH 6.5 compared to pH 7.4. observed [34]. Karakeçili et al also created a drug delivery system for the treatment of bone infections such as osteomyelitis. They incorporated vancomycin as an antibiotic into ZIF8 nanocrystals for controlled pH release (ZIF8/VAN). According to their finding, chitosan scaffolds release vancomycin-loaded ZIF8 nanocrystals under controlled pH. They also showed that around 70% of vancomycin was released within 8 hours at pH 5.4, while the value was around 55% at pH 7.4, which refers to that vancomycin release is further favored in acidic conditions [35].

Millions of people have been spared the death penalty from numerous infectious diseases thanks to antibiotics over the span of more than 50 years. But because of its abuse, bacterial resistance has grown, which presents a significant obstacle in the fight against infectious diseases. Research on hitosan nanogels loaded with Vancomycin for targeted MRSA treatment is now growing, showing some encouraging outcomes [36, 37]. Following comprehensive examination of the studies covered under this review, nearly half of the studies covered under this article fall under the category of metallic nanoparticles. Further, since MRSA secretes acids at the point of infection, similar to most bacteria, there have been researchers who have designed pH-sensitive NPs so that they attack them there. Thus, medicaments can be released in acidic environments without upsetting the body's pH. This method applies to 21% of the research that are part of this research. It is noteworthy, nevertheless, that acidic conditions are not solely the product of bacteria. Hence future evaluation of nanoparticle anti-biofilm activity is equally crucial.

Moreover, intracellular MRSA infections were not examined in any of the investigations previously described. It has been evident in recent years that *S. aureus* has a significant intracellular component to its infection cycle, which can result in infections that are challenging to cure. It has been demonstrated that *S. aureus* uses liver Kupffer cells (KCs) to hide from immune cells and drugs when it is infected in the circulation in vivo [38, 39]. The findings of the present study show that cell death caused by MRSA exposure is by both necrosis and apoptosis as well as by several different mechanisms. It can cause intracellular toxicity, but the production of excess reactive oxygen species (ROS) are the most severe reasons for cytotoxicity in the liver. Although our results have been shown in many similar research studies, comparison of newly synthesized NPs as new procedures is challenging due to variations in the structure, experimental conditions, and chitosan nanogels vancomycin loaded. According to Kang et al., dose-dependent apoptosis rate increase in a mouse dendritic cell line treated with polyvinylpyrrolidine-coated AgNPs, up to 2.6% [40]. According to Krętowski et al., apoptosis rate in a glioblastoma cell line treated with silica nanoparticles was 5-70%, and this was dose- and time-dependent. The general principle of the earlier research is that duration and exposure dose are accountable for the effect of Chitosan nanogels on necrosis and apoptosis. But since differences have been indicated by research, there must be a study of the toxicity effect of each newly synthesized Chitosan nanogels [41]. No toxic alterations in blood parameters or liver injury due to elevated levels of ALT and AST enzymes, according to our investigation. Freese et al. revealed no visible activities of 20 nm-scaled PEGylated Gold (Au) NPs against endothelial cells once they got into the blood stream, which is similar to our experiment [42]. The size regulates uptake, distribution, and elimination from cells because experiments have demonstrated it regulates NP treatment in the endocytic process efficiency and the uptake of cell.

CONCLUSIONS

In conclusion, our research confirms the strong antibacterial properties of chitosan nanogels based on Nano chelating sick mice. Additionally, the current study shown that chitosan nanogels based on Nano chelating generate a modest degree of apoptosis/necrosis in mice and impact a number of clinical parameters, including body weight, liver enzymes, and blood parameters, without clearly exhibiting any hazardous effects. We think that these data contributed significantly to our understanding of the biological activity of INF-ɣ and chitosan nanogels, which will be assessed in subsequent clinical research.

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The authors declared no conflict of interest in this study. The dataset presented in the study is available on request from the corresponding author during submission or after publication.

Conflict of interests

The authors report no declarations of interest

REFERENCES

1. Hassoun A., P.K. Linden., 2017. Incidence, prevalence, and management of MRSA bacteremia across patient populations—a review of recent developments in MRSA management and treatment. Critical care. 21, 1-10.

2. Barrett J.F., 2005. MRSA–what is it, and how do we deal with the problem? Expert Opinion on Therapeutic Targets. 9, 253-265.

3. Balasubramanian D., Lamia H., 2017. Staphylococcus aureus pathogenesis in diverse host environments. Pathogens and disease. 75, ftx005.

4. Al-Mebairik N.F., EL-kersh T., 2016. A review of virulence factors, pathogenesis, and antibiotic resistance in Staphylococcus aureus. Reviews and Research in Medical Microbiology. 72, 50-56.

5. Fergestad M.E., Stamsås G.A., 2020. Penicillin-binding protein PBP2a provides variable levels of protection toward different β-lactams in Staphylococcus aureus RN4220. Microbiologyopen. 9(8), e1057.

6. Shalaby M.A.W., Dokla E.,Rabah.A.T S., 2020.Penicillin binding protein 2a: An overview and a medicinal chemistry perspective. European Journal of Medicinal Chemistry. 199, 112312.

7. Zhang L., X GU J., Langer R.,2008. Nanoparticles in medicine: therapeutic applications and developments. Clinical pharmacology & therapeutics. 83(5), 761-769.

8. Yetisgin A.A., Cetinel S., Zuvin M.,2020. Therapeutic nanoparticles and their targeted delivery applications. Molecules. 25(9), 2193.

9. Haleem A., Javaid M., Pratap Singh R., Suman R.,2023. Applications of nanotechnology in medical field: a brief review. Global Health Journal. 7(2), 70-77.

10. Roszek B., De Jong W.H., Geertsma R.E.,2005. Nanotechnology in medical applications: state-of-the-art in materials and devices.

11. Costa B., Alves P.M., Fonseca D.R., Monteiro A.C., Martins M.C.L., 2024. Dhvar5-chitosan nanogels and their potential to improve antibiotics activity. International Journal of Biological Macromolecules. 134059.

12. Asadi K., Heidari R., Hamidi M. Ommati M.M., Gholami A., Hashemzaei M , 2024.Trinitroglycerin-loaded chitosan nanogels: Shedding light on cytotoxicity, antioxidativity, and antibacterial activities. International Journal of Biological Macromolecules. 265, 130654.

13. Zhang Y.,Zhang J., Chen W., Angsantikul P., Zhang L., Gao W., 2017. Erythrocyte membrane-coated nanogel for combinatorial antivirulence and responsive antimicrobial delivery against Staphylococcus aureus infection. Journal of Controlled Release. 263,185-191.

14. Walvekar P., Gannimani R., Salih M., Makhathini S., Govender T.,2019.Self-assembled oleylamine grafted hyaluronic acid polymersomes for delivery of vancomycin against methicillin resistant Staphylococcus aureus (MRSA). Colloids and Surfaces B: Biointerfaces.182, 110388.

15. Hibbitts A., Lucía A., Matteis L.D., McArthur M.,2019 Co-delivery of free vancomycin and transcription factor decoy-nanostructured lipid carriers can enhance inhibition of methicillin resistant Staphylococcus aureus (MRSA). PLoS One. 14(9), 84.

16. Farag R.K., Mohamed R.R., 2013. Mohamed, Synthesis and characterization of carboxymethyl chitosan nanogels for swelling studies and antimicrobial activity. Molecules. 18(1), 190-203.

17. Li X., Hetjens L., Wolter N., Li H., Shi X., 2023.Charge-reversible and biodegradable chitosan-based microgels for lysozyme-triggered release of vancomycin. Journal of Advanced Research. 43, 87-96.

18. Rasool M., Malik M., Saleem S., 2019. Assessment of circulating biochemical markers in mice receiving cinnamon and glycyrrhizin under carbon tetrachloride induced hepatic injury. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences. 89(1), 105-111.

19. Ray S., Chakraborty S., Pandit B., Das S., 2011.Cyclophosphamide-Induced Lipid Peroxidation and Changes in Cholesterol Content: Protective Role of Reduced Glutathione. Iranian Journal of Pharmaceutical Sciences. 7(4), 255-267.

20.Tang H., Long N., Lin L., Liu V.,Dai M., Sun F.,2018. Effect of MRSA on CYP450: dynamic changes of cytokines, oxidative stress, and drug-metabolizing enzymes in mice infected with MRSA. Infection and drug resistance. 229-238.

21. Shankar P., Prasanna kumar B.R., khallel M., 2011. Hepatoprotective Activity of Momordica diocia Roxb Fruits in CCl4-Induced Hepatotoxicity in Rats. Iranian Journal of Pharmaceutical Sciences. 7(4), 279-282.

22. Zhao C., Fan J.,Liu Y.,Guo W.,Cao H., Xiao J.,Wang Y.,2019.Hepatoprotective activity of Ganoderma lucidum triterpenoids in alcohol-induced liver injury in mice, an iTRAQ-based proteomic analysis. Food chemistry. 271, 148-156.

23. He Y., Xia Z.,Yu D., Wang G.,2019. Hepatoprotective effects and structure-activity relationship of five flavonoids against lipopolysaccharide/d-galactosamine induced acute liver failure in mice. International Immunopharmacology. 68, 171-178.

24. Karatayli E., 2020. Il-22 and Rantes upregulation in a new mouse model of bacterial infection related acute-on-chronic liver injury. Zeitschrift für Gastroenterologie. 2020. 58(01). 10.1055/s-0039-3402252.

25. He X., Lee H., Jin Y.,2020.Apoptotic Bodies Activate Macrophages and Promote Lung Inflammation in Septic Mice via AB-Containing MicroRNAs. American Journal of Respiratory and Critical Care Medicine. A5262-A526.

26. Chávez de Paz L.E., Resin A., Howard K.A., Wejse P.L.,2011.Antimicrobial effect of chitosan nanoparticles on Streptococcus mutans biofilms. Applied and Environmental Microbiology. 77(11), 3892-3895.

27. Aliasghari A., Khorasgani M.R., Vaezifar S.,Rahimi F.,Younesi H.,2016. Evaluation of antibacterial efficiency of chitosan and chitosan nanoparticles on cariogenic streptococci: An in vitro study. Iranian Journal of Microbiology. 8(2), 93.

28. Dey S., Bishayi B., 2015. Killing of Staphylococcus aureus in murine macrophages by chloroquine used alone and in combination with ciprofloxacin or azithromycin. Journal of Inflammation Research. 29-47.

29. Turner, M.D., Nedjai B., Hurst T., Pennington D.,2014. Cytokines and chemokines: At the crossroads of cell signalling and inflammatory disease. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research. 1843(11), 2563-2582.

30. Popa C., Netea M.G., Stalenhoef A.F.,2007. The role of TNF-α in chronic inflammatory conditions, intermediary metabolism, and cardiovascular risk. Journal of Lipid Research. 48(4), 751-762.

31. Nalos M., Nalos M., Parnell G.,Tang B., Nanan R., 2012. Immune effects of interferon gamma in persistent staphylococcal sepsis. American Journal of Respiratory and Critical Care Medicine. 185(1), 110-112.

32. Barin J.G.,Talor M.V., Schaub J.A., Diny N.L., Hou X., Hoyer M., Čiháková D., 2016. Collaborative interferon-γ and interleukin-17 signaling protects the oral mucosa from Staphylococcus aureus. The American Journal of Pathology. 186(9), 2337-2352.

33. Smith R.P., 2010.IFN-γ enhances killing of methicillin-resistant Staphylococcus aureus by human monocytes more effectively than GM-CSF in the presence of daptomycin and other antibiotics. Cytokine. 2010. 51(3), 274-277.

34. Kalhapure R.S., Jadhav M., Rambharose S., Mocktar C., Govender T., 2017. pH-responsive chitosan nanoparticles from a novel twin-chain anionic amphiphile for controlled and targeted delivery of vancomycin. international journal Colloids and Surfaces B . 650-657.

35. Karakeçili A., Topuz B., Korpayev S., Erdek M., 2019. Metal-organic frameworks for on-demand pH controlled delivery of vancomycin from chitosan scaffolds. Materials Science and Engineering. 105, 110098.

36. Mancuso G., Midiri A., Gerace E., Biondo C.,2021. Bacterial antibiotic resistance: the most critical pathogens. Pathogens. 10(10), 13-10.

37. Mba I.E., Nweze E.I.,2021. Nanoparticles as therapeutic options for treating multidrug-resistant bacteria: research progress, challenges, and prospects. World Journal of Microbiology and Biotechnology. 37, 1-30.

38. Hommes J.W., Surewaard B.G., 2022. Intracellular habitation of Staphylococcus aureus: molecular mechanisms and prospects for antimicrobial therapy. Biomedicines. 10(8), 1804.

39. Surewaard B.G., Deniset J.F., Zemp F.J., Amrein M., Otto M.,Conly J., Kubes P., 2016. Identification and treatment of the Staphylococcus aureus reservoir in vivo. Journal of Experimental Medicine. 213(7), 1141-1151.

40. Kang K., Jung H., Seok Lim J ,2012. Cell death by polyvinylpyrrolidine-coated silver nanoparticles is mediated by ROS-dependent signaling. Biomolecules & therapeutics. 20(4), 399.

41. Krętowski R., Kusaczuk M., Naumowicz M., Kotyńska J., Szynaka B., 2017. The effects of silica nanoparticles on apoptosis and autophagy of glioblastoma cell lines. Nanomaterials. 7(8), 230.

42. Freese C., Anspach L., Deller R.C., Richards S.J., Unger R.E., 2017. Gold nanoparticle interactions with endothelial cells cultured under physiological conditions. Biomaterials Science. 5(4), 707-717.