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Anthelmintic Activity of Chitosan and its Derivatives

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Abstract. *Chitosan is a biodegradable and biocompatible derivative of chitin which is the second most abundant biopolymer after cellulose. It is mainly found in the exoskeletons of crustaceans and mollusks, as well as in fungi and insect cuticles. Due to its cationic nature and biological safety, chitosan is of a great interest for biomedical and veterinary applications. Numerous studies have demonstrated that chitosan and its derivatives possess anthelmintic, antimicrobial, and antifungal properties. When applied alone, chitosan has been reported to reduce the number of animal and plant helminths, indicating its direct and indirect suppressive effects. In addition, chitosan is widely used as a nano-coating and delivery agent to improve the oral bioavailability and therapeutic efficacy of conventional anthelmintic drugs. In animal studies, most applications achieved the desired outcomes, including reductions in helminth burden or egg counts; however, depending on the concentration and formulation, some cases reported mild host-related side effects or limited toxicity against helminth. Overall, chitosan represents a promising and versatile tool for sustainable helminth management, although its efficacy and safety are strongly dose-dependent.*

Keywords: *chitosan, nano-derivatives, anthelminthic, nematode, cestode, trematode*

Introduction

Chitin is considered the second most abundant polysaccharide on Earth and it appears in Nature as ordered macrofibrils in the exoskeleton of mollusks and crustaceans, as well as in fungi and insect cuticles (Jiménez-Gómez & Cecilia, 2020). Chitosan is produced from chitin mainly through demineralization, deproteination, and deacetylation. Few studies proved presence of decolorization as a minor step (Thambiliyagodage et al., 2023). To produce chitosan natural or synthetic chitin is used as a raw material. Chitosan has attracted considerable scientific interest in recent years because of its several properties. Chitosan has bioactivity because of the primary amino groups in the chitosan's main chain. Therefore, the chitosan is widely used in the biomedical fields such as drug and gene delivery, in the industrial fields such as water treatment (for example, harmful algae control), or against bacterial, fungal and other pathogen invasions (Choi et al., 2016). Chitosan has several biologically convenient features, such as nontoxicity, biocompatibility, biodegradability, bioactivity etc. The synthesis of chitosan with inorganic nanoparticles has used for controlled drug delivery (Li et al., 2018; Parhi, 2020).
Chitin can be degraded by internal enzymes (lysozymes and chitosanases) and are subsequently absorbed by the body. Despite of these features, chitin has not been extensively utilized in the clinic due to its low solubility and poorly learned mechanical properties (Li et al., 2018). Positive charged amino groups of chitosan can interact with various negatively charged components.

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And some of these negatively charged components are found on the microbial membrane, creating antimicrobial properties of chitosan which is of great interest nowadays (Ke et al., 2021; Chandrasekaran et al., 2020). Additionally, there are 2 types of chitosan: low-molecular weight chitosan (LWC) and high-molecular weight of chitosan (HWC). Usually, HMW can't penetrate into cell, and its antimicrobial activity is due it changes cell permeability and control nutrient uptake and intake (Hu et al., 2009; Kong et al., 2010).

However, LWC can intervene intracellular activity, change RNA, protein synthesis to provide antimicrobial properties which makes it highly toxic for different pathogens such as bacteria, fungi, even helmiths (Rabea et al., 2003). Not only chitosan itself, but its derivatives, especially chitosan nanoparticles are of great interest and increase known potential of chitosan into another level. Nanoparticles provide more reactive surface to chitosan which increases its antipathogenic ability several times (Chandrasekaran et al., 2020; Kravanja et al., 2019). Antipathogenic effectivity of chitosan and its nano-derivatives are dependent on several factors like molecular weight, pH, temperature, type of pathogen and so on (Chandrasekaran et al., 2020; Kong et al., 2010; Kravanja et al., 2019; Li & Zhuang, 2020).

Our article examines the antihelmintic property of chitosan and its nano-derivatives. Particular attention is given to their underlying mechanisms of action and the parameters affecting their biological effectivity of chitosan and its derivatives against animal helminths. Furthermore, recent advances and new studies in this area are reviewed to outline current progress and future research directions.

Antipathogenic nature of chitosan

Mode of action

Previous studies have demonstrated significant antipathogenic activity of chitosan; however, the underlying molecular mechanisms remain largely unclear. There are several hypotheses. First idea is binding of chitosan and its derivatives to bacterial DNA which leads to inhibition of mRNA synthesis. Generally, it is known that chitosan has an ability to bind DNA or RNA because of its positively charged amino groups. This ability of chitosan and its derivatives also used in gene and drug delivery. And some scientists suggest that LWC can penetrate into pathogen and interrupt synthesis of vital proteins (Cao et al., 2019; Li et al., 2018). Another and mostly accepted idea are disruption of cell wall of pathogen because of polycationic nature of chitosan (Divya et al., 2017).

The presence of amine groups in each glucosamine monomer allows chitosan to interact with negatively charged surface components of many microorganisms, including bacteria, fungi etc. This causes alterations to the cell surface components, leading to leakage of intracellular substances that results in cell death (Divya et al., 2017; Ganan et al., 2009; Raafat et al., 2008; Ardila et al., 2017). Researches show that lipopolysaccharide in Gram-negative bacteria and teichoic acid in Gram-positive bacteria play a major role in binding of chitosan (Raafat et al., 2008; Ardila et al., 2017). Other idea is disruption of Electron Transport Chain in mitochondria which has not proven yet (Chandrasekaran et al, 2020).

Records of anthelmintic activity of chitosan in animals

There are a lot of studies describes study on anthelmintic activity of chitosan and its nano-derivatives against animal nematodes. In Egypt, some of the scientists had done research on 240 domestic pigeons that 97% of them had single or mixed gastrointestinal parasites. They were determined 8 species of helminths and *Ascaridia columbae* was most predominant gut parasite between helminths. Therefore, it was subjected to *in vitro* and *in vivo* treatment with chitosan nanoparticles. Their results demonstrated that chitosan nanoparticles reduced the severity of clinical signs, prevented mortality, and induced repair of intestinal tissues. However, they revealed that chitosan nanoparticles caused shrinkage in the worm moth part and induced destructive damage to bird's body (Salem et al., 2022).

Another application of chitosan against intestinal nematode was done by Egyptian scientists. *Syphacia muris* mainly infects laboratory rats and had adverse effect on their immune systems. Different biochemical, and histological analysis showed that chitosan treatment reduced the worm number in infected rats. Overall, their findings proved an anthelmintic effect of chitosan against *Syphacia muris* (Mostafa et al., 2024).

Chitosan is a natural, biodegradable polymer with strong potential for pharmaceutical use mainly due to its biocompatibility and low toxicity (Yadav et al., 2023; Thambiliyagodage et al., 2023; Jimenez-Gomez & Cecilia, 2020). Therefore, chitosan and its nano-derivatives are widely used as coating and delivery agents for anthelmintic drugs to enhance their transport to the host and improve efficacy against nematodes and other helminths. Priotti et al. (2017) used chitosan to coat albendazole microcrystal formulations, drug against internal nematodes to increase its solubility ability. They had used chitosan, cellulose derivatives, and poloxamer as a coating agent, but chitosan had the best results among them according to the ANOVA analysis. In vitro evaluation of anthelmintic activity of this complex against adult *Trichinella spiralis* also proved that the S10A formulation (albendazole + chitosan complex) was the most effective, and it was therefore selected for the next in vivo therapeutic studies (Priotti et al., 2017).

Another study evaluated *in vitro* and *in vivo* anthelmintic effect and toxicity of chitosan encapsulated bromelain in goats. Bromelain is obtained from pineapple and have anthelmintic activities. But its anthelmintic activity is decreased by the low pH in stomach of ruminants. Chitosan encapsulated bromelain solution was used against *Haemonchus contortus* nematode both *in vivo* and *in vitro*. They had determined that chitosan encapsulated bromelain showed a higher *in vivo* fecal egg count reduction in compared to the plain bromelain *in vivo* studies (Wasso et al., 2020). For the further investigation, 20 healthy male goats naturally infected with gastrointestinal nematodes (GIN) and coccidia, and they used nanoencapsulated bromelain for 60 days. Fecal egg counts (FEC) and fecal oocyst counts (FOC) was decreased at 7th day of experiments. At 28 days of treatment, The FEC and FOC were zero in goats treated with nanoencapsulated bromelain and they hadn't observed any lesions or damage in the organs of goats (Ahmota et al., 2023).

Trichinella spiralis causes trichinellosis disease in humans and Eid et al. (2020) used chitosan coated nanostructured lipid carriers to carry albendazole into mice via oral administration. Albendazole (ABZ) is a drug against intestinal worms and is used in treatment of trichinellosis. However, it has limited bioavailability. To enhance the dissolution rate and oral bioavailability of ABZ, they used albendazole suspension, coated and uncoated nanostructured lipid carriers. They observed reduced larval count by 62.9, 99.6 and 89.5%, respectively (Eid et al., 2020).

Another study learned the effect of chitosan particles on human intestinal cestode, *Hymenolepis nana* to see if it kills this cestode and increases host's immunity against *H. nana*. They have studied worm burden, egg output, histopathology, oxidative stress markers and other parameters to address the aim correctly. Their results showed that treatment significantly reduced adult worms and egg counts. It also improved tissue damage and oxidative stress. Chitosan downregulated pro-inflammatory and Th1-related markers (iNOS, IFN-α, IFN-γ, TNF-α, IL-9) while upregulating MUC2, IL-4, and SCF (intestinal cytokine genes) expression, with normalization of goblet and mast cell numbers (Abdel-Latif et al., 2017).

Firouzeh et al. (2021) evaluated chitosan nanoparticles against hydatid cysts caused by *Echinococcus granulosus*. This cestode causes cystic echinococcosis in human, and their purpose was to find a special chemical that has an ability to kill cysts of *E. granulosus* during operations which is called as scolicidal agent. They used different concentrations (125–1000 µg/ml) and tested at various exposure times (10–180 min) *in vitro*. The nanoparticles showed notable scolicidal effects compared with the control, with activity increasing in a dose- and time-dependent manner. The highest killing effect was observed at 1000 µg/ml after 180 minutes of exposure. Importantly, no significant hemolytic activity was detected during *in vitro* chitosan nanoparticle application (Firouzeh et al., 2021).

Another study learned the effect of chitosan–curcumin nanoparticles on *E. granulosus*. 0.25, 0.05, 1, 2, and 4 mg/mL concentrations of nanoparticles were used and the highest mortality (68%) was observed at 4 mg/mL. They determined that nanoparticles also decreased protoscolex size (Napooni et al., 2019). Effect of albendazole–chitosan microspheres was evaluated on another species of same genus (Abulaihaiti et al. 2015). They assessed pharmacological and antiparasitic efficacy of the substance against metacestodes of *Echinococcus multilocularis* in experimentally infected mice. Oral treatment for 12 weeks exhibited that effectively reduced parasite tissue weight and strongly suppressed metacestode growth. Histological essays revealed severe structural damage to cyst layers and a shift toward a protective Th1 immune response.

Egypt scientists examined 453 *Oreochromis niloticus* and showed a high prevalence (40.8%) of fish-borne zoonotic trematodes, mainly *Clinostomum* spp. and *Prohemistomum vivax*. Chitosan, silver, and selenium nanoparticles were synthesized and tested *in vitro* against these trematodes. Chitosan nanoparticles exhibited the highest antiparasitic efficacy, producing the lowest LC50 and LC90 values for both parasite species and they acted faster than silver and selenium nanoparticles. Ultrastructural analysis by SEM confirmed that chitosan caused the most severe tegumental destruction, including ridge loss, shrinkage, and bleb formation. These results clearly indicate that chitosan nanoparticles are the most effective agent against trematodes (Mahdy et al., 2024).

Biomphalaria alexandrina is an intermediate host for the trematode, *Schistosoma mansoni*. This trematode's eggs trigger chronic inflammation and liver fibrosis in human, and lead to severe complications. It also affects millions of people in endemic areas, causing long-term illness and economic burden. El-Menyawy et al. (2021) used three treatment methods to prevent this cycle: hymoquinone, chitosan nanoparticles and hymoquinone loaded with chitosan nanoparticles (El-Menyawy et al., 2021). They found that hymoquinone loaded with chitosan nanoparticles were the most effective against trematode without giving damage to snail itself. There is another study were scientists used *Orobanche aegyptiaca* (Egyptian broomrape) + chitosan nanocomposite to fight against *Biomphalaria alexandrina* itself and had successful results in disruption of cycle (Abdel-Khalek et al., 2025).

Taken together, these findings indicate that further experimental studies are required to validate the efficacy and safety of chitosan-based formulations. Nevertheless, the current evidence consistently highlights chitosan as a highly promising molecule with significant potential for anthelmintic applications.

We study the pathomorphological changes caused by the accumulation and bioaccumulation of a number of biologically active and newly synthesized substances, as well as some nanoparticles, in living organisms at the ultrastructural level (Ahmadov et al., 2018; Hajiyeva et al., 2019; Hajiyeva et al., 2023; Nasirov et al., 2024). Despite the fact that poultry and fish products currently play a major role in satisfying the population's demand for meat, helminths that seriously affect the quality of their meat are still found (Rzayev et al., 2021; Rzayev 2021a,b; Seyidli et al., 2022). Therefore, there is a need to study the anthelmintic properties of chitosan against fish and bird parasites.

Conclusion

Chitosan and its derivatives are safe, biodegradable, and versatile agents with anthelmintic, antimicrobial, and antifungal properties. They can reduce helminth burden both directly and indirectly, and serve as effective nano-coating carriers for anthelmintic drugs. While most animal studies report positive outcomes, their efficacy and safety are dose- and formulation-dependent, highlighting the need for optimized application strategies in sustainable nematode management. Its large-scale use is still limited by low solubility, variable properties, production challenges, and incomplete understanding of its mechanisms. Further research in these areas will be essential to fully move chitosan from laboratory studies to practical applications.

Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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