

Histological Changes in Liver and Cardiac Rat Tissues after Exposure to Chitosan Nanoparticles Orally

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Abstract

Background: As safe natural biopolymer, chitosan is resulting from chitin deacetylation. Owing to its antimicrobial and antifungal effects, chitosan and/or its biological derivatives have gained extensive interest. The antibacterial activity of chitosan exhibits only in a low pH medium since its solubility above pH 6 is poor. Several factors affect chitosan antibacterial action such as chitosan type, polymerization degree, and certain other chemico-physical features. **Objectives:** The current study was intended to inspect the chitosan injury on hepatic and myocardial cells in rats in different concentrations. **Materials and Methods:** Chitosan was purchased and prepared at various concentrations. Laboratory Wistar albino rats were orally fed with different concentrations of chitosan. Histological examination of rat liver and cardiac tissues was performed accordingly. **Results:** A noticeable increase in animal weight is seen as the concentration of chitosan increases. Normal histological appearance with slight hemorrhaging and abnormal histological appearance with more abundant hemorrhaging and cellular vacuolation were present in liver tissues. Profound histological damage with more abundant hemorrhaging and lymphocytic infiltration along with sinusoid enlargement in the liver as well as distended nuclei was also present. Cardiac tissues were less affected by changes in chitosan concentration. Liver histological changes are attributed to the metabolic breakdown of chitosan in the liver. A noticeable decrease in vascular thickness is seen in both cardiac and liver vascular networks. **Conclusion:** The study found that chitosan has robust cytotoxic influences on certain organs. However histological damage is more prominent and is seen in rat liver tissues. Histological damage is confirmed by the abnormality of histological and cellular damage seen.

Keywords: Cardiac, chitosan, hepatic, histological changes, oral

INTRODUCTION

Chitosan is a natural linear polysaccharide derived from chitin that has biocompatible recyclable, non-toxic features^[1] with recognizable immune enhancer effects, anticancer, and antimicrobial bioactivities. As well, it is being used as a stabilizing molecule that acts to modify nano-composite characteristics or long-term nanoparticles stability by avoiding particle clustering.^[2] Bhumkar *et al.* established a new approach to exploit the implication of chitosan as a reducing factor for the synthesis of gold-nanoparticles (AuNPs). Remarkably, chitosan-AuNPs exhibited bactericidal effects against strains of antibiotic-resistant *Pseudomonas aeruginosa* and *Staphylococcus aureus*.^[3,4] The experimental studies of the cytotoxicity of

NPs have conflicting results and there are still insufficient data on their effects.

In this perspective, the authors examined the chitosan-AuNPs cytotoxicity on the cell lines of HeLa (carcinoma of the human cervix) and C26 (carcinoma of the murine colon). The effectiveness of many therapies is often restricted by their ability to reach the target sites. In general (using the standard dosage forms), only a trivial amount of administered drug doses reaches their targets, whereas

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most of the remaining drug molecules distribute all over the body tissues according to their pharmacokinetic properties. Consequently, introducing a new drug delivery system that improves the *in vivo* pharmacological action while decreasing the toxicity of a new drug is a challenging job. The usage of colloidal drug carriers is one of the current approaches that can deliver site-specific or targeted-drug-delivery as well as optimum drug release profiles.^[5] The idea of administration of minute drug-loaded bullets as a drug delivery system was considered and established over a century ago, and initially suggested.

Liposomes and nanoparticles (NPs) have been considered most widely as drug delivery systems. Various technical restrictions were found in liposomes such as poor stability and low levels of the entrapped drug. Nowadays, several low molecular weight medications are available in the markets that apply such technology. Bio-polymeric NPs, which have superior reproducibility and stability than liposomes, have been introduced as substitute drug carriers that overcome many of these problems. NPs are solid colloidal molecules of around 1–1000 nm diameter, comprised macromolecular materials used medically as vaccine-adjuvant or drug carriers, thereby the active constituent is dissolute, entrapped, encapsulated, or chemically attached.

Nano-sized particles can be given intravenously because the smallest capillary has a diameter of around 4 μ m. The distribution of NPs can vary according to the surface charge, size, and hydrophobic ability of the NPs.^[6] Particles of a diameter larger than 100 nm are readily entrapped by the reticuloendothelial system (RES), while other NPs with smaller-size may have a sustained circulation time. NPs with negative-charge are eliminated quicker than positive-charged or neutral NPs.^[1] Generally, opsonins (RES proteins) favor adsorbing to hydrophobic instead of hydrophilic particle surfaces. Production of a hydrophilic coat on a hydrophobic carrier considerably increases their circulatory time.^[7] Collectively, these data propose that generating NPs with a surface that shows hydrophilic but neutral charge, is a practical approach to reduce phagocytosis and thus increase the therapeutic value of loaded drug molecules. The most hopeful drugs that have been widely considered for delivery by the such route are antitumor agents. Following intravascular administration, many-particle systems such as chitosan-NPs displayed an obvious tendency to gather in several tumors.^[7] One probable reason for this phenomenon may include tumor vascular leakage.^[8] After intravenous administration, doxorubicin-loaded chitosan-NPs induce regression in the growth of tumor mass and improve the survival rate of experimental rats. Furthermore, chitosan-NPs constitute one of the most extensively studied particles among available water-soluble polymers. At the beginning of this century, chitosan-NPs have been widely developed and presented for pharmacological uses. Hence, this

review concentrated on the technology of chitosan-NP preparation uses and the mechanism of cellular entry.

MATERIALS AND METHODS

Experimental animal sampling

From the animal house belonging to the biology department, University of Kufa, 20 Wistar albino rats (adult male), weighing up between 200 and 250 g, were gotten during the period between January and March 2021. The rats were preserved in accustomed laboratory circumstances (25 °C and 60 $\pm$ 10% humidity). Rats are allowed freely to ordinary rat chow and water. The “Guidelines of the Ethical Committee of the animal Research” were monitored, which adapts “the recommendations on Health Guide for Care and Use of Laboratory Animals.” The rats were separated into three groups: the control group (N5), group one (N5) (received 125 mg/kg chitosan), group two (N5) (received 250 mg/kg chitosan), and group three (N5) (received 500 mg/kg chitosan). Every group was nourished and followed for 14 days.

Dose formulation

The chitosan was obtained from “Sigma Aldrich, UK.” Within all groups, every animal was fed one milliliter of chitosan dispensed in distilled water to a particular concentration as mentioned earlier, whereas the control rats were fed one milliliter of “PBS food.” Each rat was weighed before feeding for the whole study period.^[9,10]

Animal sacrifice and tissue processing

The animals were sacrificed and dissected 14 days after being on chitosan feeding. The hepatic and heart tissues were removed and put in formaldehyde 10% and treated with “hematoxylin and eosin” and processed as described.^[11]

Venous measurements

Histological dimensions were completed using “Image J Software (image processing and analysis in java).” Standardized pictures were arranged before measurements. The image of each vein was calculated accordingly and 30 measurements were gotten from every rat group. The statistical examination was completed employing Microsoft Excel.

RESULTS

Weight changes

The average weight of each animal was recorded at the beginning of the experiment and repeated daily. A noticeable increase in animal weight is seen as the concentration of chitosan increases [Figure 1].

Histological and cellular examination

The results of the present study show histological changes in both liver and cardiac tissues. Figure 2 shows a normal

histological appearance with a slight hemorrhaging present. The figure shows an abnormal histological appearance with more abundant hemorrhaging and cellular vacuolation present in liver tissues. The figure also shows profound histological damage with more abundant hemorrhaging and lymphocytic infiltration along with sinusoid enlargement in the liver as well as distrupted nuclei present. Cardiac tissues were less affected by changes in chitosan concentration. Liver histological changes are attributed to the metabolic breakdown of chitosan in the liver.

Vascular changes

Chitosan plays a vital role in blood hemostasis. Vascular changes in orally fed rats have been examined in this study. A decrease in vascular thickness of hepatic tissues is seen

in comparison to tissues in control animals. Cardiac veins showed no significant changes in vascular thickness as shown in Figure 3.

DISCUSSION

The results of this study show that there is a significant histological change that takes place during exposure to chitosan. A study by Zhang *et al.*^[1] showed significant histological differences in rat liver injected with a weight-specific dose of cyclophosphamide. In their study, hepatic-destructive changes were gradually increased from slight hemorrhaging to lymphocytic infiltration indicating the cytotoxic effect of cyclophosphamide injected intraperitoneally. The current study showed

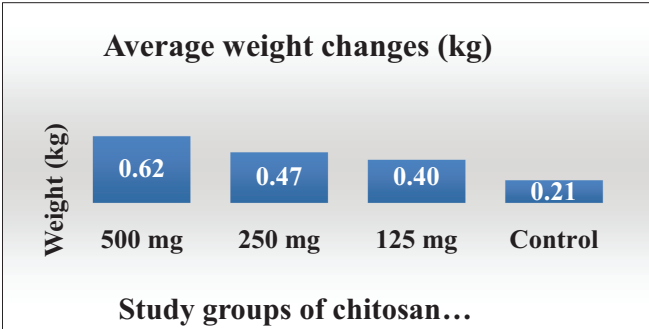


Figure 1: Average weight changes in animals after receiving oral chitosan diet

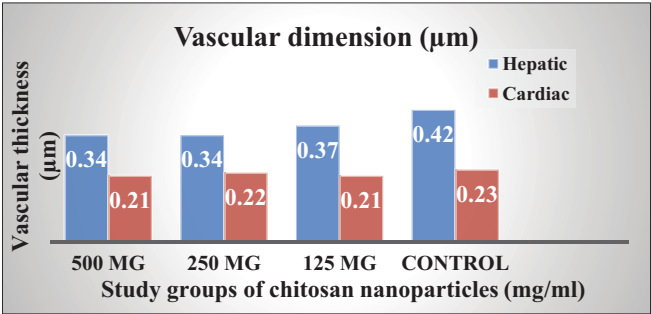


Figure 3: Vascular dimensions (veins) in rat liver and cardiac tissues. A noticeable decrease in vascular thickness is seen in both cardiac and liver vascular network is seen in all chitosan exposed animal cardiac and hepatic vascular dimensions

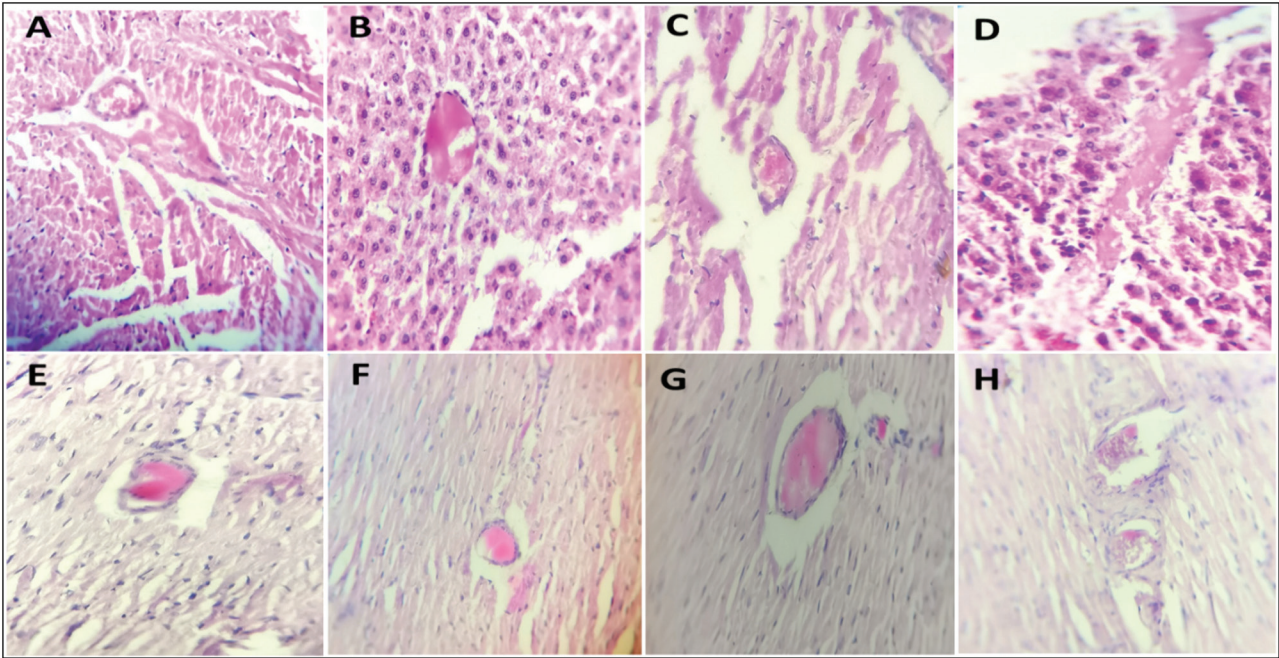


Figure 2: Histological tissue shows profound histological damage in rat liver (A—control, B—125 mg/kg, C—250 mg/kg, D—500 mg/kg). Cardiac tissues show no apparent histological damage due to changes of chitosan concentration (E—control, F—125 mg/kg, G—250 mg/kg, H—500 mg/kg) × 400

similar results to that conducted by Alhowail and his team who demonstrated that the profound effect on histomorphological changes in various rat organs. Histological damage present in the current study is concurrent with much scientific evidence which demonstrated the cytotoxic and histological damage caused by cyclophosphamide injection. Histological changes through the use of chemical agents such as cyclophosphamide in two different deliver methods was recently compared. Delivery of cyclophosphamide intraperitoneally and subcutaneously achieved noticeable histological changes.^[11-15] A study by Hassan *et al.*^[16] showed the ability to regenerate damaged tissues using PRP-loaded nanoparticles. Their study included cellular examination of epidermal cells and the efficiency of nanoparticles as a delivery molecule.

CONCLUSION

The results of the study concluded that chitosan has a robust cytotoxic impact on certain organs. However, histological damage is more prominently seen in rat liver tissues. Histological damage is confirmed by the significant decrease in vascular dimension in both liver and cardiac tissue which indicates that chitosan has a strong effect on the vascular architecture of rat tissues. Due to its metabolic effect on fatty tissues, chitosan has a profound effect on intrahepatic damage leading to both vascular and cellular damage.

Ethical approval

Not applicable.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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