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Assessment of Histological Damage to Rat Liver Induced by Gold Nanoparticles Derived from Bacteria: Preliminary Investigation on Dose-Dependent Effects

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Abstract

Background: Gold nanoparticles (GNPs) have garnered considerable attention in various biomedical applications due to their unique physicochemical properties. Understanding the dose-dependent histological effects of GNPs on liver tissue in animal models is crucial for evaluating their safety profile and optimizing their therapeutic applications. This study aimed to evaluate the hepatotoxicity of bacterially derived GNP on rat liver for proceeding into a further study. **Material and Methods:** Male Wistar rats were divided into four groups: control, 25, 50, 100, and 200 mg/kg GNP, and the rats were given a 15-day acclimatization period. The rats were subjected to lipid peroxidation, oxidative stress, and liver histology tests. **Results**: The levels of ALT, AST, and albumin significantly differed from the control group ($P<0.001$), as revealed in Fig. 1. The results revealed that the rats treated with 50 mg/Kg GNPs had the most beneficial effects, while GSH levels were higher in the group treated with 100 mg/GNPs than in the groups treated with 20 mg/L GNPs. In addition, GNPs-treated rats showed an increase in inflammation in the liver, with the presence of inflammatory cells. **Conclusion**: These findings emphasize the importance of thorough safety evaluations in GNPs for proceeding the coming research.

Keywords: Gold, nanoparticle Liver cancer, Histological , damage.

1.Introduction

Gold nanoparticles (GNPs) have garnered considerable attention in various biomedical applications due to their unique physicochemical properties [1]. However, concerns regarding their potential toxicity, particularly to vital organs such as the liver, have raised significant interest. Understanding the dose-dependent histological effects of GNPs on liver tissue in animal models is crucial for evaluating their safety profile and guiding their clinical applications [2]. This literature review explores the research on the dose-dependent histological injury to rat liver induced by GNPs, highlighting key findings, mechanisms, and implications for biomedical research and nanomedicine [3].

GNPs exhibit size-dependent properties, making them promising candidates for drug delivery, imaging, and therapeutic applications [4]. However, their interaction with biological systems, including uptake, distribution, and clearance, can lead to adverse effects, particularly in the liver, a significant site for nanoparticle accumulation and metabolism. Studies have shown that GNPs can induce dose-dependent hepatotoxicity, characterized by alterations in liver histology, oxidative stress, inflammation, and impaired liver function [5].

Several preclinical studies have investigated the dose-response relationship between GNPs and histological changes in rat liver tissue. These studies have reported dose-dependent alterations in liver morphology, including hepatocyte degeneration, necrosis, vacuolation, and inflammatory cell infiltration, following exposure to GNPs of varying sizes, coatings, and concentrations. Furthermore, dose-dependent effects on liver enzymes, such as alanine transaminase (ALT) and aspartate transaminase (AST), have been observed, reflecting hepatocellular damage and impaired liver function [6].

The mechanisms underlying GNPs-induced liver injury are multifactorial and involve oxidative stress, inflammation, apoptosis, and fibrosis. GNPs can induce the generation of reactive oxygen species (ROS), leading to oxidative damage to cellular macromolecules and activation of pro-inflammatory pathways. Additionally, GNPs may disrupt mitochondrial function, trigger apoptotic pathways, and stimulate hepatic stellate cell activation, contributing to liver fibrosis and tissue remodelling [7].

Understanding the dose-dependent histological effects of GNPs on rat liver is essential for evaluating their safety profile and optimizing their therapeutic applications. While GNPs offer tremendous potential in biomedicine, careful consideration of dose, size, surface chemistry, and route of administration is necessary to mitigate potential toxicity and maximize therapeutic efficacy [8]. Future research should focus on elucidating the underlying mechanisms of GNPs-induced liver injury, developing strategies to enhance biocompatibility and targeting specificity, and conducting comprehensive toxicity assessments in animal models and clinical trials. Therefore, our study aimed to evaluate the dose-dependent of GNPs and their toxicity on rat liver for proceeding into a further study.

2.Material and methods

Animal

The oversized stainless steel cages held twenty Wistar rats weighing 165 ± 10 g, obtained from the animal house at Alexandria University. Under the University of Benha Institutional Animal Care and Use Committee (IACUC) accreditation number, animal care and experimental methods were conducted following its recommendations. The rats were kept at 20 ± 2 °C with a relative humidity of 50 ± 15%, and they were housed in typical laboratory settings with a 12/12 hour light/dark cycle. Regular food and unlimited water were given to them. The Regional Ethical Committee approved all experimental methods, and the research was carried out following national norms for the care and use of laboratory animals.

Four groups of five rats each—Control, 25, 50, 100 and 200—were created, and the rats were given a 15-day acclimatization period. Then, using a single caudal injection that allows for quick and direct vascular access to every organ, GNPs dosages were dissolved in saline and were given to each group. An injection of saline alone was given caudally to the control group as previously described [9].

Collection of samples

The rats were given an overnight fast fifteen days after the caudal injection and then put to death. 10% (w/v) chloral hydrate (3 mL/kg body weight) was injected intraperitoneally to produce anaesthesia. Blood samples were taken from the abdominal aorta, and the plasma was separated for biochemical analysis and oxidative stress evaluation by centrifuging the samples for 15 minutes at 3000g. After the kidneys, liver, and brain were removed, they were carefully cleaned with ice-cold 0.1 M phosphate-buffered saline (PBS; pH 7.4). 10 L of ice-cold, ten mM phosphate-buffered saline was used to homogenize tissue samples using an Ultrapure homogenizer (Bioflick Scientific, Illich, France) [10], [11]. The homogenates were then centrifuged for 15 minutes at 4 °C at 6000g, and the supernatants were separated for redox marker analysis. A sharp razor was used to carefully remove the histological samples, which were then preserved in a 10% formaldehyde solution [12].

Biochemical parameters assessment

Biochemical parameters such as albumin [13], AST [14], and ALT [15] were determined using enzymatic assay kits obtained from Sigma Chemical Co., based in St. Louis, MO.

Oxidative stress assessment

Indicators of oxidative stress in liver tissues were evaluated [16]. The reduction of 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) by reduced glutathione, which results in the formation of a yellow molecule, was the basis for the technique used to measure glutathione (GSH) levels. The resultant chromogen's absorbance was measured at 405 nm, and it was shown to be directly associated with the GSH content. Thiobarbituric acid reacted with malondialdehyde (MDA), a marker of lipid peroxidation, to determine its presence in plasma and tissues [17].

Liver Histopathology evaluation

The animal tissues were removed, and different alcohol concentrations were used to dry the tissues. After dehydration, the samples were cleared in two xylene changes. After being impregnated with two different batches of melted paraffin wax, the tissue samples were embedded and sealed. After using a microtomy to slice the tissues, they were adhered to glass slides for ensuing staining and microscopic inspection. Glass slides for microscopes were mounted with sections stained with hematoxylin and eosin (HE) at a thickness of 3 μM. Then, using an optical microscope (AX80, Olympus, Tokyo, Japan), HE-stained tissue slices were examined and photographed. Microscopic observations included evaluations of tissue architectural abnormalities and tissue structures exhibiting degeneration, necrosis, inflammation, and portal fibrosis [18], [19].

3.Results

Mortality study

No rats died throughout the trial period of GNPs therapy, and no obvious aberrant clinical symptoms or behavioral changes were seen in the animals. This included evaluations of eye changes, diarrhea, breathing from the abdomen, skin and hair problems, and food intake. Furthermore, no discernible differences between the experimental group and the control group were found for average body weight, weight growth, or relative weight (exact data withheld).

Biochemical Findings

Higher doses of GNPs (100 and 200 mg/kg) in rats were associated with indications of liver impairment manifested by decreased albumin levels. The levels of ALT, AST, and albumin significantly differed from the control group ($P<0.001$), as revealed in **Fig. 1**.

Oxidative and antioxidant assessment

There was higher lipid peroxidation in groups treated with 100 mg and 200 mg/Kg GNPs, while GSH levels were lower than in groups treated with 50 mg. Lipid peroxidation and GSH levels were higher in the group treated with 50 mg/Kg GNPs, indicating that this dosage had the most beneficial effects. Consequently, 50 mg/Kg GNPs appears to be the optimal dosage for inhibiting lipid peroxidation and increasing GSH levels, as revealed in **Fig. 2**.

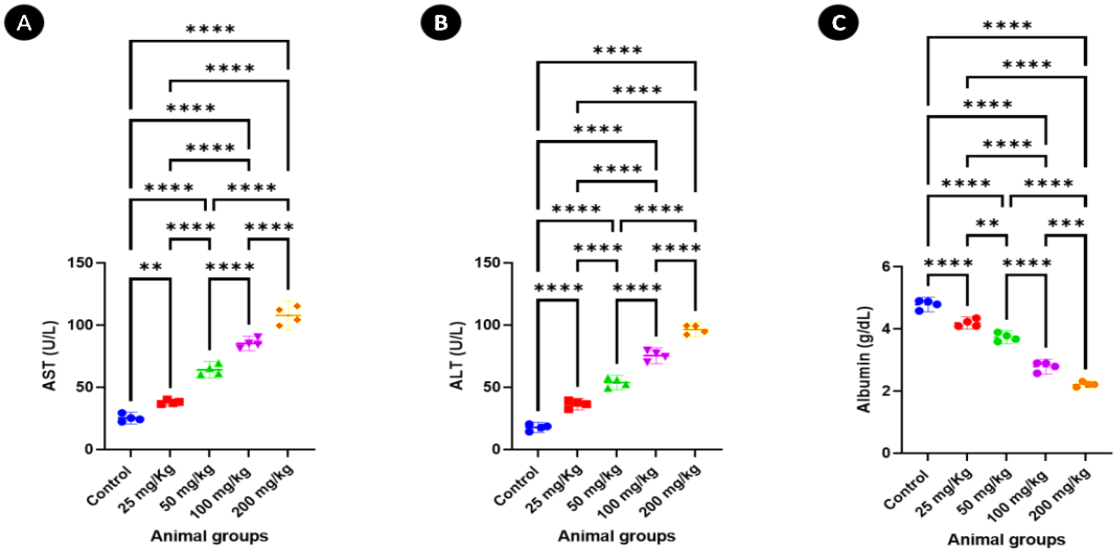

Fig. (1) Biochemical parameters ALT, AST, and Albumin in different rat-treated GNPs groups.

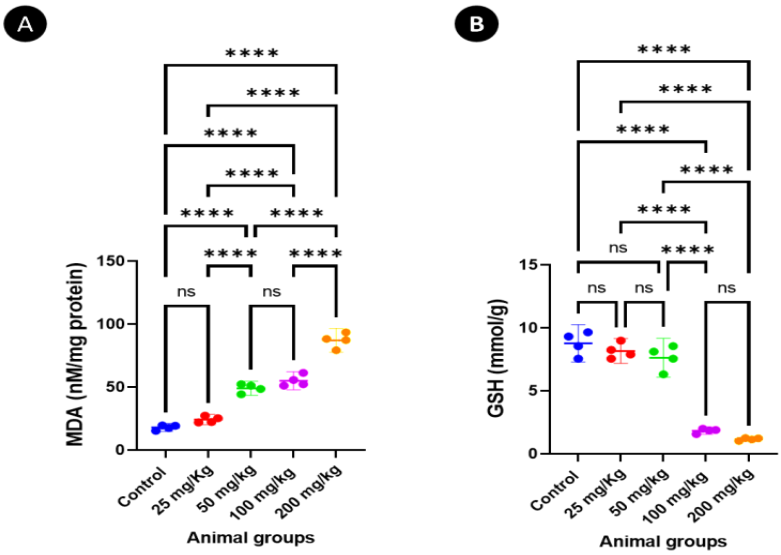

Fig. (2) Oxidative marker MDA and antioxidant marker GSH in different rat-treated groups.

Histopathology studies

According to the histological examination, there were no signs of liver damage in the rats treated with 50 mg/kg GNPs, as revealed in **Fig. 3a**. In addition, rats treated with 100 and 200 mg/kg GNPs showed significant liver damage, characterized by engorged vessels, tubular degeneration, and necrosis. In addition, the rats treated with 200 mg/kg GNP also revealed an increase in inflammation in the liver, with the presence of inflammatory cells **Fig. 3b-c**.

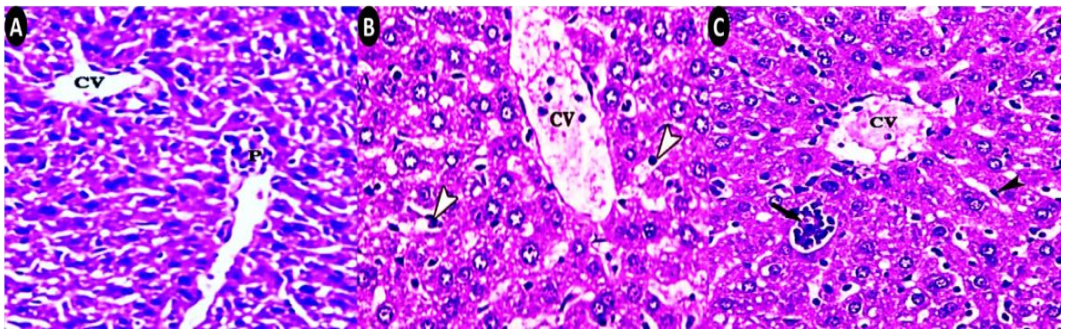

Fig. (3) Histopathology examination of different GNPs doses in Liver histology.

4.Discussion

The potential of nanoparticles (NPs) in various fields, such as medicine, electronics, and environmental remediation, is enormous. The unique physicochemical properties of GNPs and their potential applications in drug delivery, imaging, and cancer therapy have contributed to their popularity [20]. They have, however, raised concerns regarding their possible toxicity, primarily when derived from biological sources [21]. As part of this preliminary investigation, GNPs derived from bacteria were administered to rats at doses ranging from zero mg/kg to 200 mg/kg to assess how they affect liver histology in rat models.

The observed dose-dependent effects of bacterially derived GNPs on liver histology underscore the importance of understanding nanoparticle toxicity and its implications for biomedical applications. Several factors contribute to the toxicity of GNPs, including size, shape, surface chemistry, dose, and route of administration. In this study, the use of bacterially derived GNPs introduces additional variables, such as the presence of biomolecules on the nanoparticle surface, which may influence their interactions with biological systems [22]. Therefore, the optimal dose-dependent of our study ranged from 20-50 mg/kg GNPs regarding the biochemical, oxidative stress, and liver histology results.

The mechanism underlying GNPs-induced hepatotoxicity is multifaceted. It may involve oxidative stress, inflammation, mitochondrial dysfunction, and perturbation of cellular signaling pathways. GNPs can generate reactive oxygen species (ROS), leading to oxidative damage and lipid peroxidation, which in turn disrupt cellular membranes and trigger inflammatory responses. Furthermore, GNPs may accumulate in the liver, impairing hepatic function and promoting fibrogenesis [23].

The observed histological changes in higher doses, including hepatocellular degeneration, inflammation, necrosis, and fibrosis, are consistent with previous studies on GNPs-induced liver injury. These findings highlight the need for comprehensive toxicity assessments to evaluate the safety of nanomaterials before their clinical translation. Moreover, understanding the dose-response relationship is crucial for establishing safe exposure limits and informing regulatory guidelines [24].

Limitations of this preliminary investigation include the small sample size, short duration of exposure, and focus solely on histological endpoints. Future studies should incorporate additional toxicity assays, such as biochemical markers of liver function, gene expression profiling, and electron microscopy, to elucidate the underlying mechanisms of AuNP-induced hepatotoxicity.

5.Conclusion

In conclusion, this preliminary investigation provides valuable insights into the dose-dependent effects of bacterially derived GNPs on liver histology in rat models. The findings underscore the potential hepatotoxicity of GNPs and emphasize the importance of thorough safety evaluations in nanomedicine research. Further studies are warranted to elucidate the underlying mechanisms and establish safe usage guidelines for GNPs and other nanomaterials in biomedical applications.

Declarations

Ethics approval and consent to participate

All experimental procedures used are carried out following Benha University's animal care guidelines and the National Science Council's Guide for the Care and Use of Laboratory Animals.

Consent for publication: Not applicable

Availability of data and material: The datasets generated and/or analysed during the current study are available from the corresponding author

Competing interests: The authors declare no competing interests.

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