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Original Article

Hepatic Impact of Different Concentrations of *Hibiscus rosa* Zinc Oxide Nanoparticles on Rats

Sattar Ali, Z[1]*

1. Department of Pharmacology and Toxicology, College of Pharmacy, University of Al-Muthana, Samawah, Iraq

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Corresponding Author: zainbsatarali@mu.edu.iq

Abstract

Nanomaterial, especially zinc oxide nanoparticles, has entered the manufacture of many materials used in daily lives. The current study aimed to assess the impact of three concentrations of *hibiscus rosa* zinc oxide nanoparticles (HrZnONPs) and *hibiscus rosa* extract (Hre) on the liver tissue and DNA fragmentation of liver cells. A total of 35 adult male Wistar rats were grouped as follows: The first group which was the control (n=7) did not receive any treatment. The remaining 28 animals were randomly assigned to four groups. Group 1 (n=7) were subcutaneously injected with 100mg\kg BW of *Hibiscus rosa* extract for 60 days; the rats in group 2 were subcutaneouslyinjected with 25 mg\kg BW of HrZnONPs for 60 days; rats in group 3 were subcutaneouslyinjected with 75mg\kg BW of HrZnONPs for 60 days; rats in group 4 were subcutaneously injected with 100mg\kg BW of HrZnONPs for 60 days. The liver biomarkers, aspartate aminotransferase (AST), alkaline phosphatase (ALP), and alanine aminotransferase (ALT) have been assessed in serum at zero time, after one month, and after two months of the experiment. At the end of the experiment, all animals were euthanized, the liver was dissected, the specimen underwent a pathohistological investigation, and the percentage of DNA fragmentation was evaluated. The results pointed out that the rats which were treated with HrZnONPs at concentrations of 75 and 100 mg\kg B.W. demonstrated a salient elevation in serum AST, ALT, or ALP activity, a modulation in hepatic tissue architecture, and an elevated percentage of high DNA damage, as compared to those treated with HrZnONPs at a concentration of 25 mg\kg B.W. On the other hand, the recorded data indicated that the administration of Hre has some ameliorative effects on AST, ALP, and ALT levels, histological cross-section, and the value of comet assay for liver cells due to the role of Hre antioxidant. In conclusion, the results of the current study demonstrated that high doses of HrZnONPs had exerted more adverse effects, compared to low doses. Moreover, the findings confirmed the ameliorative impact of Hre on liver biomarkers, a histological cross-section of the liver, and DNA damage.

Keywords: Genotoxic, Hepatotoxicity, *Hibiscus rosa* (*Hibiscus rosa-Sinensis*), Liver biomarkers, Zinc oxide nanoparticles

1. Introduction

The novel properties of nanoparticles have made them effective substances to be used in a wide range of applications (1). Zinc oxide nanoparticles (ZnO NPs) are considered one of the widely used nanoparticles in many fields and are versatile platforms for biomedical applications and therapeutic intervention (2). The ZnO NPs were introduced as active ingredients in the manufacture of sunscreens and cosmetic lotions since they efficiently absorb UV light and do not disperse visible light (3). The ZnO NPs have been also used in biomedical imagery, as a chemotherapeutic agent for cancer, and in the food industry where entered in food packaging and synthetic textile fibers due to theirproperties, such as antimicrobial activity, their potential fungicides, and their ability to absorb ultraviolet radiation (4, 5).

Recently, ZnO NPs have been broadly used in industry, and since these nanoparticles are mostly insoluble in water, they can be utilized as an additive in rubber, glass, adhesives, ceramics, paints, and toothpaste (6). The widespread application of ZnO NPs-containing products increases people's exposure to these nanoparticles in environmental and occupational settings. It has been well documented that under certain conditions, these products exert hazardous effects on living cells (7). These adverse effects on humans, aquatics, and the ecosystem have given serious cause for concern among scientific and public communities (7, 8).

The toxicity of ZnO NPs was evaluated in various biological systems, such as mammalian cells *in vitro* and *in vivo* models and bacteria (9). The ZnO NPs demonstrated to have toxic effects on mammalian cells and cause cell injuries, DNA damage, and consequently apoptosis or inflammatory response (10). Previous studies have pointed out that the NPs which were ingested orally by laboratory animals accumulated in the liver and caused several hepatic damages (11). Nonetheless, no studies have highlighted the side effects of ZnO NPs on the human liver so far. Moreover, there is a dearth of studies on their side effects on the mammalian liver which is the fundamental organ in metabolism. The ZnO NPs may be immediately ingested when utilized in food; moreover, the workers who participate in ZnO NPs synthesis can be exposed through unintended nanomaterial transfer by hand-to-mouth (12). In light of the aforementioned issues, the current study was designed to investigate the effect of different concentrations of ZnONPs on liver function tests, histology change, and DNA fragmentation in liver cells of rats.

2. Materials and Methods

2.1. Animals

A total of 35 95-day-old adult male Wistar rats with a mean weight of 150±2 g were utilized in this study. Before the experimental onset, the rats were kept for 10 days in an animal house.

2.2. Plant Extraction and Nps Synthesis

The collection of *Hibiscus rosa*: Aqueous extract of *Hibiscus ros*awas prepared as depicted by Ali and Khudair (13). The synthesis of *Hibiscus rosa* zinc oxide nanoparticles was prepared as depicted by Devi and Gayathri (14).

2.3. Study Design

The animals were assigned to five groups. The first group (control (n=7)) did not receive any treatment. The remaining 28 animals were randomly allocated to four groups. The rats in group 1 (n=7) were subcutaneously injected with 100mg\kg BW of *Hibiscus rosa*extract for 60 days; rats in group 2 received a subcutaneous injection of 25 mg\kg BW of HrZnONPs for 60 days; rats in group 3 were subcutaneously injected with 75mg\kg BW of HrZnONPs for 60 days, and rats in group 4 were subcutaneously injected with 100mg\kg BW of HrZnONPs for 60 days. The animals were anesthetized by intramuscular injection of xylazine and ketamine (90 mg/kg and 40mg/kg, respectively). Subsequently, the blood samples were obtained from them by the orbital sinus technique. The biomarkers of liver function in serum were assessed as previously described by Reitman et al. and Kaplan et al. for the Aspartate transaminase (AST) assay (15), Alkaline phosphatase assay (ALP) (16), and alanine aminotransferase assay (ALT) (15), respectively, for all experimental groups three times (at zero time, after one month, and after two months) during the experiment. After euthanasia, the rats were dissected, and the liver specimen was obtained for more evolutions of DNA fragmentation percentage by comet assay kits according to Olive, Banáth (17), and the pathophysiology study of this specimen was performed as described by Luna (18).

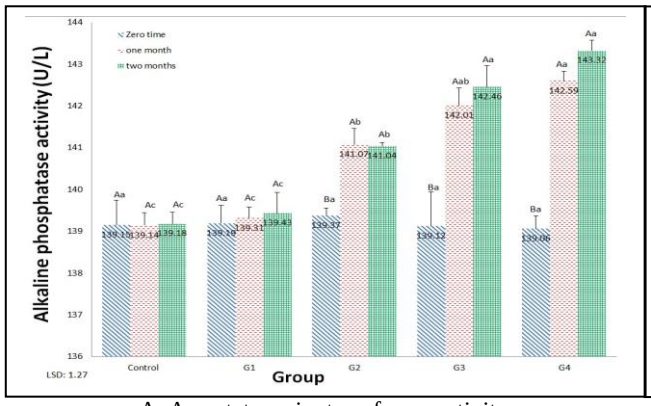
2.4. Statistical Analysis

The obtained data were tested using the two-way ANOVA and the least significant differences to compare the mean values (19). A p-value less than 0.05 was considered statistically significant.

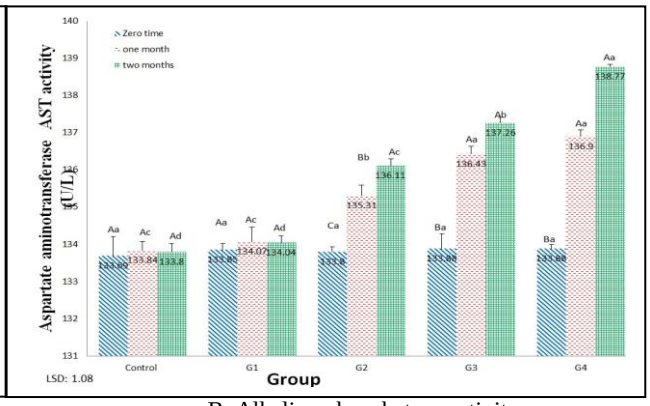
3. Results

The values of AST in serum as illustrated in Figure (1A) disclosed a significant ($P \leq 0.05$) elevation in AST activity in animals treated with HrZnONPs at concentrations of 25, 75, and 100 mg/kg B.W, as compared to the animals treated with *Hibiscus rosa* extract. In a similar vein, a

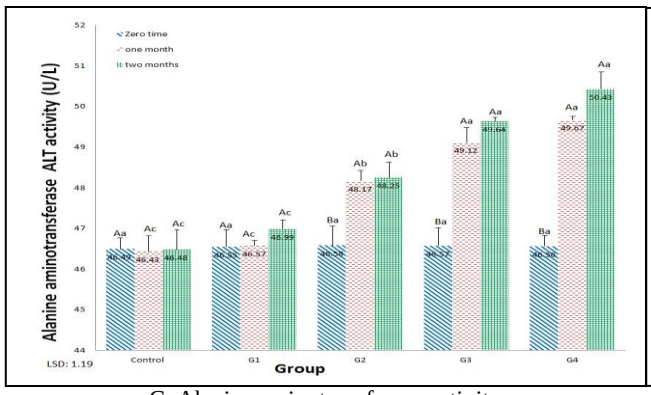
significant variation ($P \leq 0.05$) was detected among the three groups treated with three concentrations of *hibiscus rosa* zinc oxide nanoparticles, where AST activity increased with an elevated dose of HrZnONPs. Figure (1B) displays the effect of *Hibiscus rosa* zinc oxide nanoparticles in three concentrations on ALP, demonstrating an increase in ALP value in males treated with concentrations of 100 and 75 mg/kg B.W (group3 and group4), in comparison with other groups. Moreover, it indicates that ALP values in all groups after one month were not significantly different, as compared to those obtained after two months within the same group.



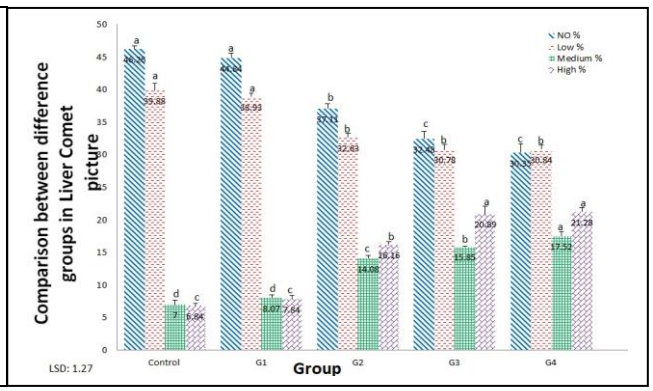
A. Aspartate aminotransferase activity



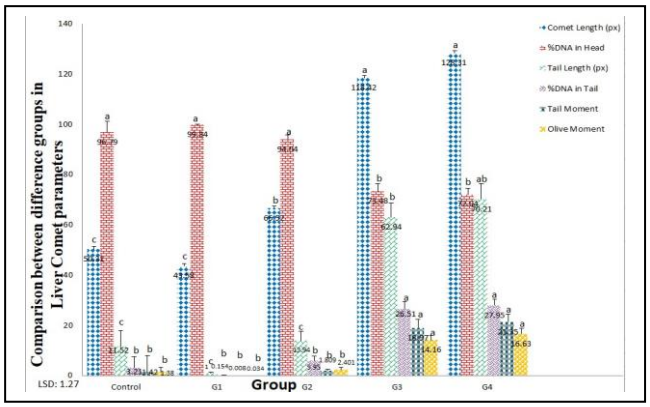
B. Alkaline phosphatase activity



C. Alanine aminotransferase activity



D. Mean of comet assay score for DNA% in liver cells



E. Characters of comet assay for DNA in liver cells

Figure 1. Effect of the three concentrations of Hibiscus rosazinc oxide nanoparticles and Hibiscus rosa extract administration on serum AST, ALP, and ALT for all experimental groups three times (at zero time, after one month, and after two months), as well as the mean score and value of comet assay % for liver cells of male rats

Numbers represent mean ± standard error.

Different capital letters indicate a significant difference ($P<0.05$) between the groups.

Different small letters denote significant differences ($P<0.05$) within the specified periods for each group.

As illustrated in Figure (1C), ALT was significantly (P<0.05) elevated in males rats treated with concentrations of 100 and 75 mg/kg B.W (group3 and group4), in comparison with the control group, whereas the male rats in group 1 treated with *hibiscus rosa* extract and those in the control group did not significantly differ in ALT value (P>0.05). Figure (1D) depicts the percentage of DNA damage in liver cells, classified as high, medium, and low. The result clarifies that rats in group 4 (Figure 2) have a low percentage of low DNA damage, compared to controls and rats injected with hibiscus rosa extract. The result also illustrates the percentage of high DNA damage in the group SC injected with HrZnONPs at a concentration of 25 mg/kg B.W, pointing to a non-significant difference between the percentage of high DNA damage in this group and that of the control group (Figure 3).

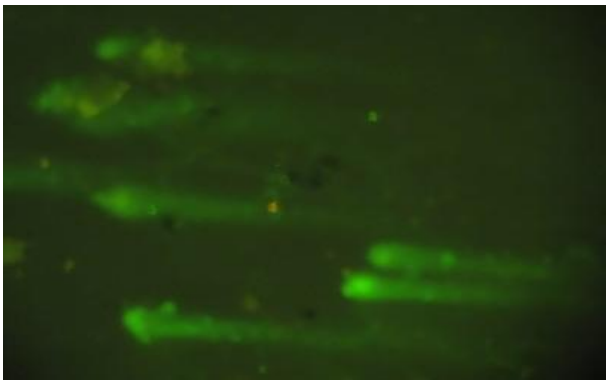


Figure 2. Comet assay (DNA damage) in liver cells for rat in Group 4

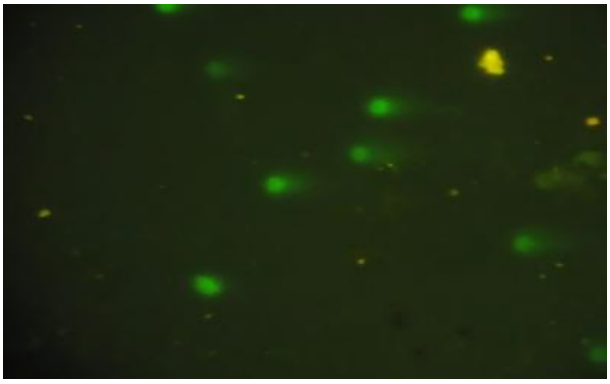


Figure 3. Comet assay (DNA damage) in liver cells for rats in Control

Figure (1E) and the images of a Fluorescent Microscope (1-5) displayed the comet assay characteristics for liver cells, suggesting a significant elevation at P<0.05 in DNA % in the tail, tail moment, head diameter, and tail length with a significant reduction in the DNA percent in the head in groups treated with 100mg\kg BW of HrZnONPs (G4) (Figure 2) and 75 mg\kg BW of HrZnONPs (G3) (Figure 4), as compared to the data in the other treated groups. The result indicated that all the aforementioned criteria were ameliorated in the group treated with hibiscus rosa extract. The subcutaneous injection of HrZnONPs at a concentration of 25 mg/kg B.W of HrZnONPs caused a significant (P<0.05) increase in DNA proportion in the head with a significant reduction (P<0.05) in the DNA proportion in the tail, tail length, and tail moment, as compared to the value obtained in group 4.

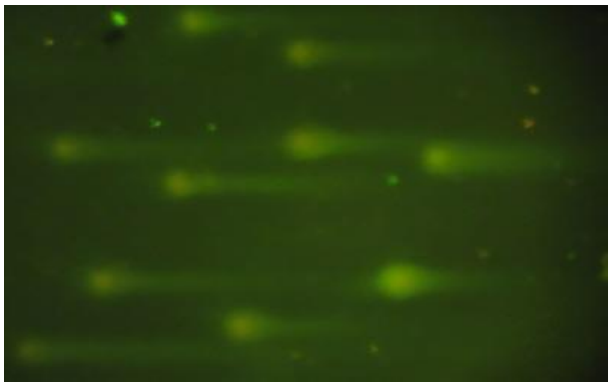


Figure 4. Comet assay (DNA damage) in liver cells for rats in Group 3

The histopathological study of the liver of rats in the control group and group SC injected with 100 mg\kg BW of *Hibiscus rosa* extract depicted in figures (5, 6) illustrates no clear lesions, when compared to the histopathological analysis of the liver in group SC injected with 25 mg\kg BW of HrZnONPs. Figure 7 displays a hepatic sinusoidal dilation and infiltration of inflammatory cells in liver tissue. While histopathological analysis of the liver in the rats which received 75 mg\kg BW of HrZnONPs by SC injection in figure 8 demonstrated that the situation is worse, signifying sinusoidal dilation, inflammatory cell

infiltration, fatty changes, and venous congestion in liver tissue. It is demonstrated that the necrotic area contains pus, inflammatory reaction, and vanishing hepatic architecture detached from the rest of the tissue by inflammatory line in rats in group 4 that received 100 mg\kg BW of HrZnONPs by SC injection in figure 9.

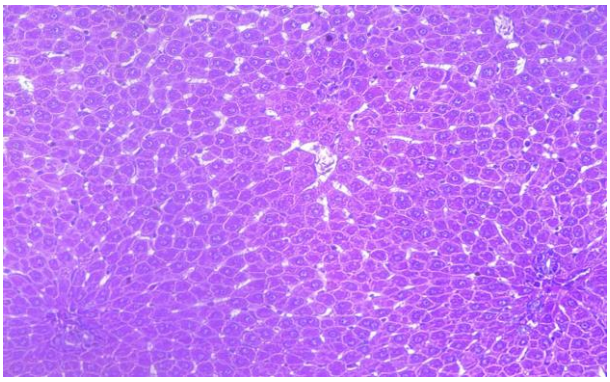


Figure 5. Histopathological section for the liver of rat in control, illustrating no clear lesions

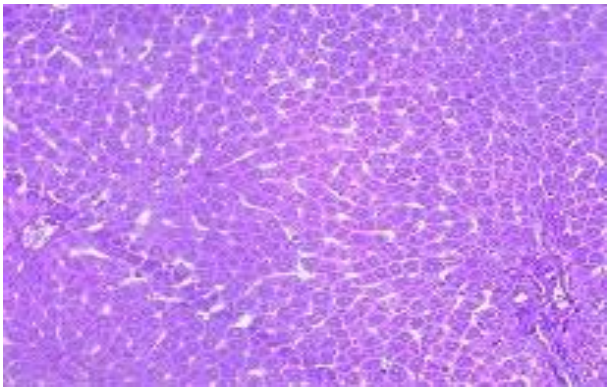


Figure 6. Histopathological section for the liver of rat in Group 1, showing no clear lesions

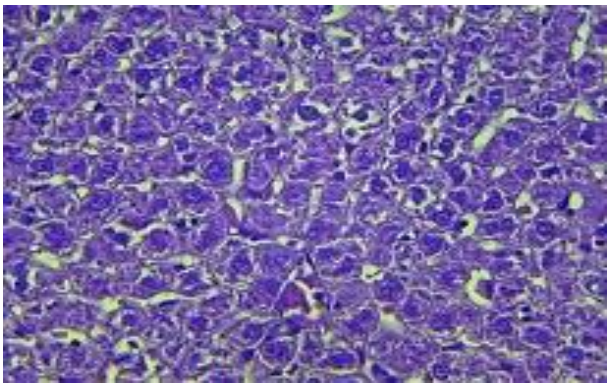


Figure 7. Histopathological section for liver of rat in Group 2, dmeonstrating a dilation in liver sinusoid and infiltration of inflammatory cells in liver tissue

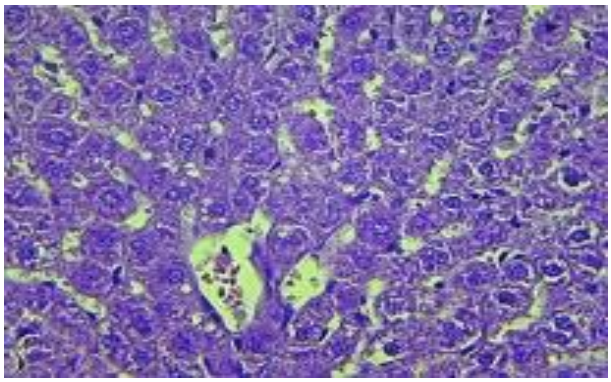


Figure 8. Histopathological section for the liver of rat in Group 3 (Figure 5), illustrating a sinusoidal dilation, inflammatory cell infiltration, and venous congestion in liver tissue

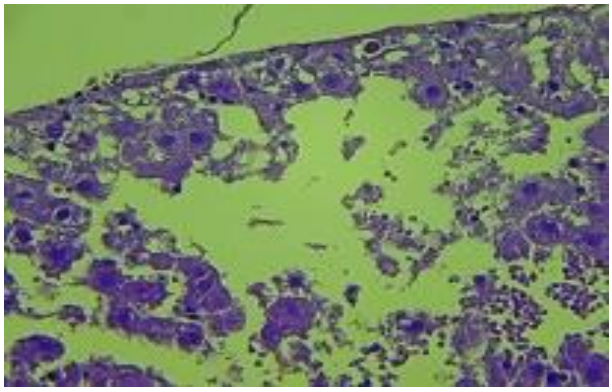


Figure 9. Histopathological section for the liver of rat in Group 4, necrotic area contain pus, inflammatory reaction, and vanishing hepatic architecture

4. Discussion

The high levels of liver enzymes, including AST, ALP, and ALT in groups 3 and 4 in this study indicated an injury in liver cells in the animals in these groups. Among the physical characteristic of zinc oxide, we can refer to its white color and insolubility in water. The ZnO2 has a wide application as an additive in food supplements, rubbers, glass, paints, and ceramics. In several studies, nanoparticles have been documented to induce toxicity in different cell lines. Aboulhoda, Abdeltawab (20) have documented that the rats which

received ZnO_2 for 14 days reported an elevation in AST and ALT activity, suggesting liver injury.

The ZnO_2 nanoparticles after oral administration and intraperitoneal injection distribute and accumulate in the kidneys, spleen, and liver (21). These liver biomarker enzymes gradually raised with an increase in the duration of treatment. This finding was confirmed by histopathological analysis of the liver of the rats in this study, where sinusoidal dilation, inflammatory cell infiltration, fatty changes, and venous congestion were observed in liver tissuein group SC injected with 75 mg\kg BW of HrZnONPs. The histopathological study of rats in group SC injected with 100 mg\kg BW of HrZnONPs demonstrated that the necrotic area contained pus, inflammatory reaction, and vanishing hepatic architecture, indicating that this concentration of HrZnONPs is more cytotoxic.

Hepatic and renal tissues have elevated metabolic needs and an intense request for mitochondria. The regulation factors for mitochondrial biogenesis involve transcription mtTFAfactor A of mitochondrial (22). Exposure to ZnO NPs exposure may lead to impaired mitochondrial biogenesis of rat liver (23). According to Attia, Nounou (24) ZnONPs exert toxic effects, such as membrane injury, apoptosis, DNA damage, and inflammatory response, on mammalian cells (24). Along the same lines, Liang, Zhang (25) linked the toxicityof ZnONPs to their role in the production of reactive oxygen species (26).

The ZnONPs induce oxidative stress in various cell lines through the production of reactive oxygen species. Oxidative stress is correlated to cell membrane leakage, lipid peroxidation, increased intracellular calcium (27), cellular injury, oxidative DNA damage, and cell apoptosis (10). In the current study, the data from comet assay for hepatic cells in rats in groups 3 and 4 demonstrated an elevated percentage of high DNA damage. The ZnO NPs are able to deactivate glutathione peroxidase, superoxide dismutase (SOD), and catalase (CAT), leading to the depletion of non-enzymatic and enzymatic cellular antioxidants. Oxidative stress generation (28, 29) led to DNA damage, caused cell death (24), and raised lipid peroxides in juvenile carp (30).

Furthermore, it is known that reactive oxygen species stimulate the mitogen-activated protein kinase (MAPK) which plays a key role in organizing numerous cellular processes and is an important mediator for signal transduction (31).The present study demonstrated a significant elevation in enzyme activities of liver biomarkers (AST, ALP, and ALT) and histopathological alteration in hepatic architecture in rats injected with 25 mg/kg B.W of HrZnONPs. Nonetheless, the activities of liver biomarkers (AST, ALP, and ALT) were less significant than those in groups 3 and 4, while the histopathological alteration and percentage of high DNA damage were less intense, as compared to those in these groups. The ZnO nanoparticles exert marked effects on many sorts of cells, such as hepatocytes, human bronchial epithelial cells, embryonic kidney cells, and osteoblast cancer cells. Furthermore, the impact of these nanoparticles may be correlated to their dosage and particle size (21).

Several studies demonstrated that low doses of ZnONp do not have toxic effects in vivo, while high concentrations could cause sudden death. In addition, ZnO nanoparticles are one of the most toxic nanoparticles, with the lowest median lethal dose value (32, 33). The increased utilization of ZnONPs and exposure to these nanoparticles elevate steadily, leading to great concerns over the extent of their potential toxicity, involving genotoxicity, proinflammatory effects, and cytotoxicity (34).

The current study demonstrated the activities of liver biomarkers (AST, ALP, and ALT). Histological cross-section of liver and data of comet assay test of liver cells in rats that were subcutaneouslyinjected with 100 mg\kg BW of Hibiscus rosa extract were similar to those in the control group. Hibiscus contains antioxidants to destroy free radicals where the raised free radicals cause damage to cells and elevate the risk of inflammation which lead to cardiovascular disease, metabolic syndrome, and cancer (35). Hibiscus extract induces testicular damage in rats, and this effect is

attributed to the existence of flavonoids and their antioxidant activity in Hibiscus rose (35).

The present study demonstrated that the toxic impact of Hibiscus Rosa zinc oxide nanoparticles depends on the duration of exposure and its toxic effect increases with time. In addition, high doses of Hibiscus Rosa zinc oxide nanoparticles exert more adverse effects, as compared to their low doses. Furthermore, the results of this study confirmed the ameliorative impact of *Hibiscus Rosa* extract on the activities of liver biomarkers, the histological cross-section of the liver, and DNA damage.

Authors' Contribution

Study concept and design: Z. S. A.
Acquisition of data: Z. S. A.
Analysis and interpretation of data: Z. S. A.
Drafting of the manuscript: Z. S. A.
Critical revision of the manuscript for important intellectual content: Z. S. A.
Statistical analysis: Z. S. A.
Administrative, technical, and material support: Z. S. A.

Ethics

All the procedures were approved by the Ethics Committee, Department of Pharmacology and Toxicology, College of Pharmacy, University of Al-Muthana, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Tsekhmistrenko S, Bityutskyy V, Tsekhmistrenko O, Horalskyi L, Tymoshok N, Spivak M. Bacterial synthesis of nanoparticles: A green approach. Biosyst Divers. 2020;28(1):9-17.
2. Wiesmann N, Gieringer R, Viel M, Eckrich J, Tremel W, Brieger J. Zinc Oxide Nanoparticles Can Intervene in Radiation-Induced Senescence and Eradicate Residual Tumor Cells. Cancers. 2021;13(12):2989.
3. Lee C-C, Lin Y-H, Hou W-C, Li M-H, Chang J-W. Exposure to ZnO/TiO2 nanoparticles affects health outcomes in cosmetics salesclerks. Int J Environ Res. 2020;17(17):6088.
4. Bera D, Pal K, Mondal D, Karmakar P, Das S, Nandy P. Gum acacia capped ZnO nanoparticles, a smart biomaterial for cell imaging and therapeutic applications. Advances in Natural Sciences: Nanoscience and Nanotechnology. 2020;11(3):035015.
5. Zhu W, Hu C, Ren Y, Lu Y, Song Y, Ji Y, et al. Green synthesis of zinc oxide nanoparticles using Cinnamomum camphora (L.) Presl leaf extracts and its antifungal activity. J Environ Chem Eng. 2021;9(6):106659.
6. Rajendiran M, Trivedi HM, Chen D, Gajendrareddy P, Chen L. Recent Development of Active Ingredients in Mouthwashes and Toothpastes for Periodontal Diseases. Molecules. 2021;26(7):2001.
7. Canta M, Cauda V. The investigation of the parameters affecting the ZnO nanoparticle cytotoxicity behaviour: A tutorial review. Biomater Sci. 2020;8(22):6157-74.
8. Singh TA, Das J, Sil PC. Zinc oxide nanoparticles: A comprehensive review on its synthesis, anticancer and drug delivery applications as well as health risks. Adv Colloid Interface Sci. 2020:102317.
9. Nagarajan M, Maadurshni GB, Tharani GK, Udhayakumar I, Kumar G, Mani KP, et al. Exposure to zinc oxide nanoparticles (ZnO-NPs) induces cardiovascular toxicity and exacerbates pathogenesis–Role of oxidative stress and MAPK signaling. Chem Biol Interact. 2022;351:109719.
10. Yu Z, Li Q, Wang J, Yu Y, Wang Y, Zhou Q, et al. Reactive oxygen species-related nanoparticle toxicity in the biomedical field. Nanoscale Res Lett. 2020;15:1-14.
11. Boey A, Ho HK. All roads lead to the liver: metal nanoparticles and their implications for liver health. Small. 2020;16(21):2000153.
12. Sruthi S, Ashtami J, Mohanan P. Biomedical application and hidden toxicity of Zinc oxide nanoparticles. Mater Today Chem. 2018;10:175-86.
13. Ali ZS, Khudair KK. Synthesis, Characterization of Silver Nanoparticles Using Nigella sativa Seeds and Study Their Effects on the Serum Lipid Profile and DNA Damage on the Rats' Blood Treated with Hydrogen Peroxide:

Zainab Sattar Ali and Khalisa Khadim Khudair. Iraqi J Vet Sci. 2019;43(2):23-37.

14. Devi RS, Gayathri R. Green synthesis of zinc oxide nanoparticles by using Hibiscus rosa-sinensis. Int J Curr Eng Technol. 2014;4(4):2444-6.
15. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol. 1957;28(1):56-63.
16. Kaplan MM. Alkaline phosphatase. Gastroenterology. 1972;62(3):452-68.
17. Olive PL, Banáth JP, Durand RE. Heterogeneity in radiation-induced DNA damage and repair in tumor and normal cells measured using the" comet" assay. Radiat Res. 1990;122(1):86-94.
18. Luna LG. Manual of histologic staining methods of the Armed Forces Institute of Pathology. 1968.
19. Snedecor GW, Cochran WG. Statistical methods, 8thEdn. Ames: Iowa State Univ Press Iowa. 1989;54:71-82.
20. Aboulhoda BE, Abdeltawab DA, Rashed LA, Abd Alla MF, Yassa HD. Hepatotoxic Effect of Oral Zinc Oxide Nanoparticles and the Ameliorating Role of Selenium in Rats: A histological, immunohistochemical and molecular study. Tissue Cell. 2020;67:101441.
21. Sudhakaran S, Athira S, Varma H, Mohanan P. Determination of the bioavailability of zinc oxide nanoparticles using ICP-AES and associated toxicity. Colloids Surf B. 2020;188:110767.
22. Yousef MI, Mutar TF, Kamel MAE-N. Hepato-renal toxicity of oral sub-chronic exposure to aluminum oxide and/or zinc oxide nanoparticles in rats. Toxicol Rep. 2019;6:336-46.
23. Guo Z, Luo Y, Zhang P, Chetwynd AJ, Xie HQ, Monikh FA, et al. Deciphering the particle specific effects on metabolism in rat liver and plasma from ZnO nanoparticles versus ionic Zn exposure. Environ Int. 2020;136:105437.
24. Attia H, Nounou H, Shalaby M. Zinc oxide nanoparticles induced oxidative DNA damage, inflammation and apoptosis in rat's brain after oral exposure. Toxics. 2018;6(2):29.
25. Liang X, Zhang D, Liu W, Yan Y, Zhou F, Wu W, et al. Reactive oxygen species trigger NF-κB-mediated NLRP3 inflammasome activation induced by zinc oxide nanoparticles in A549 cells. Toxicol Ind Health. 2017;33(10):737-45.
26. Piperigkou Z, Karamanou K, Engin AB, Gialeli C, Docea AO, Vynios DH, et al. Emerging aspects of nanotoxicology in health and disease: from agriculture and food sector to cancer therapeutics. Food Chem Toxicol. 2016;91:42-57.
27. Lam P-L, Wong R-M, Lam K-H, Hung L-K, Wong M-M, Yung L-H, et al. The role of reactive oxygen species in the biological activity of antimicrobial agents: An updated mini review. Chem Biol Interact. 2020;320:109023.
28. Hamza RZ, Al-Salmi FA, El-Shenawy NS. Evaluation of the effects of the green nanoparticles zinc oxide on monosodium glutamate-induced toxicity in the brain of rats. PeerJ. 2019;7:7460.
29. Abdel-Halim KY, Osman SR, Abdou GY. In vivo evaluation of oxidative stress and biochemical alteration as biomarkers in glass clover snail, Monacha cartusiana exposed to zinc oxide nanoparticles. Environ Pollut. 2020;257:113120.
30. Hao L, Chen L. Oxidative stress responses in different organs of carp (Cyprinus carpio) with exposure to ZnO nanoparticles. Ecotoxicol Environ Saf. 2012;80:103-10.
31. Schattauer SS, Bedini A, Summers F, Reilly-Treat A, Andrews MM, Land BB, et al. Reactive oxygen species (ROS) generation is stimulated by κ opioid receptor activation through phosphorylated c-Jun N-terminal kinase and inhibited by p38 mitogen-activated protein kinase (MAPK) activation. Biol Chem. 2019;294(45):16884-96.
32. Danabas D, Ates M, Tastan BE, Cimen ICC, Unal I, Aksu O, et al. Effects of Zn and ZnO Nanoparticles on Artemia salina and Daphnia magna organisms: Toxicity, accumulation and elimination. Science of The Total Environment. 2020;711:134869.
33. De Angelis I, Barone F, Zijno A, Bizzarri L, Russo MT, Pozzi R, et al. Comparative study of ZnO and TiO2 nanoparticles: physicochemical characterisation and toxicological effects on human colon carcinoma cells. Nanotoxicology. 2013;7(8):1361-72.
34. Elje E, Mariussen E, Moriones OH, Bastús NG, Puntes V, Kohl Y, et al. Hepato (Geno) Toxicity assessment of nanoparticles in a HepG2 liver spheroid model. Nanomaterials. 2020;10(3):545.
35. Jeffery TD, Richardson ML. A review of the effectiveness of hibiscus for treatment of metabolic syndrome. J Ethnopharmacol. 2021;270:113762.