Histological Changes of Male Westar Rats liver Following the Ingestion of Zinc Oxide Nanoparticles With special Emphasis on the Histochemical Alterations

Mosaid Abdullah Zaid Alferah*

Department of Biology, College of Science and Arts, Unaizah, Qassim University, Saudi Arabia.

*Correspondence: frh@qu.edu.sa

Abstract
The present investigation was carried out on forty apparently healthy mature male Westar rats weighing between 120-200 gm with average three months age. The rats were obtained from laboratory animal unite in the faculty of pharmacy, King Saud University and were divided randomly into four groups (10 rats/group). Group I (G1) was kept as a control, Group II (G2) was obtained ZnO NPs in a dose 100 mg/kg body weight, Group III (G3) was obtained ZnO NPs in a dose 250 mg/kg BW, Group IV (G4) was obtained ZnO NPs in a dose 500 mg/kg BW and all treated groups received ZnO NPs by oral gavage for 21 days. G1 showed normal histological structure of all hepatic tissues without any abnormalities. G2 and G3 showed mild to moderate steatosis, necrosis with focal scattered of inflammatory cells infiltration with fibrous tissue proliferation and moderate hepatocytes glycogen depletion. Meanwhile, G4 showed severe steatosis, diffuse degeneration of hepatic tissues with loss of the hepatic architectures, sever fibrous tissue proliferation with anti-inflammatory cells infiltration as well as severe congestion with the hepatic artery, sinusoids and Portal vein. In addition, sever sinusoidal dilatation accompanied by Kupffer cells hyperplasia in between the hepatic cords were clarified. Furthermore, with PAS stain, sever hepatocytes glycogen depletion were claimed. The present investigation was concluded that the ZnO NPs have potential oxidative stress in the hepatic tissues that may affect the function of the liver.

Keywords: Zinc oxide, nanoparticles, hepatotoxicity, histochemical, ZnO NPs

Introduction
Nanoparticles (NPs) are materials with a size range of approximately 1-100 nm [1] that have a very specific chemical and physical properties of size, shape and high ratio of surface area to volume. These qualities have made their suitable application for many medical and biological cases [2,3]. And also, Because of their small size, nanoparticles can cross many barriers and filters [4].

Zinc oxide nanoparticles (ZnO NPs) are an inorganic compound with the formula ZnO. It is a white powder that is insoluble in water, and it is widely used as an additive in numerous materials and products including rubbers, plastics, ceramics, glass, cement, lubricants, paints, ointments, adhesives, sealants, pigments, foods, batteries, ferrites, fire retardants, and first-aid tapes. Although it occurs naturally as the mineral zincite, most zinc oxide is produced synthetically. Furthermore, Zinc oxide (ZnO) nanopowders are available as powders and dispersions and exhibiting antibacterial, anti-corrosive, antifungal and UV filtering properties [5]. Currently, nano-ZnOs are widely used in personal care products; cosmetics and sunscreens [6].

ZnO NPs are one of the most widely used in consumer products. They are extensively used in cosmetics and sunscreens because of their efficient UV absorption properties. ZnO NPs are being used in the food industry as additives and in packaging due to their antimicrobial properties. They are also being explored for their potential use as fungicides in agriculture and as anticancer drugs and imaging in biomedical applications [7]. In addition, ZnO NPs have important application in the industry of electronic devices and paint industry. Moreover, these particles have been incorporated in polymeric matrices,
Animals and housing

Aim of work

The present study was performed to investigate and evaluate the hepatotoxicity of zinc oxide nanoparticles of male Westar rats.

Materials and method

Animals and housing

Forty apparently healthy mature male westar rats weighing between 120-200 gm with average three months age were obtained from laboratory animal unite in the faculty of pharmacy, King Saud University. The rats were randomly divided into four groups and kept in galvanized standard cages, ten animals/cage, under hygienic conditions and left for one week before starting the experiment for accommodation. Feed and water were available ad libitum. Temperature was recorded continuously, and maintained between (20 and 23°C) along the experimental period. A cycle of 14 h of light and 10 h of dark was fixed throughout the experiment. All animals were handled and all experiments were conducted in accordance with the protocols approved by King Saud University Animal Care Ethical Committee while the experimental procedures were carried out in accordance with international guidelines for care and use of laboratory animals.

Supplements (Nanoparticles)

Well-dispersed ZnO NPs (average particle size 10-30 nm) at 50 wt% in distilled water (Sigma, Aldrich) were used in the present study. The nanoparticles dispersion had the following characterization: concentration 50 wt.% in H2O; pH 5.5±0.1; density 1.7 g/ml ±0.1 g/ml.

Experimental design

Forty rats were divided randomly into four groups (10 rats/group) and subjected for 21 days to one of the following treatments:

- **Group I(G1)** kept as a control and fed with a basal diet without ZnO NPs for 21 days.
- **Group II(G2)** was obtained ZnO NPs in a dose 100 mg/kg body weight by oral gavage for 21 days.
- **Group III(G3)** was obtained ZnO NPs in a dose 250 mg/kg body weight by oral gavage for 21 days.
- **Group IV(G4)** was obtained ZnO NPs in a dose 500 mg/kg body weight by oral gavage for 21 days.

Histological and histochemical processing

At the end of experiment, cervical dislocation of rats and for histological and histochemical studies, livers were separately and small pieces from them were taken, fixed in neutral buffered formal in 10 %, dehydrated, cleared, and paraffin ionized for paraffin blocks and 5 micron sections were obtained, mounted on a glass slides and stained with Hematoxylin and Eosin (H&E), Periodic acid–Schiff (PAS) and Mercuric bromophenol blue according to Bancroft and Gamble.

Results

The histopathological examination of the liver in the control group (G1) revealed normal hepatic architecture; hepatic parenchyma, hepatic lobulation, hepatic cord, portal triad, hepatocytes and hepatic sinusoids. The liver parenchyma of these groups was observed very homogenous, intact and consisting of numerous hepatic lobules that were difficult demarcated from each other's by a very thin connective tissue septa or trabeculae in between, so the hepatic lobulations were not clear (Plate 1: Fig. A). Furthermore, the hepatic lobule appeared hexagonal in shape and had a central vein in their center. The major compartment of each hepatic lobule were the hepatocytes that represent about 80% of its structure and appeared irregular polygonal or polyhedral shaped cells typically with single, central, large vesicular nucleus with fine dispersed chromatins in most cases, however, some of them appeared occasionally bi-nucleated (Plate 1: Fig. B).
Hepatocytes were dorsally radiating from the central vein towards the periphery; the portal areas (portal triad) forming the hepatic cords. Moreover, the hepatic sinusoids were observed distributing in between the hepatic cords supplying the hepatocytes with normal, intact lining epithelium (Plate 1: Fig. B). Each hepatic lobule was bounded peripherally with portal triad that housing branches from portal vein, hepatic artery, lymph vessel and bile ductules (Plate 1: Fig. C and D). And also, strongly PAS positive reaction of the hepatic parenchyma was clarified where the glycogen contents were observed filling almost of the hepatocytes cytoplasm (Plate 1: Fig. E and F).

Plate 1. Sections of male Westar rats liver of control group (G1).
Figure (A): showing normal, homogenous, intact hepatic parenchyma; hepatic lobules, with normal central vein. H&E Obj. x10: Oc.x10. (B): Higher magnification of fig. A showing normal and intact hepatic lobule with normal, regular radiated hepatic cords (arrow) from the central vein (V) to the periphery of lobule. H&E Obj.x40 : Oc.x10 (C): showing normal and intact portal area. H&E Obj.x10 : Oc.x10
(D): Higher magnification of fig. C showing intact portal area; portal vein (V), hepatic artery (A), lymph vessel (L) and bile ductules (arrow). H&E Obj.x20 : Oc.x10 (E): showing strongly PAS positive reaction of the hepatocytes. PAS Obj.x10 : Oc.x10 (F): Higher magnification of fig. E showing the glycogen contents that filling almost of the hepatocytes cytoplasm. PAS Obj.x40 : Oc.x10.

Mean while, G2 treated with ZnO NPs in a dose of 100 mg/kg, bwt and G3 treated with ZnO NPs in a dose of 250 mg/kg, bwt by oral gavage for 21 days. Microvesicular steatosis; accumulations of small lipid droplets within the hepatocytes cytoplasm were also observed (Plate 2: Fig. B and C). In addition, focal scattered of inflammatory cells infiltration with fibrous connective tissue proliferation was demonstrated within the hepatic tissues especially portal areas (Plate 2: Fig. D, E and F). And also, moderate congestion within the portal vein in the portal triad was observed (Plate 3: Fig. G and H). Furthermore, moderate sinusoidal dilatation and congestion in between the hepatic cords were clarified (Plate 3: Fig. I). Moreover, with PAS stain, pale scattered patches of glycogen depletion within the hepatic parenchyma were demonstrated (Plate 3: Fig. J, K and L).

On the other hand, liver of G4 treated with ZnO NPs in a dose of 500 mg/kg, bwt showed severe microvesicular steatosis; accumulation of small lipid droplets in hepatocytes cytoplasm (Plate 4: Fig. B and C). And also, diffuse degeneration and necrosis of hepatic tissues with loss of the hepatic architectures were clearly observed (Plate 4: Fig. A). Sever fibrous tissue proliferation with anti-inflammatory cells infiltration; plasma cells, mast cells, kupper cells, lymphocytes and eosinophils were observed within the hepatic parenchyma especially portal triad (Plate 4: Fig. D and E). Hexagonal lobules are centered on the central vein that exhibited sever congestion and also within the portal triad, the portal vein was also showed sever congestion that overfilled with erythrocytes and some lymphocytes (Plate 4: Fig. F and Plate 5: Fig. G, H and I).
Furthermore, disorganization of hepatic cords was observed. In addition, severe degenerative changes which were evident in numerous hepatocytes; enlarged cells, had light and foamy cytoplasm filled with vacuoles of variable size that were tended to form cystic degeneration were claimed. Hepatocytes necrotic changes were evident; a small, pyknotic cellular nuclei with condensed chromatin, lack of nucleolus and acidophilic cytoplasm were recognized (Plate 4: Fig. A and C). Moreover, severe sinusoidal dilatation with congestion accompanied by Kupffer cells hyperplasia in between the hepatic cords were clarified (Plate 5: Fig. I). And also, with PAS stain, pale hepatic parenchyma with severe glycogen depletion within the hepatocytes were noticed (Plate 5: Fig. J, K and L).

Discussions
The present investigation revealed that the liver of the control group (G1) revealed normal hepatic architecture; hepatic parenchyma, hepatic lobulation, hepatic cord, hepatic portal triad, hepatocytes and hepatic sinusoids. Meanwhile, G2 treated with ZnO NPs in a dose of 100 mg/kg. bwt and G3 treated with ZnO NPs in a dose of 250 mg/kg. bwt showed mild to moderate steatosis and necrosis with moderate disorganization of hepatic cords. In addition, focal scattered of inflammatory cells infiltration with fibrous connective tissue proliferation was demonstrated within the hepatic tissues especially portal areas. Furthermore, moderate sinusoidal dilatation in between the hepatic cords and hepatocytes glycogen depletion were also observed.

With increasing dose of ZnO NPs, the liver toxicity became more obvious where, the liver of G4 treated with ZnO NPs in a dose of 500 mg/kg. bwt by oral gavage for 21 days. (A): showing sever disorganization of the hepatic cords, severe necrosis in the hepatocytes with pyknotic cellular nuclei and condensed chromatin. H&E Obj.x20 : Oc.x10. (B and C): showing sever microvesicular steatosis (arrow head). C) Higher magnification of fig. B showing the same. B, C) H&E Obj.x10 : Oc.x10 C) Obj.x40 : Oc.x10. (D and E): showing sever inflammatory cells infiltration with sever fibrous connective tissue proliferation within the hepatic parenchyma (arrow) with sever dilatation and congestion within the hepatic sinusoids (arrow head). D, E) H&E Obj. x20 : Oc.x10. (F): showing sever congestions within the portal vein in the portal triad (arrow head) and central vein (arrow). H&E Obj.x4 : Oc.x10.

Furthermore, disorganization of hepatic cords was observed.
Sever fibrous tissue proliferation with anti-inflammatory cells infiltration; plasma cells, mast cells, kupper cells, lymphocytes were observed. These investigations are coinciding with the description of Almansour et al., [27] who observed that the liver of ZnO NPs treated rats demonstrated sinusoidal dilatation accompanied by Kupffer cells activation and enlarged that lining the walls of sinusoids. This abnormality was also demonstrated in the toluidine blue stained semi thin sections. This vascular alteration was characterized by widening of capillaries lining the hepatic strands.Moreover, our findings are also goes hand in hand with Oligny and Lough [28] and Neyrinck [29] who reported that the sinusoidal dilatation in the liver of rats treated with ZnO NPs might be resulted from an injury of their sinusoids endothelia. On the other hand, Kupffer cells hyperplasia might be a sort of defense mechanism of detoxification contributed to hepatic oxidative stress induced by these particles.

Our investigation of Kupffer cells activation and hyperplasia in the hepatic cords were supported by the description of Hanley et al., [30] who clarified that Zinc oxide NPs were affect monocytes and macrophages by initiating production of interferon tumor necrosis factor by the peripheral blood monocytes. With PAS stain, sever hepatocytes glycogen depletion were observed. These investigations are in coincidence with the description of Almansour et al., [27] who observed that PAS stain exhibited partial hepatocytes glycogen content depletion. The later, was mainly observed in the degenerative hepatocytes. Moreover, Prussian blue reaction demonstrated precipitation of hemosiderin pigments in the hepatic tissues of ZnO NPs treated group.

The detected apoptosis in the liver of rats treated with ZnO NPs might be resulted from intercellular stress induced by these fine particles [31]. Apoptosis might be followed by mitochondrial swelling, endoplasmic reticulum dilatation and lysosomal rupture before shrinking and dissolution of nuclei [32].

Conclusion
From our results, we can conclude that the ZnO NPs have potential oxidative stress in the hepatic tissues that may affect the function of the liver.

Competing interests
The author declares that he has no competing interests.

Acknowledgements
I wish to express my deepest gratitude and sincere thanks to:
1) Dr. Wael A.M. Ghonimi, Professor Assistant of Histology and Cytology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt for hishelp, support and efforts in the histological examination, images capture, result reading and language editing.
2) Mr. Tayseir Rashed Almunizel, laboratory technician at the Faculty of Pharmacy, King Saud University, Riyadh, Saudi Arabia
who contributed to the care of animals during the experiment
3) Mr. Khalid Elfaki Ibrahim, technician at the Central
Laboratory, Department of Zoology, King Saud
University, Riyadh, Saudi Arabia who contributed to
the histological cutting and staining of sections.
4) Qassim University, for its financial support for this research.

Publication history
EJC: Gaetano Giuseppe Magro, University of Catania, Italy.
Received: 24-Dec-2017 Final Revised: 12-Jan-2018
Accepted: 18-Jan-2018 Published: 27-Jan-2018

References
1. Geneva, Switzerland. Nanotechnologies—Terminology and Definitions
for Nano-objects—Nanoparticle, Nanofibre and Nanoplate.
2. Erb U, Aust KT and Palumbo G.  
8. Ma H, Williams PL and Diamond SA.  
of different-sized ZnO nanoparticles in Caco-2 cells. Nanoscale Res Lett.
22. De Angelis J, Barone F, Zijno A, Bizzarri L, Russo MT, Pozzi R,
Franchini F, Giudetti G, Ubaldi C, Ponti J, Rossi F and De Berardis B.
Comparative study of ZnO and TiO2(2) nanoparticles: physicochemical
characterisation and toxicological effects on human colon carcinoma
23. Yin H, Casey PS, McCall MJ and Fenech M. Effects of surface chemistry
on cytotoxicity, genotoxicity, and the generation of reactive oxygen
species induced by ZnO nanoparticles. Langmuir. 2010; 26:13399-408. | Article | PubMed
24. Bancroft JD and Gamble M. Theory and practice of histological
25. Khorsandi L, Mansouri E, Oraziadeh M and Jozi Z. Curcumin
Attenuates Hepatotoxicity Induced by Zinc Oxide Nanoparticles in
and Brain JD. Effects of zinc oxide nanoparticles on Kupffer cell
phagosomal motility, bacterial clearance, and liver function. Int J
27. Olgyn LL and Lough J. Hepatic sinusoidal ectasia. Hum Pathol.
consequences on hepatic metabolism. Bull Mem Acad R Med Belg.
29. Almansour MI, Alferah MA, Shraideh ZA and Jarrar BM. Zinc oxide
nanoparticles hepatotoxicity: Histological and histochemical study
30. Johar D, Roth JC, Bay GH, Walker JN, Kroczak TJ and Los M.
Inflammatory response, reactive oxygen species, programmed
(necrotic-like and apoptotic) cell death and cancer. Rocz Akad Med

Citation: