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.

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, severe 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, severe 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 female 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, cerebral dislocation of rats and for histological and histochemical studies, livers were separately and small pieces from them were taken, fixed in neutral buffered formalin 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 [22].

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 lobe 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).

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 showed mild to moderate disorganization of the hepatic cords with moderate necrosis within the hepatocytes. (Plate 2: Fig. A). Microvesicular steatosis; accumulation of small lipid droplets in hepatocytes cytoplasm were also observed (Plate 2: Fig. B and C). 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). Severe fibrous tissue proliferation with anti-inflammatory cells infiltration; plasma cells, mast cells, kuppfer 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 severe congestion and also within the portal triad, the portal vein was also showed severe 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, sever degenerative changes which were evident in numerous hepatocytes; enlarged cells, had light and foamy cytoplasm filled with vacules 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, sever 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 sever 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.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.
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. Hexagonal lobules are centered on the central vein that exhibited moderate to severe congestion with the hepatic artery, sinusoids and Portal vein. These investigations are coinciding with Landsiedel et al., [25] and Watson et al., [26] who claimed that the histological analysis of liver sections showed areas of necrosis accompanied by significant inflammatory cell infiltration near the hepatic portal triad at day 7 of exposure. By this time, the engulfed ZnO ENPs would have dissolved, releasing zinc ions, which may have caused toxicity to the surrounding hepatocytes.

Furthermore, disorganization of hepatic cords was observed. In addition, sever 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 observed were recognized. These results are in parallelism with the result of Almansour et al., [27] who observed that the liver of ZnO NPs treated rats for 21 days demonstrated well-defined necrotic hepatocytes. And also, hepatocytes cytoplasmic vacuolation with partial cytoplasmic swelling was well demonstrated. Moreover, several forms of nuclear abnormality were exhibited: binucleation, nuclear vesiculation, anisokaryosis, karyolysis and nuclear membrane irregularity and apoptosis.

Sever sinusoidal dilatation accompanied by Kupffer cells hyperplasia in between the hepatic cords were clarified. These findings are in agreement with the results of Almansour et al., [27] who showed that the liver of ZnO NPs treated rats exhibited 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 between 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.

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