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Article · February 2017

DOI: 10.1016/j.etap.2017.02.015

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Zinc oxide nanoparticles hepatotoxicity: Histological and histochemical study

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ARTICLE INFO

Article history:
Received 27 June 2016
Received in revised form 11 February 2017
Accepted 13 February 2017
Available online 16 February 2017

Keywords:
Nanotoxicity
ZnO NPs
Hepatotoxicity
Hydropic degeneration
Glycogen depletion
Hemosidrosis

ABSTRACT

Zinc oxide nanoparticles (ZnO NPs) are widely used in industry and cosmetic products with promising investment in medical diagnosis and treatment. However, these particles may reveal a high potential risk for human health with no information about hepatotoxicity that might be associated with their exposure. The present work was carried out to investigate the histological and histochemical alterations induced in the hepatic tissues by naked 35 nm ZnO NPs. Male Wistar albino rats were exposed to ZnO NPs at a daily dose of 2 mg/kg for 21 days. Liver biopsies from all rats under study were subjected to histopathological examinations. In comparison with the control rats, the following histological and histochemical alterations were demonstrated in the hepatic tissues of rats exposed to ZnO NPs: sinusoidal dilatation, Kupffer cells hyperplasia, lobular and portal triads inflammatory cells infiltration, necrosis, hydropic degeneration, hepatocytes apoptosis, anisokaryosis, karyolysis, nuclear membrane irregularity, glycogen content depletion and hemosidrosis. The findings of the present work might indicate that ZnO NPs have potential oxidative stress in the hepatic tissues that may affect the function of the liver. More work is needed to elucidate the toxicity and pathogenesis of zinc oxide nanoparticles on the vital organs.

1. Introduction

Nanoparticles (NPs) are biologically reactive due to their small size and larger surface area to volume ratio (Lanone and Boczkowski, 2006; Yu et al., 2011, 2012). The available information indicate that small size NPs have easier clearance from the site of injection, longer circulating residue and slower passage to the interstitial spaces than the large size ones (Hussain et al., 2005; Wang et al., 2010; Yu et al., 2011). In addition, NPs with all sizes can induce oxidative stress and generate free radicals that could cause damage to tissues, cells and macromolecules where smaller particles are more toxic than the larger ones (Abdelhalim and Jarrar, 2011, 2012).

Zinc oxide NPs are currently being produced in high tonnage and utilized in various cosmetic and makeup products. These particles are utilized in sunscreens, ointments, tooth pastes and in some coatings for protection from UV radiation (Xia et al., 2008; Zvyagin et al., 2008; Smijs and Pavel, 2011; Vanderiel and Jong 2012). Also, ZnO NPs have recently received much attention due to their possible applications in cancer therapy (Rasmussen et al., 2010). These fine particles exhibited selective apoptosis in some cancer cells via p53 pathway mediation (Hanley et al., 2008; Guo et al., 2008; Zhang et al., 2008; Nair et al., 2009; Hackenberg et al., 2010; Akhtar et al., 2012). In addition, ZnO NPs have important application in the industry of electronic devices and paint industry. Furthermore, these particles have been incorporated in polymeric matrices, packaging materials and food systems to provide antimicrobial activity (Vanderiel and Jong 2012; Tayel et al., 2011). Zinc oxide NPs have bactericidal effects on both Gram-positive and Gram-negative bacteria with activity against spores that are resistant to high temperature and high pressure (Arabi et al., 2012).

Zinc oxide NPs toxicity was found to be related to their solubility and ability to generate free radicals (Osmond and McCall, 2010). In addition, these particles have the ability to cross the cell membrane and some blood vital organs barrier. Moreover, some reports indicated that ZnO NPs could exhibit cytotoxicity, genotoxicity, oxidative stress, mitochondrial dysfunction, apoptosis, neurotoxicity and inflammatory response (Yang et al., 2009; Yuan et al., 2010;

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http://dx.doi.org/10.1016/j.etap.2017.02.015
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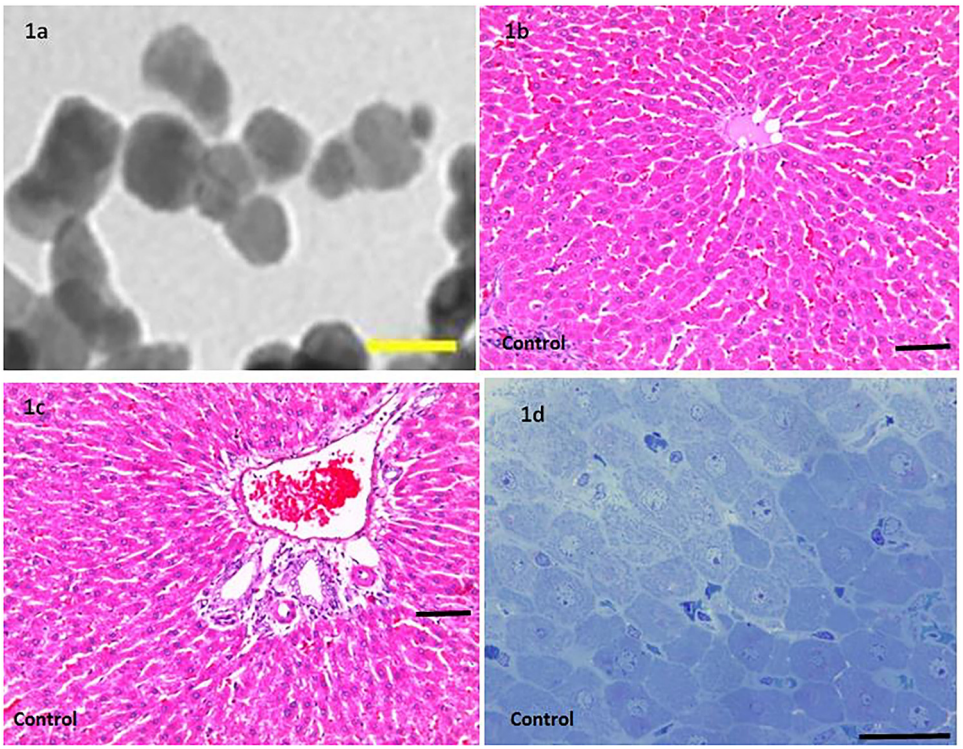

Fig. 1. (a–d) (a) Scanning electron micrograph demonstrating morphology and size of the used ZnO NPs, (Bar = 40 nm). (b) Section in the liver of control rats demonstrating normal hepatic architecture, hepatic strands and hepatocytes. H&E stain. (Bar = 40 μm). (c) Section in the liver of control rats demonstrating normal portal triads. H&E stain, (Bar = 40 μm). (d) Section in the liver of control rats demonstrating Normal sinusoids and Kupffer cells. Toluidine blue stain, (Bar = 15 μm).

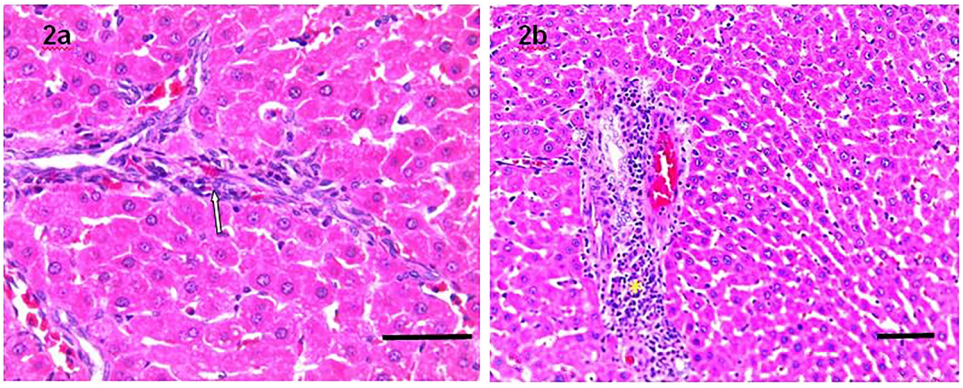

Fig. 2. (a–b) Light photographs of section in the liver of ZnO NPs treated rats demonstrating: (a) Lobular inflammatory cells infiltration (arrow). H&E stain. (Bar = 20 μm) (b) Microgranulomus inflammatory infiltration (star). H&E stain. (Bar = 40 μm).

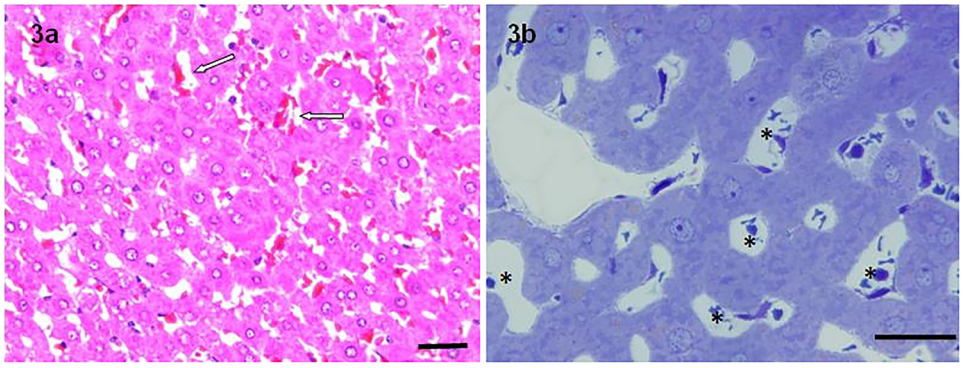

Fig. 3. (a–b) Light photographs of section in the liver of ZnO NPs treated rats demonstrating: (a) Sinusoidal dilation (arrows). H&E stain, (Bar = 30 μm) (b) Sinusoidal dilation (stars). Toluidine blue stain, (Bar = 10 μm).

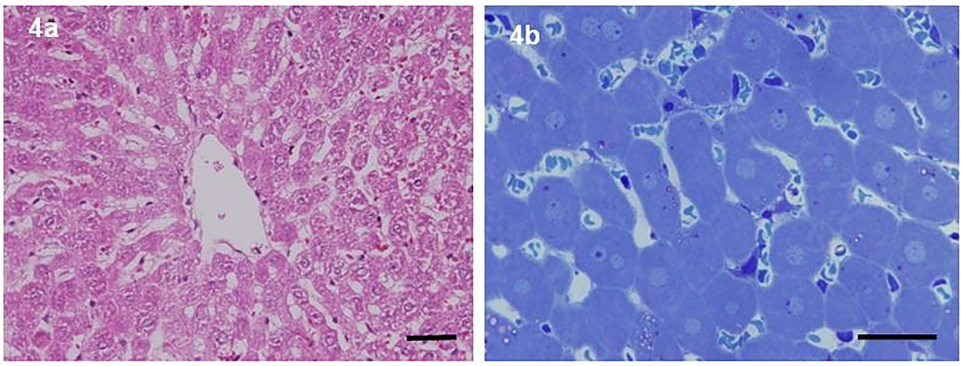

Fig. 4. (a–b) Light photographs of sections in the liver of ZnO NPs treated rats demonstrating: (a) Kupffer cells hyperplasia. H&E stain, (Bar = 20 μm) (b) Kupffer cells enlargement. Toluidine blue stain, (Bar = 10 μm).

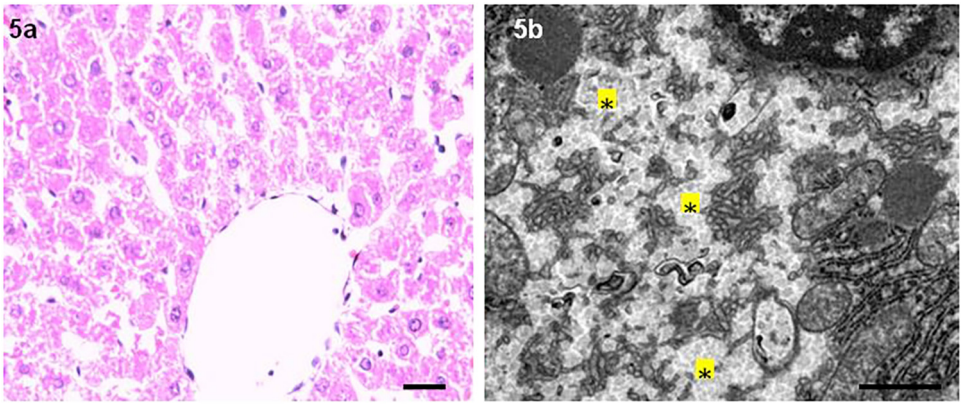

Fig. 5. (a–b) Light photograph of section in the liver of ZnO NPs treated rat demonstrating necrotic hepatocytes. H&E stain, (Bar = 20 μm) (b) Transmission electron micrographs of ZnO NPs treated rats demonstrating necrotic hepatocytes (stars), (Bar = 1 μm).

Sharma et al., 2011; Pasupuleti et al., 2010). Other studies indicated potential risks of ZnO NPs on the liver, spleen, lung, kidney and heart as target organs (Jachak et al., 2012; Li et al., 2012). Furthermore, some previous reports showed that ZnO nanorods were more toxic than spherical ones and the smaller ones were more toxic than the larger ones (Hsiao and Huang, 2011).

Zinc oxide NPs are rapidly taken into the circulatory system with the highest accumulation in the vital organs (Lanone and Boczkowski, 2006). Some in vivo reports indicated that inhalation of ZnO NPs induced bronchoalveolar inflammation together with damage in the tissue of liver and kidney (Wang et al., 2010; Yu et al., 2012; Warheit et al., 2009). In addition, exposure to ZnO NPs (50–70 nm) induced potent but reversible inflammation while 10 nm of these particles resulted in granulomatous inflammation (Sayes et al., 2007; Cho et al., 2012). Other studies showed that exposure to ZnO NPs induced proliferation of airway epithelial cells, goblet cells hyperplasia and pulmonary fibrosis (Cho et al., 2011). Moreover, ZnO NPs significantly altered levels of serum alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total protein, creatine kinase, and lactate dehydrogenase compared to the unexposed controls (Wang et al., 2010).

It is expected that the investment of ZnO NPs will grow and human body will be increasingly exposed to these nanomaterials via inhalation, ingestion and to lesser extent via dermal exposure. In parallel to the widespread use and rapid commercialization of ZnO NPs, a concern has been growing regarding their toxicity. The knowledge of ZnO NPs potential risks in human health is limited with a need to be identified. Full attention must be given to safety and toxicological issues of these nanoparticles on the tissue, cells and macromolecule of human body. To the best of the authors knowledge, limited information is available on the histological and histochemical alterations induced by ZnO NPs on the vital organs. Therefore, the present study was carried out to explore the alterations that might be induced by these particles in the hepatic tissues.

2. Materials and methods

2.1. Nanoparticles

Well-dispersed ZnO NPs (average particle size 35 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 H_2O; pH 5.5 ± 0.1; density 1.7 g/ml ± 0.1 g/ml. To evaluate the veracity of the manufacturer's specification, the particle size was assessed by using Jeol transmission electron microscope at 80 kv (JEM-1011, Japan) in the Research Center, College of Science at King Saud University (Fig. 1a).

Zinc oxide NPs have rapid dissolution in acidic condition (pH 5.5). Accordingly, nanoparticles dispersion was disaggregated by ultrasonication before being diluted with sterile acidic distilled water (pH 5.5) at 37 °C immediately before use. Nanoparticles solution was prepared so that the necessary dose could be administered i.p. in a volume of 400 μl.

2.2. Animals and conditions

Twenty healthy male Wistar albino rats from King Saud University colony of the same age (10–12 weeks old) weighing 210–230 g

were used. The animals were housed at 24 ± 1 °C, on 12 h dark/light cycle, randomly assigned and separately caged to one test group and a control one (10 rat each).

2.3. Experimental protocol

The control animals received single i.p. injection of 400 μl of the distilled water. Each rat of the test group received a daily i.p. injection with a dose of 2 mg/kg bw of 35 nm ZnO NPs for 21 days. Administration volume was adjusted based on body weight measured each week.

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.

2.4. Histological and histochemical processing

All members of both groups were euthanized by cervical dislocation after two days of the final administration. Fresh liver biopsies from each rat of both groups were cut rapidly, fixed in neutral buffered formalin, dehydrated with ascending grades of ethanol (70, 80, 90, 95 and 100%), cleared in xylene, impregnated then embedded and blocked out in paraffin wax. Paraffin sections (4–5 μm) of the control and ZnO NPs treated rats were stained with the following histological and histochemical stains: hematoxylin and eosin (H&E), periodic acid-Schiff (PAS), Mallory trichrome, reticulin stain and Prussian blue reaction. Stained sections of control and treated mice were examined for alterations in the architecture, portal triads, hepatocytes, sinusoids and for histochemical alterations.

2.5. Semithin sections preparation

Small pieces of liver from each rat minced into small cubes of 1 mm in length were fixed in 2.5% glutaraldehyde fixative in 0.1 M phosphate buffer (pH 7.4) for 24 h at 4 °C. Specimens were then post fixed in 2% osmium tetroxide (OsO_4) in cacodylate buffer for 90 min at room temperature. Tissues were washed in the buffer and dehydrated at 4 °C through a gradual series of acetone and embedded in Epon-araldite resin mixture. Semithin sections (500–1000 nm) were obtained in a Leica EM UC6 ultramicrotome mounted on glass slides and stained with toluidine blue stain.

2.6. Microscopic examination

Histological sections of all rats under study were examined by using Leica photomicroscope equipped with Leica Las EZ digital camera.

3. Results

3.1. Control rats

Microscopic examination of the control rat liver sections demonstrated normal well preserved and kept intact lobular architecture and zonal accentuation together with normal hepatocytes, sinusoids, Kupffer cells and hepatic portal triads (Fig. 1b–d). No histological or histochemical abnormalities were detected in the hepatic tissue of any member of this group of rats.

3.2. Rats exposed to 35 nm ZnO NPs

In comparison with the control group, microscopic examination of hepatic tissues of rats exposed to 2 mg/kg daily dose of 35 nm ZnO NPs for 21 days showed the following histological and histochemical alterations:

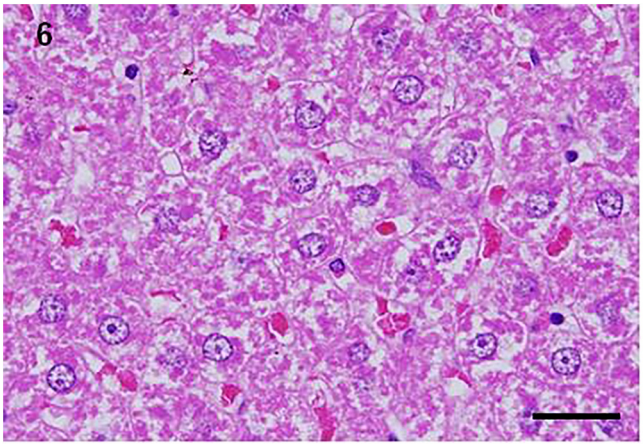

Fig. 6. Light photograph of section in the liver of ZnO NPs treated rat demonstrating hydropic degenerated hepatocytes. H&E stain, (Bar = 30 μm).

3.2.1. Inflammatory cell infiltration

Lobular and portal triads focal scattered inflammatory cells infiltration was demonstrated in the hepatic tissues of this group of rats (Fig. 2a–b). Inflammatory cells were seen intervening the hepatic strands and surrounding degenerative hepatocytes. Lobular inflammation was mostly seen in the form of microgranulomus one. In addition, pericentral vein inflammation was also seen.

3.2.2. Sinusoidal dilatation

The liver of ZnO NPs treated rats exhibited sinusoidal dilatation accompanied by Kupffer cells activation (Fig. 3a). This abnormality was also demonstrated in the toluidine blue stained semithin sections (Fig. 3b). This vascular alteration was characterized by widening of capillaries lining the hepatic strands.

3.2.3. Kupffer cells hyperplasia

Enlarged and prominent Kupffer cells lining the walls of sinusoids were seen in the liver of rats subjected to 35 nm ZnO NPs (Fig. 4a). This abnormality was also noticed in the liver semithin sections of all members of this group (Fig. 4b).

3.2.4. Necrosis

The liver of ZnO NPs treated rats for 21 days demonstrated well-defined necrotic hepatocytes (Fig. 5a–b). Some of the insulted hepatocytes exhibited eosinophilic amorphous cytoplasm with occasional apoptotic characterization.

3.2.5. Hepatocytes hydropic degeneration

Hepatocytes cytoplasmic vacuolation with partial cytoplasmic swelling was well demonstrated (Fig. 6). This alteration was mainly observed in the pericentral and midzonal hepatocytes.

3.2.6. Nuclear alterations

Several forms of nuclear abnormality were exhibited by the hepatic tissues of this group of rats. Nuclear changes were demonstrated in the form of binucleation, nuclear vesiculation, anisokaryosis, karyolysis and nuclear membrane irregularity and apopotosis (Fig. 7a–c).

3.2.7. Hepatocytes glycogen depletion

In comparison with the control liver sections where PAS stain exhibited normal hepatocytes content of glycogen, partial hepatocytes glycogen content depletion was demonstrated in rats exposed

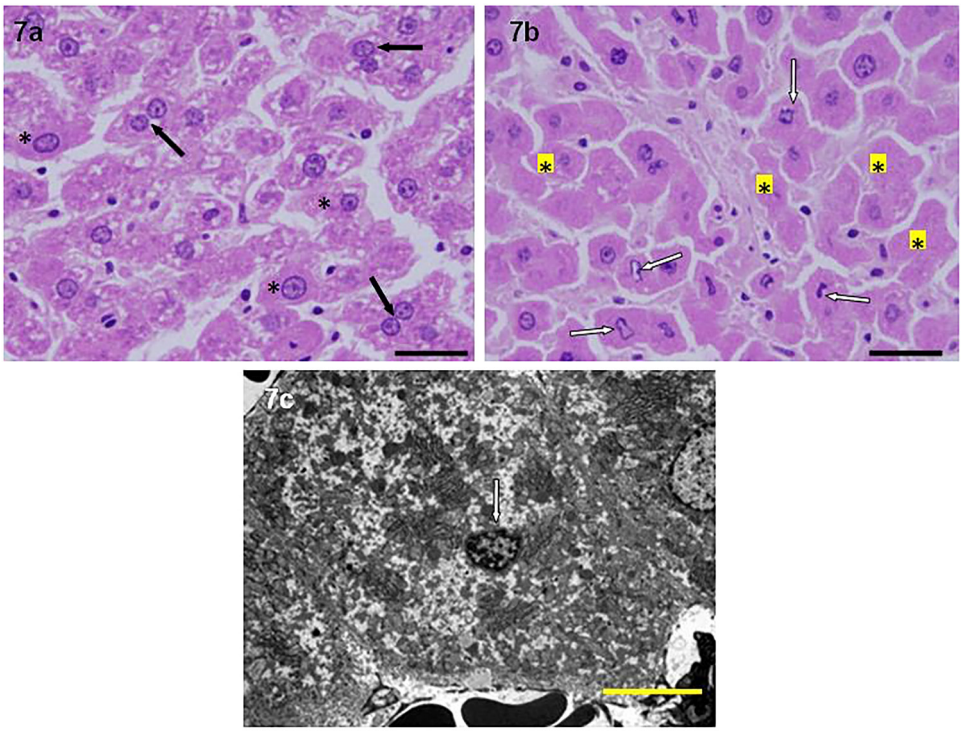

Fig. 7. (a–c) (a) Light photographs of section in the liver of ZnO NPs treated rats demonstrating binucleation (arrows) and anisokaryosis (stars). H&E stain, (Bar = 30 μm) (b) Light photographs of section in the liver of ZnO NPs treated rats demonstrating karyolysis (yellow stars) and nuclear membrane irregularity (arrows). H&E stain, (Bar = 30 μm) (c) Transmission electron micrographs of ZnO NPs treated rats demonstrating apoptotic hepatocytes (arrow), (Bar = 5 μm). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

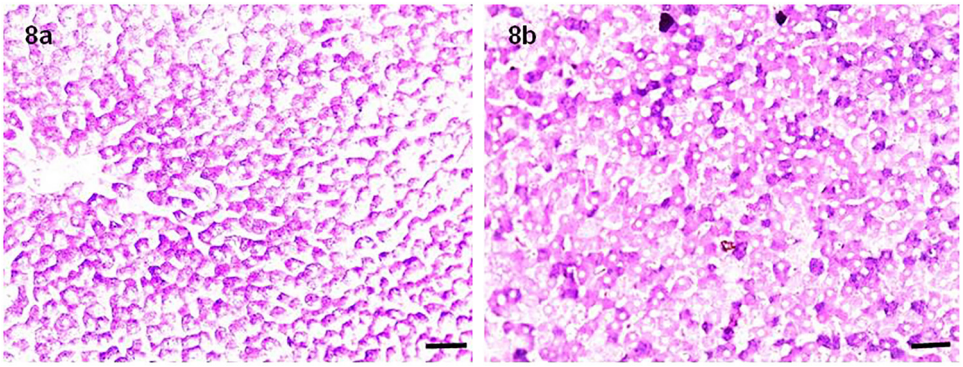

Fig. 8. (a–b) (a) Light photographs of section in the liver of control rats demonstrating normal hepatocytes glycogen content. PAS stain, (Bar = 40 μm) (b) Light photographs of section in the liver of ZnO NPs treated rat demonstrating hepatocytes partial glycogen content depletion. PAS stain, (Bar = 40 μm).

to 35 nm ZnO NPs (Fig. 8a–b). This glycogen depletion was mainly observed in the degenerative hepatocytes.

3.2.8. Hemosiderin pigments precipitation

Prussian blue reaction demonstrated precipitation of hemosiderin pigments in the hepatic tissues of this group of rats while no precipitation was seen in those of the control rats (Fig. 9a–b).

No bile-duct hyperplasia, fibrosis, cirrhosis or fatty change was detected in the liver of any member of the ZnO NPs treated group. In addition, reticulin stain demonstrated no alterations in the net of the hepatic reticular fibers of these rats.

4. Discussion

The central role of liver in detoxification, metabolism and excretion of drugs and xenobiotics, making this vital organ highly susceptible to their adverse and toxic effects (Singh et al., 2011). Some previous studies indicated that liver is one of the target organs of ZnO NPs (Vanderiel and Jong, 2012). Oral and intraperitoneal administration of 100 nm ZnO NPs accumulated these particles in the liver, lung, spleen and kidney (Li et al., 2012). Moreover, inhalation of 20 nm ZnO NPs by rats twice daily for 3 days resulted in increased zinc content in the liver (Vanderiel and Jong, 2012). Some reports showed that ZnO NPs could cause damage in liver, kidneys and lungs tissues together with collagen loss (Landsiedel et al., 2010; Wang et al., 2010).

Some of the hepatic histological alterations as demonstrated by the findings of the present work are online with those of previous studies, who reported inflammation, hepatic necrosis and degeneration induced by ZnO NPs (Landsiedel et al., 2010). In addition, the findings of the present study showed glycogen depletion and hemosiderin pigments precipitation in the hepatic tissues due to ZnO NPs exposure. These together may indicate negative impact on

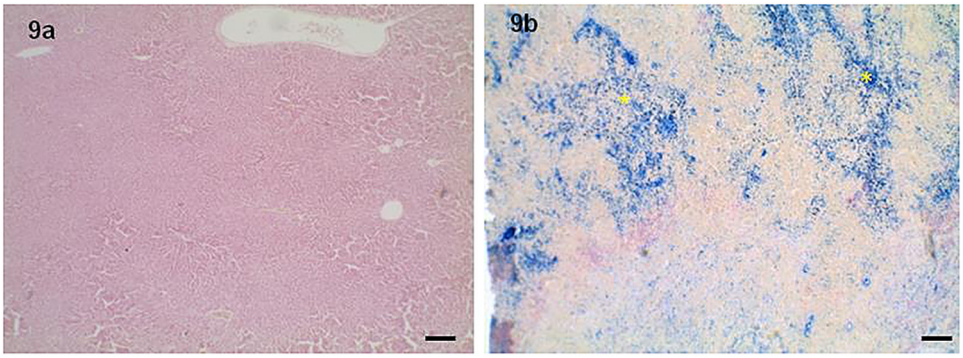

Fig. 9. (a–b) (a) Light photographs of section in the liver of control rat demonstrating absence of hemosiderin preciptation. Prusian blue reaction, (Bar = 40 μm) (b) Light photographs of section in the liver of ZnO NPs treated rat demonstrating hemosiderin precipitation (stars). Prussian blue reaction, (Bar = 40 μm).

the structure and function of the liver induced by these nanoparticles.

The findings of the present study illustrated that ZnO NPs dilated sinusoids and activated Kupffer cells. Sinusoidal dilatation is a sort vascular alteration characterized by focal widening of sinusoidal spaces resulted from hepatocytes atrophy and necrosis (Oligny and Lough, 1992). In addition, 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 (Neyrinck, 2004).

Hepatocytes of ZnO NPs-treated rats exhibited hydropic degeneration characterized by vacuolization, cloudy swelling and ballooning degeneration with pale cytoplasm and poorly delineated and displaced nuclei. This cytoplasmic injury might be resulted from disturbances of hepatocytes membranes function that lead to massive influx of water and Na^+ due to ZnO NPs effects. Moreover, hepatocytes ballooning degeneration might be accompanied by leakage of lysosomal hydrolytic enzymes that lead to cytoplasmic degeneration (Del Monte, 2005).

The current work showed that ZnO NPs induced hepatocytes necrosis. This finding may indicate recent hepatotoxicity injury resulted from the cessation of protein synthesis due to subjection to these nanoparticles. Necrosis is induced by toxicants that attack the cell organelles specially the mitochondria, endoplasmic reticulum and nucleus thus disturbing their activity (Singh et al., 2011). Cell lines of human bronchial and alveolar epithelial cells were necrotized by nano-size zinc oxide and showed low mitochondrial membrane potential and loss of membrane integrity (George et al., 2010). In addition, necrosis was evidenced by glutathione deletion, reduction in catalase and superoxide dismutase activity, and inner mitochondrial membrane depolarization (Sharma et al., 2011; Moos et al., 2010; De Berardis et al., 2010). Zinc oxide NPs were reported to affect monocytes and macrophages by initiating production of interferon tumor necrosis factor by the peripheral blood monocytes (Hanley et al., 2009).

The detected apoptosis in the liver of rats treated with ZnO NPs might be resulted from intercellular stress induced by these fine particles. Zinc oxide NPs were reported to induce apoptosis, p53 unregulation, dermal fibrosis and increased cell cycle progression (Meyer et al., 2011). Apoptosis might be followed by mitochondrial swelling, endoplasmic reticulum dilatation and lysosomal rupture before shrinking and dissolution of nuclei (Johar et al., 2004).

The inflammatory cells infiltration induced by ZnO NPs may suggest that these particles might interact with the interstitial hepatic tissues leading to immune response resulted from ROS generation (Johar et al., 2004). Some studies showed that ZnO NPs could increase neutrophils numbers with affinity to some proteins such as immunoglobulin and lipoproteins (Landsiedel et al., 2010; John et al., 2010). Moreover, some previous reports showed that inhaled ZnO NPs can escape macrophages to the interstitium resulting in inflammation and energy metabolism disturbance (Osmond and McCall, 2010; Yan et al., 2012).

The rats subjected to ZnO NPs demonstrated hemosiderosis and hepatocytes depletion of glycogen storage. Hepatic hemosiderin deposition is an indicator of negative impact on the iron recycling capacity of the liver. Iron overload disorder may lead to hemochromatosis and/or micronodular cirrhosis. On the other hand, glycogen depletion by ZnO NPs may indicate negative impact on carbohydrate absorption or on the enzymes involved in the process of glycogenesis or/and glycolysis.

Oxidative dissolution of ZnO NPs and releasing of Zn^{2+} in the hepatic tissue could deplete hepatocytes dissolved oxygen leading to ROS generation (Sharma et al., 2009, 2011; Kao et al., 2012). Zinc is a component of many enzymes and transcription factors (John et al., 2010). The entrance of ZnO NPs into the cell increases Zn^{+2} content in the cytosol leading to disruption of cellular zinc homeostasis (Osmond and McCall, 2010; Kao et al., 2012). The induced alterations by ZnO NPs might be related to increased intracellular zinc ions, inducing oxidative stress and interaction with the tissues components (Vanderiel and Jong, 2012; Landsiedel et al., 2010).

One might conclude from the results of the present study that exposure to ZnO NPs produces hepatic histological and histochemical alterations that might affect the functions of the liver. This hepatotoxicity might be resulted from the induced oxidative stress due to disturbance in the pro-oxidant/antioxidant of the hepatic tissues leading to hepatic tissues damage. Moreover, the results of current work may raise the concerns about the potential risk on human health that might be related with numerous applications of ZnO NPs. More work is needed to elucidate the potential risks of these particles on the vital organs and their pathogenesis. The present study may suggest finding out whether coating or capping of ZnO NPs can reduce their toxicity.

Competing interest

The authors declare that they have no competing interests of any type.

Acknowledgment

The authors would like to extend their sincere appreciation to the Deanship of Scientific Research at King Saud University for funding this research project (RG135-040).

References

Abdelhalim, M., Jarrar, B., 2011. Gold nanoparticles administration induced prominent inflammatory, central vein intima disruption, fatty change and Kupffer cells hyperplasia. Lipids Health Dis. 10, 113.

Abdelhalim, M., Jarrar, B., 2012. Histological alterations in the liver of rats induced by different gold nanoparticles sizes, doses and exposure duration. J. Nanobiotechnol. 10, 5.

Akhtar, M., Ahmad, M., Kumar, S., Khan, M., Ahmad, J., Alrokyan, S., 2012. Zinc oxide nanoparticles selectively induce apoptosis in human cancer cells through reactive oxygen species. Int. J. Nanomed. 7, 845–857.

Arabi, F., Imandar, M., Negahdary, M., Masoud, N., Noughabi, M., Akbari-dastjerdi, H., Fazilati, M., 2012. Investigation anti-bacterial effect of zinc oxide nanoparticles upon life of Listeria monocytogenes. Ann. Biol. Res. 3 (7), 3679–3685.

Cho, W., Duffin, R., Howie, S., Scotton, C., Wallace, W., Macnee, W., Bradley, M., Megson, I., Ken Donaldson, K., 2011. 'Progressive severe lung injury by zinc oxide nanoparticles; the role of Zn^{2+} dissolution inside lysosomes. Part. Fibre Toxicol. 8, 27.

Cho, W., Duffin, K., Poland, C., 2012. Differential pro-inflammatory effects of metal oxide nanoparticle and their soluble ions in vitro and in vivo: zinc and copper nanoparticles, but not their ions, recruit eosinophils to the lung. Nanotoxicology 6 (1), 22–35.

De Berardis, B., Civitelli, G., Condello, M., Lista, P., Pozzi, R., Arancia, G., Meschini, S., 2010. Exposure to ZnO nanoparticules induces oxidative stress and cytotoxicity in human colon carcinoma cells. Toxicol. Appl. Pharmacol. 246 (3), 116–127.

Del Monte, U., 2005. Swelling of hepatocytes injured by oxidative stress suggests pathological changes related to macromolecular crowding. Med. Hypotheses 64 (4), 818–825.

George, S., Pokhrel, S., Xia, T., Gilbert, B., Ji, Z., Schowalter, M., Rosenauer, A., Damoiseaux, R., Bradley, K., Mädler, L., Nel, A., 2010. Use of a rapid cytotoxicity screening approach to engineer safer zinc oxide nanoparticles through iron doping. ACS Nano 4 (1), 15–29.

Guo, D., Wu, C., Jiang, H., Li, Q., Wang, X., Chen, B., 2008. Synergistic cytotoxic effect of different sized ZnO nanoparticles and daunorubicin against leukemia cancer cells under UV irradiation. J. Photochem. Photobiol. B 93, 119–126.

Hackenberg, S., Scherzed, A., Kessler, M., Froelich, K., Ginzkey, C., Koehler, C., Burghartz, M., Hagen, R., Kleinsasser, N., 2010. Zinc oxide nanoparticles induce photocatalytic celldeath in human head and neck squamous cell carcinoma cell lines in vitro. Int. J. Oncol. 37 (6), 1583–1590.

Hanley, C., Layne, J., Punnoose, A., Reddy, K.M., Coombs, I., Coombs, A., Feris, K., Wingett, D., 2008. Preferential killing of cancer cells and activated human T cells using zinc oxide nanoparticles. Nanotechnology 19, 295103–295113.

Hanley, C., Thurber, A., Hanna, C., Punnose, A., Zhang, J., Wingett, D., 2009. The influences of cell type and ZnO nanoparticle size on immune cell cytotoxicity and cytokine induction. Nanoscale Res. Lett. 4 (12), 1409–1420.

Hsiao, I., Huang, Y., 2011. Effects of various physiochemical characteristics on the toxicities of ZnO and TiO_2 nanoparticles toward human lung epithelial cell. Sci. Total Environ. 409 (7), 1219–1228.

Hussain, S., Hess, K., Gearhart, J., Geiss, K., Schlager, J., 2005. In vitro toxicity of nanoparticles in BRL-3A rat liver cells. Toxicol. In Vitro 19, 975–983.

Jachak, A., Lai, S., Hida, K., Suk, J., Markovic, N., Biswal, S., Breysse, P., Hanes, J., 2012. Transport of metal oxide nanoparticles and single-walled carbon nanotubes in human mucus. Nanotoxicology 6, 614–622.

Johar, D., Roth, J., Bay, G., Walker, J., Kroczak, T., Los, M., 2004. Inflammatory response, reactive oxygen species, programmed (necrotic-like and apoptotic) cell death and cancer. Rocz. Akad. Med. Bialymst. 49, 31–39.

John, E., Laskow, T., Buchser, W., Pitt, B., Basse, P., Butterfield, L., Kalinsk, P., Lotz, M., 2010. Zinc in innate adaptive tumor immunity. J. Transl. Med. 8, 118.

Kao, Y., Chen, Y., Cheng, T., Chiung, Y., Liu, P., 2012. Zinc oxide nanoparticles interfere with zinc ion homeostasis to cause cytotoxicity. Toxicol. Sci. 125 (2), 462–472.

Landsiedel, R., Ma-Hock, L., Van Ravenzwaay, B., Oesch, F., 2010. Gen toxicity studies on titanium dioxide and zinc oxide nanomaterials used for UV-protection in cosmetic formulation. Nanotoxicology 4, 364–381.

Lanone, S., Boczkowski, J., 2006. Biomedical applications and potential health risks of nanomaterials: molecular mechanisms. Curr. Mol. Med. 6, 651–663.

Li, C.H., Shen, C., Cheng, Y., Huang, S., Wu, C., Kao, C., Liao, J., Kang, J., 2012. Organ distribution, clearance and genotoxicity of oral administered zinc oxide nanoparticles in mice. Nanotoxicology 6 (7), 746–756.

Meyer, K., Rajanahalli, P., Ahmed, M., Rowe, J., Hong, Y., 2011. Nanoparticles induces apoptosis in human dermal fibroblasts via p53 and p38 pathways. Toxicol. In Vitro 25 (8), 1721–1726.

Moos, P., Chung, K., Woessner, D., Honeggar, M., Cutter, N., Veranth, J., 2010. ZnO particulate matter requires cell contact for toxicity in human colon cancer cells. Chem. Res. Toxicol. 23 (4), 733–739.

Nair, S., Sasidharan, A., Divya, F., Rani, V., Menon, D., Nair, S., Manzoor, K., Raina, S., 2009. Role of size scale of ZnO nanoparticles and microparticles on toxicity toward bacteria and osteoblast cancer cells. J. Mater. Sci. Mater. Med. 20, 235–241.

Neyrinck, A., 2004. Modulation of Kupffer cell activity: physio-pathological consequences on hepatic metabolism. Bull. Mem. Acad. R. Med. Belg. 159 (5–6), 358–366.

Oligny, L., Lough, J., 1992. Hepatic sinusoidal ectasia. Hum. Pathol. 23, 953–956.

Osmond, M., McCall, M., 2010. Zinc oxide nanoparticles in modern sunscreens: an analysis of potential exposure and hazard. Nanotoxicity 4 (1), 15–41.

Pasupuleti, S., Alapati, S., Ganapathy, S., Anumolu, G., Pully, N., Prakhya, B., 2010. Toxicity of zinc oxide nanoparticles through oral route. Toxicol. Ind. Health 28 (8), 675–686.

Rasmussen, J., Martinez, E., Louka, P., Wingett, D., 2010. Zinc oxide nanoparticles for selective destruction of tumor cells and potential for drug delivery applications. Expert Opin. Drug Deliv. 7 (9), 1063–1077.

Sayes, C., Reed, K., Warheit, D., 2007. Assessing toxicity of fine and nanoparticles: comparing in vitro measurements to in vivo pulmonary toxicity profiles. Toxicol. Sci. 97 (1), 163–180.

Sharma, V., Shukla, R., Saxena, N., Parmar, D., Das, M., Dhawan, A., 2009. 'DNA damaging potential of zinc oxide nanoparticles in human epidermal cells. Toxicol. Lett. 185 (3), 211–218.

Sharma, V., Anderson, D., Dhawan, A., 2011. Zinc oxide nanoparticles induce oxidative stress and genotoxicity in human liver cells (HepG2). J. Biomed. Nanotechnol. 7 (1), 98–99.

Singh, A., Bhat, T., Sharma, O., 2011. Clinical biochemistry and hepatotoxicity. J. Clin. Toxicol. S4, 001.

Smijs, T., Pavel, S., 2011. Titanium oxide and zinc oxide nanoparticles in sunscreens: focus on their safety and effectiveness. J. Nanotechnol. Sci. Appl. 4, 95–112.

Tayel, A., El-Tras, W., Moussa, S., El-Baz, A., Maahrous, H., Salem, M., Brimer, L., 2011. Antibacterial action of zinc oxide nanoparticles against foodborne pathogens. J. Food Saf. 31 (2), 211–218.

Vanderiel, R., Jong, W., 2012. A review of mammalian toxicity of ZnO nanoparticles. Nanotechnol. Sci. Appl. 5, 61–71.

Wang, L., Ding, W., Zhang, F., 2010. Acute toxicity of ferric oxide and zinc oxide nanoparticles in rats. J. Nanosci. Nanotechnol. 10 (12), 8617–8624.

Warheit, D., Sayes, C., Reed, K., 2009. Nanoscale and fine zinc oxide particles: can in vitro assay, accurately forecast lung hazards following inhalation exposure? Environ. Sci. Technol. 43 (20), 7939–7945.

Xia, T., Kovochich, M., Liong, M., Madler l. Gilbert, B., Shi, H., Yeh, J., Zink, J., Nel, A., 2008. Comparison of the mechanism of toxicity of zinc oxide and cerium oxide nanoparticles based on dissolution and oxidative stress properties. ACS Nano 2 (10), 2121–2134.

Yan, G., Huang, Y., Bu, Q., Ly, L., Deng, P., Zhou, J., Wang, Y., Yang, Y., Liu, Q., Cen, X., Zhao, Y., 2012. Zinc oxide nanoparticles cause nephrotoxicity and kidney metabolism alterations in rats. J. Environ. Sci. Health A Tox. Hazard. Subst. Environ. Eng. 47 (4), 577–588.

Yang, H., Liu, C., Yang, D., Zhang, H., Xi, Z., 2009. Comparative study of cytotoxicity, oxidative stress and genotoxicity induced by four typical nanomaterials: the role of particle size, shape and composition. J. Appl. Toxicol. 29 (1), 69–78.

Yu, J., Baek, M., Chung, H., Choi, S., 2011. Effects of phsicochemical properties of zinc oxide nanoparticles on cellular uptake. J. Phys. Conf. Ser. 304 (1), 012007.

Yu, M., Park, J., Jon, S., 2012. Targeting strategies for multifunctional nanoparticles in cancer imaging and therapy. Theranostics 2 (1), 3–44.

Yuan, J., Chen, Y., Zha, H., Song, L., Li, C.Y., Li Xia, X., 2010. Determination, characterization and cytotoxicity on HELF cells of ZnO nanoparticles. Colloids Surf. B Biointerfaces 76 (1), 145–150.

Zhang, Y., Chen, W., Wang, S.P., Liu, Y., Pope, C., 2008. Phototoxicity of zinc oxide nanoparticles conjugates in human ovarian cancer. J. Biomed. Nanotechnol. 4, 432–438.

Zvyagin, A.V., Zhao, X., Gierden, A., Sanchez, W., Ross, J.A., Roberts, M.S., 2008. Imaging of zinc oxide nanoparticles penetration in human skin in vitro and in vivo. J. Biomed. Opt. 13 (6), 064031.