Nanoscience and Nanotechnology Letters
Vol. 7, 763–769, 2015

Ultrastructural Hepatic Alterations Induced by 35 nm Zinc Oxide Nanoparticles

Mansour Almansour[1], Laszlo Sajti[2], Walid Melhim[3], and Bashir Jarrar[4,*]

[1] *Zoology Department, College of Science, King Saud University, 11451, Saudi Arabia*
[2] *The Nanoparticle Group, Department of Nanotechnology, Hannover University, 30419, Germany*
[3] *Electron Microscopy Unit, College of Medicine, King Faisal University, 31982, Saudi Arabia*
[4] *Department of Biological Sciences, College of Science, Jerash University, 26150, Jerash, Jordan*

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 high potential risk for human health with little information is available about their toxicity. The present study was carried out to investigate the ultrastrcutural alterations induced in the hepatic tissues by ZnO NPs. Male Wistar albino rats were exposed to ZnO NPs at a daily dose of 2 mg/kg for 21 days. Furthermore, liver biopsies from all rats under study were subjected and processed for transmission electron microscopy and ultrastructural examination. Exposure to ZnO NPs has induced the following ultrastructural alterations: sinusoidal dilatation, Kupffer cells enlargement and activation, mitochondrial crystolysis and swelling, endoplasmic reticulum dilatation and vesiculation, myelin figures formation, karyopyknosis, nuclear membrane irregularity, chromatin fragmentation and glycogen depletion. These findings may suggest that ZnO NPs can induce ultrastructural alterations in the hepatic tissues resulted from disturbance of the pro-oxidant/antioxidant system leading to cellular alterations and affecting the liver functions. The results raise the concerns about the safety associated with ZnO NPs applications and highlight on the need to elucidate probable nanotoxicity that might be induced by these particles in the vital organs.

Keywords: Zinc Oxide Nanoparticles, Hepatocytes, Nanotoxicity, Oxidative Stress, Ultrastructural Alterations.

1. INTRODUCTION

Nanoparticles (NPs) are biologically reactive due to their small size and larger surface area to volume ratio.[1,2] Small size of these particles have longer circulating residue and slower passage to the interstitial spaces from the vasculature than the large size ones.[3–5] In addition, NPs can induce oxidative stress that could cause damage to tissues, cells and macromolecules where smaller particles are more reactive and more toxic than the larger ones.[6–8]

Zinc oxide nanoparticles (ZnO NPs) are currently being produced in large scales and utilized in various cosmetic products such as makeup, sunscreen, ointments, foot care and others.[9,10] Moreover, these particles are used in some products invested for the protection from UV radiation together with other ones that might be inhaled or ingested.[11,12] Furthermore, ZnO NPs have promising application in cancer therapy due to their ability to exhibit apoptosis in cancer cells via p53 mediation pathway.[13,14] In addition, ZnO NPs have been incorporated in the industry of electronic devices, paint industry to provide antimicrobial activity in many applications including the packaging materials and food systems.[12,15] 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.[16]

Zinc oxide NPs have the ability to cross the cell membrane and may also have the potential to cross barriers to the vital organs. It is also 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 by skin contact. Some studies reported that ZnO NPs could exhibit cytotoxicity, genotoxicity, oxidative stress, mitochondrial dysfunction, membrane damage, apoptosis, neurotoxicity and inflammatory response.[17,18] Other studies showed that the toxicity of ZnO NPs was determined mainly by their solubility and generation of the reactive oxygen species (ROS).[19] On the other hand, some investigations indicated potential risks of ZnO NPs on the liver, spleen, lung, kidney and hearts as target organs with more accumulation was

*Author to whom correspondence should be addressed.

produced by the smaller particles.[20] Other reports indicated that ZnO nanorods were more reactive than the spherical ones and the smaller particles were more toxic than the larger ones.[21]

Zinc oxide NPs are rapidly taken into the circulatory system with highest accumulation in the vital organs and in tissues with high blood flow.[22, 23] Some *in vivo* reports indicated that inhalation of ZnO NPs could induce bronchoalveolar inflammation together with damage in the tissues of liver and kidneys.[24, 25] In addition, exposure to ZnO NPs (10–70 nm) induced potent inflammation together with goblet cells hyperplasia and pulmonary fibrosis.[26–28] 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.[23]

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 almost lacking with a need to be identified. To the best of the authors knowledge, limited information is available on the ultrastrucural alterations induced by ZnO NPs on the vital organs. Therefore, the present work was carried out to explore the ultrastructural alterations induced by ZnO NPs in the hepatic tissues.

2. MATERIALS AND METHODS

2.1. Nanoparticles

Zinc oxide NPs was purchased from Sigma, Aldrich and were used in the present study as received. According to the manufacturer, the nanoparticles dispersion had the following characterization: average particle size 35 nm; concentration 50 wt.% in H_2O; pH 5.5 ± 0.1; density 1.7 g/ml $\pm$ 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. Dose chosen was based on data from previous studies.[20, 23]

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 ip 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 gramme 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 rats each).

2.3. Experimental Protocol

The control animals received a daily intraperitoneal (ip) injection of 400 μl sterile acidic distilled water (pH 5.5). Each rat of the test group received a daily ip injection with a dose of 2 mg/kg 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 animal care bioethical committee, King Saud University. Furthermore, the experimental procedures were carried out in accordance with international guidelines for care and use of laboratory animals.

2.4. Electron Microscopic Investigation

Small pieces of liver from each rat were minced into small cubes of 1 mm in length and fixed in 2.5% glutaraldehyde 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 minutes at room temperature. Tissue blocks were washed in the buffer and dehydrated at 4 °C through a gradual series of acetone and embedded in Epon-araldite resin mixture.[29–31] Semithin sections (500–1000 nm thick) were obtained with glass knife on a Leica EM UC6 ultramicrotome, stained with toluidine blue (1%), examined and photographed using light microscopy. Ultrathin sections (60 nm thick) were obtained with a diamond knife on Leica EM UC6 ultramicrotome and stained with 0.5% uranyl acetate and 3% lead citrate using Leica automated EM stainer.[32]

2.5. Ultramicroscopic Examination

Examination of the ultrathin sections was carried out by using Joel transmission electron microscope (JEM-1011, Japan) at 80 kv.

3. RESULTS

After 21 days of consecutive ZnO NPs administration, no mortality occurred in any of the two experimental groups. In addition, no significant difference was observed in food consumption and water intake between ZnO NPs treated rats and the control ones. The average body weight gain in the treated rats was significantly lower in comparison with the control ones.

3.1. Control Rats

Semithin and ultrathin sections of the control rats demonstrated normal hepatocytes as well as normal sinusoids lined with Kupffer cell with no signs of abnormality. The hepatocytes revealed normal nucleoplasm with round nuclei surrounded by even distinct regular nuclear envelop demonstrating fine granular euchromatin and dense heterochromatin. The cytoplasm of these hepatocytes appeared crowded with organelles mainly the mitochondria, endoplasmic reticulum (ER), free and bounded

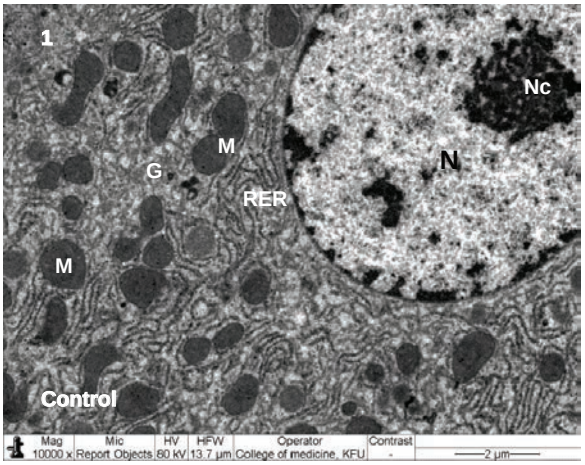

Fig. 1. Transmission electron micrograph of control rat demonstrating normal ultrastructure of hepatocytes, with rounded nucleus (N), prominent nucleolus (Nc) numerous of mitochondria (M), glycogen particles (G), rough endoplasmic reticulum (RER) loaded with bounded ribosomes. ×10000.

ribosomes, lysosomes and glycogen particles (Fig. 1). Furthermore, the control hepatocytes demonstrated mitochondria in orthodox conformation together with regular parallel organized ER.

3.2. Exposed Rats

In comparison with the control group, electron microscopic examination of the hepatic tissues of rats exposed to 2 mg/kg daily dose of 35 nm ZnO NPs for 21 days showed the following remarkable ultrastructural alterations.

3.2.1. Sinusoidal Dilatation

Examination of semithin sections stained with toluidine blue revealed marked to moderate hepatic sinusoidal dilation in all rats treated with ZnO NPs with variable degree of sinusoidal widening (Figs. 2(a)–(b)). Reactive Kupffer sinusoidal cells were also encountered with this architectural alteration.

3.2.2. Kupffer Cells Alterations

Some Kupffer cells showed ball-like rounding, ruffling, process retraction, vacuolization, multiple lysosomes and deformed nuclei. These macrophages displayed phagocytic debris and minute electron dense bodies in their cytoplasm (Figs. 3(a)–(c)).

3.2.3. Mitochondrial Alterations

Hepatocytes of ZnO NPs treated rats demonstrated mitochondrial pleomorphism in the form of enlargement, elongation, angulations, swelling and cristolysis. Some degenerated mitochondria demonstrated widening of mitochondrial intercristae spaces while other lacked cristae. Moreover, some affected mitochondria showed ruptured membranes (Figs. 4(a)–(b)).

3.2.4. Alterations in the Endoplasmic Reticulum

Some hepatocytes of rats subjected to ZnO NPs showed changes in the arrangement and distribution of the ER in the form of dilatation, denudation and vesiculation (Figs. 5(a)–(b)). Moreover, partial destruction and reduction of ER profile together with a loss of parallel arrays and stacks shortening were demonstrated by hepatocytes of the exposed rats. In addition, ribosomal dropping was observed in the hepatocytes of this group of rats.

3.2.5. Multilammellar Myelin Bodies Formation

Membranous phagolysosomed multilayered myelin figures were demonstrated in some affected hepatocytes (Figs. 6(a)–(b)).

3.2.6. Glycogen Depletion

While the control hepatocytes demonstrated normal glycogen content, partial glycogen contents depletion was seen in the degenerated hepatocytes of the rats subjected to ZnO NPs (Figs. 7(a)–(b)).

3.2.7. Nuclear Alterations

Nuclear ultrastructural changes in the hepatocytes of rats exposed to ZnO NPs were observed in the form

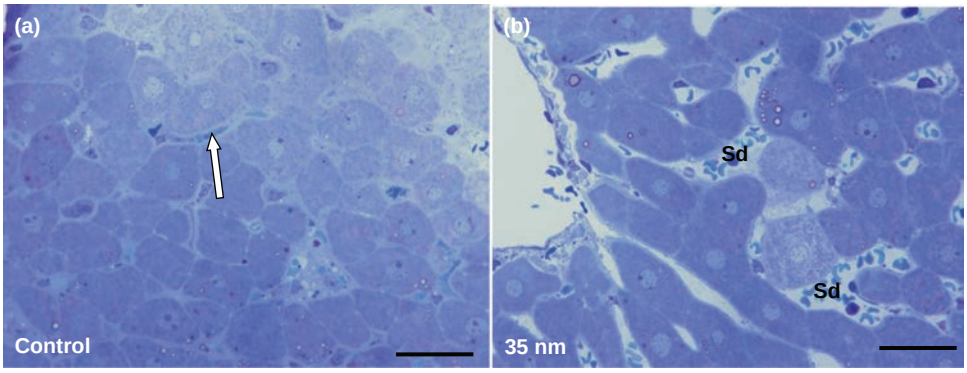

Fig. 2. (a) Semithin section of control rat demonstrating normal sinusoids (arrow). Toluidine blue stain, (Bar = 10 μm) (b) semithin section of ZnO NPs-treated rat demonstrating dilated sinusoids (Sd). Toluidine blue stain, (Bar = 10 μm).

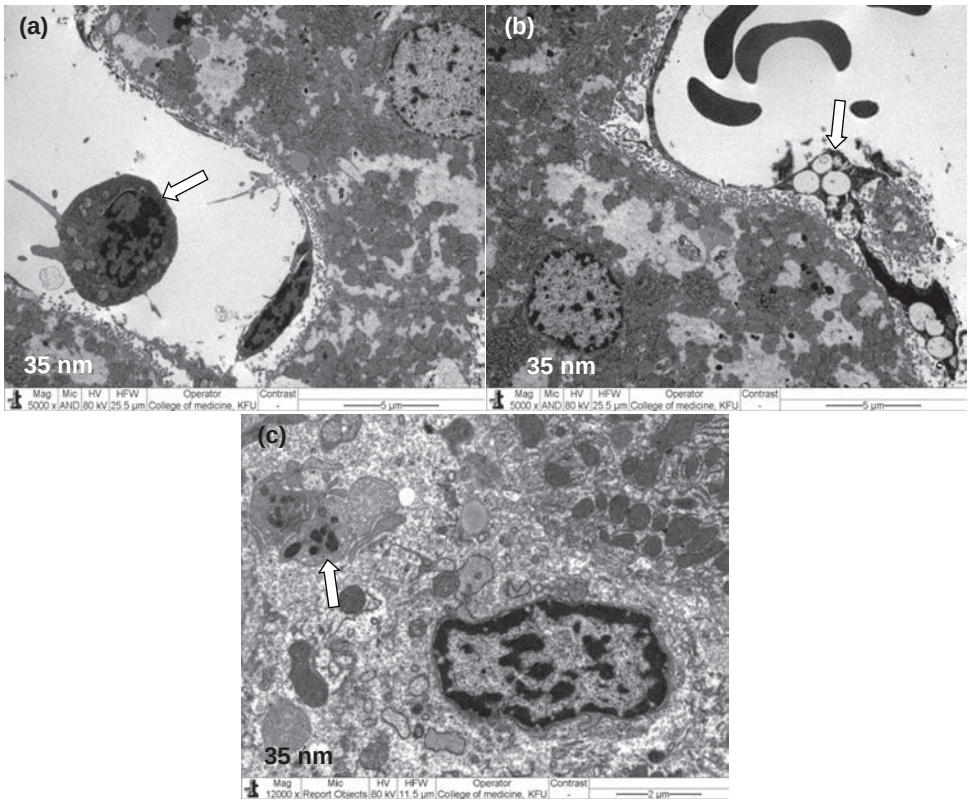

Fig. 3. Transmission electron micrographs of ZnO NPs treated rats demonstrating: (a) Ball-like rounding and cellular process retraction (arrow). ×5000 (b) Cytoplasmic vacuolization (arrow). ×5000 (c) Phagolysosomed dense bodies (arrow). ×12000.

karyopyknosis, nuclear membrane irregularity with indentation and chromatin fragmentation. Hepatocytes shrunken micronuclei with reticular-pattern chromatin condensation and apoptotic activity were also observed. Furthermore, it was noticed that hepatocytes with apoptotic nuclei demonstrated almost normal organelles (Figs. 8(a)–(b)).

No significant ultrastructural changes concerning the cytoplasmic vacuoles, number of lysosomes and lipids droplets accumulation were observed in the hepatocytes of ZnO NPs-treated rats.

4. DISCUSSION

The liver is the site of detoxification and homeostasis and plays central role in the metabolism and excretion of drugs and xenobiotics that make it highly susceptible to their adverse and toxic effects.[33] Several previous studies indicated the liver as one of the target organs of ZnO NPs where these particles accumulated in the hepatic tissues and induced damage to this vital organ.[12, 20, 23]

The findings of the present study illustrated that ZnO NPs could induce dilated sinusoids and activated Kupffer

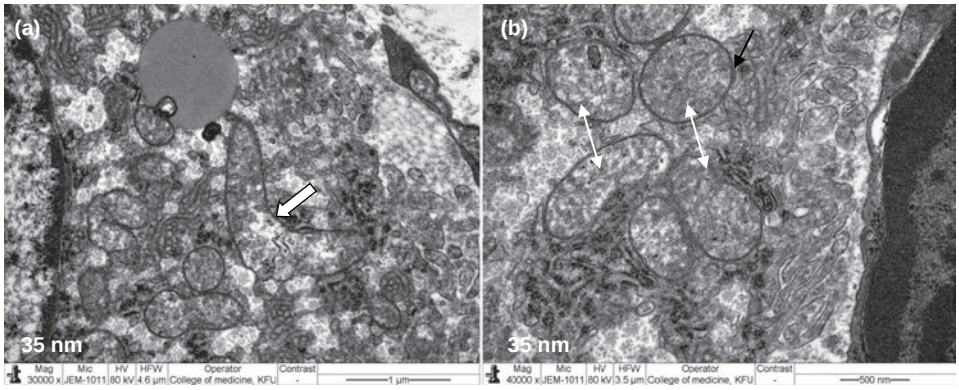

Fig. 4. Transmission electron micrographs of ZnO NPs treated rats demonstrating: (a) mitochondrial enlargement, elongation and angulations (arrow). ×30000 (b) Mitochondrial swelling and cristolysis (double-headed arrows). ×40000.

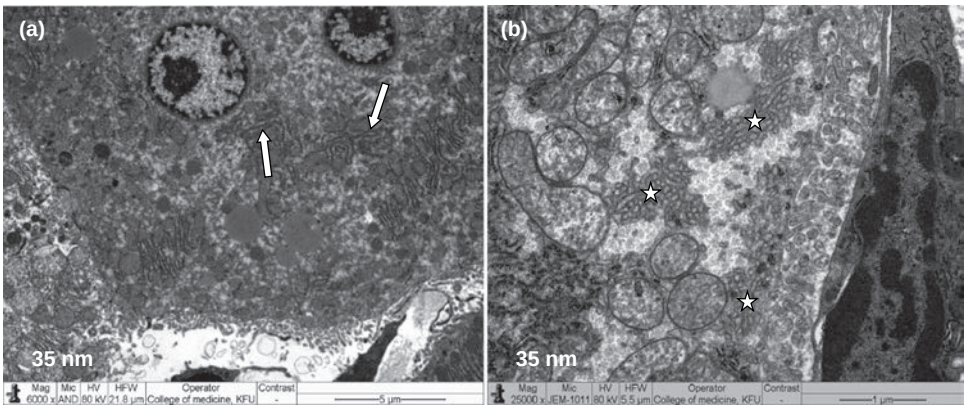

Fig. 5. Transmission electron micrographs of ZnO NPs treated rats demonstrating: (a) endoplasmic reticulum dilatation and loss of parallel arrays and stacks shortening (arrows). ×6000 (b) Endoplasmic reticulum vesiculation (stars). ×25000.

cells. Sinusoidal dilatation is a sort of vascular alteration associated with hepatocytes atrophy and necrosis.[34] The sinusoidal dilatation in the liver of rats treated with ZnO NPs might be resulted from deterioration of these sinusoids endothelia. On the other hand, the resulted Kupffer cells activation might be a sort of defense mechanism of detoxification contributed to hepatic oxidative stress induced by ZnO NPs subjection.[35]

The mitochondria play an essential role in the hepatic metabolism, ROS homeostasis and in cell regulation where most liver chronic diseases are associated with hepatocytes damaged mitochondria. The findings of the present work illustrated that the mitochondria were the most affected organelles by the toxicity of ZnO NPs. The mitochondrial damage was demonstrated in the form of enlargement, swelling, crystolysis and pleomorphism confirming some previous reports indicated the mitochondrion as a target organelle of ZnO NPs toxicity.[20] The resulted mitochondrial swelling might indicate response to acute injury induced by ZnO NPs associated with alteration in the hepatocyte osmolarity caused water and salt influx to the mitochondrion matrix affecting the integrity of the inner membrane. Furthermore, crytolysis induced by these particles might impair oxidative phosphorylation, energy production and reduce the efficiency of the mitochondrial electron transport system. Several reports indicated that high level of ROS in the mitochondria could cause damage and depolarize the membranes of this organelle.[11, 36–38] Some cell line studies indicated that nano-size zinc oxide could reduce the mitochondrial membrane potential leading to membrane integrity loss, glutathione deletion and reduction in the activity of catalase and superoxide dismutase.[39, 40]

The findings of the present work showed that ZnO NPs could affect hepatocytes endoplasmic reticulum in the form of destruction, loss of array, vesiculation and stack shortening. This ultrastructural alteration might be resulted from hepatocytes redox disturbance induced by ZnO NPs in the ER membranes and their associated enzymes. These ER abnormalities would indicate that subjection to ZnO NPs could decrease the surface density of these organelles that might negatively impact proteins and lipids synthesis of the affected hepatocytes.

Moreover, the ultrastructural findings of the present study showed that ZnO NPs could induce sequence of alterations leading to hepatocytes necrosis and apoptosis.

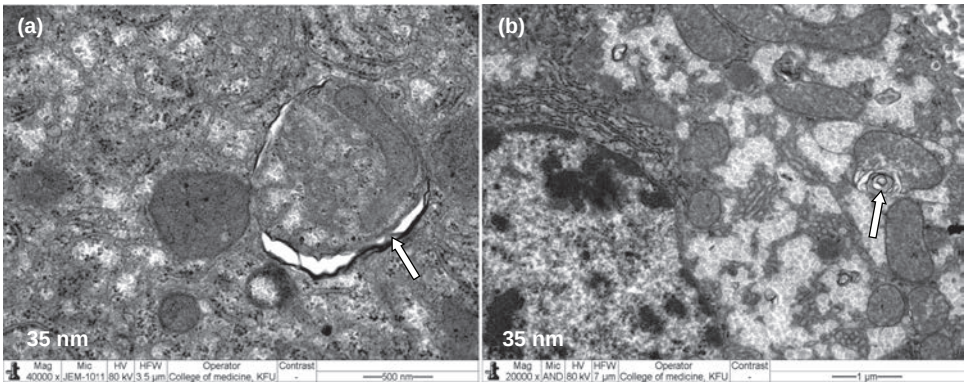

Fig. 6. Transmission electron micrographs of ZnO NPs treated rat demonstrating: (a) myelin figure surrounding angulated mitochondrion (arrow). ×40000 (b) Electron-dense multilayered myelin like material adjacent to the outer mitochondrial membrane (arrow). ×20000.

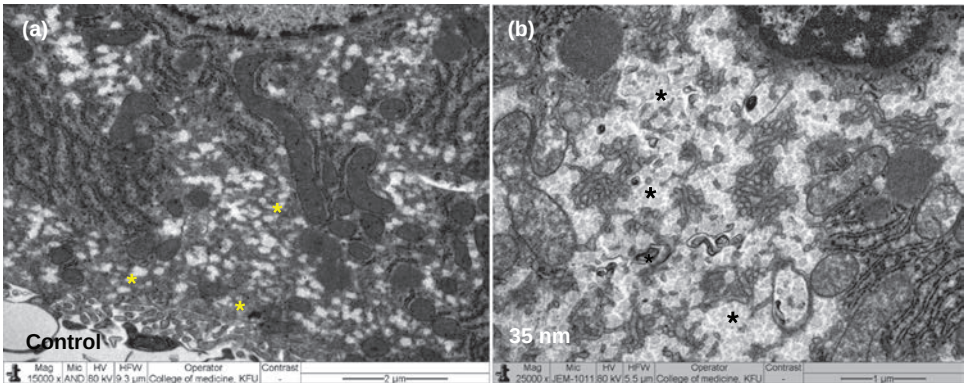

Fig. 7. Transmission electron micrographs of: (a) control hepatocytes demonstrating normal glycogen content (yellow stars). ×15000 (b) Hepatocyte of exposed rat demonstrating glycogen content depletion (stars). ×25000.

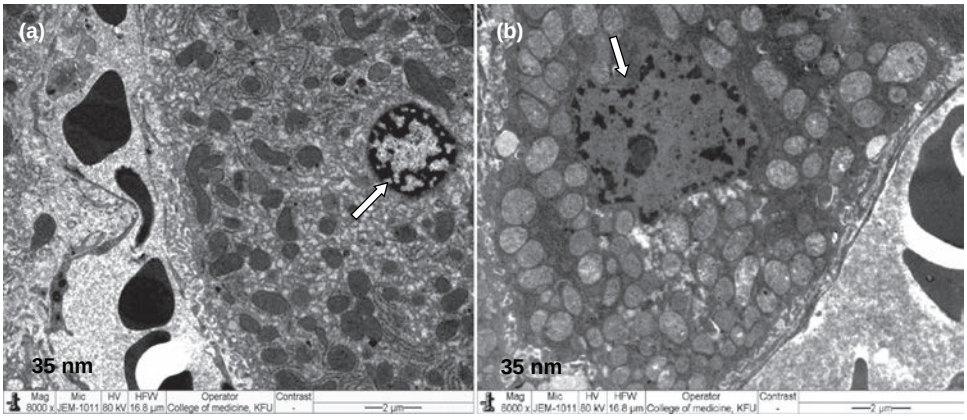

Fig. 8. Transmission electron micrographs of ZnO NPs treated rats demonstrating hepatocytes with: (a) shrunken micronucleus with reticular-pattern chromatin (arrow). Note that this apoptotic hepatocyte demonstrates almost normal organelles. ×8000 (b) Nuclear membrane irregularity with indentation (arrow). ×8000.

These alterations proceed towards cellular aging, removing and/or replacing the injured cells. Some reports showed that apoptosis was resulted from the disturbance in the activity of the mitochondrion, endoplasmic reticulum and the nucleus.[33] On the other hand, necrosis is a nonprogrammed form of cell death resulted from consequent energy depletion due to metabolic failure and the cessation of protein synthesis. The detected apoptosis in the hepatic tissue of rats treated with ZnO NPs might be due to intercellular stress induced by these fine particles. Apoptosis might be followed by mitochondria swelling, endoplasmic reticulum dilatations and rupture of lysosomes before shrinking and dissolution of nuclei.[41] Hepatocytes necrosis induced by ZnO NPs exposure might indicate oxidative stress on these cells leading to organelles damage and glutathione depletion.

Multilayered myelin like materials were demonstrated by the hepatocytes of rats subjected to ZnO NPs. Electron-dense lamellar myelin bodies have lysosomal character consisting mainly of phospholipids, with autophagic activity signing acute cytotoxicity.[42,43] These intracellular myeloid bodies are considered to be pathologic and morphologic hallmark of phospholipidosis and are seen in some lysosomal storage diseases.[44]

The seen ultrastructural alterations induced by ZnO NPs subjection might be resulted from the oxidative dissolution of these particles and releasing of zinc ions. Previous reports indicated that zinc ions could deplete cellular dissolved oxygen and could generate more intercellualr ROS.[12,45,46] Other studies showed that the entrance of ZnO NPs into the cell might increase cystolic and mitochondrial zinc ions content leading to disruption of cellular zinc homeostasis.[19,47,48] The induced ultrastructural pathology by the nanoparticles under study might be due to the interaction of the zinc ions with the hepatocytes components together with probable negative impact on the activity of zinc ions dependent enzymes.[12]

5. CONCLUSION

One might conclude from the results of the present study that exposure to ZnO NPs would produce hepatic tissue ultrastructural alterations that might affect the functions of the liver. These changes might be resulted from the induced oxidative stress due to disturbance in the hepatic

tissues pro-oxidant/antioxidant balance leading to hepatocytic ultrastructural alterations. In addition, 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 nanomaterials on the vital organs and their pathogenesis on the level of tissues, cells and macromolecules in relation to dose, size, shape and time of exposure.

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 (IRG14-06).

References and Notes

1. J. H. Yuan, Y. Chen, H. X. Zha, L. J. Song, C. Y. Li, J. Q. Li, and X. H. Xia, *Colloids Surf. B Biointerfaces* 76, 145 **(2010)**.
2. L. E. Yu, L. Yung, C. Ong, Y. Tan, K. Balasubramaniam, and D. Hartono, *Nanotoxicology* 1, 235 **(2007)**.
3. S. M. Hussain, K. L. Hess, J. M. Gearhart, K. T. Geiss, and J. J. Schlager, *Toxicol. In Vitro* 19, 975 **(2005)**.
4. J. Yu, M. Baek, H. E. Chung, and S. J. Choi, *J. Phys. Conf. Ser.* 304, 2007 **(2011)**.
5. M. K. Yu, J. Park, and S. Jon, *Theranostics* 2, 3 **(2012)**.
6. M. A. Abdelhalim and B. M. Jarrar, *Lipids Health and Dis.* 10, 113 **(2011)**.
7. M. A. Abdelhalim and B. M. Jarrar, *J. Nanobiotechnol.* 10, 5 **(2012)**.
8. Q. Jarrar, A. Battah, F. Obeidat, K. Battah, and J. Brit, *Pharmac. Res.* 4, 2670 **(2014)**.
9. A. V. Zvyagin, X. Zhao, A. Gierden, W. Sanchez, J. A. Ross, and M. S. Roberts, *J. Biomed. Opt.* 13, 064031 **(2008)**.
10. T. G. Smijs and S. Pavel, *J. Nanotech. Sc. Appl.* 4, 95 **(2011)**.
11. T. Xia, M. Kovochich, M. Liong, I. Madler, B. Gilbert, H. Shi, J. I. Yeh, J. I. Zink, and A. E. Nel, *ACS Nano.* 2, 2121 **(2008)**.
12. R. J. Vanderiel and W. H. Jong, *Nano Sci. Appl.* 5, 61 **(2012)**.
13. S. Hackenberg, A. Scherzed, M. Kessler, K. Froelich, C. Ginzkey, C. Koehler, M. Burghartz, R. Hagen, and N. Kleinsasser, *Int. J. Oncol.* 37, 1583 **(2010)**.
14. M. J. Akhtar, M. Ahmad, S. Kumar, M. Khan, J. Ahmad, and S. Alrokyan, *Int. J. Nanomed.* 7, 845 **(2012)**.
15. A. A. Tayel, W. F. El-Tras, S. Moussa, A. F. El-Baz, H. Maahrous, M. F. Salem, and L. Brimer, *J. Food Saf.* 31, 211 **(2011)**.
16. F. Arabi, M. Imandar, M. Negahdary, N. Masoud, M. T. Noughabi, H. Akbari-dastjerdi, and M. Fazilati, *Annals. of Biolog. Res.* 3, 3679 **(2012)**.
17. V. Sharma, D. Anderson, and A. Dhawan, *J. Biomed. Nanotechnol.* 7, 98 **(2011)**.
18. S. Pasupuleti, S. Alapati, S. Ganapathy, G. Anumolu, N. R. Pully, and B. M. Prakhya, *Toxicol. Ind. Health.* 28, 675 **(2012)**.
19. M. J. Osmond and M. J. McCall, *Nanotoxicity* 4, 15 **(2010)**.
20. C. H. Li, C. C. Shen, Y. W. Cheng, S. H. Huang, C. C. Wu, C. Kao, J. W. Liao, and J. J. Kang, *Nanotoxicology* 6, 746 **(2012)**.
21. I. L. Hsiao and Y. J. Huang, *Sci. Total Environ.* 409, 1219 **(2011)**.
22. S. Lanone and J. Boczkowski, *Curr. Mol. Med.* 6, 651 **(2006)**.
23. L. Wang, W. Ding, and F. J. Zhang, 10, 8617 **(2010)**.
24. D. B. Warheit, C. M. Sayes, and K. L. Reed, *Environ. Sci. Technol.* 43, 7939 **(2009)**.
25. G. Yan, Y. Huang, Q. Bu, L. Ly, P. Deng, J. Zhou, Y. Wang, Y. Yang, Q. Liu, X. Cen, and Y. Zhao, *J. Environ. Sci. Health Tox. Hazard Subst. Environ. Eng.* 47, 577 **(2012)**.
26. C. M. Sayes, K. I. Reed, and D. B. Warheit, *Toxicol. Sci.* 97, 163 **(2007)**.
27. W. Cho, R. Duffin, S. Howie, C. Scotton, W. Wallace, W. MacNee, M. M. Bradley, I. Megson, and K. Ken Donaldson, *Part. Fibre Toxicol.* 8, 27 **(2011)**.
28. W. S. Cho, K. Duffin, and C. A. Poland, *Nanotoxicology* 6, 22 **(2012)**.
29. J. J. Bozzola and L. D. Russell, Electron Microscopy: Principles and Techniques for Biologists, 2nd edn., Jones and Bartlett, Toronto **(1999)**, pp. 16–120.
30. J. A. Mascorro and J. J. Bozzola, Electron microscopy, methods and protocols, Methods in Molecular Biology, edited by J. Kuo, Human Press, Boston **(2007)**, pp. 57–72.
31. J. Ayache, L. Beaunier, J. Boumendil, G. Ehret, and D. Laub, Sample Preparation Handbook for Transmission Electron Microscopy, Methodology, Springer, London **(2010)**, Vol. 2, pp. 425–517.
32. M. A. Hayat, Principles and Techniques of Electron Microscopy, Biological Applications, Fourth edn., Cambridge University Press, Cambridge **(2000)**, pp. 307–366.
33. A. Singh, T. Bhat, and O. Sharma, *J. Clin. Toxicol.* S4, 001 **(2011)**.
34. L. L. Oligny and J. Lough, *Hum. Pathol.* 23, 953 **(1992)**.
35. A. Neyrinck, *Bull. Mem. Acad. R Med. Belg.* 159, 358 **(2004)**.
36. G. Lenaz, *IUBMB Life* 52, 159 **(2001)**.
37. L. Braydich-Stolle, S. Hussain, J. J. Schlager, and M. C. Hofmann, *Toxicol. Sci.* 88, 412 **(2005)**.
38. C. Sioutas, R. J. Delfino, and M. Singh, *Environ. Health Perspec.* 113, 947 **(2005)**.
39. V. Sharma, R. K. Shukla, N. Saxena, D. Parmar, M. Das, and A. Dhawan, *Toxicol. Lett.* 185, 211 **(2009)**.
40. S. George, S. Pokhrel, T. Xia, B. Gilbert, Z. Ji, M. Schowalter, A. Rosenauer, R. Damoiseaux, K. A. Bradley, L. L. Mädler, and A. E. Nel, *ACS Nano* 4, 15 **(2010)**.
41. G. Pandey, D. Srivastava, and S. Madhuri, *Toxicol. Int.* 15, 69 **(2008)**.
42. R. G. Urich and C. T. Cramer, *Toxicol. In Vitro* 5, 239 **(1991)**.
43. G. Schmitz and G. J. Muller, *Lipid Res.* 32, 1539 **(1991)**.
44. N. Anderson and J. Borlak, *FEBS Lett.* 580, 5533 **(2006)**.
45. B. De Berardis, G. Civitelli, M. Condello, P. Lista, R. Pozzi, G. Arancia, and S. Meschini, *Toxicol. Appl. Pharmacol.* 246, 116 **(2010)**.
46. R. Landsiedel, L. Ma-Hock, B. Van Ravenzwaay, and F. Oesch, *Nanotoxicology* 4, 364 **(2010)**.
47. E. John, T. C. Laskow, W. J. Buchser, B. R. Pitt, P. H. Basse, L. H. Butterfield, P. Kalinsk, and M. Lotz, *J. Translational Med.* 8, 118 **(2010)**.
48. Y. Y. Kao, Y. C. Chen, T. J. Cheng, Y. M. Chiung, and P. S. Liu, *Toxicol. Sci.* 125, 462 **(2012)**.

Received: 8 July 2015. Accepted: 28 July 2015.