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Vitamins E and C attenuates liver injury induced by copper nanoparticle in Rats (*Rattus norvegicus)*

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Abstract

Nanoparticles are widely used in many different fields, and production. However, the ingestion of nanoparticles from environmental pollution may have significant health impacts, which are currently poorly understood. In this study we aimed to investigate the cellular toxicity of copper nanoparticles on liver tissue of Rat, and also investigate the role of vitamin E and C, separately or together, in reducing the cellular damage that may be caused by copper nanoparticles. In this study 56 (5–6) weeks old rats with a weight of 120 ± 10 grams were divided into seven groups, treated with copper nanoparticles for four weeks, then treated with vitamins, either alone or together, for another four weeks where is control.

Rats were treated with copper nanoparticles showed changes in the ultrastructure of the liver tissue, in which there was a clear decrease in the number of cellular organelles in hepatocytes. Which show small in their size, and had irregular nuclear envelopes, swelling of the mitochondria, shortening of their cristae and effects on their matrix, an increase in the size of lysosomes, an increase in the number of lipid droplets, and an expansion of the Disse spaces. The treatment with vitamins C or E or both, marked improvement was found in the size and number of organelles. The cells returned to almost their normal state. We concluded that vitamins C and E have a positive role in reducing the toxic effects of copper nanoparticles. We recommended their use in doses of 250 mg/kg/day.

Key words : copper nanoparticles, hepatocyte, cellular effects, vitamin E,

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1. Introduction

Nanotechnology is a technology, in the most important sciences. It is important in many different branches of science, including fields related to human biology, Nanotechnology has become important in physics, chemistry, engineering, industrial fields, electronics, environmental studies, and many other fields. This technology raises the possibility of considerable progress in the near future. Hamzeh and Sunahara.,[1]. Sau and Rogach.,[2]. Dziendzikowska *et al*.,[3]. Goncalves *et al*.,[4]. In recent years, different types of nanomaterials have been applied in the fields of medicine and the food industry, because of their unique physical and chemical properties. Petros and Joseph,[5]. Xu *et al*., [6]. Amin *et al*., [7].

This diversity in the properties of nanoparticles in terms of chemical composition, surface area, and size have made them useful for many purposes. They are increasingly used in materials such as paints, sunscreens, ceramics, coatings, building materials, hardware, gas sensors, textile processing, baby powders, and anti-foaming shampoo. Zhang *et al*.,[8]. Although the benefits of nanotechnology are wide-ranging, the increased public and occupational exposure to these materials may cause general concern about their potential negative effects when they are released into the environment. Chen *et al*.,[9]. Nanoparticles may enter the body from water, food, cosmetics, or medicine Hoet *et al*.,[10]. Oberdörster *et al*.,[11]. by ingestion, inhalation, or through the pores of the skin. so it is important to study the biological effects of different nanoparticles, in living organism organ. The effects of nanomaterials on biological systems, and particularly their health effects, are poorly understood. Chen and Schluesener.,[12]. Carlson *et al*.,[13]. Their small size enables them to invade the lungs or skin, and move directly through the circulatory and lymphatic systems, causing disruption of cellular systems. Ma *et al*.,[14]. Nanomaterials may cause pulmonary infection, hepatotoxicity, nephrotoxicity, immunotoxicity, neurotoxicity, and testicular damage in animal models. Chou *et al*.,[15]. El-Sayed *et al*.,[16]. In addition, nanomaterials can also damage DNA and apoptosis cells Ahamed *et al*.,[17].

2. Materials and Methods

This study was conducted according to the criteria set by the Scientific Research Ethics Committee at King Saud University, Ethical Approval No. KSU-SE-19-32.

Fifty-six male rats *Rattus norvegicus,* (5–6) weeks old with weigh 120 ± 10 g, were obtained from the Animal House of the College of Science of King Saud University in Riyadh. The animals were kept at a temperature of 25°C ± 5°C with a relative humidity of 45% ± 5%, and a 12/12-hour light/dark cycle.

2.1. Experimental design

Copper nanoparticles were obtained from Sigma-Aldrich, with a size of 10 nm and a purity of 99.9%. The nanoparticles were orally administered to rats for four weeks at a dose of 100 mg/kg/day. The rats were then given vitamins C and E at a dose of 250 mg/kg/day for another four weeks. The rats were divided into seven groups, of eight, which were treated as follows:

The first group was considered as the control group, and were given only drinking water without any additives. The second group was given vitamin E, and the third group was given vitamin C, both at a dose of 250 mg/kg/day. The fourth group was dosed with copper nanoparticles at a dose of 100 mg/kg/day. The fifth group was dosed with both copper nanoparticles and vitamin E, and the sixth group was dosed with both copper nanoparticles and vitamin C. The seventh group was dosed with copper nanoparticles, vitamin E, and vitamin C, at doses of 250 mg/kg/day.

Following treatment, the rats were dissected in two stages. The first stage included four rats from each group at the end of the first month. In the second stage, the rest of the rats were dissected at the end of the second month. The animals were dissected directly after anesthesia, and cellular sectors were prepared for the samples.

2.2. Electron microscopic investigation :

Preparation of tissue samples for examination by transmission electron microscopy (TEM): The stages of preparing sectors for samples as described by. Eisenman and Alfert,[18]. can be summarized as follows:

Samples were taken from livers in the form of cubes about (1 mm 3) in size and quickly applied to the primary stabilizer Glutaraldehyde 3% and the secondary stabilizer Osmium tetroxide 1%. Samples were washed with a buffer between primary and secondary fixation and the washing solution was the same as the buffer (0.1M Na-Cacodylate buffer). The cells have been dehydrated with progressive concentrations of ethyl alcohol (30%, 50%, 70%, 80%, 90%, 96%, 100%). The samples were left at each concentration for half an hour except for the last concentration (100% absolute alcohol). for an hour. Transtition fluid was done by using propylene oxide (without dilution) for the purpose of withdrawing ethyl alcohol. Infiltration was gradually by making equal proportions of (propaline oxide: resins). Then the samples were embedded in capsules for making plastic molds. After that Sectioning and its purpose are to obtain very thin sectors measured in nanometers to facilitate the penetration of electrons through the thin sector. For this, glass knives are used. The thin sections were loaded onto the copper grids and examined by a transmission electron microscope and imaged.

3. Results

The liver tissue of animals treated with copper nanoparticles and their comparison with the control group and with the groups treated with copper particles and vitamin C, E separately or together was examined using transmission electron microscopy. Changes in the composition of the liver tissue were observed, as follows:

- In animals treated with copper nanoparticles, a clear reduction of cellular organelles in the hepatocytes, were observed, In addition to its small size. The role of vitamin C and E separately or together in improving the number of organelles as well as restoring the normal size of the nucleus was evident.
- The cellular sectors of the animals treated with nanoparticles also showed mitochondrial swelling, shortening of the cristae, and disintegration on their matrix. Vitamin C and E, separately or together, had a clear role in returning the mitochondria almost to their normal form.
- An increase in the size of the lysosomes was observed in the cytoplasm of the hepatic cells of animals treated with nanoparticles. This increase was not observed in the sectors treated with copper nanoparticles together with vitamins C and E, separately or together.
- An increase in the number of lipid droplets was observed in the cytoplasm of the hepatocytes of animals treated with copper nanoparticles. Vitamin C and E, separately or together, limited the increase in the number of lipid droplets.
- An expansion of the space of Disse was observed in the sectors treated with nanoparticles, and the presence of a tumor cell as well as a pit cell, but no increase in the number of Kupffer cells was observed.

These effects are illustrated in Figures 1 to 6.

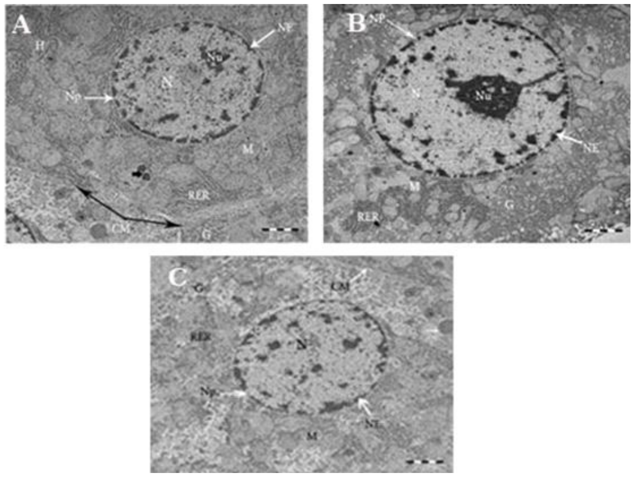

Fig. 1: Transmission electron micrographs of liver tissue. (A): The control group shows a hepatic cell (H), in which the nucleus (N) appears circular and regular and contains a clear nucleolus, and the nuclear envelope (NE) and nuclear holes (Np) are observed, as are the cell membranes (CM), mitochondria (M), and the rough endoplasmic reticulum. (RER). There are a few lysosomes (Ly) (short black arrow), and glycogen granules (G), and an absence of lipid droplets, Magnification 2000X.(B): Following treatment with vitamin E, a hepatocyte has a nucleus (N) that appears circular and regular, and contains a clear nucleolus and nuclear envelope (NE), as well as mitochondria (M) and the rough endoplasmic reticulum (RER), which indicates cell activity. (G) glycogen granules are present and lipid droplets absent. Magnification 2000X.(C): Following treatment with vitamin C, a hepatocyte appears, its nucleus (N) is circular and regular in shape and has a nuclear envelope (NE) and the nuclear holes (Np) are clear as observed in the cell membrane (CM), as well as the mitochondria (M) and the rough endoplasmic reticulum (RER), which indicates cell activity. Glycogen granules are present (G) and lipid droplets absent. Magnification 2000X.

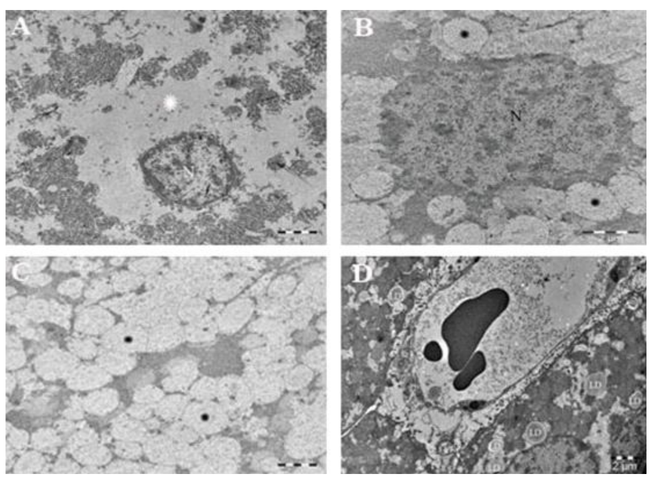

Fig. 2: Transmission electron micrographs of liver tissue from the group that was treated with copper nanoparticles where (A): A hepatic cell appears deficient in cell organelles (asterisk) and a small nucleus (N) is visible, 2000X. (B): shows an irregular nucleus (N), mitochondrial bulge, and decomposition of cristae and matrix (star) 3000X. (C): shows mitochondrial swelling and decomposition of cristae and matrix (star) 3000X. (D) shows an increase in the number of lipid droplets in the cytoplasm of the hepatocyte 2000X.

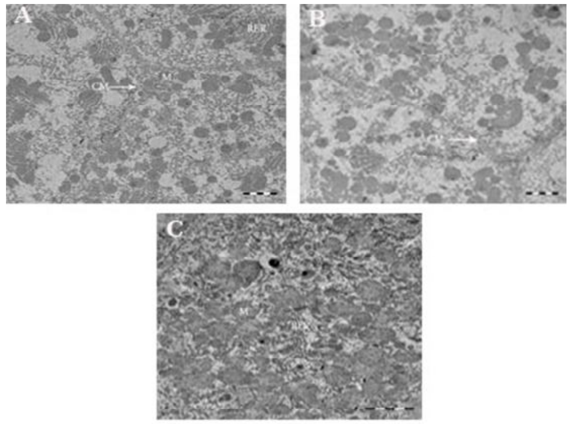

Fig. 3: Transmission electron micrographs of liver tissue. (A): The group treated with copper nanoparticles and vitamin E shows an improvement in the number of cellular organelles compared to the group treated with copper nanoparticles 2000X.(B): The group treated with copper nanoparticles and vitamin E showed an improvement in the number of cellular organelles compared to the group treated with copper nanoparticles. 3000X.(C): The group treated with copper nanoparticles and vitamin E, showing improvement in the number of cellular organelles compared to the group treated with copper nanoparticles 2000X.

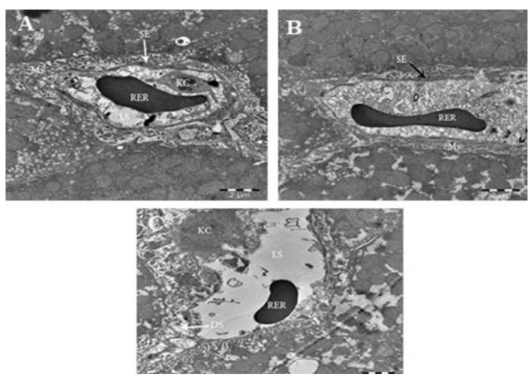

Fig. 4: Transmission electron micrographs of liver tissue. (A): The control group in the liver sinusoids A red blood cell (RBC) can be seen. Kupffer cell (KC) 2500X. (B): Vitamin E, in the liver sinusoids, notice there the presence of a red blood cell (RBC) can be seen. Disse space containing the microvilli (Mv) and some endothelial cells (SE) 2500X. (C): Vitamin C, in the area of the liver sinusoids a red blood cell (RBC) can be seen. Disse space containing the microvilli (Mv) and a Kupffer cell are observed (KC) 2500X.

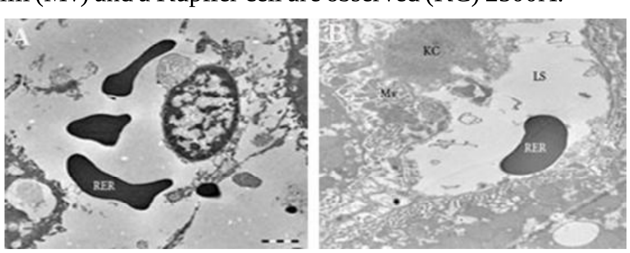

Fig. 5: Transmission electron micrographs of liver tissue of a group of copper nanoparticles, in the liver sinusoids. Red blood cells (RBC) are present. Disse space ruptured and almost devoid of microvilli (Mv). A tumor cell can be seen, and by the lysosome attempts to devour it 2000X.

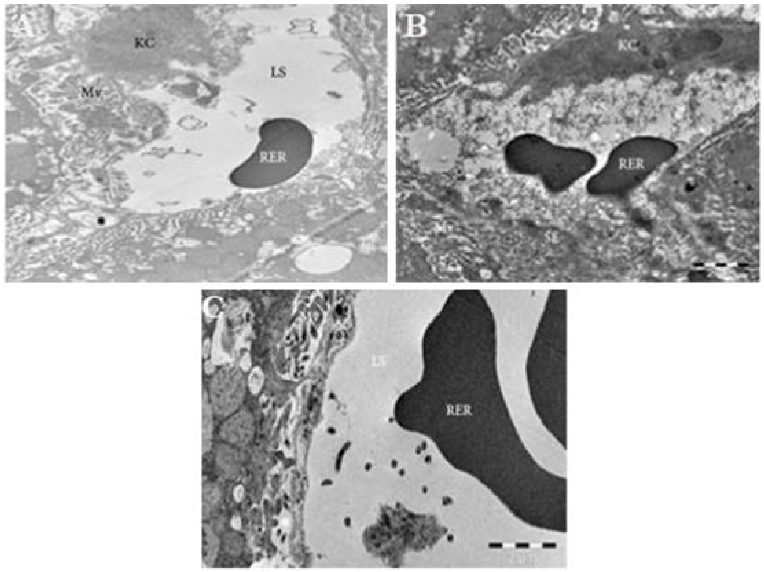

Fig. 6: Transmission electron micrographs of liver tissue. (A): group of copper nanoparticles and vitamin E, (B): group of copper nanoparticles and vitamin C. In the liver sinusoids, the role of vitamin E and C in the improvement of the hepatic sinus is clear, and red blood cells (RBC) are present. There is a clear Disse space (Asterisk) and microvilli (Mv), and a Kupffer cell is observed (KC) 2000X. (C): A group of copper nanoparticles and vitamin C and E. The role of vitamins C and E in the improvement of the liver sinusoids is apparent, there are no noticeable ruptured, and red blood cells (RBC) are present. There is a clear Disse space (asterisk), microvilli (Mv), and endothelial cells (SE) 3000X.

4. Discussion

Pollution has become a global problem in all environments. Nanoparticles have long been recognized as a serious pollutant of aquatic environment and becoming a matter of global concern Aruoja *et al.*,[19]. Sun *et al.*, [20]. and their accumulation over a long period in the body has significant health effects Oberdörster *et al.*,[11]. Liver is the first organ to encounter ingested nutrients, drugs and environmental toxicants that enter the hepatic portal vein from digestive system and liver can be injury resulting from acute or chronic exposure to toxicants Kermanizadeh *et al*.,[21]. In the present study hepatocytes of *Rattus norvegicus* dosed with nanoparticles showed an increase in the lysosomal number and in the lipid droplets with presence of cytoplasmic vacuoles of different sizes. Similar observation was found by Sarhan and Hussein,[22]. who shows manifestation of cytotoxicity caused by Silver nanoparticles. Terentyuk *et al.,*[23]. Asharani *et al.,* [24]. also showed that animal exposed to Gold nanoparticles caused the expansion of capillaries, blood congenstion between hepatocytes and with irregular nuclei. The present study agree with the finding of. Sardari *et al.,*[25]. who found that rats exposed to Silver nanoparticles caused swelling of liver cells and enlargement of nucleus and mitochondria. The RER and nuclear membrane also appeared irrergular. The RER and the nuclear membrane also appeared irregular. Almansour *et al*.,[26]. showed that mitochondria were the main organelles affected by the toxicity of nanoparticles, with the nuclei of Kupffer cells being enlarged and irregular in shape. Large lysosomes and fatty droplets were present in the cytoplasm. Liver damage can be explained by the deposition of nanoparticles in Kupffer's hepatic cells and the endothelial cells lining the liver sinusoids. Costa *et al*., [27]. or by inhibition of the mitochondrial respiratory chain that normally produces energy for the cells Asharani *et al.,* [24]. Chen and Schluesener,[12]. or it may be via the generation of ROS associated with inflammatory, oxidative and cytotoxic effects, and the induction of apoptosis. Kim *et al*., [28]. Johnston *et al*.,[29].

Administration of vitamins E and C protected the organism tissues treated with nanoparticles as evidenced by an appearance of normal histological structure, due to its antioxidant properties. Based on the results obtained in the present investigation, it can be suggested that administration of vitamins E and C might alleviate the liver biochemicals disturbances and histopathological alterations from the oxidative stress produced by copper nanoparticles. Shotop and Al-Suwiti,[30]. When *Rattus norvegicus* treated with vitamins E and C, many lysosomal and lipid droplets disappeared and a number of organelles increased. The nucleus returned to almost it's normal size, with a recoganized nuclear envelope. The mitochondria also returned to almost normal shape and size. The hepatoprotective effect of vitamins may be due to its antioxidant activity. Many studies are required to clarifiy the mechanism action of vitamins as an agent against nanoparticles toxicity.

5. Conclusions

This study confirmed that vitamins are ideal antioxidants to increase tissue protection from toxicant materials. Thus, we recommend that vitamins C and E should be used to reduce the harmful effects of nanoparticles and doing more studies to reveal the role of vitamins in ridding the body of the harmful effects of other nanoparticles.

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References

1. Hamzeh, M., and Sunahara, G. I. In vitro cytotoxicity and genotoxicity studies of titanium dioxide (TiO_2) nanoparticles in Chinese hamster lung fibroblast cells. Toxicol. in Vitro, *27:* 864–873 (2013).
2. Sau, T.K., and Rogach, A.L. Nonspherical noble metal nanoparticles: colloid-chemical synthesis and morphology control. Adv. Mat. Res., *22:* 1781–1804 (2010).
3. Dziendzikowska, K., Gromadzka Ostrowska, J., Lankoff, A., Oczkowski, M., Krawczyńska, A., Chwastowska, J., and Kruszewski, M. Time dependent biodistribution and excretion of silver nanoparticles in male Wistar rats. J Appl. Toxicol., *32:* 920–928 (2012).
4. Goncalves, D.M., Chiasson, S., and Girard, D. Activation of human neutrophils by titanium dioxide (TiO_2) nanoparticles. Toxicol. in Vitro, *24:* 1002–1008 (2010).
5. Petros, R. A., and DeSimone, J. M. Strategies in the design of nanoparticles for therapeutic applications. Nat. Rev. Drug. Disc., *9:* 615 (2010).
6. Xu, L., Li, X., Takemura, T., Hanagata, N., Wu, G., and Chou, L.L. Genotoxicity and molecular response of silver nanoparticle (NP)-based hydrogel. J. Nanobiotechnol., *10:* 16 (2012).
7. Amin Eltokhy, R. A., Abdelsalam, M. H., Elzoheiry, E. A., Mohamed, M. I., Rashed, L. A., & Elnemr, R. S. Histopathological Assessment of Hepato-Nephrotoxicity Induced by Nano Copper Particles in Adult Male Albino Rats and the Potential Protective Effect of Alpha Lipoic Acid: A Chronic Study. Indian Journal of Forensic Medicine & Toxicology, *14(2)* (2020).

8. Zhang, X.D., Wu, H.Y., Wu, D., Wang, Y.Y., Chang, J.H., Zhai, Z.B., and Fan, F.Y. Toxicologic effects of gold nanoparticles in vivo by different administration routes. Int. J. Nanomedicin, *5:* 771 (2010).
9. Chen, Z., Meng, H., Xing, G., Chen, C., Zhao, Y., Jia, G., and Chai, Z. Acute toxicological effects of copper nanoparticles in vivo. Toxicol. Lett., *163:* 109–120 (2006).
10. Hoet, P.H., Brüske-Hohlfeld, I., and Salata, O.V. Nanoparticles–known and unknown health risks. J. Nanobiotech., *2:* 12 (2004).
11. Oberdörster, G., Oberdörster, E., and Oberdörster, J. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environ. Health. Perspect., *113:* 823–839 (2005).
12. Chen, X. and Schluesener, H.J. Nanosilver: a nanoproduct in medical application. Toxicol. Lett., *176:* 1–12 (2008).
13. Carlson, C., Hussain, S.M., Schrand, A.M., Braydich-Stolle, L., Hess, K. L., Jones, R.L., and Schlager, J.J. Unique cellular interaction of silver nanoparticles: size-dependent generation of reactive oxygen species. J. Phys. Chem. B. *112:* 13608–13619 (2008).
14. Ma, L., Zhao, J., Wang, J., Liu, J., Duan, Y., Liu, H., & Hong, F. The acute liver injury in mice caused by nano-anatase TiO_2. Nanoscale research letters, *4(11),* 1275 (2009).
15. Chou, C. C., Hsiao, H. Y., Hong, Q.S., Chen, C.H., Peng, Y.W., Chen, H.W., and Yang, P.C. Single-walled carbon nanotubes can induce pulmonary injury in mouse model. Nano. Lett., *8:* 437–445 (2008).
16. El-Sayed, Y.S., Shimizu, R., Onoda, A., Takeda, K., and Umezawa, M. Carbon black nanoparticle exposure during middle and late fetal development induces immune activation in male offspring mice. Toxicology, *327:* 53–61 (2015).
17. Ahamed, M., Posgai, R., Gorey, T.J., Nielsen, M., Hussain, S.M., and Rowe, J.J. Silver nanoparticles induced heat shock protein 70, oxidative stress and apoptosis in Drosophila melanogaster. Toxicol. Appl. Pharmacol., *242:* 263-269 (2010).
18. Eisenman, E.A., Alfert, M. A new fixation procedure for preserving the ultrastructure of marine invertebrate tissues. J. Micr., *125:* 117–120 (1982).
19. Aruoja, V., Dubourguier, H.C., Kasemets, K., and Kahru, A. Toxicity of nanoparticles of CuO, ZnO and TiO_2 to microalgae Pseudokirchneriella subcapitata. Sci. Total Environ., *407:* 1461–1468 (2009).
20. Sun, T., Kang, Y., Liu, J., Zhang, Y., Ou, L., Liu, X., & Shao, L. Nanomaterials and hepatic disease: toxicokinetics, disease types, intrinsic mechanisms, liver susceptibility, and influencing factors. Journal of Nanobiotechnology, *19(1),* 1-23 (2021).
21. Kermanizadeh, A., Powell, L. G., & Stone, V. A review of hepatic nanotoxicology–summation of recent findings and considerations for the next generation of study designs. Journal of Toxicology and Environmental Health, Part B, *23(4),* 137-176 (2020).
22. Sarhan, O.M.M., and Hussein, R. M. Effects of intraperitoneally injected silver nanoparticles on histological structures and blood parameters in the albino rat. Int. J. Nanomed., *9:* 1505 (2014).
23. Terentyuk, G.S., Maslyakova, G.N., Suleymanova, L.V., Borodulin, V.B., Dudakova, Y.S., Khlebtsov, N.G., and Tuchin, V.V. Morphological and biochemical changes after intravenous injection of gold nanoparticles. In Seventh International Conference on Photonics and Imaging in Biology and Medicine, Vol. *7280,* p. 728005, (2009). Int. Society Optics Phot.
24. Asharani, P.V, Hande, M.P. and Valiyaveettil, S. Anti-proliferative activity of silver nanoparticles. BMC

Cell. Biol., *10:* 65 (2009).

25. Sardari, R.R.R., Zarchi, S.R., Talebi, A., Nasri, S., Imani, S., Khoradmehr, A., and Sheshde, S.A.R. Toxicological effects of silver nanoparticles in rats. Afr. J. Microbiol. Res., *6:* 5587–5593 (2012).
26. Almansour, M., Sajti, L., Melhim, W., and Jarrar, B.M. Ultrastructural hepatocytic alterations induced by silver nanoparticle toxicity. Ultrastruct. Pathol. *40:* 92–100 (2016).
27. Costa, C. S., Ronconi, J.V.V., Daufenbach, J.F., Gonçalves, C.L., Rezin, G.T., Streck, E.L., and da Silva Paula, M.M. In vitro effects of silver nanoparticles on the mitochondrial respiratory chain. Mol. Cell. Biochem. *342:* 51–56 (2010).
28. Kim, H.R., Kim, M.J., Lee, S.Y., Oh, S.M., and Chung, K.H. Genotoxic effects of silver nanoparticles stimulated by oxidative stress in human normal bronchial epithelial (BEAS-2B) cells. Mutat. Res. Genet. Toxicol. Environ. Mutagen., 726: 129–135 (2011).
29. Johnston, H.J., Hutchison, G., Christensen, F.M., Peters, S., Hankin, S., and Stone, V. A review of the in vivo and in vitro toxicity of silver and gold particulates: particle attributes and biological mechanisms responsible for the observed toxicity. Crit. Rev. Toxicol., *40:* 328–346 (2010).
30. Shotop, Y. M., & Al-Suwiti, I. N. The possible role of vitamins E and C in reducing the toxicity of copper nanoparticles in the kidney and liver of the rats (Rattus norvegicus). Journal of King Saud University-Science, *33(2),* 101357 (2021).