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The Effects of ZnO Nanoparticles and ZnO/Chitosan NCs on Liver Histology and Serum Parameters in Rats

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Abstract

There are conflicting and confusing reports about prooxidant/ activities of Zinc oxide nanoparticles and Zinc oxide NCs. This study aimed to resolve these discrepancies by examining the effects of these compounds on liver histopathology in healthy rats. Materials and Methods: 42 adult male Wistar rats were divided into 7 groups. Rats in the treatment group received intraperitoneal injections of ZnO nanoparticles 10, 20 and 40 mmol/ml) and ZnO nanocomposites (NCs) (10, 20 and 40 mmol/ml) for 28 days. Control rats received distilled water. At the end of the study, the following parameters were assessed: serum liver enzymes (ALT and AST), the activity of serum catalase (CAT) and superoxide dismutase (SOD), serum BUN and serum creatinine and liver histology. Intraperitoneal injection of ZnO nanoparticles at a concentration of 10 mmol/ml/day had no significant effect on serum liver enzymes but at 20 and 40 mmol/ml/day significantly decreased serum catalase and SOD activity compared with the control group ($P<0.05$). ZnO NCs at the concentrations of 20 and 40mmol/ml/day decreased serum catalase activity and SOD activities and significantly elevates serum liver enzymes. Furthermore, both ZnO nanoparticles and ZnO NCs had no significant effect on serum BUN and creatinine levels. Both nanoparticles induced severe histological changes at the two higher doses (20 and 40 mmol/ml). The results suggest that proper concentrations of ZnO nanoparticles and ZnO NCs have no toxic effects on the liver while the higher doses can induce severe histological changes.

Keywords: ZnO nanoparticles, ZnO NCs, serum biochemical parameters, Rat

1 Introduction

Zinc is an essential component of many cytoplasmic enzymes that participates in the metabolism of carbohydrates, cytoplasmic proteins, lipids, and nucleic acids. This element induces many biological, physiological, and behavioral effects (1). Zinc is a necessary element for the growth of the body and zinc deficiency is associated with impaired immune function, irritability and stunting of growth. Specific age groups including infants, children, and adults are more susceptible to Zinc deficiency (2). Zinc is stable in the environment but is seldom abundant in food chains. Natural sources of zinc include meats, fruits, legume, and seeds. Major anthropogenic sources of zinc in the world are usually arising from municipal, industrial and agricultural activities (3). The brain, hematopoietic, hepatic and renal systems are the most important organs affected by Zinc deficiency (4). Nowadays there is an increasing trend toward the use of Zn in nanotechnology in the form of Zinc nanoparticles. Nanotechnology is a field of science which is about the preparation of nanoscale materials (5-7). Nanoparticles exhibit new and improved characteristics such as morphology and size distribution respecting larger particles from which they are made (8-25). Zinc nanoparticles are widely used in agriculture, cosmetic industry and medicine. ZnO nanoparticles, due to their unique optical, chemical, mechanical and electrical properties, have attracted scientific attention (26). Based on previous reports, ZnO NPs can increase or decrease reactive oxygen species (ROS) generation and subsequently oxidative stress in different parts of the body (27). Reactive oxygen species are short-lived organic compounds with uneven number of electrons that stabilize themselves by oxidizing biological molecules (28). Normally, the reactive oxygen-containing molecules and other free radicals can be quickly removed by natural defense mechanisms such as Glutathione peroxidase, superoxide dismutase, and catalase (29). Nanochamposites are usually synthesized by using ionic gelation of pentasodium tripolyphosphate and chitosan. Applications of these compounds have also become more widespread regarding their chemical properties (30). Adding chitosan to Zinc structure affects its biological and physicochemical properties (31) So in the present study, we decided to examine whether adding chitosan to zinc can decrease or increase the and pro-oxidant activities of Zinc.

2 Material and Methods

2.1 Animals

Wistar rats (201- 234 g) obtained from Laboratory Animal Center University of Zabol were used in the current study. Animals were maintained in well-ventilated

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rooms at a constant temperature of 20–23 ◦C and 12 (h) light/dark cycles with free to standard rodent food (Javaneh-Khorasan, Iran) and tap water. they were handled in accordance with the Animal Ethics Committee of the University of Zabol, Zabol- Iran, and Guide for the Care and Use of Laboratory Animals (National Institutes of Health (NIH) publication 86–23; revised 1985. 42 adult male Wistar rats were divided into 8 groups (7 rats in each group: one control group and six treatment group. Rats in treatment group received intraperitoneal injections of ZnO nanoparticles (10, 20 and 40 mmol/ml/day) and ZnO NCs (10, 20 and 40 mmol/ml/day) for 28 days. Control rats received 1 ml of distilled water intraperitoneally for 28 days. At the end of our study, blood samples were collected via retro-orbital puncture and centrifuged (3000 rpm for 10 minutes) for serum separation. The serum samples were immediately frozen at −80◦ C. Following blood collection, rats were sacrificed by cervical dislocation and whole-brain tissues were isolated. The fresh brain tissues were immediately washed with 0.9% Na Cl and stored at −20°C for further determination of lipid peroxidation in MDA form.

3.1 Serum biochemical parameters

Analyses of serum ALT, AST and ALP levels were performed by using the Selectra pro, M auto analyzer, (Vital Scientific, SpanNeren, Netherlands) with Pars Azmoon reagents kit (Pars Azmoon. Co., Tehran, Iran). Serum creatinine and BUN were measured using commercial kits (Pars Azmoon Lab, Iran), according to manufacturer's instructions. Serum antioxidant enzymes were measured using commerciall kits (Zell Bio Germany) and according to the company instructions.

3.2 Histopathological examination

After animals were euthanized by diethyl ether, liver specimens were separated and washed in water. The liver specimens were then sliced and preserved in a 10% neutral buffered formalin solution (NBF). After paraffin embedding and block making of tissue samples, serial sections were prepared by using the hematoxylin-eosin method and were examined under a light microscope (Olympus, Tokyo, Japan).

2.2 Statistical analysis

The collected data were analyzed with SPSS software (version 20.0) expressed as mean ± SD. All Multiple comparisons were performed by using one-way analysis of variance (ANOVA) followed by posthoc Tukey's test. Statistical significance was set at $P < 0.05$.

3 Results

Intraperitoneal injection of ZnO NPs at a concentration of 10 mmol/ml had no significant effect on serum biochemical parameters ($P > 0.05$). Also, liver histology was not affected by this dose (fig.2). However, ZnO NPs at 20 and 40 mmol/ml significantly decreased serum catalase and superoxide dismutase activity compared with the control group ($P < 0.05$) (fig.1). As seen in fig.2, serum liver enzymes ALT and AST were increased by ZnO NPs administration ($P>0.05$). Furthermore, intraperitoneal injection of two low concentrations of ZnO NPs did not affect serum creatinine and BUN fig.3. As seen in fig 3, ZnO NPs intraperitoneal injection at a concentration of 40 mmol/ml caused a non-significant difference in kidney function tests (BUN and serum creatinine) fig.3 ($P>0.05$). ZnO NCs at a concentration of 40 mmol/ml decreased serum catalase and SOD activity ($P < 0.05$). Intraperitoneal injection of ZnO NCs also increased serum liver enzymes (fig 2), while serum BUN and creatinine levels were not affected by ZnO NCs administration fig.3. As expected, ZnO nanoparticles and ZnO NCs had no significant effect on serum BUN and creatinine fig.5. Liver histological investigation of rats received a low dose of ZnO NCs showed normal architecture. However, the rats received the 20 mmol/ml and 40 mmol/ml of NCs showed signs of necrosis and hemorrhage (fig.5).

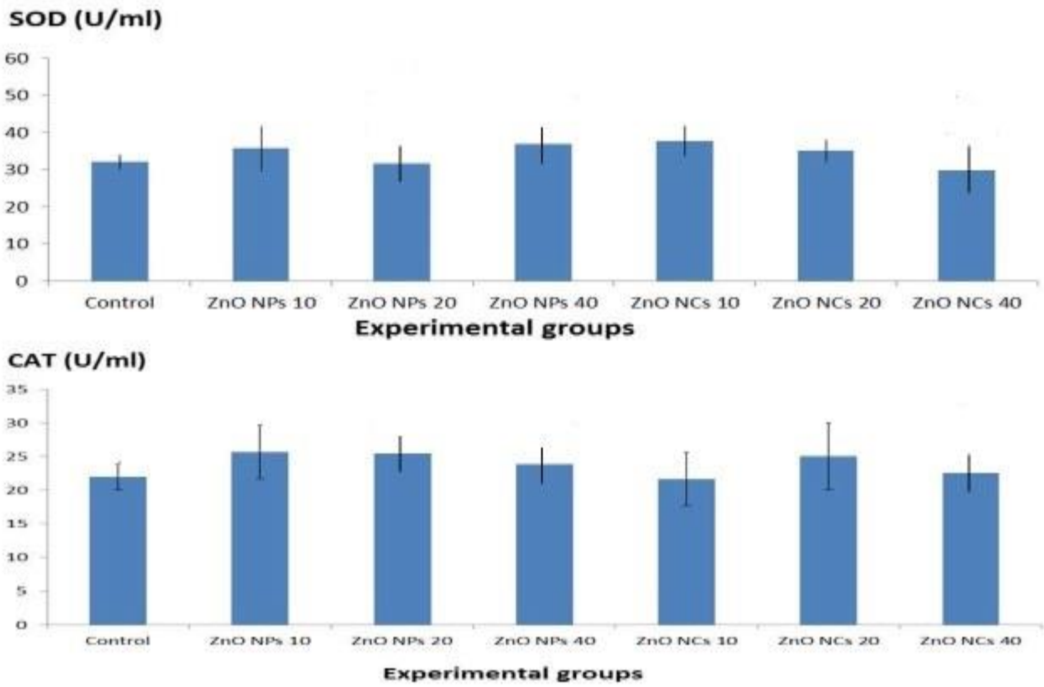

Fig. 1: Serum CAT and SOD activities in rats treated with *ZnO NPs (*X ±SD, n=10). *P < 0.05, compared to control group.

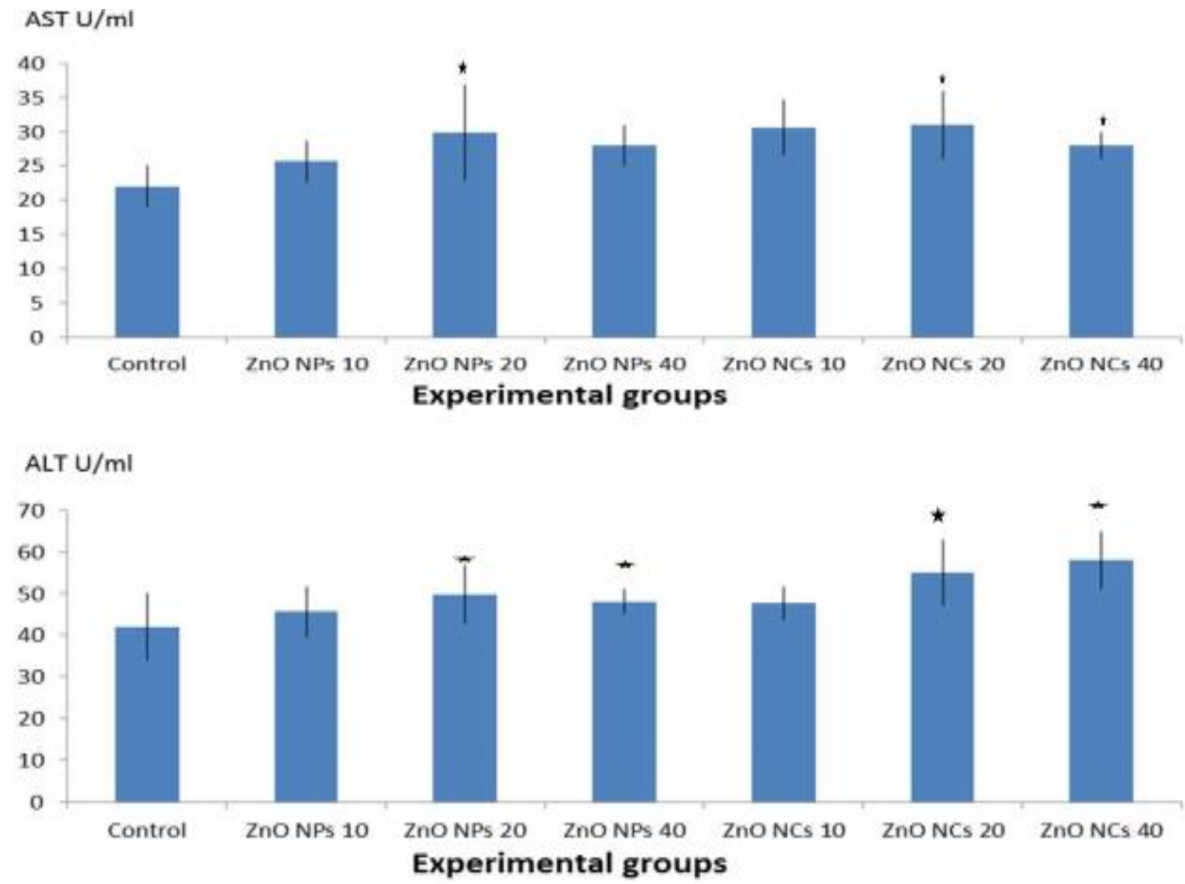

Fig. 2: Serum ALT and AST activities in rats treated with *ZnO NPs* (X ±SD, n=10). *P < 0.05 compared to control group

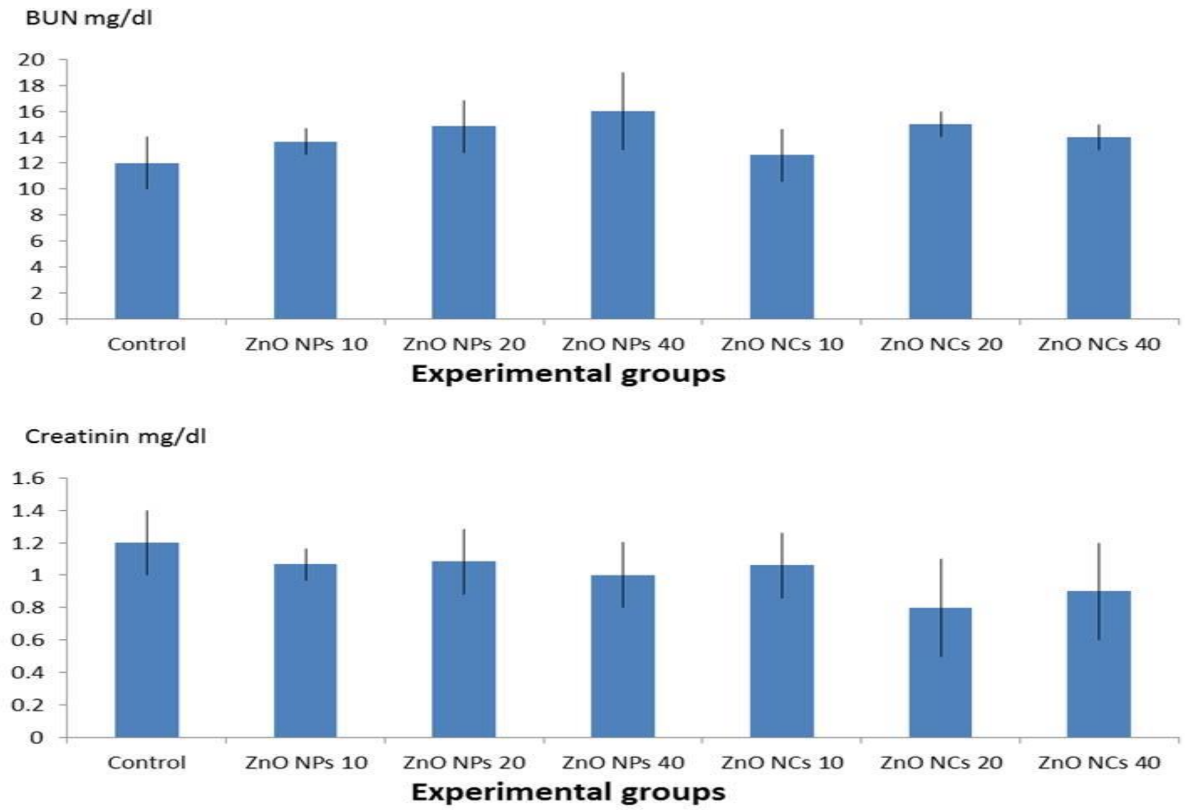

Fig. 3: Serum BUN and creatinine levels in rats treated with *ZnO NPs* (X ±SD, n=10). *P < 0.05compared to control group.

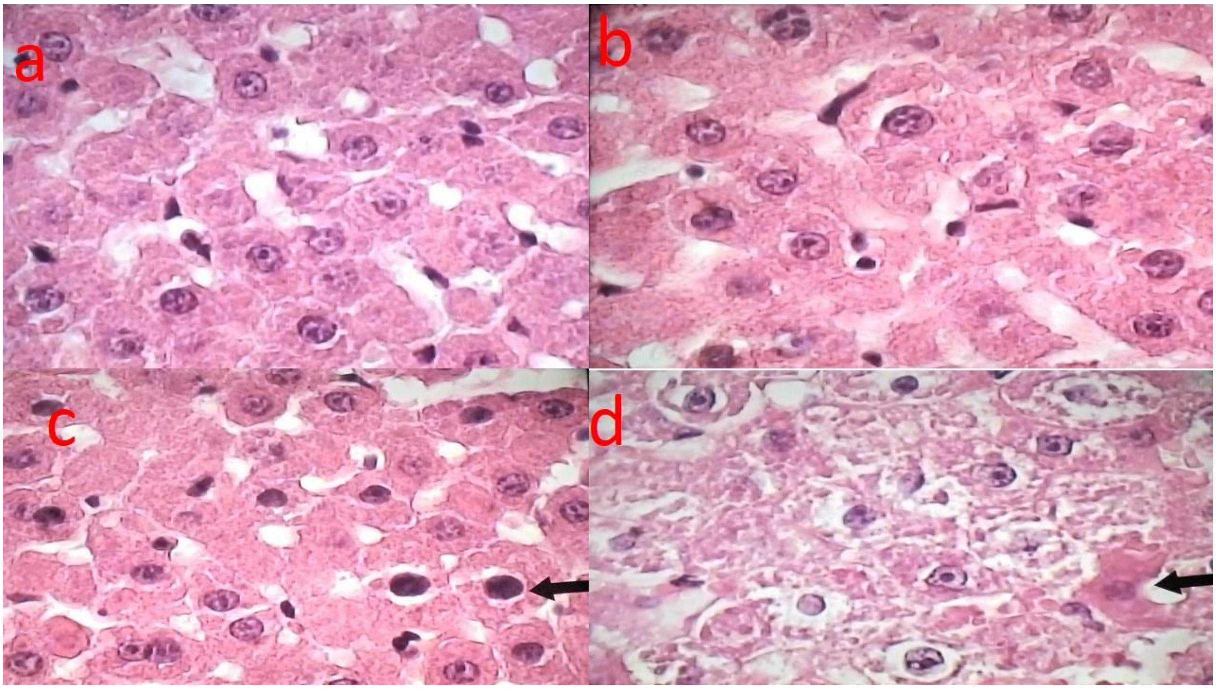

Fig. 4: Liver histology in control rats (a): Normal liver architecture with intact hepatocytes and sinusoids, rats received ZnO nanoparticles 10 mmol (b): normal hepatocytes, rats received ZnO nanoparticles 20 mmol (c): necrosis (arrow), rats received ZnO nanoparticles 30 mmol (d): necrosis (arrow) and fatty changes. . H&E staining (× 40)

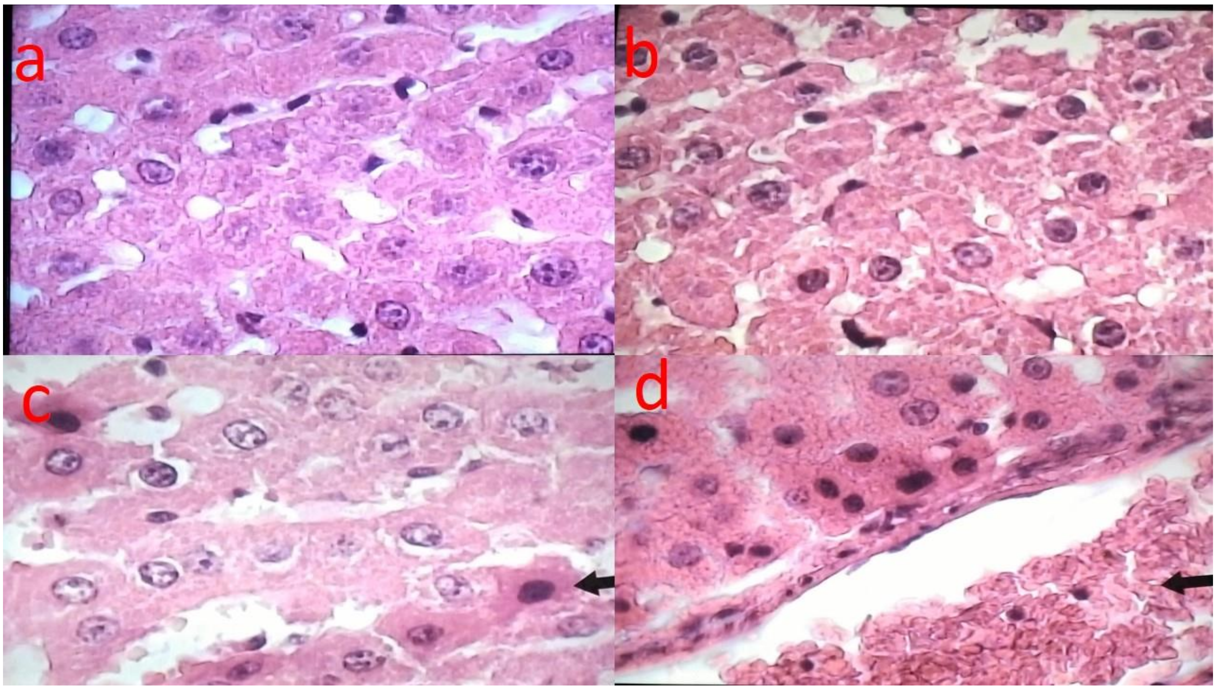

Fig. 5: Liver histology in control rats (a): healthy hepatocytes and well-arranged sinusoids, rats received ZnO nanoparticles 10 mmol (b): normal liver histological pattern, rats received ZnO nanoparticles 20 mmol (c): necrosis (arrow), rats received ZnO nanoparticles 30 mmol (d): necrosis (arrow) and bleeding. . H&E staining (× 40).

4 Discussion

The effects of ZnO NPs on serum oxidative stress biomarkers have not been fully elaborated yet. Some studies had reported the properties of ZnO nanoparticles (32) while other studies indicated oxidative damage caused by ZnO nanoparticles (33). As seen in this study, administration of ZnO nanoparticles decreased serum enzyme activities. These results were in line with previous studies about the biological effects of ZnO nanoparticles (34, 35) It can be concluded that the decreases in serum catalase and superoxide dismutase activity is an indicator of pro-oxidant activities of ZnO nanoparticles (36). Superoxide radical anion , peroxyl radicals which are the major reactive oxygen species generated during oxidative stress, may induce liver fibrosis and fatty changes by activating type I procollagen synthesis enzyme. This process may led to the generation of lipid peroxidation end-products. Our eukaryotic cells have a broad types of oxidative defense mechanisms, like enzymatic molecules such as CAT, SOD, glutathione peroxidase and molecular scavengers such as vitamin A, reduced glutathione (GSH), ascorbic acid, vitamin E (alpha tocopherol) and N-acetyl-5-methoxytryptamine (melatonin) (37). In this investigation, decrease in the activities of SOD and CAT might be due to increased generation of reactive oxygen species or increased availability of NADPH that is required to maintain oxidative defenses mechanisms. These results suggest that the ZnO nanoparticles have free radical producing effect and can reduce the activity of the endogenous enzymes. In this study, a significant increase in serum AST and ALT levels was observed in rats treated with ZnO nanoparticles. These results were in line with other studies indicating hepatic damage caused by ZnO nanoparticles or with studies indicating hepatoprotective potential of these nanoparticles (38, 39). As previously noted, damage to liver tissues releases the AST, ALP and ALT into the serum, and, hence, elevation of serum activities of these enzymes is considered a valuable marker of liver damage (40). Serum creatinine and blood urea nitrogen are used as indicators of glomerular filtration rate and renal function (41). ZnO nanoparticles or ZnO nanochitosans had no significant effect on serum kidney function markers. These findings were in contrast with previous studies that reported significant increase in serum kidney markers following treatment with ZnO nanoparticles (8-23, 42). This result might be due to the low doses of ZnO nanoparticles or ZnO nanochitosans used in this study. This observation led us to conclude that low concentrations of these compounds have potential without any toxicity in vivo.

5 Conclusion

Our results indicate that ZnO nanoparticles have similar effects compared to ZnO NCs. Further studies are required to elucidate the effects of these compounds in laboratory animals.

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Conflicts of interest

There are no conflicts of interest.

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