American Journal of Biomedical Sciences
ISSN: 1937-9080
nwpii.com/ajbms

Study the Oral Toxicity of Zinc Oxide Nanoparticles in Adult Mice

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Received:14 October 2020; | Revised:25 October 2020; | Accepted:06 March 2021

Abstract

ZnO-NPs suspended in distilled water were administered to mice at dose of 100 mg/ kg body weight through oral gavage. The mean body weight gain in mice given ZnO-NPs was similar to this of control group during the two weeks. The effects of ZnONPs on RBCs have been investigated in context with the osmotic fragility, all of the RBCs did not hemolyze at the same time when treated with ZnONPs, some RBCs took more time, and some became crenated while some remained turgid for relatively longer duration. ZnONPs can spread more in blood, kidney, liver and other organs,and this was supported by the histopathological section showed that ZnONPs is able to induce changes in tissues of kidney and liver and brain in mice. The toxic effects of zinc oxide nanoparticles on the central nervous system include cytotoxicity, inflammation, and oxidative stress induction that result in neurodegeneration.

Keywords: ZnO-NPs, Osmotic fragility, Oxidative stress

1. Introduction

Recently, rapid advances in nanotechnology have contributed to manufacture and control of engineered nanoparticles, which are generally defined as particles in the size range of 1-100 nm in one dimension[1].

Nanoparticles have special physical and chemical properties and unusual shape, size and surface area to volume ratio. Mentioned features make these materials unique for biological, medical and industrial applications. One of the most useful features is high surface area that causes nanoparticle's widespread application in medical sciences and production of Nano based drugs for some of the Incurable disease[1]. On the other hand, this material distributed in all of body rapidly after injection by circulation and reached to the all of the organs and tissues[2]. So pollution of our living environment by nanoparticles is very dangerous because they can interact with macromolecules in living cells, while we don't have enough information and suitable model for the action of these materials in the body.[3]

Zinc oxide (ZnO) is one of the most commonly utilized materials in diverse industrial fields such as dyes, paints, pigments, metallurgy additives, rubber, alloys, ceramics, chemical fibers, electronics,

catalyst, medical diagnosis, sunscreens, cosmetics, personal care products, and food additives [4-5]. The wide range of applications of ZnO is attributed to their unique characteristics, including semiconducting, electrical, optical, catalytic, magnetic, antimicrobial and ultraviolet light absorption properties.[6-7]

Zinc oxide nanoparticles (ZnONps) are semiconductor metal oxide nanoparticles that are widely used in biomedical fields as an anticancer drug, a tool for imaging biological systems, and also in cosmetics. [7]

Therefore the aim of this work is to evaluate the oral toxicity of zinc oxide nanoparticles on the red blood cells (fragility test) and the changes of the liver, kidney& brain of mice.

2. Materials and Methods

2.1 Preparation of Zinc oxide nanoparticles

Zinc sulfate heptahydrate and sodium hydroxide were used in the experiment, and deionized water is used for the preparation of solutions. To the aqueous solution of zinc sulfate, sodium hydroxide solution was added slowly drop wise in a molar ratio of 1:2 under vigorous stirring, and the stirring was continued for 12 h. The precipitate obtained was filtered and washed thoroughly with deionized water. The precipitate was dried in an oven at 100° C and ground to fine powder using agate mortar. The powder obtained from the above method was calcined at 72° C for 24 hours.

2.2 Characterization

2.2.1 X-ray diffraction (XRD) of ZnONPs

The crystallinity was determined by XRD powder diffraction. Analysis was performed by using an XRD SHIMADZU 6000 diffractometer equipped with a Cuk α (K=1.54 A°) source, maintaining applied voltage of 40 kV and current at 30 mA. About 0.3 g of dried ZnO-NPs was deposited as a randomly oriented powder into a plexiglass sample container and the XRD patterns were recorded between 5° and 50° angles, with a speed of 5.0° /min.

The crystalline domain Diameter (D) was obtained from XRD peaks using the following Scherrer's equation $D=K * \lambda / \beta * \cos \theta$, where λ is the wavelength of the incident X-ray beam; Θ the Bragg's diffraction angle; β the width of the X-ray pattern line at half peak-height in radian and the dimensionless shape factor (K) has a typical value of 0.89, but varies with the actual shape of the crystalline [8].

2.2.2 Transmission electron microscopy (TEM) of ZnO-NPs

The size distribution of ZnO-NPs in suspension was measured using a submicrometer particle size analyzer (Nicomp,Port Richey, FL) and was confirmed to be within the designated range. Images of NP in the vehicle were also obtained by TEM (JEM-2010, JEOL, Tokyo, Japan).

2.3 Experimental animals

Twenty albino male mice obtained from the animal house. The age of mice was 3 weeks, and the weight was 20-25 gm. Animals cages were kept in standardized conditions (25 ± 5 ° C, 12 hrs. light/ dark cycle). In this study, the male albino mice were divided into two groups (each group comprises 10 mice).

The First group (control group) received general food and water.

The second group was oral gavages by (100 mg/ml) body weight of ZnO Nps.

All animals were sacrificed after two weeks at the end of experiment.

A small piece of liver and kidney and brain were taken and fixed in 10% formalin solution, after that routine histological preparation was conducted[9].

Use of experimental animals in the study protocol will be carried out with the ethical guidelines of the Medical Research Institute, Alexandria University (Appendix 2, Guiding Principles for Biomedical Research Involving Animals, 2011).

For the two previous groups the followings were done.

2.4 Osmotic fragility Study of the RBC' s

Whole blood samples were collected in EDTA from the heart, preferably the ventricle, which can

be accessed either via the left side of the chest, through the diaphragm, from the top of the sternum. Voiles and treated with RBC diluting fluid to lyse the WBCs. In order to lyse the RBCs the resultant mixture was treated with varied concentrations between 0.1 to 1.2 % NaCl[10].

The supernatant absorption was measured using spectrophotometer at 540 nm using UV-Spectrophotometer (Shimadzu, UV-1800, UV-Spectrophotometer, Japan)

The morphological changes of the RBC's were examined using Bright field microscopy.

V Histopathological studies

Specimens from each of the followings organs (liver, kidney & brain) were subjected to the general routine technique for tissue preparation.

3. Results

3.1 X-ray Diffraction of ZnONPs

Figure (1) represents the XRD pattern of ZnONPs . A definite line broadening of XRD peaks indicates that the prepared material consists of particles in the Nano scale range. From this XRD patterns analysis, we determined peak intensity, position and width. Diffraction lines of ZnO were broadened and diffraction broadening was found dependent on Miller indices of the corresponding sets of crystal planes. For most samples the diffraction line 4000 is narrower than the line 8000 and 8000 narrower than the line 5000. The average crystallite size of samples S2 (700 ° C) and S3 (900 ° C) were determined by the Debye-Scherer formula 0.9 λ /Bcos (θ) and were found to be 26.74 nm and 28.93 nm, respectively. Comparing the XRD report of three samples it has been concluded that the samples calcined at 700 ° C and 900 ° C gives a high intensity fine peaks.

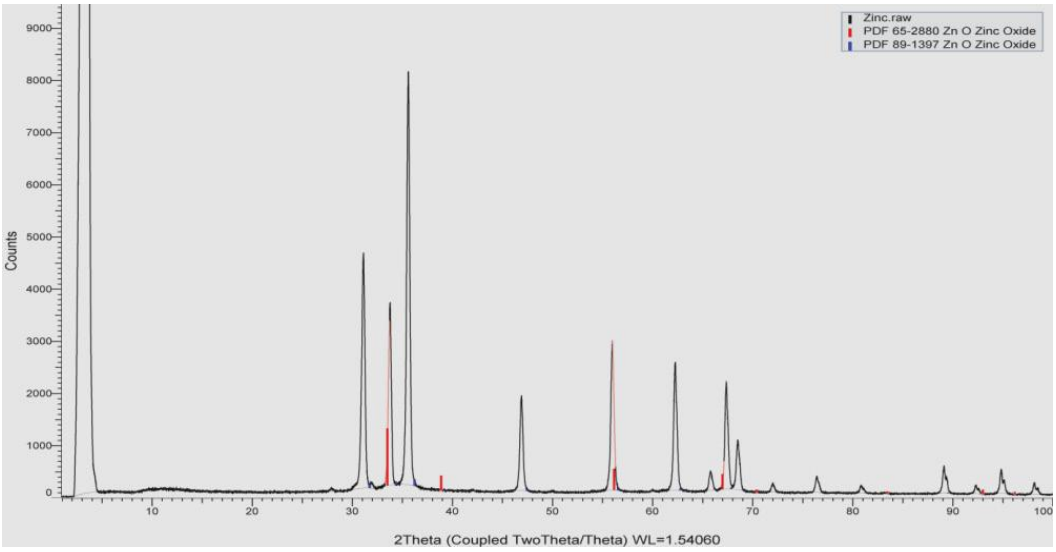


Figure 1: XRD pattern of ZnONPs synthesized at calcining temperatures 500°C

3.2 Transmission electron microscopy (TEM) of ZnO-NPs

Figure (2) shows the TEM images and selected area electron diffraction patterns of ZnO-NPs annealed at 700 ° C and 900 ° C. This image reveals that the product consists of spherical particles with the average size of<100 nm. The selected Area Electron Diffraction (SAED) shows the crystalline structure, complexity for variable calcination. It indicates that ZnO-NPs are not single crystals, rather are the aggregation of several single crystals.

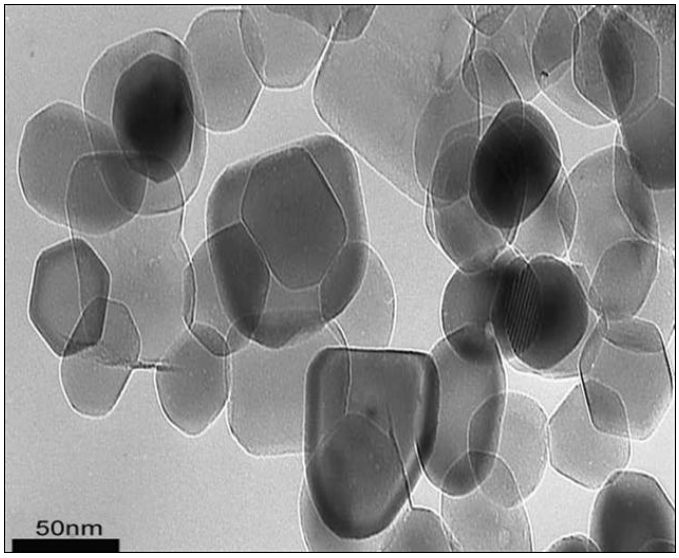
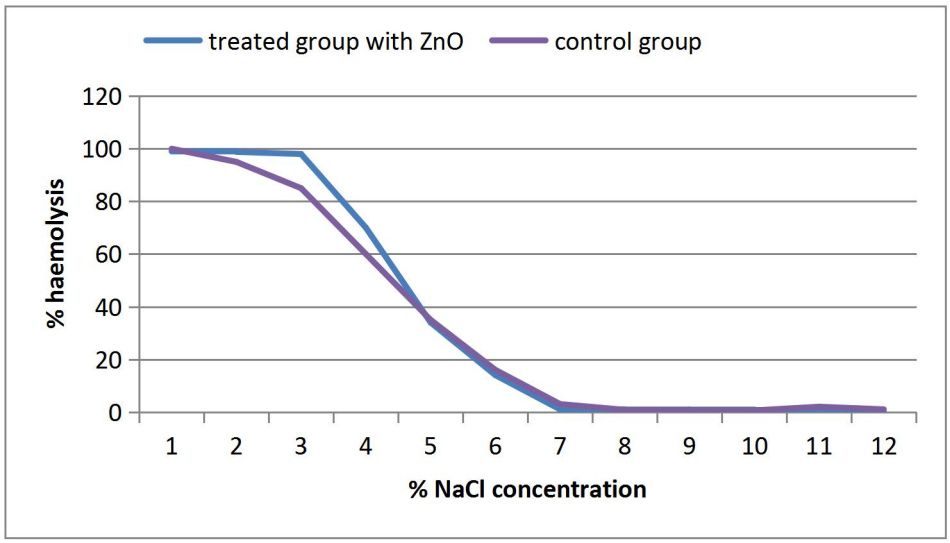


Figure 2: Transmission electron microscopy (TEM) images of synthesized ZnO nanoparticles



The Figure (3): Osmotic fragility curve for RBCs for both control & treated group

The RBCs of the ZnO treated group begins to lyse approximately at the same time to the normal cell

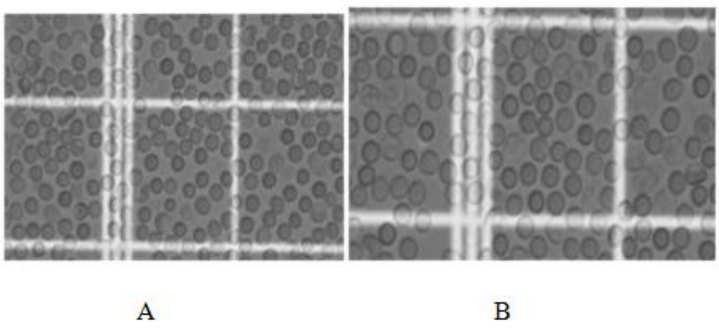


Figure 4: The morphological changes of RBC's under Bright field microscopy for both control group A, treated group with ZnO nanoparticles B

A:normal round shapes of RBCs. B:RBCs. of the treated group with nanoparticles showing swollen, crenation and lysed cells.

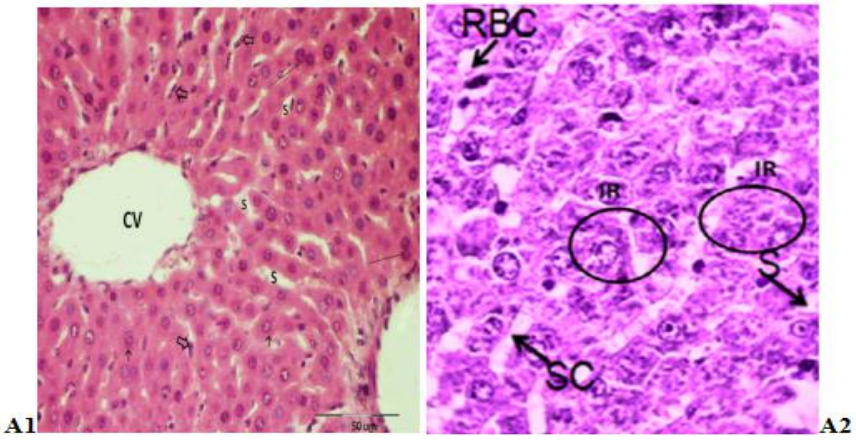


Figure 5: Histopathology of liver tissues in rats treated with ZnO-NPs (100 mg/Kg) for 5 consecutive day

(A1)Control group showing normal hepatocytes with central vein (CV). A2 pathological alterations in the liver of ZnO-NPs (100 mg/kg) treated rats manifested by sinusoidal congestion (SC) and RBC (Red Blood Cells) deposit and inflammatory response (IR),Sinusoide (S) mononucleated (↑) and binucleated hepatocytes (thin arrow) A1 and A2magnification x 400

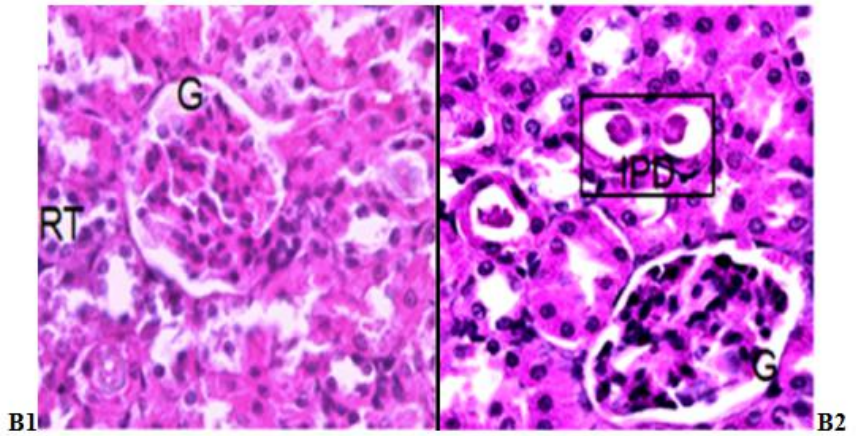


Figure 6: Histopathology of kidney tissues in rats treated with ZnO-NPs (100 mg/Kg) for 5 consecutive days

B1 control group showing normal architecture of renal corpuscles with their glomeruli (G) and renal tubules (RT); B2 treated group showing intratubular protein deposition (IPD) but no significant glomerular changes (G). B1 and B2 magnification x 400.

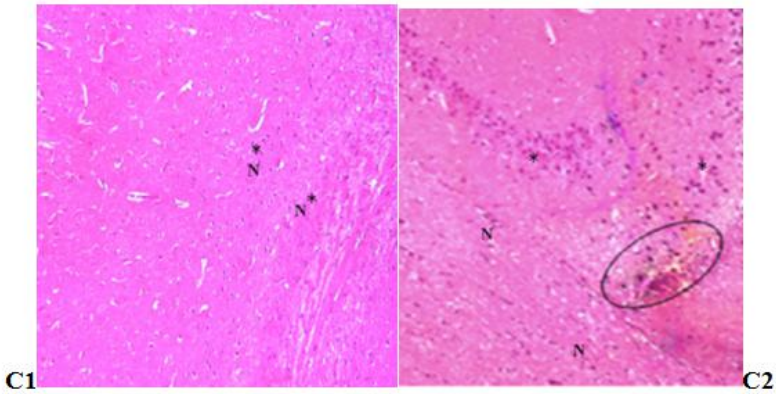


Figure 7: Histological analyses in the brain tissues in rats treated with ZnO-NPs (100mg/kg) for 5 consecutive days

C1control group, the histological analyses in the brain tissues did not show significant changes in morphology and nuclei of neurons were observed (N).C2 treated group showing congestion of vascular (asterisk) and edema (round). C1, C2 magnification x400.

4. Discussion

The small sizes of the particles can enter and damage the organism allows to penetrate the physiological barriers traveling with circulatory systems [11]. The study found that oral exposure to nano-forms was more toxic than micro-counterparts [12].

In the present study, the effects of ZnONPs on RBCs have been investigated in context with the osmotic fragility, hemolysis and morphological features of RBCs. Fragility of RBCs represents stress on the membrane due to osmotic and mechanical stress; osmotic stress is due to the pressure caused by the flow of hypotonic solution; osmotic fragility is also related to the composition, integrity, size of the cell and/or surface area to volume ratio. The fragility of red blood cells is related to some of the diseased conditions like hereditary spherocytosis, hypernatremia, anemia, thalassemia, and sickle cell anemia.[13,14] Mechanical fragility of RBCs refers to the stress caused due to some kind of shear stress during diagnostic testing of blood, handling devices, manipulation of blood during dialysis or intra-operative auto transfusion, storage of red blood cells (storage lesions) and/or applications during blood transfusion and blood bank.[15,16]

In current study, all of the RBCs did not hemolyzed at the same time when treated with ZnONPs, some RBCs took more time, and some became crenated while some remained turgid for relatively longer duration (Fig 4A, 4B) Similarly in this study ZnONPs were found to influence the membrane of red blood cells without breaking it i.e. degree of hemolysis increased slightly with increase in the concentration of NaCl under isotonic condition.

Percentage hemolysis was found at 0.1%, 0.2%, 0.3% and 0.4% of NaCl, as reference points. These concentrations of NaCl were involved while studying the effects of ZnO NPs on hemolysis of red blood cells.

The reason why erythrocytes do not show immediate damage when exposed to toxic substances is because they have a system of antioxidant defense that includes non-enzymatic antioxidants such as glutathione and antioxidant enzymes such as catalase and peroxiredoxin-2[17,18] The presence of this defense system could explain their resistance to the damage induced by nanoparticles, indicating that these cells are not as sensitive to the toxic effects of nanoparticles.

ZnONPs can spread more in blood, kidney, liver and other organs.[19] This study agrees with Sharma V. et al results[20] showed that oral acute exposure to ZnONPs causes apoptosis in mice liver cells and induces severe oxidative stress. Histopathological section showed that ZnONPs is able to induce changes in tissues of kidney and liver in albino male mice. Liver damage could be induced due to excess oral ZnONPs, and this agrees with another study in 2012.[21]

Additionally, the histopathological examination may be helpful in detecting organs abnormalities after ZnONPs uptake by the gastrointestinal tract. The results of this experimental study indicated that ZnONPs in mentioned concentration(100mg/kg) don't show significant effect on the body weight gain and the relative organs weight. This is in accordance with our previous finding indicating the absence of toxic signs and mortality in adult rats exposed to ZnO-NPs. [21,22]

Sharma et al.(23)showed that nanoparticles were mainly found to be retained in the liver after 14 day of sub-acute oral exposure to ZnONPs at a higher dose (300 mg/kg). However, Matsumoto et al[23]suggested that repeated oral gavage of nanoparticles reached the gastro-intestinal tract as agglomerates and were mostly excreted via faeces but no investigations and results to support this suggestion were presented. However histopathological analysis of the kidney showed intratubular protein deposition (IPD) but no significant glomerular changes.

In the current study, oral exposure to ZnONPs did not cause significant changes in the activity of mice. This result is in accordance with our previous study indicating the absence of correlation between zinc accumulation in brain following the oral administration of ZnONPs and the behavioral performances of rodents.[24]

Our results revealed many histopathological and ultrastructural changes in the brain and spinal cord such as congestion and mild degeneration to severe degeneration associated with ultrastructural disturbance in the neuronal cells and apoptotic

degenerative cells. These results are consistent with Migliore and colleagues.[25] who reported that zinc oxide nanoparticles cause neurodegeneration in the brain tissues, and in agreement with Win-Shwe and Fujimaki[26] who showed that the toxic effects of zinc oxide nanoparticles on the central nervous system include cytotoxicity, inflammation, and oxidative stress induction that result in neurodegeneration.

According to Nguyen, H.[27] the mechanism of zinc oxide nanoparticle toxicity depends on the oxidative stress generation that causes an inflammation process based on the activation of inflammation related genes. In the same context, it has been shown that the oxidative stress of zinc oxide nanoparticles have the ability to affect the integrity and permeability of blood-brain barrier via inducing endothelial cell leakiness and pro inflammatory mediators, which is in agreement with Setyawati, M.,& Giovannia, M.[28,29]

Neurotoxicity may also occur via a direct effect on the brain itself or by an indirect systemic inflammatory effect. In the contrasting context, Zheng,Y. et al[30] confirmed that zinc oxide nanoparticles don't have any toxic effect on the brain tissues, which is in agreement withWang,et.al.[31] The results of the present study together with those of previous studies of ZnONPs is not clear, but may involve probably the impairment of the active transport processes or ions exchange mechanisms in the brain.

5. Conclusion

The results of the present study together with those of previous investigations showed that effects of oral nanoparticles exposure are less remarkable than those observed for parenteral administration. The oral route is probably one of realistic way of modeling nanoparticles exposure in humans. In the present investigation oral exposure to moderate dose of ZnO-NPs has no significant main effect on the behavior of the rodents and causes subtle signs of toxicity.

Further characterization of nanoparticles in the gastrointestinal tract, including both absorption and excretion in response to ZnONPs intake are needed.

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