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The Impact of Zinc Oxide Nanoparticles (ZnO-NPs) on the Kidney Structure of Male Albino Mice

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Abstract. According to the distinctive physical and chemical properties, zinc oxide nanoparticles (ZnO-NPs) are widely used in a multitude of applications such as medical diagnosis, preparation of pharmaceutical products anti-sunshine, deliver drugs to target parts in the body and cosmetics. The objective of this study was to investigate the impact of ZnO-NPs on the kidney structure of male albino mice by using intra-peritoneal injection. Animals were injected with 0.1 ml of 150 mg kg-1 of ZnO-NPs (25-45 nm size) for a period of 7 and 14 days. The results showed a decrease in the average body weights of animals after 14 days of injection with ZnO-NPs when compared with control group ($p \leq 0.05$), while, the period of seven days did not caused any alteration in the mice average body weights. A decrease was recorded in the diameter of renal glomerular after 14 days of injection with 150 mg/kg ZnO-NPs, and an increase was recorded in the space of Bowmann's capsule after 7 and 14 days of injection with 150 mg kg-1 ZnO-NPs compared with the control animals ($P \leq 0.05$). The diameter of proximal and distal tubules showed statistically significant increase ($P \leq 0.05$) after injection with 150 mg kg-1 ZnO-NPs for 7 and 14 days compared with control groups. The histological examination revealed injuries in the kidney structure after 7 and 14 days of exposure to ZnO- NPs which were involved accumulation of inflammatory cells close to the blood vessel, sloughing and degeneration of lining epithelium in renal tubule, necrosis, foci of nucleus hypertrophy, congestion of blood vessels, lose and atrophy of glomerulus, intratubular calcium deposition and shrinkage of glomerulus. However, the histopathological changes in the kidney after 14 days of injection were more severe than at day 7. We conclude that ZnO- NPs could have serious kidney structure and functional toxicological impacts.

INTRODUCTION

Humans can exposed to a different kind of natural and man-made nanomaterials in the air via inhalation, food via the ingestion, and medical applications via injection [1]. Zinc oxide nanoparticles (ZnO-NPs) are among the most popular metal oxide with unique physical and chemical properties, such as high chemical stability, elevated coefficient of electrochemical coupling, broad variety of radiation intake and elevated photostability[2]. According to these properties, ZnO-NPs are commonly used in various industrial areas such as paints, dyes, pigments and coatings, catalysts, rubbers, alloys, cosmetic material, food additives, electronics, medical diagnostics, etc. [3,4]. These particles are nonetheless toxic to mammals, and may cause oxidative DNA harm, inflammation and apoptosis in the brain of rats following oral exposure [5] or cytotoxic impacts in the cell line [6].[7] found that oral gavage of 10 mg kg^{-1} body weight of ZnO-NPs for 5 days in rats caused pathological changes in the liver (sinusoidal congestion, deposition of red blood cells in the vein and inflammatory response), kidney (vascular congestion and deposition of protein in intra-tubular) and brain (edema and vascular congestion).

Kidney is a significant organ for body waste material excretion through electrolyte and water balance regulation. The assessment of histological changes in the kidney provides helpful data about this organ's health. [8] Discovered that 333.33 mg kg^{-1}/day ZnO-NPS oral administration caused glomeruli segmentation, hydropic degeneration in lining epithelial cell, necrosis and enlargement of epithelial cell in renal tubules. The goal of this study is to explore the impacts of ZnO-NPs on the histological structure of albino mice's kidney. Whereas a study by [16] disclosed that oral gavages of 10 mg^{-1} kg of ZnO-NPs in rats for five consecutive days can cause minor changes in the composition of the kidney (intratubular protein deposition and vascular congestion) without altering the biochemical parameters (creatinine, uric acid and glucose levels).

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MATERIALS AND METHODS

Preparation of ZnO-NPs

The white powder of ZnO-NPs was produced by Sky Spring company Nanomaterials, Inc. USA with a diameter of 10- 30 nm and a purity of 99. 8%. This powder was dissolved in saline solution at the concentrations of 150 $mg^{-1}kg^{-1}BW$. The shape and size of nano ZnO have been defined using Transmission Electron Microscopy (TEM), in AL-Nahrin University, Faculty of medicine. The results showed the shape of ZnO-NPs was hexagonal that may appear as individual particle or in clusters whereas the size was ranged between 25-35 nm (Fig. 1). Whereas, the purity of ZnO-NPs was detected by using Energy Dispersive X-Ray Spectroscopy (EDS) device that found in the Ministry of Science and Technology/Department of materials research. The ZnO-NPs purity was 99.2% with a 0.8% of impurities as shown in Fig. (2).

Stock suspensions of 150 mg kg^{-1} ZnO-NPs were prepared in saline solution (sodium chloride 0.9%)) and sonicated for half hour (Norin Optech, France) and stirred (Daihan.Lab.tech, korea)(for 15 min in a low-density polyethylene (LDPE) plastic container to prevent the agglomeration of ZnO-NPs stock solution. Stocks were further stirred prior to injection every day through the experiment to ensure reasonably dispersed material.

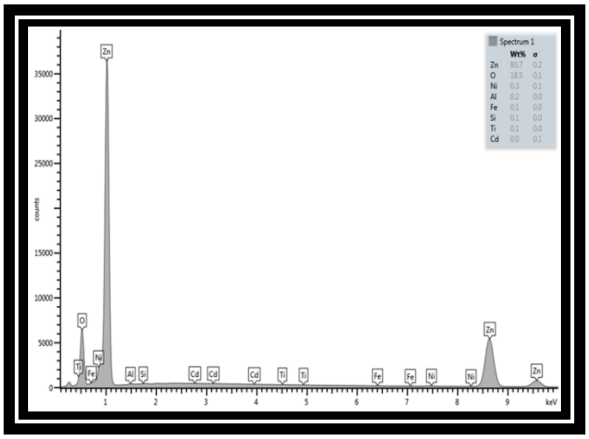

FIGURE 1. Showed the shape and size of ZnO-NPs by using TEM.X180000.

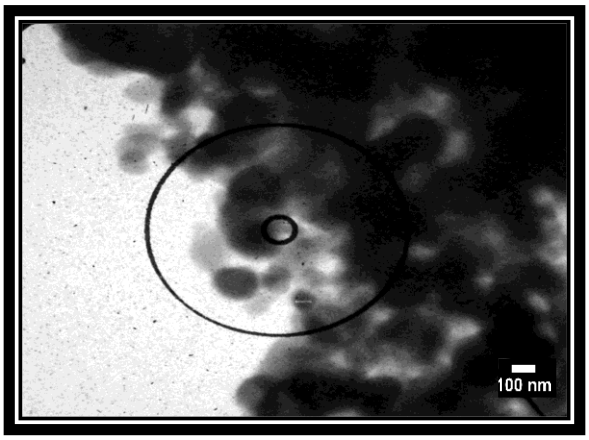

FIGURE 2. Showed the purity of ZnO-NPs by using Energy Dispersive X - ray Spectroscopy (EDS). The purity percentage of ZnO-NPs was 99.2% and impurities was 0.8%.

Experimental Animals

Male albino mice (28.80 ± 0.80 g, Mean ± S.E., n= 24) were obtained from Iraqi Center for Cancer Research and Medical/Genetic in Al-Mustansiriyah University. Animals were kept under controlled temperature, 12 h light: 12 h dark conditions for 7 days (1 week) before starting the experiments for adaption to laboratory conditions. Animals were distributed into four groups, each one contains 6 animals. The first and second group was a control that intra-peritoneal injection with normal saline for 7 and 14 days, respectively. The third and fourth groups was injected with 0.1 ml of 150 mg kg^{-1} ZnO-NPs for 7 and 14 days, respectively. Mice were fed *ad libitum* with commercial pellets and drunk tap water every day. Seven and fourteen days after injection, male

mice were sacrificed by cervical dislocation approach. For histological preparing, kidneys were gathered. At room temperature, a specimen of kidney was fixed overnight in buffered neutral formaldehyde. The samples were then dehydrated in an increasing series of ethyl alcohol, after that treated with xylene and then embedded in paraffin wax. Rotary microtome were used to prepare histological sections of 5 µm thickness and then chosen slides were stained with hematoxylin-eosin. Sections were then examined by optical light microscopy with Olympus digital camera (Sony, Japan). A semi-quantitative measurements (Biometric) were made by using image J programme to determine the structural changes in the kidney.

Statistical Analysis

All current result was written as a Mean ± Standard error and analyzed using one-way variance assessment (ANOVA) by using SPSS version 21. A statistical significant difference was regarded to be $p \leq 0.05$ for all tests.

RESULTS

Body Weights Alteration

The results showed significantly decrease in the average of animal body weights after 14 days of injection with ZnO-NPs when compared with control group ($p \leq 0.05$) (Fig. 3). Whereas, the period of seven days did not shown significant difference ($p > 0.05$) in the average of body mice weights when compared with the control (Fig. 3).

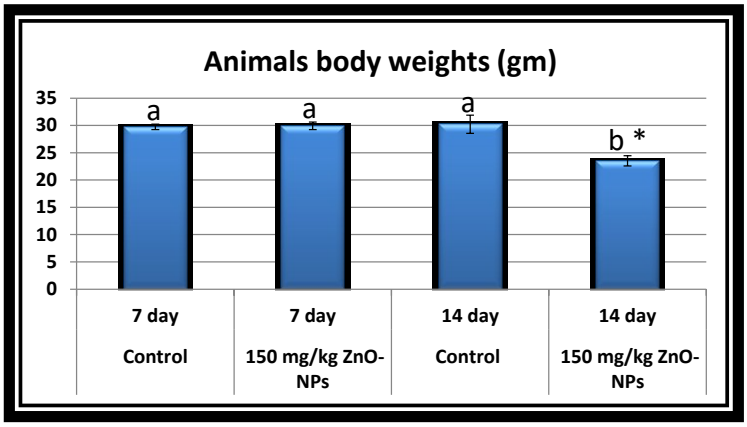

FIGURE 3. illustrate average body weights of mice after injection with 150 mg/kg/BW of ZnO-NPs for 7 and 14 days. Data refers means ±S.E.M. (n = 6/ treatments). Similar letters proclaim no significant difference ($p > 0.05$). Different letters proclaim significant differences ($p < 0.05$). * proclaim significant difference between day 7 and 14.

Biometric Alteration

The current results illustrated alteration in the diameter of renal corpuscle's that involved a decrease in the diameter of glomerular after 14 days of injection with 150 mg kg^{-1} ZnO-NPs when compared with control group (Table 1). Bowman's space distance showed a statistically significant elevation ($P \leq 0.05$) after 7 and 14 days of injection with 150 mg kg^{-1} ZnO-NPs when compared with the control (Table 1).

The diameter of renal tubules (proximal and distal tubules) illustrated statistically significant increase ($P \leq 0.05$) after injection with 150 mg kg^{-1} ZnO-NPs for 7 and 14 days when compared with control groups (Table 2).

TABLE 1. Alterations in the diameter of glomerular corpuscles and Bowmann's space distance after 7 and 14 days of intra-peritoneal injection with 150 mg kg^{-1} ZnO-NPs in mice.

Treatments	Duration of injection (day)	Glomerular diameter (µm)	Bowmann's Space (µm)
Control	7	53.68 ± 2.03a	9.12 ± 0.56 a
150mg/kg ZnO-NPs	7	53.96 ± 1.69 a	14.54 ± 1.51 b
control	14	61.86 ± 3.09 a	7.74 ± 0.99 a
150 mg/kg ZnO-NPs	14	49.91 ± 3.60 b	15.10 ± 1.18 b

Values refers mean ± S.E.M (n=6/treatments)
Similar vertical letters proclaims no significant alterations ($P > 0.05$)
Different vertical letters proclaims significant alterations ($P \leq 0.05$)

TABLE 2. Alterations in the diameter of proximal and distil tubules after 7 and 14 days of intra-peritoneal injection of mice with 150 mg kg^{-1} ZnO-NPs.

Treatments	Duration of Injection (day)	Diameters of (µm)	
		Proximal tubule	Distil tubule
Control	7	34.65 ±0.66 a	35.59 ±1.94 a
150 mg/kg ZnO-NPs	7	40.49 ±1.03 b	40.57 ±0.82 b
Control	14	36.91 ±1.03 a	36.95 ±0.89 a
150 mg/kg ZnO-NPs	14	40.61 ±1.79 b	40.85 ±2.12 b

Values refers mean ± S.E.M (n=6/treatments)
Similar vertical letters proclaims no significant alterations ($P > 0.05$)
Different vertical letters proclaims significant alterations ($P < 0.05$)

Histopathological Examination

Histological examination of the kidney tissues in control groups revealed no important differences from the ordinary histological structure (Fig.4-A). The intraperitoneal injection of 150 mg kg^{-1} of ZnO- NPs for 7 days caused infiltration of inflammatory cells, sloughing and damaging of lining epithelium in renal tubules, epithelial cells necrosis, foci of nucleus hypertrophy, congestion of blood vessels, lose and atrophy of glomerulus, intratubular calcium deposition (Fig.4-B, C, D). Shrinkage of glomerulus and more deposition of intratubular calcium of were found after 14 days of injection (Fig. F). However, the histopathological changes in the kidneys after 14 days of injection were more severe than at day 7 (Fig. E and F).

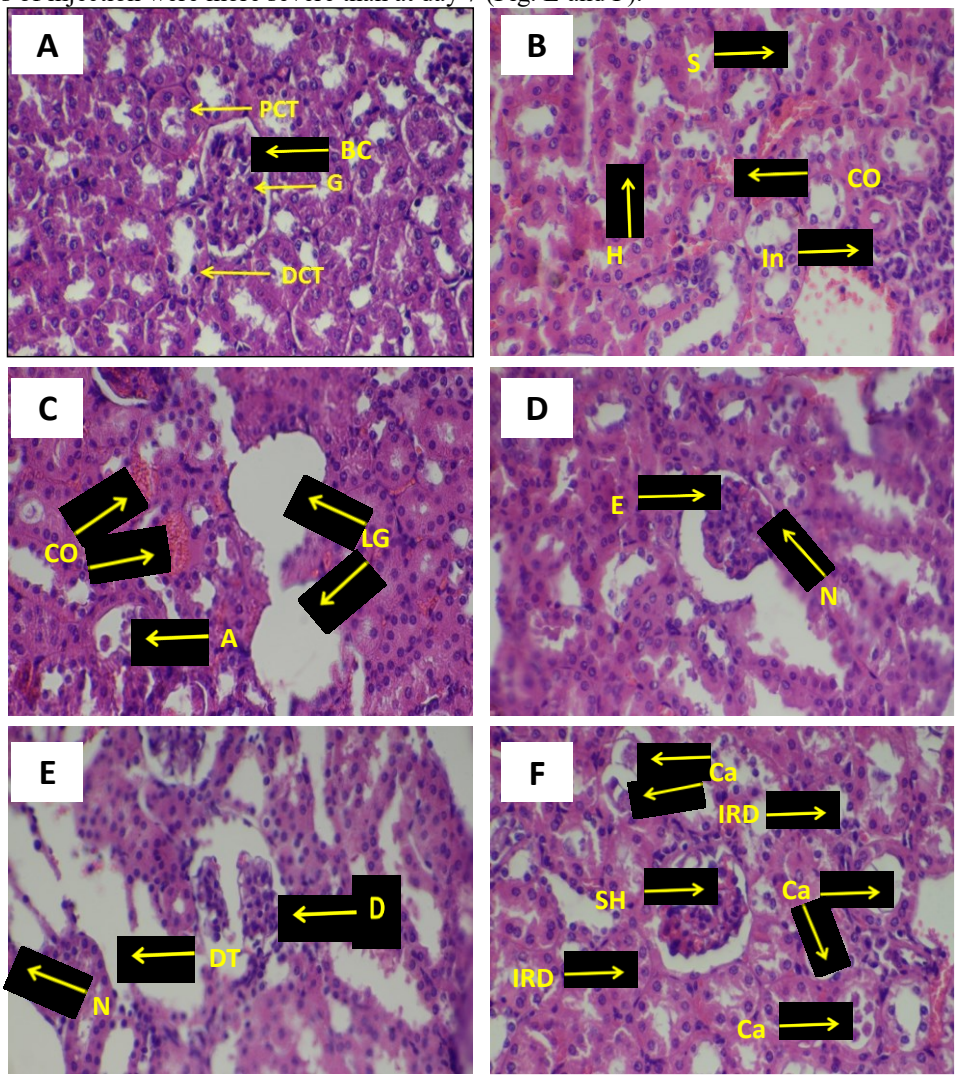

FIGURE 4. Transverse sections of kidney in male albino mice in different groups. (A) The kidney cortex of control group showed renal corpuscle with normal structure of glomerulus (G), the proximal (PCT) and distal convoluted tubules (DCT); (B, C, and D) The kidney abnormal structure in mice that were injected with 150 mg^{-1} kg of ZnO-NPs for seven days; (E and F) The kidney abnormal structure in mice that were injected with 150 mg/kg of ZnO-NPs for 14 days. The injected groups

illustrate lesions which involved infiltration of Inflammatory cells (In), Sloughing of lining epithelium from the basement membrane (S), Necrosis and degeneration of lining epithelium in renal tubule (N), Nucleus Hypertrophy (H), Blood vessels congestion (CO), Expansion of Bowmann's capsule space (E), Lack of glomerulus (LG), (D) Degeneration of renal corpuscle and renal tubule (RT), (SH) Shrinkage of glomerulus, and deposition of calcium in lumen of renal tubule (Ca).(H +E stained, X400).

DISCUSSIONS

After multiple exposure paths (inhalation, ingestion or intravenous injection), metal nanoparticles (M-NPs) were discovered to achieve systemic circulation. They are known to be distributed to various organs, including liver, spleen, kidneys, and brain as well as the heart [9-10-11]. Kidney is an oranges that play an significant role in removing xenobiotics from the body and thus, M-NPs that absorbed in the circulatory system can be evacuated by renal clearance [12-13]. Translocation, however, relies on the physicochemical properties of M-NPs, and their migration to remote sites is a major toxicity problem. This research was therefore targeted at investigating the impact of ZnO –NPs on albino mice's kidney structure to highlight their prospective toxicity and/or biological responses to this material.

Animal Body Weights

The intra-peritoneal injection of ZnO-NPs illustrated a decrease in the average body weight of animals after 14 days, and this might be due to the gastrointestinal dysfunction or loss of appetite to get a food because of the accumulation of nanomaterial in the stomach or intestine or to liver disfunction. This result is consistent with a study of [14] that found a decline in the average body weight of animal after 7 and 14 days of oral gavage with 100 and 200 mg kg^{-1} of ZnO-NPs.

Biometric Measurements and Histopathological Alterations

Renal exposure to metal nanoparticles should be anticipated with the key role of the organ in xenobiotics excretion. The biometric measurements in the current study support our finding in the histopathological portion, which illustrated the elevation in the diameter of renal tubules and the distance of Bowmann's space as well as a decline in the diameter of renal glomeruli. However, histopathological observation revealed that intra-peritoneal injection of ZnO-NPs for 7 and 14 days caused severe renal toxicological effect through causing an increase in the inflammatory cell infiltration, blood vessel congestion, formation of calcium molds within the renal tubules, nuclei hypertrophy, foci of necrosis and degeneration in the lining epithelial cells of renal tubules, shrinkage or loss of renal glomeruli, sloughing epithelial cells from the basement membrane in renal tubule. The current results is consistent with a study of [15] that found the oral gavage of 100, 250, and 500 mg kg^{-1} of nano ZnO in rats produced kidney injuries such as necrosis in the renal corpuscle and renal tubules with dilatation of renal tubules that associated with sloughing and degeneration of its lining epithelium and severe inflammatory cells infiltration after 21 days of exposure.

Several studies have shown histopathological alteration in mice kidney that were exposed to ZnO-NPs via different routes of exposure [8-16-17]. [17] illustrated that intra-peritoneal or intravenous injection of 1 and 2 mg kg^{-1} of ZnO-NPs cause tubular dilatation, brush borders losing and epithelial cell flattening, decline of Bowman’s space and elevated cellularity in the glomeruli of ZnO NP-treated mice. Whereas, [8] found that oral gavages of 333.33 mg kg^{-1}/day ZnO-NPs in mice caused injuries in the lung, liver and kidney. These injuries include serous inflammation, edema and severe alveoli hyperemia in the lung; cellular necrosis, blood vessel congestion and glycogen accumulation in liver; enlargement of epithelial cells in proximal tubules, segmentation of glomeruli, hydropic degeneration of epithelial cells and necrosis in the kidney tubules.

The alteration in the structure of renal tissue that found in the current study could occur due to the oxidative changes. In vitro study, [18] conclude that the potential of nephrotoxicity [using glomerular mesangial cell line (IP15) and proximal epithelial tubular cell line (HK-2)] of ZnO-NPs and CdS-NPs is occurred due to the creation of reactive oxygen spices (ROS) and induction of oxidative stress (OS). [19] discovered that ZnO-NPs can cause apoptosis in cancer cells by mediating ROS through p53, caspase and bax/bcl-2 pathways. On the other hand, [20] illustrated that 25, 50, 75 and 100 µg ml^{-1} of ZnO-NPs (50 nm) can induce cellular morphological abnormalities, mitochondrial dysfunction, DNA damage and decrease of superoxide dismutase as well as depletion of glutathione. However, [20] suggested that oxidative stress could contribute to the cytotoxicity of ZnO-NPs. In fact, oxidative stress and lipid peroxidation play a major role in the toxicity of ZnO-NPs that stimulate the creation of the pro- inflammatory cytokines, IFN-γ, TNF-α, and IL-12 [21]. The observation of inflammatory cells infiltration in the current study is agreement with the results of [22] who found infiltration of mononuclear cells in the interstitial tissues with severely degenerated and vacuolated renal tubular epithelium in

the kidney of rabbit after feeding with ZnO-NPs. Additionally, the findings of [23] in mice are completely in agreement with our finding result where they elucidated that the kidney structure is not normal in the treated groups and considerably several histopathological changes have been observed, such as accumulation of inflammatory cells in glomerular capillaries and degeneration of proximal and distal tubules after single injection of ZnO-NPs.

Another important histopathological alteration that observed in the current study is the formation of calcium molds in the lumen of renal tubules. This pathological alteration may be cause due to the high level of hydrogen peroxide (H2O2) in the kidney tissue which resulting from the depletion of glutathione enzyme level that responsible for the transformation of H2O2 to H2O. The elevation of H2O2 can cause nephrotoxicity, which causes alteration in the permeability transition of the mitochondrial membrane, then the amount of Ca^{2+} uptake by the mitochondrial membrane in the presence of ROS was increased. Thus, the degeneration of mitochondrial was associated with the elevation of intra-mitochondrial calcium level in the renal tissue [24].

CONCLUSION

In conclusion, 150 mg kg^{-1}/day intra-peritoneal administration of ZnO-NPs for 7 and 14 days can trigger many deleterious impacts in the kidney tissue, including infiltration of inflammatory cells, sloughing and degeneration of lining epithelium in renal tubule, foci of necrosis and nucleus hypertrophy, blood vessels congestion, lose and atrophy of glomeruli, intratubular calcium deposition and shrinkage of glomerulus. Nonetheless, these effects were more severe at day 14 than at day 7. However, the ZnO-NPs have potential nephrotoxicity that may cause kidney dysfunction or disorder in the glomerular filtration rate

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