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Zinc oxide nanoparticles (ZnO NPs) induced nephrotoxicity in male sprague dawley rats

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Abstract

Nano size particles (<100nm) have various applications in electronics, coating, cosmetics, packaging and biotechnology. Zinc Oxide nanoparticles (ZnO NPs) are being used in ceramics, leather manufacturing, plastics, rubber, glass, fire retardants and batteries along with having antimicrobial and anticancerous properties. In present research, 25 post weaning male Sprague Dawley rats of similar weight were procured from the animal house of Government College University Faisalabad after approval of the ethical committee on animal experimentation. Rats were kept in 5 cages (n=5) and varying levels of ZnO NPs were injected intraperitoneally (i.p.) for 28 days on alternate days to treated groups at the dose of either 10 or 20 or 30 mg/kg and named as group one (G1), two (G2) and three (G3), respectively for the assessment of toxicity for better understanding of precautionary measures in near future. Without any treatment groups i.e., control (C) and saline(S) received normal diet and saline water (0.9% sodium chloride), respectively. Histological changes were investigated in kidney tissues of all groups. Groups receiving 10 and 20mg/kg of NPs showed moderate pathological changes like atrophic glomerulus, inter-tubular space, degeneration of tubular epithelium and tubules and accumulation of ZnONPs. While, G3 group showed congestion, accumulation of RBCs and hemorrhages in kidney tissues along with above noticed variations. Whereas, no alterations were seen in control groups (C &S). It is concluded that ZnO NPs at higher concentration are more toxic to Sprague Dawley rats than at lower concentrations.

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Introduction

Nanotechnology uses matter less than 100nm in diameter for the formation of nano size particles of distinct properties. Nanoparticles have unique physical and chemical properties than bulk salt. These nano size particles have various functions in electronics, coating, cosmetics, packaging, and biotechnology (Khatoon *et al.*, 2017). NPs can be introduced in the environment and living system through various pathways such as spillage, effluent, disposal and consumer products. Living things can filter its lower concentration but higher concentration leads to damages and ultimately mortality.

Zinc oxide nanoparticles (ZnO NPs) have distinct benefits and are added in the diet of livestock as a supplement (Noori *et al.*, 2014). It is a necessary ingredient of various enzymes, ointment (pain and itch relief) and sun screens (Siddiqi *et al.*, 2017). These are being used in ceramics, leather manufacturing, plastics, rubber, glass, fire retardants and batteries along with having antimicrobial and anticancerous properties. They are chemically stable and more soluble and are useful in the biomedicine, agriculture and food additives (Nawaz *et al.*, 2011; Wang *et al.*, 2016; Haq *et al.*, 2017). Its effect on biological processes is based on its morphology, exposure time, particle size, pH, concentration and biocompatibility (Siddiqi *et al.*, 2017).

Zinc is one of the essential elements needed for the body growth and important physiological processes. It is required for the enzymes activity (250 to 300 enzymes) and takes part in several metabolic and enzymatic functions in the body of animals (Ahmadi *et al.*, 2013).

NPs can be introduced in the living organisms by various routes such as ingestion, inhalation and injection etc.. They can also mixes with blood leading to severe biological alternations in living organs. Moreover, kidney is specifically vulnerable to the xenobiotics because of its more blood supply and its ability to filter toxins. They significantly reduced the glutathione concentration compared with control, which expresses severe injuries to tissues of kidney. Recent research confirmed that ZnO NPs disturbed energy metabolism and damage mitochondria along with injuries to cell membranes of kidney (Lin *et al.*, 2015). These can't penetrate in the body by skin but can be ingested through lipsticks and sunscreens accidentally, and also through food chain (Khorsandi *et al.*, 2018). Therefore it is very necessary to evaluate its toxic potential for better health and for manufacturing of valuable materials. Our research was based on confirmation of dose dependent toxicity by ZnO NPs to assess lethal dose.

Materials and methods

Chemicals

High quality chemicals of analytical grade were used for histological study. ZnO NPs of size range between 50-100nm were purchased from Sigma-Aldrich.

Stock solution of ZnO NPs

ZnO NPs suspension was prepared in 0.9% saline solution of sodium chloride. The solution was ultrasonicated (sonicator: 4000) for thirty minutes to dissolved the material. Afterward, severe shaking of suspension was done by using vortex for one minute. Then these fully dissolved nanoparticles were injected intraperitoneally (i.p.) in male albino rats in different concentrations as required.

Animal housing and sample collection

In present research 25 post weaning male Sprague Dawley rats of similar weight were procured from the animal house of Government College University Faisalabad after approval of the ethical committee on animal experimentation of Government College University Faisalabad. Rats were kept in 5 cages (n=5) and were acclimatized for normal light, temperature and diet for 7 days. Then experimental trial was started. First cage represented control group (C) and received normal diet. Second one represented saline group (S) received saline water (0.9% sodium chloride) for the equivalency of shock. Treated groups named as G1, G2 and G3 received intraperitoneal injection of varying levels of ZnO NPs on alternate day for 28 days at the dose of either 10 or 20 or 30

mg/kg of b.wt., respectively. At the end of experimental period, animals were dissected and small pieces (2-3 mm) of kidneys were fixed in sera solution for histological analysis.

Histological protocol

The samples of kidney were fixed in sera formed of 30 ml formaldehyde, 10 ml acetic acid (glacial) and 60 ml alcohol. Ethanol of different concentrations such as 70-90, 95 and 100 percent were used for dehydration purpose. Tissues were cleared by using cedar wood oil. Then tissues were kept in paraplast. Paraplast were replaced after thirty minutes and processed kidney tissues were again kept in an incubator for twelve hours at sixty degrees. Process was repeated three times. The blocks of kidney samples were prepared and mounted into plastic casters (HAION Caster Industries Co. Ltd, Taiwan). The kidney tissues (3–4µm) were sectioned by using microtome (SLEE Rotary Microtome CUT5062 by Nikon Instruments Europe). Afterward, slides of samples were heated for twenty four hours. De-parafinization, rehydration along with staining were carried out by using xylene, ethanol (50-100%) hematoxylin and eosine, respectively. Dehydration was carried out by absolute alcohol. Two drops of DPX (histology Mountant) was poured on every slide which was spread by using cover slip.

The slides were studied under light microscope (Nikon E200 POL) and photographed by a digital camera attached with the microscope.

Results and discussion

In the present research, control group showed normal glomerulus, bowman's capsule, tubules and bowman's space in renal cortex (Fig. 1A). Normal vasa recta, collecting ducts, loop of henle and ascending loop were noted in medulla region of kidney (Fig. 1B).

Normal renal cortex and medulla part were observed in saline group as well (Fig. 1C-D). G1, G2 and G3 groups receiving 10 mg/kg, 20 mg/kg and 30mg/kg of ZnO NPs showed atrophic glomerulus, intertubular space, nanoparticles accumulation, degenerated tubules (atrophic tubules) in a dose dependent manner with more severity at higher dose (Fig. 2-4; Table 1).

Table 1. Histopathological alterations in kidney of male Sprague Dawly rats among different groups.

Abnormalities	C	S	G1 (10MG/Kg)	G2 (20mg/Kg)	G3 (30mg/Kg)
Degenerated-tubules	-	-	+	+	++
NPs- accumulation	-	-	+	+	++
Degenerated-Epithelium	-	-	+	+	++
Degenerated- Epithelium	-	-	-	-	++
Congestion	-	-	-	-	++
Hemorrhage	-	-	-	-	++
RBC's-accumulation	-	-	-	-	++

In table - represents no abnormality, + represents for moderate abnormalities and ++ represents highest pathological changes in kidney tissues.

This study is in accordance with the study of Mosaid (2015) who also explained renal toxicological condition in rats after administration of ZnO NPs.

He investigated atrophic renal corpuscles (glomerulus and bowman's capsule) and degeneration of tubules creating intertubular space in between tubules. Baek *et al.* (2012) revealed deposition of ZnO NPs in kidney, liver and lungs analyzed by TEM (Transmission Electron Microscopy), and analyzed results were in good agreement with our research in which all three groups have shown the deposition of NPs. G2 group (20mg/kg) also showed damaged epithelium of tubules in medulla region and findings are parallel with work of Mosaid (2015), who evaluated toxicity and noted damaged epithelium oftubules and brush border.

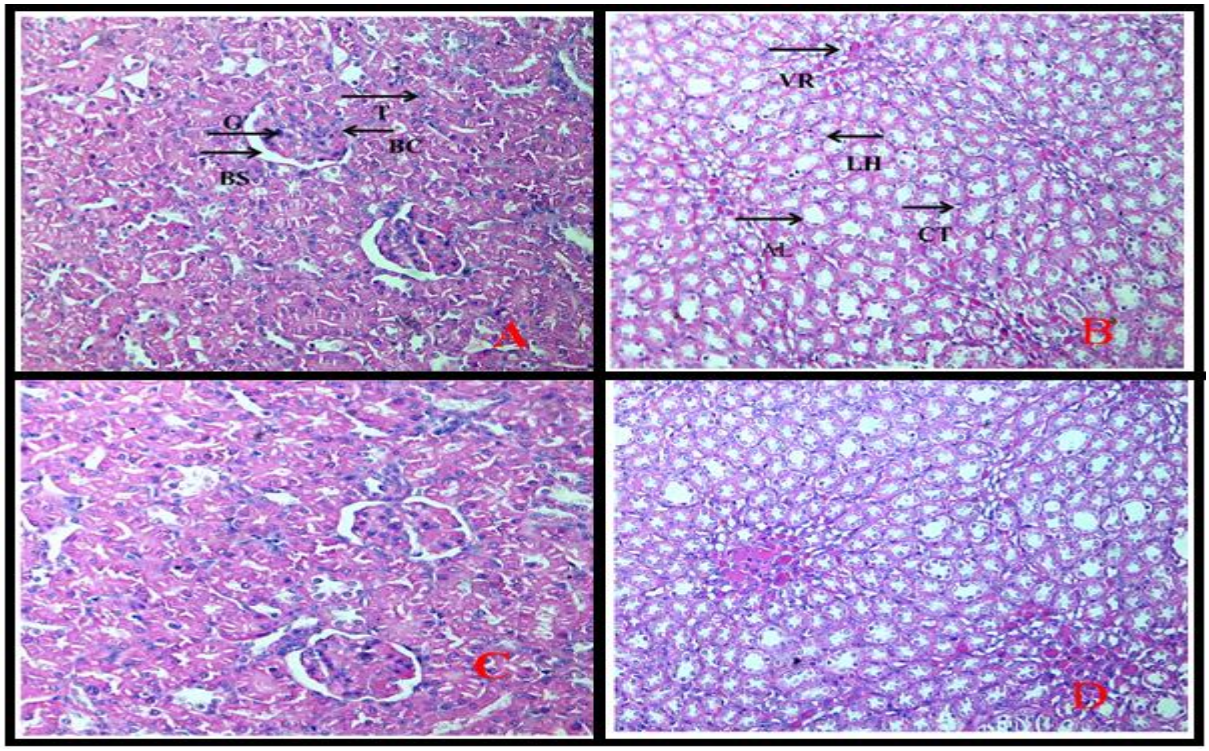

Fig. 1. Photomicrograph (H&E; X200) of control group showing normal Glomerulus (G), Bowman's capsule (BC), Tubules and Bowman's space in renal cortex (A). Normal vasa recta (VR), collecting ducts (CD), Loop of henle (LH) and ascending loop (AD) in medulla region of kidney (B). Normal architecture of renal cortex and medulla were also observed in saline group (C and D).

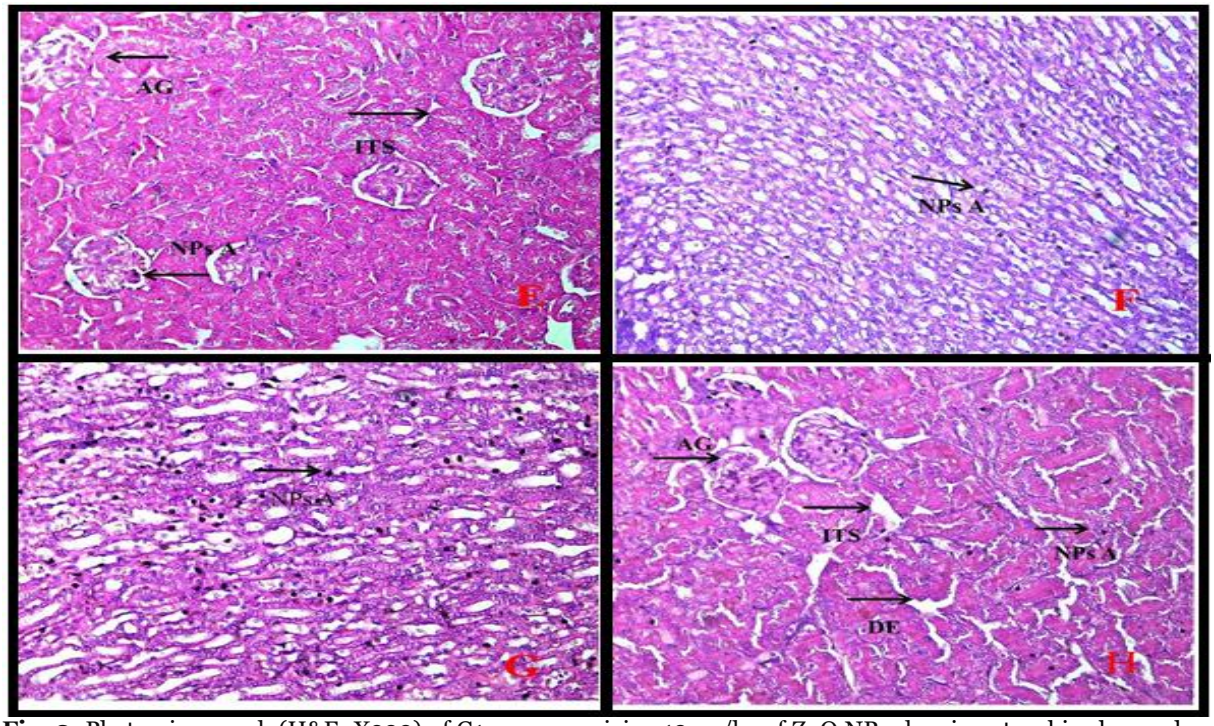

Fig. 2. Photomicrograph (H&E; X200) of G1 group receiving 10mg/kg of ZnO NPs showing atrophic glomerulus (AG), intertubular space (ITS), nanoparticles accumulation (NP's A) in medulla and cortex region of kidney (E and F). Photomicrograph (H&E; X200) of G2 group receiving 20mg/kg of ZnO NPs exhibited atrophic glomerulus (AG), intertubular space (ITS), damaged epithelium (DE), nanoparticles accumulation in cortex and medulla region of kidney (C and D).

G3 group receiving 30mg/kg of ZnO NPs exhibited highest histological changes such as congestion, hemorrhage and changes in glomerulus space other than above pathological changes. Congestion by RBC's in tubules and cortex region were according to the research of Khorsandi *et al.* (2018), who proved toxicity of ZnO NPs and RBC's congestion along with other pathological changes in kidney. Reddy *et al.* (2014) used naringenin to eliminate pathological states of kidney by ZnO NPs and reported hemorrhage and dilation of vessels and congestion in between them.

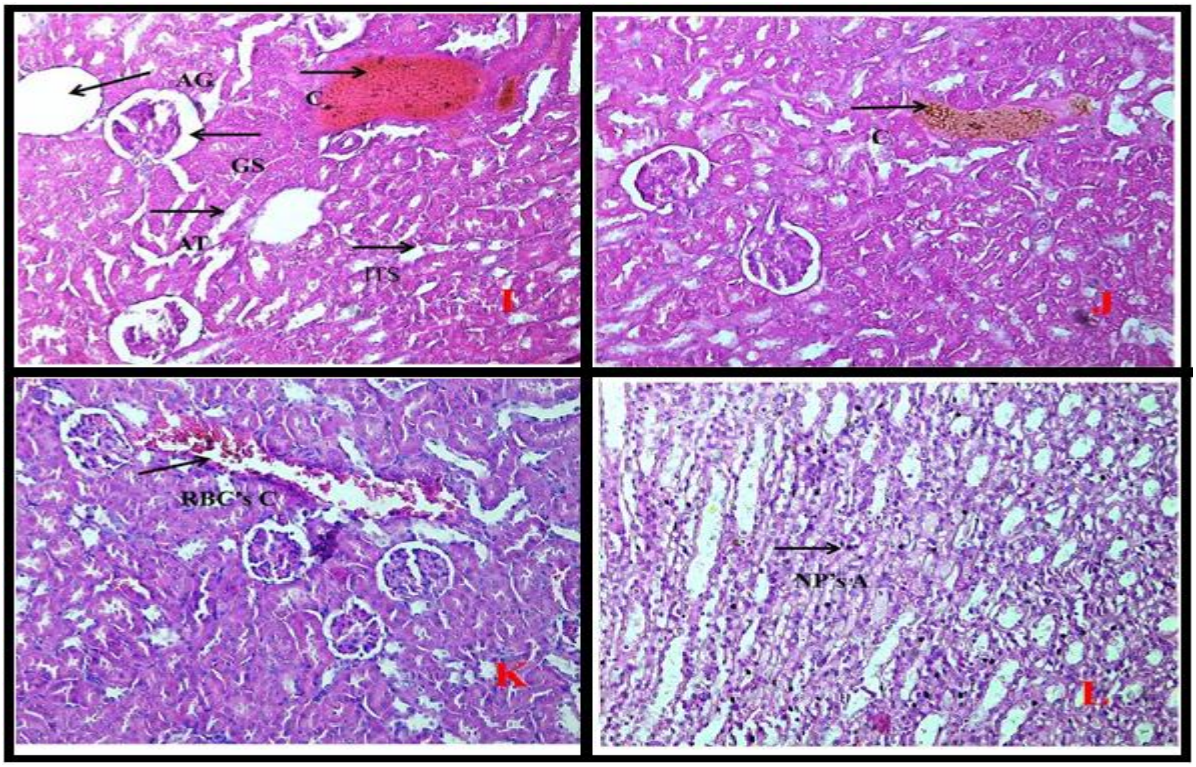

Fig. 3. Photomicrograph (H&E; X200) of G3 group receiving 30mg/kg of ZnO NPs exhibited severe histological changes. Atrophic glomerulus (AG), glomerulus space (GS), atrophic tubules (AT), intertubular space (ITS), congestion (C) and red blood cells accumulation (RBC's A) were observed in medulla region of kidney. Nanoparticles accumulation (NPs A) was observed in medulla region of kidney (NP's A) (I, J, K, L).

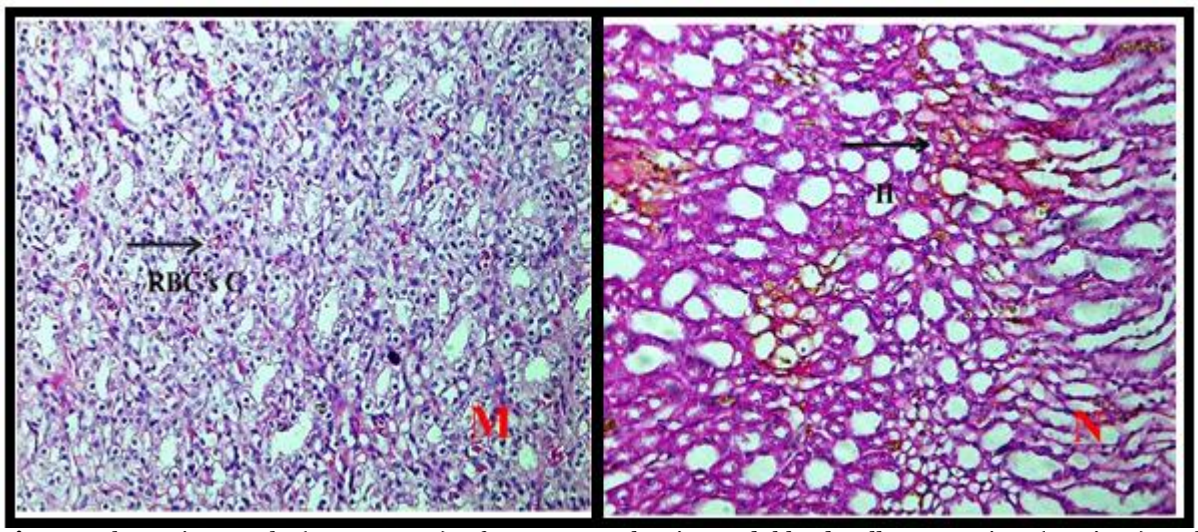

Fig. 4. Photomicrograph (H&E; X200) of G3 group showing red blood cells congestion (RBC's C) and hemorrhage (H) were also noticed in collecting ducts (RBC's C) and in think and thick loop of henle.

The toxicity of nanoparticles at higher dose for kidney was also evaluated by Abbasalipourkabir *et al.* (2015) while working on male rats. The findings of Esmaeillou *et al.* (2013) are in agreement with our results in which ZnO NPs caused toxicity to kidney tissues.

Conclusion

In this study dose dependent ZnO-NPs Toxicity was observed in Sprague Dawley rats. At low and moderate doses of ZnO-NPs less sever histopathological abnormalities were observed as compared to control and saline groups. While exposure at high dose of ZnO NPs the histopathological aberrations were also high as compared to other groups. This study investigated that at higher concentration ZnO NPs are toxic and must be used with care.

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