

Renal Toxicity of Zinc Oxide Nanoparticles (ZnONPs) of Male Westar Rats

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Abstract: *Background:* 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. Nano particles have the ability to enter, penetrate physiological barriers, and travel within the circulatory systems to all body organs affecting the health of human and animals. Understanding various adverse effects of ZnO NPs on cellular and organs functions is necessary to provide better approaches for them. *Aim of work:* In the present study, we evaluated the potential toxicity of ZnONPs in kidney of Westar rats. *Methods:* The current study was carried out on forty apparently healthy mature male Westar rats weighing between 120-200 gm with average three months age. The rats were divided randomly into four groups (10 rats/ group). Group I (G1) was kept as a control and fed with a basal diet only. Group II (G2), Group III(G3) and Group IV(G4) were obtained ZnO NPs in a dose 100, 250 and 500 mg/ kg body weight respectively by oral gavage for 21 days. At the end of experiment, cervical dislocation of rats and the kidneys were separated for the histopathological studies. *Result:* The present investigation revealed that the kidney of the control group (G1) showed normal renal parenchyma without any abnormalities. Meanwhile, G2 and G3 showed mild to moderate necrosis of the renal corpuscle and renal tubules with moderate accumulations of dense acidophilic material in intra tubular parts. With increasing dose of ZnO NPs, the renal toxicity became more obvious where, the kidney of G4 showed severe necrosis in the renal corpuscles with severe dilatation of the renal tubules accompanied with sloughing and degeneration of its lining epithelium, with severe accumulation of dense acidophilic material in intra and inter tubular parts, severe nephritis with severe inflammatory cells infiltration and fibrous tissue proliferation. *Conclusion:* The present investigation was concluded that the ZnO NPs have potential renal toxicity that may affect the function of the kidney.

Keywords: Nephritis, Renal, Toxicity, Zinc Oxide, Nanoparticles, ZnO NPs

1. Introduction

Nanotechnology and nanoparticles could be risk factors for neuropathological and toxicological processes. Along with extensive application of ZnO nanoparticles in the industrial field, it is conceivable that the human body may be intentionally or unintentionally exposed to nanoparticles via several possible routes, including oral ingestion, inhalation, intravenous injection, and dermal penetration. Among these, uptake of nanoparticles by the gastrointestinal tract is one of the most important routes (Hillyer and Albrecht, 2001; Soheili et al., 2013). Retention of metal oxide nanoparticles in the environment and food chain is high and continuous exposure to them may affect human health (De Berardis et al., 2010). Very small particles have the ability to enter, translocate within, and damage living organisms. This ability, results primarily from their small size, which allows them to penetrate physiological barriers, and travel within the circulatory systems (Wang et al., 2007). Earlier studies have shown that nano-forms of different particles are more toxic than their micro-counterparts after acute exposure via the oral route (Dhawan and Sharma, 2010).

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 (Djurisic and Leung, 2006; Fan and Lu, 2005). 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 (Fan and Lu, 2005; Kumari and Li, 2010). 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. To date, a great deal of attention has been focused on nanosized ZnO particles because they possess unique features, which are completely different from bulk-sized ZnO nanoparticles. As the particle size of ZnO decreases, its chemical reactivity, transparency, ultraviolet-filtering efficiency, and dispersion properties also increase, which are favorable features for commercial application (Sokohara and Ishida, 1998; Baek et al., 2011)

In the recent years, global interest in using nanotechnology increased as nanoparticles contain a high number of atoms at their surface which leads to increase surface area and reaction. Among different nanoparticles, zinc oxide nanoparticles (ZnO NPs) have significant benefits and are used as a dietary supplement for a livestock (Noori et al., 2014).

The same properties that make nanoparticles useful can potentially make them harmful to the environment and health of human and animals. Understanding various adverse effects of ZnO NPs on cellular and organs functions is necessary to provide better approaches for them (Fazilati, 2013).

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

Aim of work

The present study was performed to investigate and evaluate the renal toxicity of zinc oxide nanoparticles of male Westar rats.

2. Materials and Methods

• Animals and housing

Forty apparently healthy mature male westar rats weighing between 120-200 gm with average three months age were obtained from laboratory animal unite in the faculty of pharmacy, King Saud University. The rats were randomly divided into four groups and kept in galvanized standard cages, ten animals/cage, under hygienic conditions and left for one week before starting the experiment for accommodation. Feed and water were available ad libitum. Temperature was recorded continuously, and maintained between (20 and 23 °C) along the experimental period. A cycle of 14 h of light and 10 h of dark was fixed throughout the experiment. All animals were handled and all experiments were conducted in accordance with the protocols approved by King Saud University Animal Care Ethical Committee while the experimental procedures were carried out in accordance with international guidelines for care and use of laboratory animals.

• Supplements (Nanoparticles)

Well-dispersed ZnO NPs (average particle size 10-30 nm) at 50 wt% in distilled water (Sigma, Aldrich) were used in the present study. The nanoparticles dispersion had the following characterization: concentration 50 wt.% in H₂O; pH 5.5 ± 0.1; density 1.7 g/ml ± 0.1 g/ml.

• Experimental design

Forty rats were divided randomly into four groups (10 rats/group) and subjected for 21 days to one of the following treatments:

Group I(G1) kept as a **control** and fed with a basal diet without ZnO NPs for 21 days.

Group II (G2) was obtained ZnO NPs in a dose 100 mg/ kg body weight by oral gavage for 21 days.

Group III(G3) was obtained ZnO NPs in a dose 250 mg/ kg body weight by oral gavage for 21 days.

Group IV(G4) was obtained ZnO NPs in a dose 500 mg/ kg body weight by oral gavage for 21 days.

• Histological and histochemical processing

At the end of experiment, cervical dislocation of rats and for histological studies, kidneys were separately and small pieces from them were taken, fixed in neutral buffered formalin 10 %, dehydrated, cleared and paraffin ionized for paraffin blocks and 5 micron sections were obtained, mounted on a glass slides and stained with Hematoxylin and Eosin (H&E), Periodic acid-Schiff (PAS) and Mercuric bromophenol blue according to **Bancroft and Gamble (2001)**.

3. Results

The kidney from the **control group (G1)** showed normal structure of the renal tissues without any histological abnormalities. The renal parenchyma was observed consisting of large number of uriniferous tubules that are consisted of nephron and the collecting tubules. The renal parenchyma consisted of two regions; the outer cortex (**Fig. A1, A2, A3**) and inner medulla (**Fig. A4, A8**). The renal cortex was consisted of numerous spherical structure; renal corpuscles; a thin walled expansion of the proximal end of nephron and deeply invaginated to form double-walled cup-shaped structure; Bowman's capsules. The concavity of the cup was occupied by a tuft of capillaries; glomerulus (**Fig. A1, A5**).

Bowman's capsules, was a double-walled cup, the internal layer; visceral layer was lined with modified cuboidal epithelial cells; podocytes that enveloping the capillaries of the glomerulus. While, the external layer; parietal layer was lined with simple squamous epithelium and was surrounded with the proximal and distal convoluted tubules and loop of henle (**Fig. A5, A6, A7**).

In cross sections, the renal corpuscles were observed surrounding with groups of tubes like structures. Some of them were lined with 3-5 simple cuboidal cells with single central spherical nucleus and acidophilic granular cytoplasm; proximal convoluted tubules. Others were lined with 5-8 simple cuboidal cells with single spherical nucleus located near the lumen and less acidophilic cytoplasm; distal convoluted tubules (**Fig. A5, A6, A7**). The renal parenchyma was centered on "medullary rays", which were bundles of straight tubules; collecting ducts with cuboidal epithelium and loops of Henle (**Fig. A4, A8**).

Regarding the histological changes in the kidney of G2 treated with ZnO NPs in a dose of 100 mg/kg. bwt and **G3** treated with ZnO NPs in a dose of 250 mg/kg. bwt showed mild to moderate degeneration and necrosis of the renal parenchyma specially renal corpuscle accompanied with damage and breakdown of the Bowman's capsules (**Fig. B1, B2, B3**). Furthermore, moderate necrosis of the renal tubules lining epithelium with loss of its brush border was observed (**Fig. B4**). In addition, focal scattered of inflammatory cells infiltration with fibrous connective tissue proliferation was demonstrated within the renal parenchyma especially in between tubular parts (**Fig. B5, B6, B7**). And also, moderate congestion in between the renal tubules was observed (**Fig. B8, B9**). Moreover, moderate accumulations of dense acidophilic material in inter and intra tubular parts were clarified (**Fig. B10, B11, B12**).

On the other hand, kidneys of **G4** treated with ZnO NPs in a dose of 500 mg/kg. bwt showed severe necrosis and degeneration in the renal corpuscles with completely loss of its continuity with completely damage of the Bowman's capsule (**Fig. C1, C2, C3**). Atrophy of some renal corpuscle was also observed (**Fig. C4**). In addition, sever dilatation of the renal tubules with sloughing and degeneration of its lining epithelium were clarified (**Fig. C5, C6, C7**). Some renal tubules lining epithelium showed sever vacuolations with variable size (**Fig. C8**). And also, sever dilatation of

some renal tubules accompanied with sever accumulation of dense acidophilic material in intra and inter tubular parts were clearly clarified (Fig. C9, C10, C12, C13, C14, C15). Some of these dilated renal tubules were observed overfilled with RBCs (Fig. C11, C12). Moreover, sever dilatation of the inter tubules blood vessels accompanied with sever congestions and over filling with RBCs were noticed (Fig. C16, C17, C18). Sever nephritis with sever inflammatory cells infiltration and fibrous tissue proliferation were observed filling almost of inter tubular spaces (Fig. C19, C20).

4. Discussions

The present investigation revealed that the kidney of the **control group (G1)** showed normal renal parenchyma; renal corpuscles with their glomeruli and renal tubules. Meanwhile, **G2** treated with **ZnO NPs** in a dose of 100 mg/kg. bwt and **G3** treated with **ZnO NPs** in a dose of 250 mg/kg. bwt showed mild to moderate pathological findings resembling necrosis of the renal corpuscle and renal tubules. In addition, focal scattered of inflammatory cells infiltration with fibrous connective tissue proliferation was demonstrated. And also, moderate accumulations of dense acidophilic material in inter and intra tubular parts were clarified.

With increasing dose of **ZnO NPs**, the renal toxicity became more obvious where, the kidney of **G4** treated with **ZnO NPs** in a dose of 500 mg/kg. bwt showed severe necrosis in the renal corpuscles with severs dilatation of the renal tubules accompanied with sloughing and degeneration of its lining epithelium and some renal tubules lining epithelium showed sever vacuolations with variable size. And also, sever dilatation of some renal tubules accompanied with sever accumulation of dense acidophilic material in intra and inter tubular parts were clearly clarified. These results are in parallelism with **Noori et al., (2014)** in mice who described that the degeneration in proximal and distal tubule walls and the accumulation of dense eosinophilic material in proximal and distal tubules (due to the congestion of eosinophils in these tubules) were observed 8 days post injection. And also, our results are goes hand in hand with **Ben-Slama et al. (2015)** in rats who claimed that the histopathological analysis of the kidney showed intratubular protein deposition (IPD).

The present investigation claimed that severs dilatation of the renal tubules accompanied with degeneration of its lining epithelium with loss of its brush border were observed. Such investigation is completely supported by the finding of **Lin et al., (2016)** in the human embryonic kidney cell line HEK-293 who observed tubular dilatation with the loss of brush borders and flattened tubular epithelium. In addition, atrophy of some renal corpuscle was also observed. This result is in parallelism with the results of **Lin et al., (2016)** in the human embryonic kidney cell line HEK-293 who observed reduction of Bowman's space.

Furthermore, the present study clarified that sever nephritis with sever inflammatory cells infiltration and fibrous tissue proliferation were observed filling almost of inter tubular spaces. These findings are in agreement with the results of

Ismail and El-Araby (2017) in rabbit who showed severe interstitial nephritis with marked mononuclear cells infiltration in the interstitial tissues with severely degenerated and vacuolated renal tubular epithelium in the rabbit kidney. Moreover, the findings of **Najafzadeh et al., (2013)** in lambs and **Noori et al., (2014)** in mice are completely in agreement with our result where they clarified that the kidney tissue is not normal in the treated groups and significantly several histopathological alterations were seen. The accumulation of neutrophils and eosinophils in glomerular capillaries (due to the capillary infiltration), infiltration of inflammatory cells in kidney indicate the ZnO toxicity

The current investigation noticed sever dilatation of the interstitial blood vessels accompanied with sever congestions and over filling with RBCs. These results are in parallelism with **Noori et al., (2014)** in mice.

5. Conclusion

From our results, we can conclude that the ZnO NPs have potential renal toxicity that may affect the function of the kidney; glomerular filtration rate.

6. Acknowledgments

- I wish to express my deepest gratitude and sincere thanks to:
- 1) Dr. Wael A.M. Ghonimi, Professor Assistant of Histology and Cytology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt for his help, support and efforts in the histological examination, images capture, result reading and language editing.
 - 2) Mr. Tayseir Rashed Almunizel, laboratory technician at the Faculty of Pharmacy, King Saud University, Riyadh, Saudi Arabia who contributed to the care of animals during the experiment
 - 3) Mr. Khalid Elfaki Ibrahim, technician at the Central Laboratory, Department of Zoology, King Saud University, Riyadh, Saudi Arabia who contributed to the histological cutting and staining of sections.
 - 4) Qassim University, for its financial support for this research

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- convoluted tubules and distal convoluted tubules. A1) H&E A2) PAS A3) Bromo phenol blue
A1, 2, 3) Obj.x10 : Oc.x10
- (A4): showing normal, intact renal medullary rays; medullary tubules (arrow head). H&E Obj.x10 : Oc.x10
- (A5, A6, A7): showing normal, intact renal corpuscle (single arrow head); Bowman's capsules and glomerulus, proximal convoluted tubules (double arrow head) and distal convoluted tubules (arrow). A5) H&E A6) PAS A7) Bromo phenol blue A5, 6, 7) Obj.x40 : Oc.x10
- (A8): showing normal, intact renal tubules with intact lining epithelium. H&E Obj.x40 : Oc.x10
- Plate 2:** Sections of *male Westar rats* kidneys of **G2** treated with **ZnO NPs** in a dose of 100 mg/kg. bwt and **G3** treated with **ZnO NPs** in a dose of 250 mg/kg. bwt by oral gavage for 21 days
- (B1, B2, B3): showing moderate necrosis and degeneration in the renal corpuscles with breakdown and damage of Bowman's capsules (arrow head). H&E Obj.x40 : Oc.x10
- (B4): showing moderate necrosis in the lining epithelium of the renal tubules with loss of its brush border. H&E Obj.x40 : Oc.x10
- (B5): showing moderate inflammatory cells infiltration with fibrous tissue proliferation in between the renal tubules (arrow). H&E Obj.x10 : Oc.x10
- (B6, B7): showing moderate inter tubular inflammatory cells infiltration with fibrous tissue proliferation (arrow). B6) H&E B7) Bromo phenol blue B6, 7) Obj.x40 : Oc.x10
- (B8, B9): showing moderate congestion of the inter tubular blood vessels (arrow head). H&E Obj.x40 : Oc.x10
- (B10, B11, B12): showing accumulation of dense acidophilic material in inter and intra tubular parts (arrow). B10, 12) Bromo phenol blue B11) H&E Obj.x40 : Oc.x10
- Plate 3:** Sections of *male Westar rats* kidneys of **G4** treated with **ZnO NPs** in a dose of 500 mg/kg. bwt by oral gavage for 21 days
- (C1, C2, C3): showing sever necrosis and degeneration in the renal corpuscles and completely loss of its architectures (arrow). C1, C2) PAS C3) H&E Obj.x40 : Oc.x10
- (C4): showing sever atrophy of the renal corpuscles (arrow). PAS Obj.x40 : Oc.x10
- (C5, C6, C7): showing sever dilatation of the renal tubules (arrow head) with sloughing and degeneration of its lining epithelium (arrow). C5, C6) PAS C7) H&E Obj.x40 : Oc.x10
- (C8): showing sever vacuolations of renal tubules lining epithelium (arrow). H&E Obj.x40 : Oc.x10

Figure legends:

Plate 1: Sections of *male Westar rats* kidneys of control group (G1);

Figure (A1, A2, A3): showing normal, homogenous, intact renal cortex; renal corpuscles (arrow head), proximal

(C9, C10, C11, C12): showing sever accumulation of dense acidophilic material in inter and intra tubular parts (arrow) accompanied with sever congestions with over filling of RBCs (arrow head). H&E Obj.x40 : Oc.x10

(C16, C17, C18): showing sever dilatation of the inter tubules blood vessels with sever congestions and over filling with RBCs (arrow). C16, 18) H&E C17) Bromo phenol blue Obj.x40 : Oc.x10

Plate 4: Sections of *male Westar rats* kidneys of **G4**

(C13, C14, C15): showing sever dilatation of renal tubules with sever filling with dense acidophilic material (arrow). H&E Obj.x40 : Oc.x10

(C19, C20): showing sever inflammatory cells infiltration with fibrous tissue proliferation in between the renal tubules (arrow). H&E Obj.x40 : Oc.x10

Plate 1

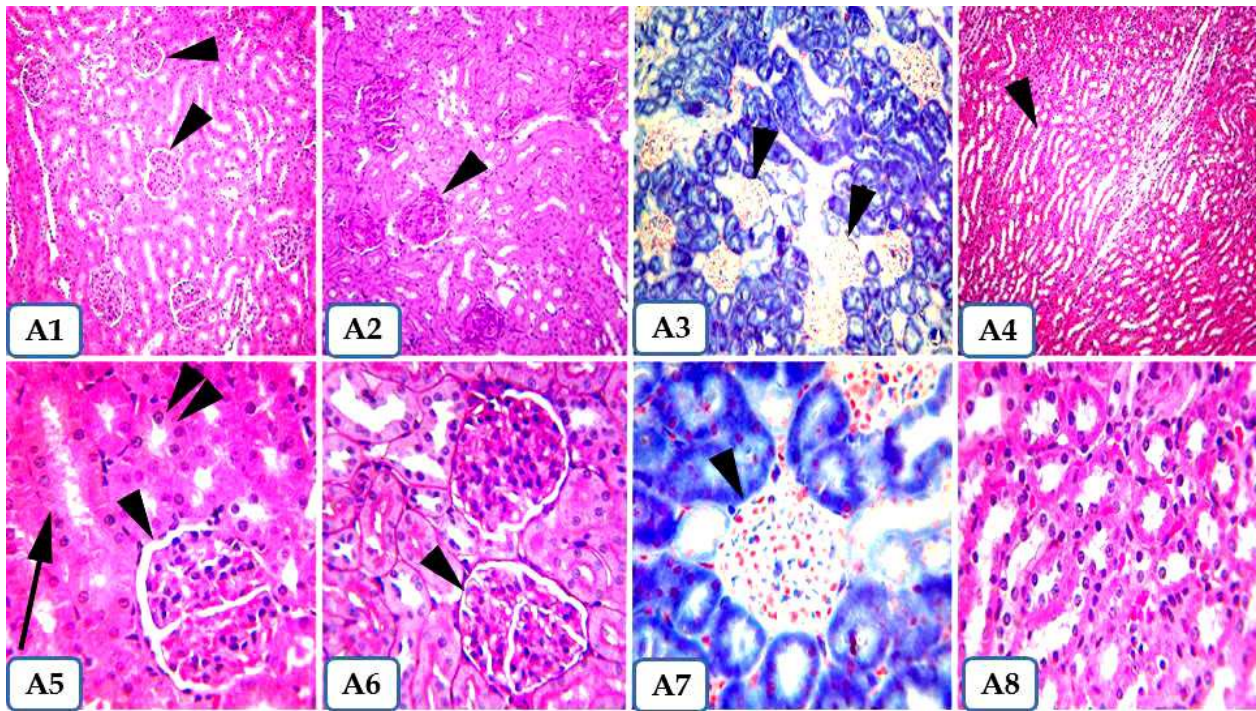


Plate 2

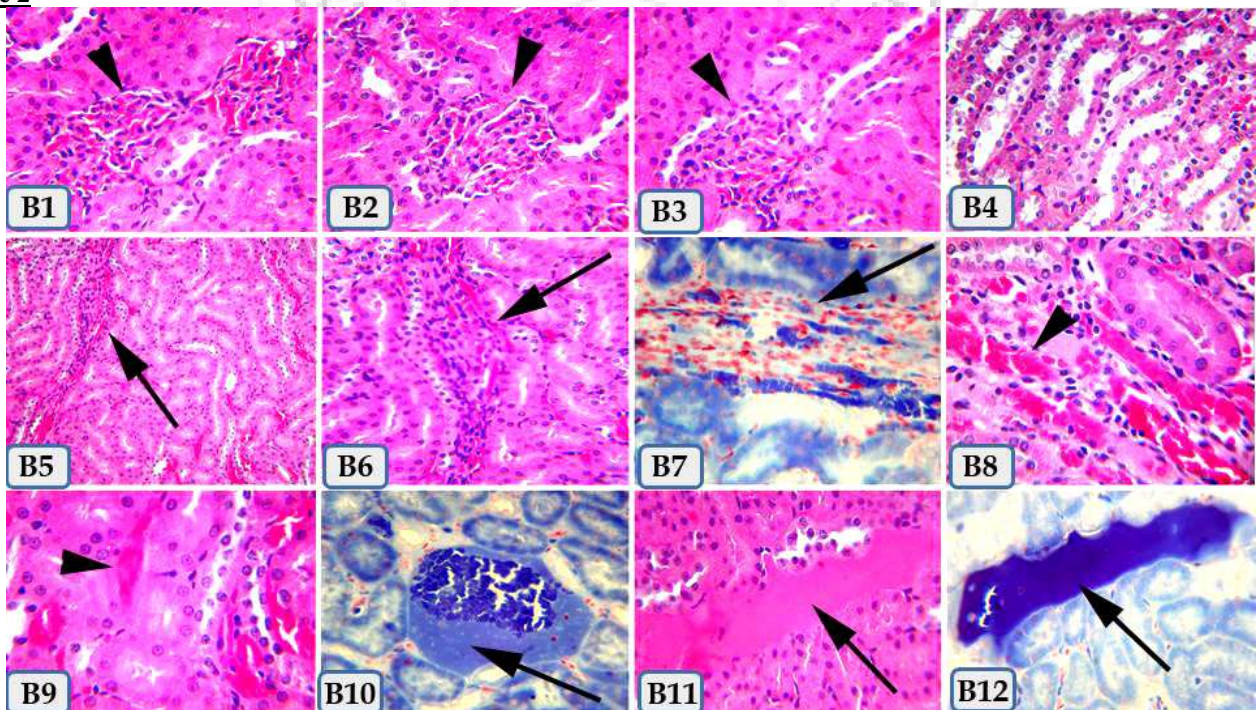


Plate 3

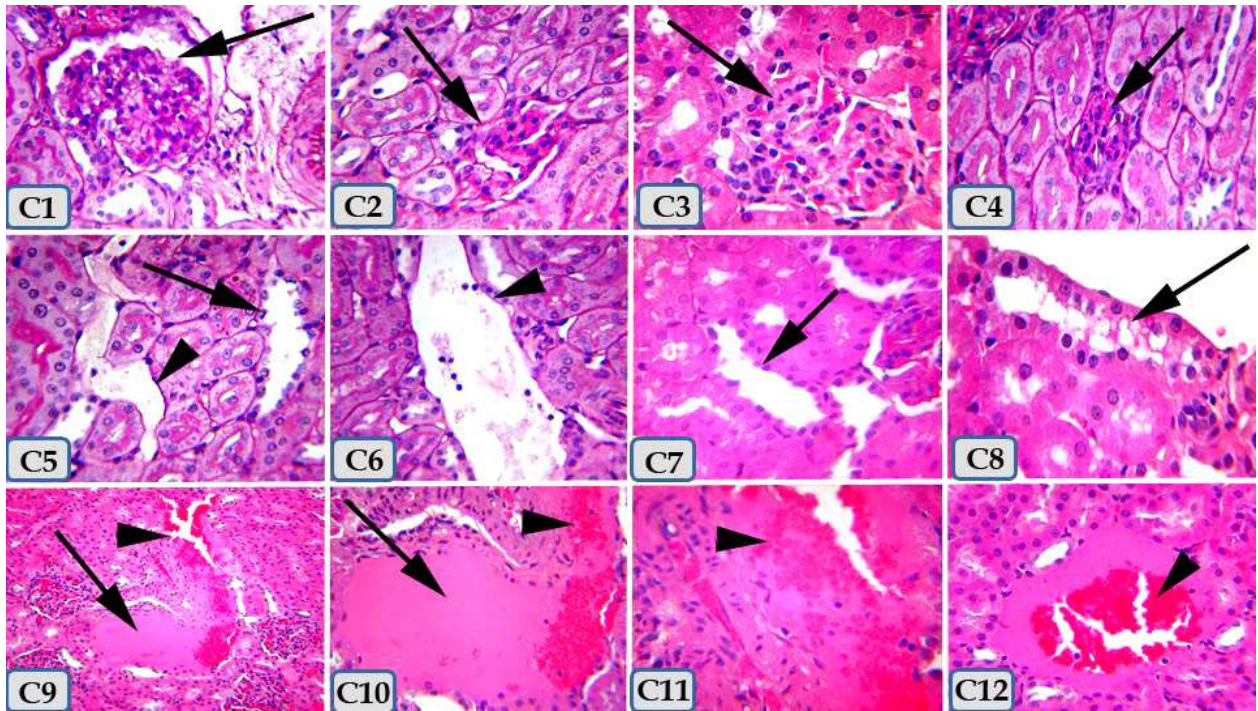


Plate 4

