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**Original Research Article**

# Nephrotoxicity Induced By Copper Oxide and Zinc Oxide Nanoparticles and Their Mixtures in Male Albino Rats

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**Abstract:** **Background:** Nanotechnology is a fast growing field that provides for the development of materials that have new dimensions, novel properties, and a broader array of applications. It is now known that the toxic behavior of NPs differ from their bulk counterparts. Even NPs that have the same chemical composition differ in their toxicological properties; the differences in toxicity depend upon size, shape, and surface covering. Hence, before NPs are commercially used it is most important that they be subjected to appropriate toxicity evaluation. **Objectives:** The aim of the present study was to evaluate the nephrotoxicity induced by CuO and/or Zn-O nano- particles in male rats. **Materials and Methods:** Twenty adult male rats were grouped randomly into four groups (n=5 each group). Group I (control): Rats were injected with saline intraperitoneally and at a dose of 1.0 ml/kg b.w. for 28 days. Group II (ZnONPs): Rats were administrated orally with ZnONPs (10 mg/kg/day) for 28 days. Group III (CuONPs): Rats were injected with CuONPs (0.5 mg/kg/day, in saline; intraperitoneally) for 28 days. Group IV (ZnONPs + CuONPs): Rats were given orally ZnONPs (10 mg/kg/day) follwed by injected with CuONPs (0.5 mg/kg/day, in saline; intraperitoneally), for 28 days. At the end of the experimental period, rats were anesthetized using light ether. Blood and kidney tissue samples were taken and prepared for biochemical and histological measurements. **Results:** Serum urea concentration was elevated due treatment of rats with CuONPs, ZnONPs and their mixture compared to control group. Also, CuONPs increased serum uric acid, while ZnONPs reduced serum uric acid and increased creatinine concentration compared to the control group. Treatements of rats with nanoparticls mixture elevated uric acid and creatinine concentration. histopathological abnormalities were observed in kidney of rats treated with CuO-NPs, ZnO-NPs, and CuO-NPs + ZnO-NPs. CuO-NPs, ZnO-NPs, and CuO-NPs + ZnO-NPs –induced histopathological changes in the kidney. The control group showed normal structure of renal glomeruli, Bowman's capsule lined by squamous epithelium, distinct urinary space, and normal renal tubules. CuO NPs treated group showed degenerated glomerulus, degenerated, desquamated and necrotic renal tubules, and inflammatory cells in the interstitum. More severe similar changes were observed in the ZnO-NPs, and CuO-NPs + ZnO-NPs–treated groups besides wide, congested blood vessels in ZnO-NPs–treated group. **Conclusion:** It can be concluded that CuONPs, ZnONPs, and their mixture were induced biochemical and morphological changes in renal tissues. Also, these results demonstrate that metal oxide nanoparticles induce a range of biological responses that vary from cytotoxic and can only be properly understood by using a tiered test strategy to study other aspects of nanoparticle toxicity. Toxicological studies must be performed before nano-particles application specially nano-oxide nano-particles. Caution should be taken in nano-particles use in worke place, preparations as well as while handling.

**Keywords:** Copper oxide nanoparticles, Zinc oxide nanoparticles, CuO & ZnO mixture, NPs, Nephrotoxicit Renal Histopathology.

## INTRODUCTION

The manufacture and use of metal oxide nanoparticles is continuously expanding. It becomes increasingly important to investigate and identify their possible toxicological effects and to identify which particles pose the greatest harm to human health (Fahmy, B., & Cormier, S. A. 2009).

Copper oxide nanoparticles (CuO NPs) are of great interest in nanoscience and nanotechnology

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because of their broad industrial and commercial applications. Therefore, toxicity of CuO NPs needs to be thoroughly understood. Akhtar *et al.*, (2012) investigated the cytotoxicity and oxidative stress induced by CuO NPs in human lung epithelial cells.

The manufacture and use of nanoparticles are increasing, humans are more likely to be exposed occupationally or via consumer products and the environment. Copper oxide nanoparticles (CuO NPs) are of great interest in nanoscience and nanotechnology because of their broad industrial and commercial applications. Therefore, toxicity of CuO NPs needs to be thoroughly understood (Karlsson, H. L. *et al*., 2008). Sizova *et al*., (2011) reported that despite an increasing application of copper nanoparticles, there is a serious lack of information concerning their impact on human health and the environment. In this study, Serum biochemical analysis and histopathological examinations of the kidney of all the rats were simultaneously performed. All the results indicated that the effects produced by nano-copper at a dose of 100 or 50 mg/kg/d were less than those induced at a higher dose of 200 mg/kg/d. Nano-copper induced overt nephrotoxicity at 200 mg/kg/d for 5 d, which mainly involved widespread renal proximal tubule necrosis. Valko *et al*., (2005) (Valko, M. M. H. C. M. *et al*., 2005) reported that metal-induced toxicity and carcinogenicity, with an emphasis on the generation and role of reactive oxygen and nitrogen species, is reviewed. Metal-mediated formation of free radicals causes various modifications to DNA bases, enhanced lipid peroxidation, and altered calcium and sulfhydryl homeostasis. Oxidative stress may be the cause of the cytotoxicity of CuO NPs in mammalian cells. However, little is known about the genotoxicity of CuO NPs following exposure to human cells. The role of reactive oxygen species, with the subsequent oxidative deterioration of biological macromolecules in the toxicities associated with transition metal ions is reviewed. Studies have shown that metals, including iron, copper, chromium, and vanadium undergo redox cycling, while cadmium, mercury, and nickel, as well as lead, deplete glutathione and protein-bound sulfhydryl groups, resulting in the production of reactive oxygen species as superoxide ion, hydrogen peroxide, and hydroxyl radical. As a consequence, enhanced lipid peroxidation DNA damage (Stohs, S. J., & Bagchi, D. 1995).

Syama *et al*., (2014) reported that engineered nanoparticles are developed for various applications in industrial, electrical, agricultural, pharmaceutical and medical fields due to their unique properties. Nanoparticles such as ZnO are widely used in cosmetics for UV protection. The toxicological investigations of ZnO NPs are highly recommended because of the increasing use in various industrial and consumer products. The toxic potential of ZnO NPs was assumed to be caused by the release of free Zinc ions in the medium. Zhang *et al*., (Zhang, H. *et al*., 2012) reported that CuO and ZnO generated oxidative stress and acute pulmonary inflammation that is not predicted by E(c) levels, the adverse biological effects of these materials could be explained by their solubility, as demonstrated by ICP-MS analysis. This predictive toxicological paradigm is also of considerable importance for regulatory decision-making about this important class of engineered nanomaterials.

Although nanozinc oxide (nano-ZnO) is applied widely in photocatalysts and gas sensors and in biological fields, it can cause serious oxidative stress and DNA damage to mammalian cells. Along with existing and emerging use of nanoscale materials, growing concerns have arisen about their unintentional health and environmental impact.

## OBJECTIVES

Presently, there is only limited knowledge concerning the toxicological effects of NPs. However, it is now known that the toxic behavior of NPs differ from their bulk counterparts. Even NPs that have the same chemical composition differ in their toxicological properties; the differences in toxicity depend upon size, shape, and surface covering. Hence, before NPs are commercially used it is most important that they be subjected to appropriate toxicity evaluation. The aim of the present study was to evaluate the nephrotoxicity induced by nano-CuO and/or Zn-O in male rats.

## MATERIAL AND METHODS

### Chemicals

Copper oxide and Zinc oxide as nanoparticles with an average size of 6 and 51 nm, respectively, were a gift from Dr. Amina El-Trass. Synthesis, characterization, optical properties and interaction with amino acids of CuO nanoparticles to confirm the negative surface of CuO nanoparticles were performed by El-Trass *et al*., (2012).

### Animals and Housing

Twenty healthy male Wistar Albino rats weighing 150 ± 10 g, were obtained from the Animal Breeding House of the National Research Centre (NRC), Dokki, Cairo, Egypt. The rats were allowed to acclimatize for a week before starting the experiments. Rats were maintained under temperature-controlled conditions (25 $^{o}$C), and a normal photoperiod of 12 h of darkness and 12 h of light. They were fed with standard food and had free access to water. Animals were randomly divided into 4 groups of five rats each, with one group assigned to be an untreated control. The housing and management of the animals and the experimental protocols were conducted as stipulated in the Guide for Care and Use of Laboratory Animals (NRC. 1996).

**Experimental Protocol**

Twenty adult male rats were grouped randomly into four groups (n=5 each group). Group I (control): Rats were injected with saline intraperitoneally and at a dose of 1.0 ml/kg b.w. for 28 days. Group II (ZnONPs): Rats were administrated orally with ZnONPs (10 mg/kg/day) for 28 days. Group III (CuONPs): Rats were injected with CuONPs (0.5 mg/kg/day, in saline; intraperitoneally) for 28 days (Liao, M., & Liu, H. 2012). Group IV (ZnONPs + CuONPs): Rats were given orally ZnONPs (10 mg/kg/day) follwed by CuONPs (0.5 mg/kg/day, in saline; intraperitoneally), for 28 days.

At the end of the experimental period, rats were anesthetized using light ether. Blood samples were taken from the vena cava of rat heart within 1 min after sacrifation. Tubes were used to compile blood drawn from the heart directly; the blood was collected in glass tubes for coagulation and serum formation, blood was allowed to set for 30 min at 4$^{o}$C to clot, then centrifuged for 5 minutes at 1000 x g. Packed cells were discarded and the supernatant serum samples were decanted and stored into capped sterile poly-ethelene tubes at -20$^{o}$C until used (within 24 hours). The abdominal cavity of each rat was opened where the liver was excised.

**BIOCHEMICAL PARAMETERS IN RAT SERUM AND TISSUES**

**Determination of Urea**

Urea was determined using a commercially available kit (Urea-kit S 180, bioMeriex Vitek, Inc. USA). Concentration of urea in the sample was calculated which is equal = ΔA sample/ΔA standard × concentration of the standard which is known and equal 50 mg/dl (Patton, C.J., & Crouch, S. R. 1977).

**Determination of Uric Acid**

Uric acid was determined using a commercially available kit (Uric acid-kit, Cat. No. 10690. Human Geselschaft, Taunusstein, Germany). Concentration of uric acid in the sample was calculated which equal = ΔA sample/ΔA standard × concentration of the standard which is known and equal 8 mg/dl (Guder, W. G. *et al*., 1996).

**Determination of Creatinine**

Creatinine was determined using a commercially available kit (CREA-kit MPR3 124192 Boenringer Mannheien). The concentration of creatinine in the sample was calculated which is equal = ΔA sample/ΔA standard × concentration of the standard which is known and equal 2 mg/dl (Henry, R.J. 1974).

**Histopathological Analysis**

Dissected kidneys were immediately fixed into 10% formaldehyde saline. Tissues were processed by embedding in paraffin. Sections were cut by rotatory microtome and mounted on glass slides. The sections were stained by conventional Hematoxylin & Eosin (H&E) stain. The sections were examined by light microscope (Drury, R., & Wallington, E. 1980).

**Statistical Analysis**

Values obtained as mean ± SEM were subjected to one-way analysis of variance (ANOVA) followed by Tukey test using GraphPad Prism version 4.0 for windows from GraphPad Software, San Diego, California, USA). Values of $P < .05$ were considered significant.

**RESULTS AND DISCUSSION**

Nanoparticles (NPs) were found to reach the systemic circulation after inhalation, ingestion or intravenous injection. They are known to disseminate to several organs such as liver, spleen, kidneys, brain or heart (Kreuter, J. 2001; Girardin, F. 2006; Oberdörster, G. *et al*., 2005; Elder, A. *et al*., 2006). Such translocation depends on the physicochemical properties of NPs, and their migration to distant sites is an important issue with regard to their toxicity.

**Effects of Copper Oxide Nanoparticles and Zinc Oxide Nano-Particles on Rats Serum Urea, Uric Acid, and Creatinine**

Over a third of the atoms in a nanoparticle are at the surface, and these are extremely reactive systems, which in some cases can generate oxygen radicals; because of the size of nanostructures, it is possible to manipulate the surface interface to allow for interactions with biological systems (Guyton, A.C., & Hall, J.E. 2001). With the correct coating, particles below 50 nm can translocate them into cells relatively easily and are able to interact with channels, enzymes, and other cellular proteins (Zhang, X. D. *et al*., 2010). This may be the mechanism that CuNPs induced its toxicity to hematological indices. This study aimed to investigate human renal cell responses to manufactured NPs in order to highlight their potential toxicity and/or biological responses. Kidneys play an important role in eliminating xenobiotics from the body and thus, NPs absorbed in the systemic circulation can be excreted by renal clearance (Burns, A. A. *et al*., 2008; Schipper, M. L. *et al*., 2009).

An abnormally elevated blood creatinine level is a specific and sensitive indicator of impaired kidney function. The urea, uric acid and blood urea nitrogen values in the CuONPs group were significantly higher compared to control ($P < 0.05$) groups, while creatinine without significant changes. ZnO NPs iduced serum urea and creatinine, while reduced uric acid compared to control. On the other hand the mixture of CuO and ZnO NPs induced significant ($P > 0.05$) changes in urea, uric acid, and creatinine (Tables 1 & Figures 1-3). The exposure to ZnO NPs significantly affected cellular viability in a dose-dependent manner and formation of reactive oxygen species (ROS) was found to be the mechanism of cellular toxicity (Syama, S. *et al*., 2014).

In this study, the biochemical compositions of serum of rats treated with nano-copper and zinc oxide indicating impairment of kidney function. Also, Lei *et al*., (2008) found that 200 mg/kg/d of nano-copper induced nephrotoxucity reflected with renal proximal tubule necrosis and elevation in creatine levels in serum. In addition, Liao and Liu (2012) and Sizova *et al*., (2011) found that nanocopper can induce widespread renal proximal tubule necrosis in rat kidneys with blood urea nitrogen and creatinine increase.

**Table (1). Effects of treatment of rats with zinc oxide** (ZnONPs)**, and copper oxide nanoparticles** (CuO NPs)**, and their mixture on serum urea, uric acid, and creatinine**

| Groups | Groups | | | |
|---|---|---|---|---|
| Parameters | Control | CuONP | ZnONP | CuO + ZnONP |
| | Mean±SE | Mean± SE | Mean± SE | Mean± SE |
| Serum urea (mg/dl) | 26.80± 4.749[bcd] | 43.0± 3.52 [a] | 40.6± 5.28 [a] | 42.2± 3.82 [a] |
| Serum uric acid (mg/dl) | 2.50 ± 0.08 [cd] | 3.11 ± 0.28 [ac] | 2.27 ± 0.15[abd] | 3.79 ± 0.69 [ac] |
| Serum creatinine (mg/dl) | 0.95± 0.02[bcd] | 0.92± 0.06[a] | 1.01± 0.05[a] | 1.03± 0.05[a] |

Significance at $P < 0.05$. [a] Comparison of control and other groups; [b] Comparison of CuONP and other groups; [c] Comparison of ZnONP and other groups; [d] Comparison of CuO+ZnONP and other groups

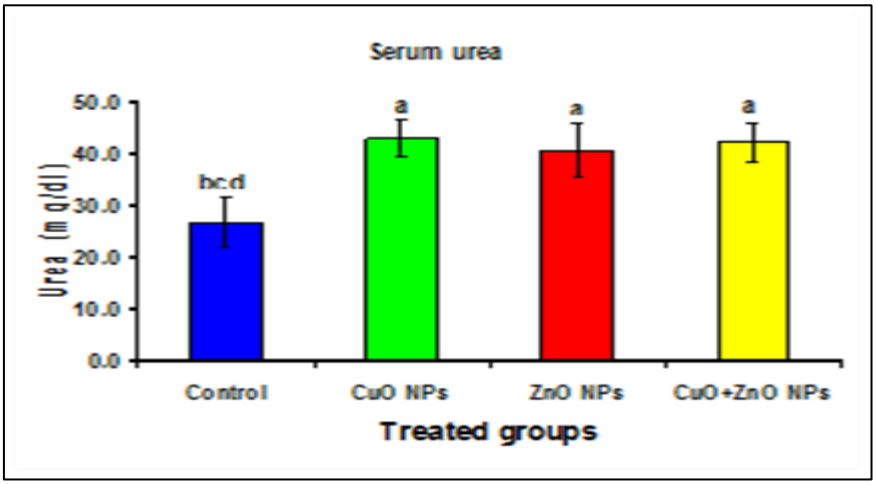


**Figure 1: Serum urea (mg/dl) of rat treated with zinc oxide (ZnO) and copper oxide (CuO) nanoparticles (NPs). Significance at P> 0.05. a Comparison of control and other groups; b Comparison of CuO NPs and other groups; c Comparison of ZnO NPs and other groups; d Comparison of CuO+ZnONPs and other groups**

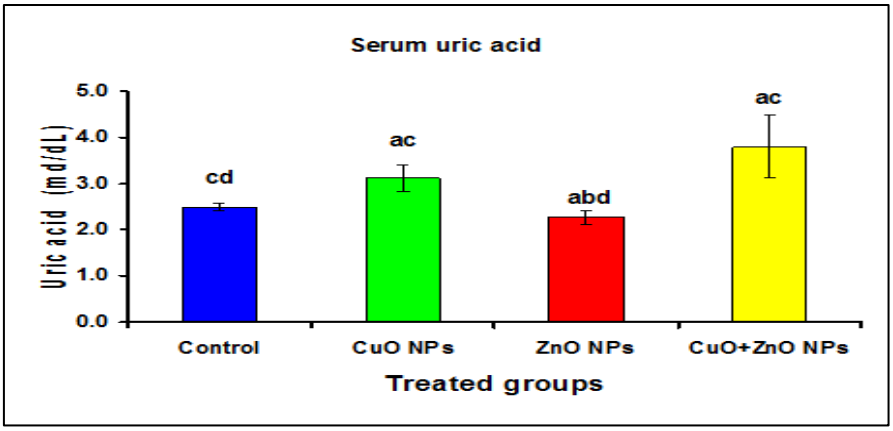


**Figure 2: Serum uric acid (mg/dl) of rat treated with zinc oxide (ZnO) and copper oxide (CuO) nanoparticles (NPs). Significance at P 0.05. Significance at P> 0.05. a Comparison of control and other groups; b Comparison of CuO NPs and other groups; c Comparison of ZnO NPs and other groups; d Comparison of CuO+ZnONPs and other groups**

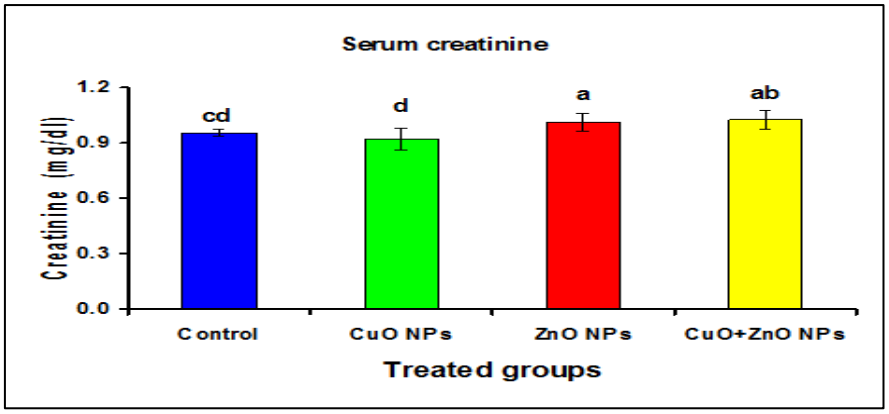


**Figure 3: Serum creatinine (mg/dl) of rat treated with zinc oxide (ZnO) and copper oxide (CuO) nanoparticles (NPs). Significance at P > 0.05. Significance at P > 0.05. a Comparison of control and other groups; b Comparison of CuO NPs and other groups; c Comparison of ZnO NPs and other groups; d Comparison of CuO+ZnONPs and other groups**

**Effects of Copper Oxide Nanoparticles (Cuo Nps) and Zinc Oxide Nano-Particles (Zno Nps) On Rats Kidney Histology**

Despite an increasing application of copper and zinc nanoparticles, there is a serious lack of information concerning their impact on human health and the environment. Elevation in serum levels of urea could also be attributed to increase in the activities of urea enzymes, ornithine carbomoyl transferase and arginase mostly associated in liver damage in many animal species, since the urea cycle is confined to the liver (James, W. *et al*., 1998). Damage to the kidney may result in reduced erythropoeitin production, resulting in high urea which may in turn be associated with low blood volume. Thereby leading to an elevation in inflammatory cells types, this usually occurs during the inflammatory process. Inflammation exposes the body organs to infections, leading to the release of high amount of white blood cells (James, W. *et al*., 1998).

Microscopic examination of organs to evaluate the toxic effects of contaminants has been used as a biomarker to evaluate the toxicity of various pollutants (Adams, S. 2002; Mela, M. *et al*., 2007). Histopathology studies of target organs along with the studies of oxidative stress would give the complete risk assessment and toxic potential of copper and zinc oxide nanoparticles (CuO NPs & ZnO-NPs). In the present study, histopathological abnormalities were observed in kidney of rats treated with CuO-NPs, ZnO-NPs, and CuO-NPs + ZnO-NPs.

Figures (4-7) demonstrate CuO-NPs, ZnO-NPs, and CuO-NPs + ZnO-NPs –induced histopathological changes in the kidney. The control group showed normal structure of renal glomeruli, Bowman's capsule lined by squamous epithelium, distinct urinary space, and normal renal tubules. CuO NPs treated group showed degenerated glomerulus, degenerated, desquamated and necrotic renal tubules, and inflammatory cells in the interstitum. More severe similar changes were observed in the ZnO-NPs, and CuO-NPs + ZnO-NPs–treated groups besides wide, congested blood vessels in ZnO-NPs–treated group.

Kidney of the different treated groups showed degeneration of tubular epithelial cells and glomerular degeneration. This may be due to the accumulation of inflammatory cells (Kurtović, B. *et al*., 2008). Venkatramreddy and Baul, (2010), Aslam *et al*., (2013), and Patel *et al*., (2014) reported that lipid peroxidation is the primary source for tubular dilation, and disintegration tubules. Necrosis is a form of cell injury that results in the premature death of cells in living tissue by autolysis (Patel, J. G. *et al*., 2014). Cells that die due to necrosis exhibit loss of cell membrane integrity and an uncontrolled release of products of cell death into the intracellular space (Proskuryakov, S. Y. *et al*., 2003). This initiates, in the surrounding tissue, an inflammatory response which prevents nearby phagocytes from locating and eliminating the dead cells by phagocytosis (Kasper, D. L., & Zaleznik, D. F. 2001). The histopathological abnormalities observed in the present study, in kidney of rats treated with CuO-NPs, ZnO-NPs, and CuO-NPs + ZnO-NPs could be explained on basis of the reported increase in lipid peroxidation, and decreased level of reduced glutathione.

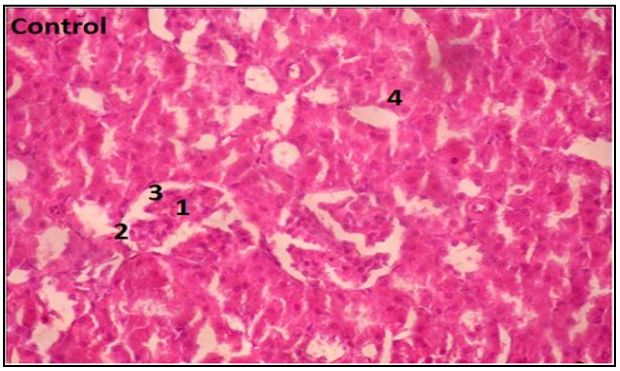


**Figure 4: Photomicrograph of section of kidney of control group showing normal structure of renal glomeruli (1), Bowman's capsule lined by squamous epithelium (2), distinct urinary space (3), and normal renal tubules (4). H & E stain x 400.**

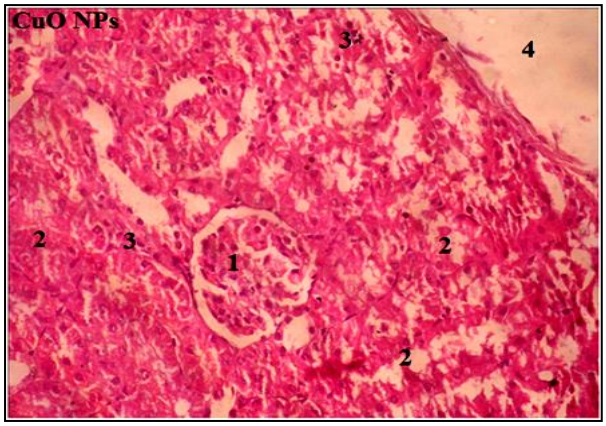


**Figure 5: Photomicrograph of section of kidney of CuO NPs treated group showing degenerated glomerulus (1), degenerated,desquamated and necrotic renal tubules (2), and inflammatory cells in the interstitum (3) (H&E X 400).**

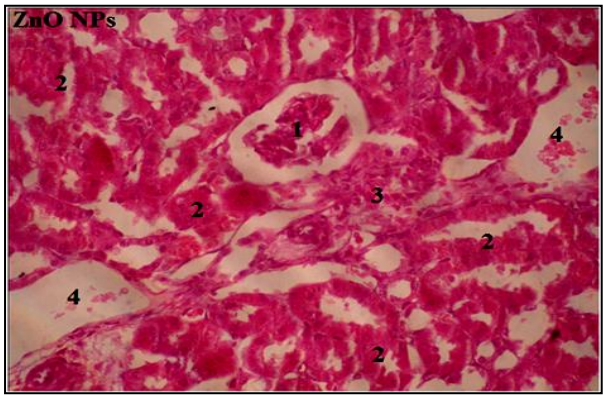


**Figure 6: Photomicrograph of section of kidney of ZnO NPs- treated group showing degenerated glomerulus (1), degenerated, atrophied, desquamated and necrotic renal tubules (2), inflammatory cells in the interstitum (3), and wide, congested blood vessels (4) (H&E X 400).**

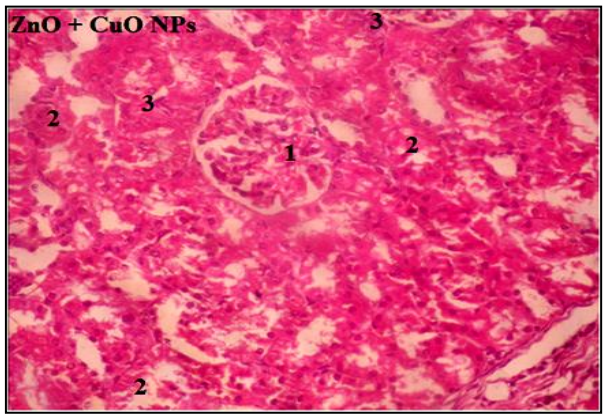


**Figure 7: Photomicrograph of section of kidney of ZnO + CuO NPs treated group showing degenerated glomerulus (1), degenerated, atrophied, desquamated and necrotic renal tubules (2), and inflammatory cells in the interstitum (3) (H&E X 400).**

Furthermore, these histological changes were in line with the reported changes in the kidney function parameters (serum creatinine, urea, and uric acid). The negative surface of CuO nanoparticles reported by El-Trass *et al*., (2012) indicates its ability to generate free radicals, and its ability to induce oxidative stress as reported by Karlsson *et al*., (2009), Song *et al*., (2013). As a result of this it could play an important role in the peroxidation of unsaturated fatty acids in the cell membranes and development of necrosis as suggested by Lloyd *et al*., (1997; 1998), and Susa *et al*., (1996). The redox alterations caused by oxidative agents have been shown to induce apoptosis and necrosis in hepatocytes and other cells (Han, D. *et al*., 2006).

## CONCLUSION

From the previous results, it could be concluded the main following points: The results obtained showed that CuONPs, ZnONPs and their mixture increased serum kidney function tests and produce morphological alterions in the kidney tissues. These results demonstrate that metal oxide nanoparticles induce a range of biological responses that vary from cytotoxic and can only be properly understood by using a tiered test strategy such as we developed for oxidative stress and adapted to study other aspects of nanoparticle toxicity. Toxicological studies must be performed before nano-particles application specially nano-oxide nano-particles. Caution should be taken in nano-particles use in work place, preparations as well as while handling.

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