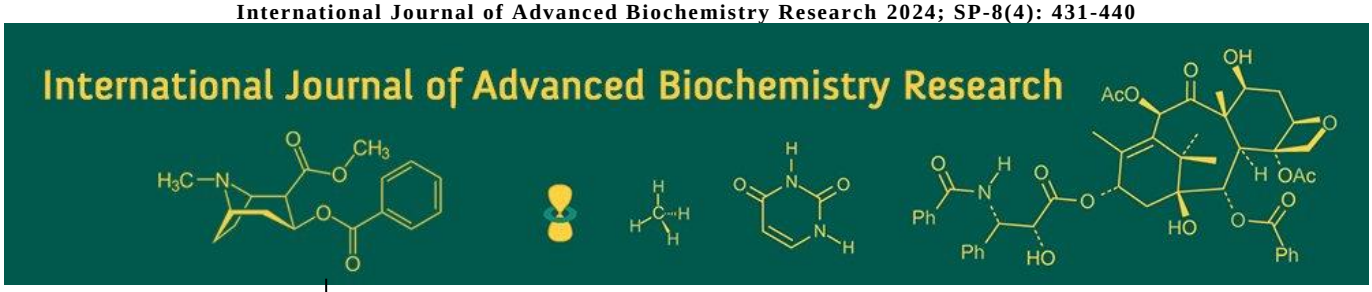

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Nephrotoxic effect of copper oxide nanoparticles in Wistar rats (*Rattus norvegicus*)

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Abstract
CuO NPs (Copper oxide nanoparticles) are among the most commonly used nanomaterials. They have applications in different fields such as medicine, catalysis, sensing and textile industry. This NPs are released and accumulate in the environment causing adverse effects on the environment. Hence the study aimed to assess the impact of Copper oxide nanoparticles (CuO NPs) on Wistar rats following oral administration of CuO NPs. Forty-eight adult Wistar rats were divided into four groups: Group C (control), Group T1 (100 mg/kg), Group T2 (200 mg/kg) and Group T3 (400 mg/kg) and administrated CuO NPs for 28 days via oral gavage. Exposure of CuO NPs leads to significant alteration in percentage of neutrophils, monocytes and lymphocytes in higher dose group of both male and female rats also CuO NPs leads to significant alteration in Total Leukocyte Count (TLC) and platelet count in 400 mg/kg treated group in both male and female rats. There was significantly increased in level of markers of kidney damage Urea, Uric acid, Creatinine and Total protein in both male and female rats treated with high dose (400 mg/kg) of CuO NPs. CuO NPs exposure leads to significant reduction in SOD, CAT activity and significant increased MDA level in kidney of T3 (400 mg/kg) and T2 (200 mg/kg) group of both male and female rats. In histopathological examination treatment related changes were observed in dose dependent manner.

Keywords: Copper oxide nanoparticles, nephrotoxicity, haematological, biochemical, histological

1. Introduction
Nanoparticles (NPs) have recently gained attention due to its numerous applications in industries such as electronics, medicinal, pharmaceuticals, cosmetics and environmental activities. Because of its great potential in this technology, investments in nanoscale applications are becoming increasingly popular all around the world (Dunphy Guzman *et al*., 2006) [11]. Nanoparticles are everywhere in our daily lives. They are unavoidably present in the air, water and soil. Due to their widespread use in everyday life, copper oxide nanoparticles (CuO NPs) stand out among other nanoparticles. CuO NPs have a brownish-black appearance with size ranges from 1 - 100 nm. When it comes into contact with hydrogen or carbon monoxide at a high temperature, it can be converted to metallic copper (Osman, 2016) [36].
Characterization of CuO NPs includes particle size, shape and charge. There is a relationship between the size of the NPs and surface-to-volume ratio; smaller the size, greater the surface-to-volume ratio and vice versa. The size of a particle determines its penetration and reactivity (Naz *et al*., 2020) [35]. Copper oxide nanoparticles were among the first engineered nanoparticles (Doudi and Setorki, 2014) [10], because of which they are having distinct physiochemical characteristics it is widely used in catalysis, batteries, gas sensors, heat transfer fluids, and solar energy (Singh *et al*., 2017; Katsumiti *et al*., 2018) [45, 22] and are being marketed for a variety of uses, such as propellants, ceramics, electronics, plastic (Pan *et al*., 2010) [38] as well as in cosmetics (Doudi and Setorki, 2014) [10]. CuO NPs are demonstrated their potential in biomedical and pharmaceutical fields, including as antibacterial, antifungal, anticancer and drug delivery agents (Saratale *et al*., 2018) [42]. Morever, they are used to remove heavy metals from wastewater (Jain *et al*., 2021) [21]. Agriculture's sustainability will increase, when CuO NPs used as fertilisers, fungicides or pesticides on soils (Pelegrino *et al*., 2020) [40].

Copper oxide nanoparticles due to their exclusive properties and various applications, human beings exposed to CuO NPs and it may cause adverse effects (Doudi & Setorki, 2014) [10]. CuO NPs are highly toxic when compared to other metal oxide nanomaterials (Peralta-Videa *et al*., 2011) [41]. Copper nanoparticles are produced on a large scale, making it likely that they will enter the human body through effluent, spills during shipping and handling and the disposal of consumer goods (Chen *et al*., 2006) [8]. They are built up in vital organs like brain, liver or kidneys through ingestion, inhalation, skin pores, reproductive organs and urinary tract (Li *et al*., 2008) [27].

Copper oxide nanoparticles cause severe hepato - nephro toxicity through exerting oxidative stress on the affected tissues (Wang *et al*., 2012) [47]. Exposure to NPs damage functional integrity of cell membrane in the liver and increase activity of intracellular enzymes like transaminases and alkaline phosphatase (Mohammadyari *et al*., 2014) [32] Additionally, Generation of reactive oxygen species (ROS) and DNA damage caused by CuO NPs, which led to histopathological abnormalities in the liver tissue (Moller *et al*., 2010) [33]. Moreover, it may also inhibit cellular antioxidant defences by decreasing the activity of catalase (CAT) and glutathione (GSH), while an increasing the activity of GSH peroxidase enzyme in cells exposed to these substances (Fahmy and Cormier, 2009) [15]. Oral administration of CuO NPs causes oxidative stress, increased ROS generation, inflammation, apoptosis and histopathological changes in liver, kidney, stomach and bone marrow (Anreddy, 2018; Bugata *et al*., 2019; De Jong *et al*., 2019; Elkhateeb *et al*., 2020) [3, 7, 9, 14].

2. Materials and Methods

2.1 Experimental animals and environment

Total 48 (24 male and 24 female) Wistar rats (7-8 weeks of age) were procured from Cadila Pharmaceuticals Limited in Dholka, Ahmedabad, Gujarat, India. Rats were maintained as per the protocol described in the publication of the Committee for Control and Supervision of Experiments on Animal guidelines. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC), College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh, KU-JVC-IAEC-SA-92-22; Dated: 08/07/2022).

The experiment was carried out at Laboratory Animal House Facility (Reg. No. 1766/GO/Re-S/ReBi-L/14/CPCSEA), College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh. The rats were housed in standard polypropylene cages with stainless steel top grill. Corn cob was used as bedding material. The cages and bedding material were changed at least twice in a week or as and when required. During the study period, all rats were maintained under standard laboratory conditions with 12 hr light and dark cycles, temperature 22 °C (± 3 °C) and relative humidity 45-75%. The rats were fed standard feed pellets (VRK Nutritional Solutions, Maharashtra) *ad libitum* during the whole study period. The values of dry matter, crude protein, ether extract, crude fiber, crude ash and NFE in feed were 91.96, 22.21, 4.29, 6.28, 8.56 and 58.76%, respectively. During the study period, filtered RO water was provided *ad libitum* to animals. Polypropylene bottles (300 mL) were used to provide drinking water to animals.

All animals were acclimatized to laboratory condition under constant observation for 7 days before commencement of the experiment.

2.2 Preparation of CuO NPs and Experimental design

CuO NPs were obtained from the Sisco Research Laboratories Pvt Ltd (CAS number1317-38-0). Daily fresh dispersion was prepared by suspending the powder in water and sonicated for 5 minutes by ultrasonic homogenizer (Model: 1-800-745-1105, USA) which involved vibration at 20 kHZ with a continuous pulse of 40% of total pulse power, resulting in a power output of 40 W. After that, during dosing time, the working suspension was subjected to vigorous vertexing as per requirements and administered through oral gavage.

Rats were divided in to 4 groups, each group includes 6 male and 6 female adult wistar rats. In which control group received only R.O. water and other 3 groups received CuO NPs at the dose rate of 100, 200 and 400 mg/kg b.w./day in water (Table 1)

2.3 Hematological evaluation

Hematological parameters like Hemoglobin, Hematocrit, Total leukocyte count, Total erythrocyte count, Differential leukocyte count, Mean corpuscular volume, Mean corpuscular hemoglobin concentration, Mean corpuscular hemoglobin were estimated by Automated hematology analyzer (Abacus Vet 5, Diatron) with ready to use suitable kits.

2.4 Biochemical evaluation

Biochemical parameters Aspartate aminotransferase, Alanine aminotransferase, Total protein, Albumin, Globulin, Creatinine, Urea, Total Bilirubin, Alkaline phosphatase, Uric acid, Calcium, Phosphorus were estimated using standard kits (Diatek kit) on automatic biochemistry analyzer (Dia-chem 240 plus, Diatek).

2.5 Oxidative stress parameters

Superoxide dismutase SOD activity was determined in liver and kidney tissue homogenates following the protocol of Marklund and Marklund (1974) [31]. The enzyme activity was expressed in units per mg protein. One unit of enzyme activity is the amount of enzyme required for 50% inhibition of pyrogallol auto-oxidation.

2.5.1 Catalase

CAT activity was measured in liver and kidney tissue homogenates following the protocol of Aebi *et al.* (1974). The activity of catalase was calculated using the molar extinction coefficient of 43.6 cm^{-1}. Catalase activity was expressed in U/mg protein.

2.5.2 Malondialdehyde

The MDA content formed as the end product of lipid peroxidation assay was determined in liver and kidney tissue homogenates following the protocol of Lykkesfeldt, (2001) [29]. The final concentration of MDA was expressed in µ mol/mg of protein.

2.6 Collection of tissue samples

All rats were humanely sacrificed using CO_2 method. For

histopathological examinations kidney were collected from rats of each group in 10% neutral buffered formalin for proper fixation.

2.7 Histopathology
The formalin-fixed tissues were embedded in paraffin and processed as per standard procedures. These tissue samples were sectioned at 5 µ thickness with an automatic section cutting machine semi-automated rotary microtome (Leica Biosystems, Germany) and were stained with hematoxylin and eosin (H & E) stain (Luna, 1968) [28]. The H & E stained slides were observed under the microscope and microscopic pathological lesions were recorded.

2.8 Statistical analysis
Statistical analyses of all the data of study were carried out using GraphPad prism 9.0. Kolmogorov- Smirnov test was used evaluate the normality of data along with Bartlett's test to confirm the equal variance. Data with normal distribution and homogeneous variance (ANOVA) followed by Tukey's HSD test. The data didn't having either normal distribution or equal variances were analyzed by Kruskal-Wallis test followed by Dunn's test. The value off $p<0.05$ (*) was considered as statistically significant and $p<0.0$l (**), $p<0.005$ (***) and $p<0.001$ (****) were considered for highly statistica1 significant difference.

3. Results
3.1 Symptomatology
The rats were regularly and closely observed throughout the experiment. The clinical symptoms were observed in mid (200 mg/kg) and high (400 mg/kg) dose of CuO NPs treated group i.e. T2 and T3. Clinical signs like loss of appetite, dullness, depression, hair falling and change in colour and consistency of the faeces, which was black in colour and soft at the dose rate of 200 mg/kg (T2) and 400 mg/kg (T3) as compared to 100 mg/kg (T1) treated group and control group in both male and female rats.

3.2 Haematological parameters
Effect of daily oral administration of Copper oxide nanoparticles on Haematological Parameters in different treatment groups of rats were calculated and presented in table 2 and 3.

The administration of CuO NPs leads significantly ($p<0.05$) reduction in hematocrit % and Mean Corpuscular Hemoglobin in male rats treated with high dose (400 mg/kg) as compared to control and low dose (100 mg/kg) of CuO NPs treated group, whereas in female rats it was non significantly decreased in dose dependent manner. Value of Mean Corpuscular Hemoglobin Concentration (MCHC) was significantly ($p<0.05$), high significantly ($p<0.01$) and very high significantly ($p<0.005$) decreased in male rats treated with CuO NPs at the dose level of 100 mg/kg, 200 mg/kg and 400 mg/kg, respectively as compared to control group, whereas in female rats showed non significantly decreased in value of MCHC in dose dependent manner. In present study Neutrophil percentage and Lymphocyte percentage was high significantly ($p<0.01$) increased in T3 group as compared to control and T1 group in male rats, while in female rat's neutrophil percentage was non significantly increased and llymphocyte percentage was high significantly ($p<0.01$) decreased in T3 group as compared to control group. Monocyte percentage was very high significantly ($p<0.001$) and high significantly ($p<0.01$) increased in T3 and T2 group, respectively when compared to control group in male rats, whereas in female rats it was very high significantly ($p<0.005$) increased in T3 group as compared to control group. The administration of CuO NPs leads to high significantly ($p<0.01$) increased in Absolute Neutrophils Count in 400 mg/kg treated group as compared to control group in both male and female rats, whereas it was significantly ($p<0.05$) increased in 200 mg/kg treated group as compared to control group in male rats. The administration of CuO NPs leads to high significantly ($p<0.01$) decreased in Absolute Lymphocyte Count in 400 mg/kg treated group as compared to control group and other treatment groups in both male and female rats.

The administration of CuO NPs produced very high significantly ($p<0.005$) increased in Absolute Monocyte Count in 400 mg/kg treated group as compared to control group in male rats, whereas it was very very high significantly ($p<0.001$) increased in females rats treated with 400 mg/kg CuO NPs and high significantly ($p<0.01$) increased in 200 mg/kg treated group as compared to control group Total. Leucocyte Count in male rats was significantly ($p<0.05$) increased in 400 mg/kg treated group as compared to control group and in other treatment group. There were no any significant changes were found in female rats. The administration of CuO NPs leads to high significantly ($p<0.01$) and significantly ($p<0.05$) increased in platelet count in 400 mg/kg treated group as compared to control group and 100 mg/kg treated group, respectively in male rats, whereas it was significantly increased ($p<0.05$) in females rats treated with 400 mg/kg treated group as compared to control group and in other groups it did not leads to any significant changes.

3.3 Biochemical parameters
Effect of daily oral administration of Copper oxide nanoparticles for 28 days on Biochemical Parameters in rats of different experimental groups were presented in table 4 and 5.

At the end of 28th day of experiment, The level of Urea was very high significantly ($p<0.005$) and significantly ($p<0.05$) increased in 400 mg/kg treated group as compared to control and 100 mg/kg treated group respectively and in female rats level was significantly ($p<0.05$) increased in 400 mg/kg treated group as compared to control and other treatment groups. Serum uric acid was high significantly ($p<0.01$) and significantly ($p<0.05$) increased in 400 mg/kg treated group when compared to the control and 100 mg/kg treated group, respectively in male rats, while in female rats it was significantly ($p<0.05$) increased in 400 mg/kg treated group as compared to control. At the end of 28th day of experiment, CuO NPs treated rats showed significantly ($p<0.05$) increased in level of Creatinine in 400 mg/kg treated group as compared to control and other treatment groups in male and female rats. The level of Total protein was significantly ($p<0.05$) increased in 400 mg/kg treated group as compared to control group in male rats, whereas in female rats there was no any significant changes were found in any treatment groups. The level of Calcium was significantly ($p<0.05$) increased in 400 mg/kg treated group as compared to control group in male and female rats. The level of Phosphorus was high significantly ($p<0.01$) and significantly ($p<0.05$) increased in 400 mg/kg treated group as compared to control and 100 mg/kg treated group,

respectively in male rats, whereas in female rats it was significantly (p<0.05) increased in 400 mg/kg treated group as compared to control group.

3.4 Antioxidant parameters

SOD, CAT activity and MDA level in kidney tissue collected from all animals on the 29th day of Copper oxide nanoparticles were evaluated and presented graphically in figure 1.

In the present study, SOD activity in kidney was high significantly (p<0.01) decreased in T3 (400 mg/kg) group and significantly (p<0.05) decreased in T2 (200 mg/kg) group as compared to control group in male rats, whereas in female rats it was high significantly (p<0.01) and significantly (p<0.05) decreased in T3 (400 mg/kg) group as compared to control and T1 (100 mg/kg) group respectively. Moreover CAT activity in kidney was high significantly (p<0.01) decreased in T3 (400 mg/kg) group and significantly (p<0.05) decreased in T2 (200 mg/kg) group as compared to control group in male rats, whereas in female rats it was significantly (p<0.05) decreased in T3 (400 mg/kg) and T2 (200 mg/kg) group as compared to control group and kidney MDA levels in male rats was high significantly (p<0.01) increased in T3 (400 mg/kg) group and significantly (p<0.05) increased in T2 (200 mg/kg) group as compared to control group, whereas in female rats MDA level was significantly (p<0.05) increased in T3 (400 mg/kg) and T2 (200 mg/kg) group as compared to control group.

3.5 Histopathology

In microscopic examination of kidney in control group (C) showed normal architecture of renal tubules, glomerulus and renal pelvis. The microscopic lesions in kidney of group T1 showed intertubular hemorrhage, mild degeneration of renal tubules and narrowing of tubular lumen. Kidney of group T2 showed congested glomeruli, intertubular hemorrhage, narrowing of tubular lumen, vacuolar degeneration of renal tubules, necrosis of renal tubular epithelium and presence of renal hyline cast, whereas in kidney of group T3 showed distorted tubules, presence of intraluminal hyline cast, intertubular hemorrhage, narrowing of the tubular lumen and enlargement in glomerulus, vacuolar degeneration and severe coagulative necrosis of tubular epithelium (Figure 2).

4. Discussion

Our finding was supported by Lei *et al*. (2008) [26], Lee *et al*. (2016a) [24] and Maciel-Magalhaes *et al*. (2020) [30], they observed the signs of anorexia, severe diarrhoea, loss of weight, changes occur in faeces colour and consistency it was black in appearance and soft in consistency in higher dose of CuO NPs treated group. This toxic manifestation might be due to release of Cu2+ ion from Cu NPs cause damage to the gastrointestinal tract. (Chen *et al*., 2006) [8].

Similar finding to the present study observed by Lee *et al*. (2016b) [25] and Yaqub *et al*. (2018) [48], They reported significant reduction in HCT %, mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), lymphocyte (%) and significantly increased in neutrophils (%), monocytes (%), Total leukocyte count (TLC) and platelet count of rats treated with CuO NPs as compared to control group. In present study dose dependent reduction in HCT% might be due to destruction of RBCs. The possible reason for RBC destruction and hemolysis is the inhibition of glucose-6-phosphate dehydrogenase induced by acute copper poisoning leading to the reduction of NADPH concentration in the red blood cells and subsequently decreased the level of reduced glutathione which leads to occurrence of hemolysis (Patel *et al*., 1976) [39]. Changes in hematology parameters like MCHC, MCH, HCT% were indicated a microcytic anemia that generally observed with iron deficiency (Hebert *et al*., 1993; Arredondo and Nunez, 2005) [19, 5]. Decreased in percentage of lymphocyte was indicated CuO NPs might have adverse effects on the immune system, which was well correlated with the reduction of cellularity seen in spleen and increased in percentage of neutrophils and monocytes due to inflammatory response. Moreover, increased in WBC count may indicate the intrinsic protection and initiation of the body's defense mechanism (Sarhan & Hussein, 2014) [43].

Increased in the level of total bilirubin, urea, uric acid and creatinine in present study were supported by Lei *et al*. (2008) [26], Lee *et al*. (2016b) [25] and Yaqub *et al*. (2018) [48]. High bilirubin levels in serum suggestive to hemolytic anemia which was correlated to decreased in total RBCs count and HCT% in present study. Serum urea concentration indicated balance between urea production in the liver and urea elimination by the kidneys in the urine. So reduced in urinary elimination of urea associated with marked reduction in glomerular filtration rate (GFR) (Higgins, 2016) [20]. In present study increased in creatinine level was indicating dysfunction in renal glomerular filtration which was confirmed by histopathologically in which widespread renal tubule cell necrosis was observed.

In support to present study, Soliman *et al*. (2021) [46] reported total protein level was significantly increased in nile tilapia (*Oreochromis niloticus*) exposed to 15 mg/L of CuO NPs as compared to control group. This might be due to fulfil the increased demand for tissue repair and to improve their immunological response following copper exposure. According to previous studies total protein level was high significantly increased in higher dose of CuO NPs treated group (Lee *et al*., 2016b; Mohammadyari *et al*., 2014; Ouni *et al*., 2020) [25, 32, 37]. Contrary to the present study El-Atrash *et al*. (2022) [13] observed significantly decreased in total protein in male rats received CuO NPs at the dose rate of 400 mg/kg intraperitoneally for 4 weeks. In present study increased in total protein is might be due damage to the liver or dehydration. Calcium and phosphorus homeostasis is the complex process which is regulated by interactions between the trace elements in the body. Increased in the serum calcium level which is reduces the absorption of copper in the body. In accordance with the present study Ouni *et al*. (2020) [37] and El-Atrash *et al*. (2022) [13] reported significantly increased in level of calcium and phosphorus in serum of rats due to exposure of CuO NPs. Contrary to the present study El-Atrash *et al*. (2022) [13] found significantly decreased in calcium level of male rats treated with CuO NPs at the dose rate of 400 mg/kg intraperitoneally for 4 weeks. In our study significant increase in calcium level might be due to kidney damage which is correlated with changes in renal biomarker, oxidative stress and histopathological changes

Nanoparticles enhanced ROS production, which resulted in elevated proinflammatory responses and oxidative stress via intracellular signaling pathways. ROS generation, which has

a stronger effect on physiology, is one of the adverse effects of CuO NPs (Sarkar *et al*., 2014) [44].

Assadian *et al*. 2018 [6] also found *in vitro* cytotoxicity of CuO-NP, which was related with a considerable increase in intracellular ROS levels and effective oxidative stress induction. One of the key causes of NP toxicity is its propensity to generate free radicals. CuONP induced hepatotoxicity and nephrotoxicity were linked to oxidative stress damage to the liver and kidney tissues via reactive oxygen species (ROS) generation (Moller *et al*., 2010; Wang *et al*., 2012) [33]. Our finding was supported by Arafa *et al*. (2017) [4], Anreddy (2018) [3], Hassanen *et al*. (2019) [18] and Ghareeb *et al*. (2021) [16] reported SOD activity and MDA level was significantly increased and CAT activity was significantly decreased in liver of rat treated with CuO NPs as compared to control group. Similar finding to present study was reported by Bugata *et al*. (2019) [7] and Elkhateeb *et al*. (2020) [14] in which SOD and CAT activity in rat kidney tissue was significantly decreased and MDA level was significantly increased in higher dose group of CuO NPs as compared to control group reported by Hassanen *et al*. (2019) [18]; Ahmed *et al*. (2022) [2].

Our finding was supported by Khalid *et al*. (2018) [23], Bugata *et al*. (2019) [7], Hassanen *et al*. (2019) [18] and El Bialy *et al*. (2020) [12] reported histopathogical changes in kidney of wistar rats which showed tubular and glomerular necrosis, hemorrhagic patterns, enlarged Bowman's space, tubular dilatation, degeneration, vacuolization, epithelial denudation and moderate interstitial nephritis.

They also found extensive renal tubular necrosis, vacuolar degeneration of the renal tubular epithelium, renal cellular and hyaline cast accumulated intraluminally, necrotic tubular epithelium with intensely eosinophilic cytoplasm and pyknotic or completely lysed nuclei appeared. There were peritubular haemorrhages, glomerular capillaries and interstitial blood vessel congestions, glomerular hypercellularity, severe coagulative necrosis, detached tubular epithelia, loss of the brush border and intraluminal hyaline casts in the cortex and medulla, intertubular haemorrhage, cloudy swelling, narrowing of the tubular lumen. The basement membrane of glomeruli showed thickening and periglomerular lymphocytic infiltration in CuO NPs treated group as compared to control group. Similarly, kidney of male albino mice revealed damage in renal capsule, enlargement in glomerulus, loses in urinary space associated with vacuolization in cytoplasm in 100 mg/kg CuO NPs treated group as compared to other groups (Haleem & Khaleel *et al*., 2020) [17]. Kidney of birds treated with dose of 5 mg/kg CuO-NPs revealed focal interstitial inflammatory cell infiltrations associated with mild to moderate granulomatous and vacuolar degenerations in the epithelial lining of the renal tubules, whereas kidney of birds given 15 mg/kg CuO-NPs revealed moderate to severe degeneration and necrosis of renal tubular epithelial cells, as well as severe interstitial haemorrhage and inflammatory cell infiltrations (Morsy *et al*., 2021) [34].

Table 1: Group of animals and different treatments

Groups	No. of Animals		Dose (Oral gavage for 28 days)
	M	F	
Control	6	6	R.O. Water
Treatment 1	6	6	100 mg/kg b.w./day CuO NPs in water
Treatment 2	6	6	200 mg/kg b.w./day CuO NPs in water
Treatment 3	6	6	400 mg/kg b.w./day CuO NPs in water
Total	24	24	

Table 2: Effect of daily oral administration of CuO NPs on hematological parameters in male rats (Day - 28)

Hematological parameters	Treatment groups (Mean ± SE)			
	Control	100 mg/kg (T1)	200 mg/kg (T2)	400 mg/kg (T3)
Hb (g/dL)	16.03 ± 0.35^{a}	16.02 ± 0.46^{a}	14.93 ± 0.64^{a}	14.60 ± 0.85^{a}
HCT (%)	49.32 ± 0.62^{a}	49.5 ± 1.15^{a}	48.99 ± 1.21^{ab}	44.64 ± 0.46^{b}
TEC (10^{6}/µL)	9.39 ± 0.12^{a}	9.41 ± 0.15^{a}	9.40 ± 0.27^{a}	9.18 ± 0.11^{a}
TLC (10^{3}/µL)	15.63 ± 1.53^{a}	16.27 ± 1.96^{a}	18.28 ± 1.16^{a}	20.70 ± 1.80^{a}
Platelets (10^{3}/µL)	649.00 ± 70.57^{c}	741.67 ± 84.84^{bc}	865.00 ± 23.53^{abc}	1418.67 ± 107.78^{a}
Lymphocyte (%)	82.12 ± 1.97^{a}	81.08 ± 1.66^{a}	71.62 ± 3.40^{ab}	44.33 ± 2.50^{b}
Neutrophils (%)	12.52 ± 2.31^{b}	12.25 ± 1.04^{b}	18.22 ± 3.19^{ab}	36.52 ± 3.04^{a}
Monocyte (%)	3.23 ± 0.79^{c}	4.52 ± 0.16^{bc}	7.15 ± 1.11^{b}	12.10 ± 0.86^{a}
Eosinophils (%)	2.13 ± 0.96^{a}	2.15 ± 1.60^{a}	3.02 ± 1.22^{a}	7.05 ± 1.27^{a}
Basophils (%)	0.00	0.00	0.00	0.00
MCV (fl)	51.67 ± 0.49^{a}	50.83 ± 0.95^{a}	51.00 ± 1.2^{a}	49.83 ± 0.70^{a}
MCH (pg)	16.88 ± 0.39^{a}	17.08 ± 0.27^{a}	16.72 ± 0.22^{ab}	15.62 ± 0.36^{b}
MCHC (g/dL)	35.12 ± 0.82^{a}	31.85 ± 0.65^{bcd}	30.98 ± 0.83^{cd}	30.52 ± 0.32^{d}
Neutrophils (10^{3}/µL)	1.20 ± 0.13^{b}	2.33 ± 0.54^{ab}	6.94 ± 0.87^{a}	8.52 ± 1.33^{a}
Lymphocytes (10^{3}/µL)	15.00 ± 0.87^{a}	12.59 ± 1.08^{ab}	10.12 ± 0.82^{ab}	8.79 ± 0.98^{b}
Monocytes (10^{3}/µL)	0.67 ± 0.12^{b}	0.98 ± 0.11^{ab}	1.42 ± 0.28^{ab}	3.46 ± 0.36^{a}

Note: Values with different superscript in a row were significantly different ($p<0.05$)

Table 3: Effect of daily oral administration of CuO NPs on hematological parameters in female rats (Day - 28)

Hematological parameters	Treatment groups (Mean ± SE)			
	Control	100 mg/kg (T1)	200 mg/kg (T2)	400 mg/kg (T3)
Hb (g/dL)	15.17 ± 0.40^{a}	15.07 ± 0.05^{a}	14.37 ± 0.21^{a}	14.05 ± 0.25^{a}
HCT (%)	45.72 ± 0.98^{a}	45.26 ± 0.87^{a}	43.54 ± 1.03^{a}	44.30 ± 0.82^{a}
TEC (10^{6}/µL)	9.15 ± 0.19^{a}	9.15 ± 0.19^{a}	8.66 ± 0.20^{a}	8.73 ± 0.14^{a}
TLC (10^{3}/µL)	8.72 ± 1.20^{b}	10.39 ± 0.62^{ab}	10.75 ± 1.71^{ab}	14.44 ± 1.76^{a}
Lymphocyte (%)	80.73 ± 0.62^{a}	77.72 ± 1.51^{a}	74.33 ± 1.76^{a}	71.17 ± 1.78^{a}
Platelets (10^{3}/µL)	827.50 ± 19.97^{b}	877.83 ± 23.17^{ab}	886.00 ± 23.13^{ab}	945.33 ± 35.40^{a}
Neutrophils (%)	11.95 ± 0.49^{a}	12.73 ± 1.72^{a}	15.13 ± 1.20^{a}	17.05 ± 1.72^{a}
Monocyte (%)	4.90 ± 0.57^{b}	6.85±0.60^{ab}	6.98 ±0.85^{ab}	8.03 ± 0.34^{a}
Eosinophils (%)	2.42 ± 0.58^{a}	2.70 ± 0.33^{a}	3.55 ± 0.49^{a}	3.76 ± 0.49^{a}
Basophils (%)	0.00	0.00	0.00	0.00
MCV (fl)	51.00 ± 0.45^{a}	50.00 ± 1.39^{a}	51.33 ± 0.95^{a}	48.33 ± 1.12^{a}
MCH (pg)	16.92 ± 0.17^{a}	16.43 ± 0.36^{a}	16.25 ± 0.26^{a}	16.28 ± 0.11^{a}
MCHC (g/dL)	34.02 ± 0.79^{a}	33.92 ± 0.66^{a}	31.80 ± 0.75^{a}	31.55 ± 0.22^{a}
Neutrophils (10^{3}/µL)	1.15 ± 0.17^{b}	1.29 ± 0.23^{b}	1.78 ± 0.23^{ab}	2.70 ± 0.06^{a}
Lymphocytes (10^{3}/µL)	12.01 ± 1.37^{a}	9.75 ± 1.39^{ab}	9.36 ± 0.47^{ab}	5.30 ± 0.50^{b}
Monocytes (10^{3}/µL)	0.41 ± 0.08^{c}	0.47 ± 0.11^{c}	0.83 ± 0.05^{b}	1.43 ± 0.03^{a}

Note: Values with different superscript in a row were significantly different (p<0.05)

Table 4: Comparison of mean values of serum-biochemical parameters in male rats following CuO NPs exposure for 28 days

Biochemical parameters	Treatment groups (Mean ± SE)			
	Control	100 mg/kg (T1)	200 mg/kg (T2)	400 mg/kg (T3)
Total protein (gm/dL)	6.84 ± 0.41^{b}	5.74 ± 1.31^{ab}	8.72±1.53^{ab}	9.42 ± 0.68^{a}
Albumin (gm/dL)	3.92 ± 0.09^{a}	4.19 ± 0.48^{a}	5.01 ± 0.73^{a}	5.68 ± 0.47^{a}
Globulin (gm/dL)	1.88 ± 0.44^{a}	2.93 ± 0.46^{a}	3.74 ± 0.46^{a}	3.88 ± 0.66^{a}
A:G ratio	2.62 ± 0.47^{a}	1.77 ± 0.45^{a}	1.37 ± 0.16^{a}	1.64 ± 0.27^{a}
Creatinine (mg/dL)	0.78 ± 0.02^{b}	0.58 ± 0.16^{ab}	1.36 ± 0.57^{ab}	1.47 ± 0.75^{a}
Urea (mg/dL)	50.26 ± 2.76^{b}	56.67 ± 3.46^{b}	86.84 ± 1.30^{ab}	251.41 ± 4.35^{a}
Uric acid (mg/dL)	5.77 ± 2.70^{b}	3.66 ± 0.79^{b}	4.03 ± 0.58^{ab}	10.93 ± 1.81^{a}
Calcium (mg/dL)	12.19 ± 0.56^{b}	11.53 ± 3.77^{ab}	13.77 ± 3.31^{ab}	17.86 ± 2.19^{a}
Phosphorus (mg/dL)	9.62 ± 1.52^{b}	10.86 ± 2.49^{b}	14.34 ± 3.10^{ab}	22.37 ± 2.06^{a}

Note: Values with different superscript in a row were significantly different (p<0.05)

Table 5: Comparison of mean values of serum-biochemical parameters in female rats following CuO NPs exposure for 28 days

Biochemical parameters	Treatment groups (Mean ± SE)			
	Control	100 mg/kg (T1)	200 mg/kg (T2)	400 mg/kg (T3)
Total protein (gm/dL)	6.16 ± 0.40^{a}	6.85 ± 0.37^{a}	6.27 ± 1.19^{a}	6.79 ± 1.42^{a}
Albumin (gm/dL)	3.86 ± 0.18^{a}	4.47 ± 0.33^{a}	4.14 ± 0.80^{a}	4.13 ± 0.76^{a}
Globulin (gm/dL)	2.29 ± 0.25^{a}	2.38 ± 0.16^{a}	2.14 ± 0.41^{a}	2.66 ± 0.73^{a}
A:G ratio	1.75 ± 0.13^{a}	1.91 ± 0.18^{a}	2.10 ± 0.26^{a}	1.95 ± 0.36^{a}
Creatinine (mg/dL)	0.62 ± 0.04^{b}	0.64 ± 0.04^{ab}	0.76 ± 0.05^{ab}	0.84 ± 0.04^{a}
Urea (mg/dL)	40.32± 4.21^{b}	44.64 ± 2.74^{ab}	51.87 ± 4.10^{ab}	59.26 ± 3.61^{a}
Uric acid (mg/dL)	2.55 ± 0.33^{b}	3.23 ± 0.64^{b}	4.90 ± 0.53^{ab}	5.91 ± 0.84^{a}
Calcium (mg/dL)	11.49 ± 0.82^{b}	12.21 ± 1.09^{ab}	14.22 ± 0.99^{ab}	15.58 ± 1.17^{a}
Phosphorus (mg/dL)	6.94 ± 0.73^{b}	9.57 ± 0.29^{ab}	9.48 ± 1.03^{ab}	11.13 ± 1.23^{a}

Note: Values with different superscript in a row were significantly different (p<0.05)

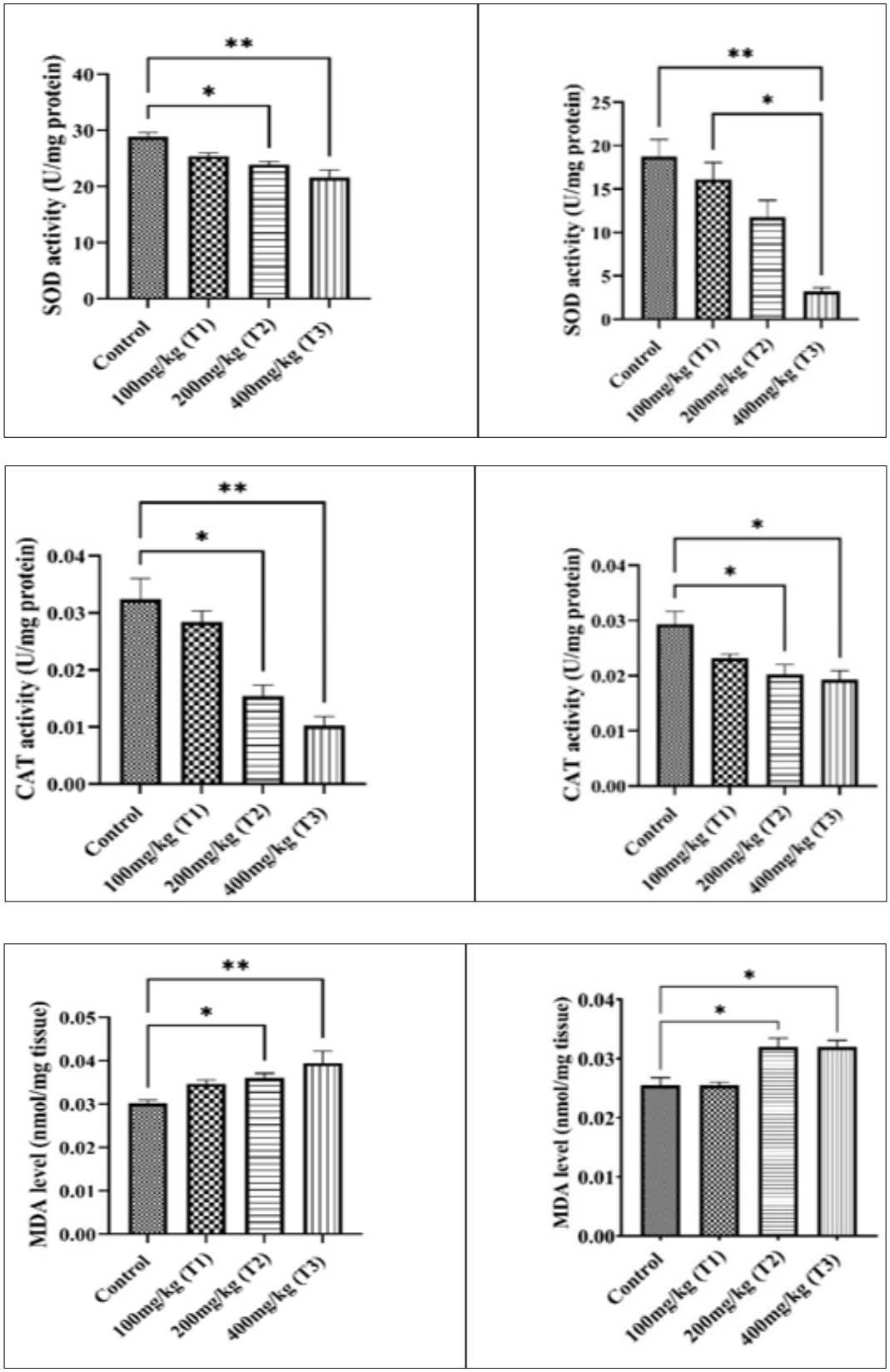

Fig 1: Comparison of oxidative stress parameters in the kidney of male (A, B, C) and female (A1, B1, C1) rats following CuO NPs exposure for 28 days. Where *indicates $p<0.05$, **indicates $p<0.01$

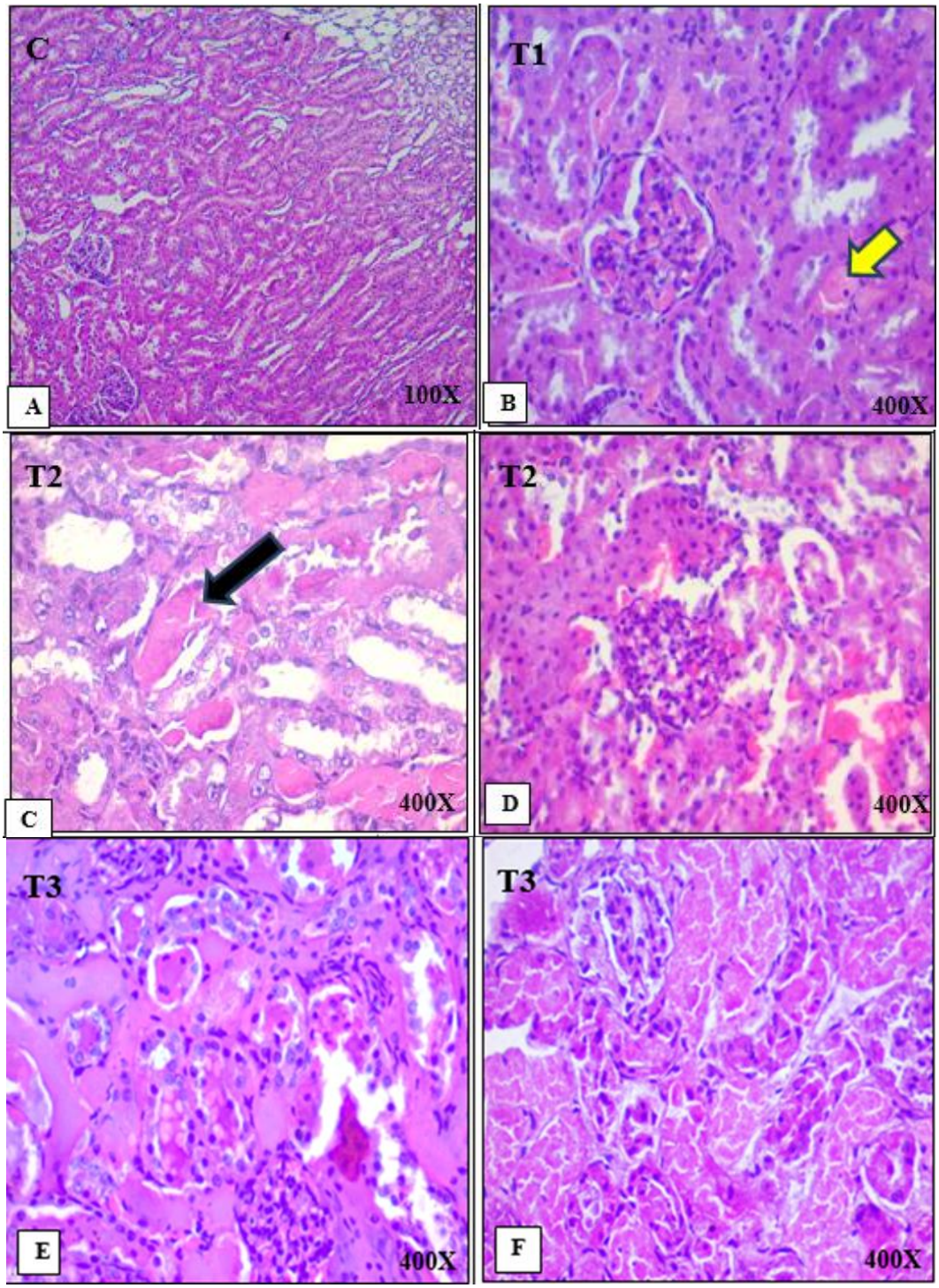

Fig 2: Histopathological changes in the kidney of Wistar rats from different groups, A: Normal architecture of renal tubules and glomerulus in control group B: Intertubular hemorrhage (Yellow arrow), mild degeneration of renal tubules and narrowing of tubular lumen in T2 group C: Kidney in T2 group showing vacuolar degeneration of renal tubules, necrosis of renal tubules and presence of renal hyline cast (Black arrow) D: Congested glomeruli, intertubular hemorrhage, narrowing of tubular lumen with degenerative changes in T2 group E: Necrosis of tubular epithelium with presence of hyline cast, cloudy swelling and narrowing of the tubular lumen were observed in T3 group F: Kidney of T3 group showing severe coagulative necrosis, intertubular hemorrhage, and enlargement of glomerulus.

5. Conclusion

The current study found that CuO NPs caused nephrotoxicity by producing ROS and oxidative stress. These effects are attributed to a weakened antioxidant defence system and alterations in hemato-biochemical parameters. Further research is needed to discover the underlying toxicity processes and develop reliable solutions for the safe use of these nanoparticles in industrial and biological applications.

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