

*Full Length Research Paper*

## **A study of two weeks administration of copper sulphate on markers of renal function and feeding pattern of Wistar rats**

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**This study aimed at determining the changes in food consumption, water intake, plasma and urine concentrations of some organic constituents which are often used in the assessment of renal function following two weeks' administration of two doses of copper sulphate to Wistar rats. Fifteen adult male Wistar rats were randomly divided into three groups of five rats each. Group I (control group) received distilled water; groups II and III were given 100 and 200 mg/kg/day *p. o* of copper sulphate for 14 days, respectively. Significant reductions in food consumption and water intake were observed in group II when compared with the control and group III rats, but their body weight increased insignificantly throughout the study. The plasma urea concentrations of the treated rats were not significantly different from the control rats. The plasma creatinine levels of the experimental rats rose slightly, but not significantly different from the control rats. The creatinine and urea concentrations in the urine fell significantly in group II when compared with the control group. This was accompanied by decrease in creatinine clearance. Photomicrographs of the kidneys of both the control and experimental rats revealed no alteration in the histology of their renal tissue. It is concluded that acute copper sulphate administration to rats induced anorexia and suppression of renal function, thereby indicating the potential toxicity of the salt if ingested for a longer period.**

**Key words:** Copper sulphate, kidney, creatinine, urea, rats.

### **INTRODUCTION**

Copper (Cu) is an essential trace element and one of the most important heavy metals capable of producing toxic

effects in man and animals when ingested acutely or chronically in excess. Copper compounds are widely used

in electrical industry, metallurgy, photography, painting, leather manufacture and water purification. Burning of copper sulphate in houses and shops (as a good luck charm and for religious activities) is a common practice among Buddhists and Hindus. Among the medicinal applications of copper is its utilization in certain types of dental amalgam and intrauterine contraceptive devices (IUCD). It appears in several enzymes, facilitates the absorption of iron, and helps to transmit electrical signals in the body. In high doses, however, the metal can be extremely toxic (Saravu et al., 2007). The circulation and proper utilization of copper in the body requires good functioning of the liver, gall bladder and adrenal glands. If any of these organs are impaired, the body cannot properly excrete and utilize copper. Initially, the copper will build up in the liver, further impairing its ability to excrete copper. As copper retention increases, it will build up in the brain, the joints and the lungs, adversely affecting the structure and function of the tissues. Copper is a powerful oxidant causing inflammation and free radical damage to the tissues. To avoid these toxic effects, it must be bound to the binding proteins, ceruloplasmin and metallothionein. These proteins can become deficient due to impaired adrenal and liver function which allows free copper to build up (Sinkovic et al., 2008).

Copper sulfate, one of the most available salts of copper, is a blue and odorless salt that is employed in various products such as fungicides, herbicides and insecticides (Blundell et al., 2003; Oldenquist and Salem, 1999). Copper sulfate is also found in chemistry laboratories as wettable powders and fluid concentrates. It can be absorbed through the gastrointestinal tract, lungs and skin causing both systemic and local toxicity including stupor, coma, convulsion, hypotension, shock, respiratory failure, pallor and jaundice (Oldenquist and Salem, 1999; Agarwal et al., 1993). Ingestion of significant quantities of copper sulphate carries a risk of multi organ failure.

Recently, the adverse effect of copper sulphate poisoning on sperm quality and testicular histopathology has been reported (Sakhaee et al., 2011). Studies carried out by Babaei et al. (2012) showed that short term administration of copper sulphate (14 days) at a dose of 100 and 200 mg/kg had deleterious effects on intracellular organelles of rat ovarian cells. Literature is scanty on the influence of short term administration of copper sulphate on the feeding pattern and renal function of rats hence, we decided to investigate the effects of acute ingestion of copper sulphate on the feeding pattern and some markers for the assessment of kidney function in Wistar rats at the same doses that have been reported to be toxic to their reproductive organs.

## MATERIALS AND METHODS

### Animal care and management

Fifteen (15) adult male Wistar rats weighing 120 - 150 g were used for this study. The rats were obtained from the Animal House of the College of Health Sciences, Obafemi Awolowo University, Ile-Ife, Nigeria. Each rat was housed in a separate metabolic cage (Ohaus R Model; Ohaus, Pine Brook, NJ, USA) during the experiment to obtain a 24 h urine sample. The rats were kept under normal environmental conditions with a natural light/dark cycle and free access to standard rodent pellet diet (Caps Feed PLC, Osogbo, Nigeria) and water *ad libitum*. They were allowed to acclimatize in the laboratory for one week before the commencement of the study. The experimental procedures adopted in this study were in strict compliance with the guidelines on Experimental Animal Care and Use of Laboratory Animals in Biomedical Research, College of Health Sciences, Obafemi Awolowo University, Ile-Ife, Nigeria.

### Experimental design

The rats were randomly divided into three groups of five rats each. Group I (control group) received distilled water; groups II and III were given 100 and 200 mg/kg/day *p.o* of copper sulphate for 14 days, respectively.

Twenty-four hours after the last dose of treatment, the rats in each group were sacrificed by cervical dislocation and blood was obtained by cardiac puncture into separate heparinized bottles for hematological analyses. The blood was centrifuged for 20 min at 4000 rpm using a cold centrifuge (Centrium Scientific, Model 8881). The plasma was separated and analyzed for organic constituents that are routinely used in the assessment of kidney function. Thereafter, the kidney of each rat was carefully excised and fixed inside 10% formo-saline for histopathological studies.

### Measurement of body weight

The body weight of the animals were measured once in a week using a weighing balance (Camry; Zhongshan Guangdong, China) during the experiment to access the weight gain or loss in each group.

### Measurement of food consumption and water intake

The food consumption and water intake of each rat were determined daily. The volume of water and weight of food given to each rat was measured with a measuring cylinder and a weighing balance respectively. The difference between the previous day volume of water and weight of food, and the left-over was taken as the daily food consumption and water intake of the rats.

### Haematological indices

The haematocrit (HCT), hemoglobin (Hb) concentration, red blood cell (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), white blood cell (WBC), granulocyte, monocytes, lymphocytes and platelet counts were measured using an auto-analyzer machine (SFRI Blood Cell Counter, H18 Light, France).

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**Table 1.** Effect of copper sulphate on food consumption (gram) of rats.

Week	I Control (Water)	II (100 mg/kg)	III (200 mg/kg)
Pre-treatment	20.51 ± 1.39	20.80 ± 1.66	21.71 ± 0.63
1	19.94 ± 1.11	13.71 ± 1.40 <sup>*#</sup>	19.57 ± 1.02 <sup>§</sup>
2	19.09 ± 1.60	15.86 ± 1.50	17.89 ± 0.98 <sup>#</sup>

Values are given as mean ± SEM (n=5). \* = Significantly different from control. § = Significantly different from group II. # = Significantly different from pre-treatment ( $p < 0.05$ ).

**Table 2.** Effect of copper sulphate on the body weight of rats (gram).

Week	I Control (Water)	II (100mg/kg)	III (200mg/kg)
Pre-treatment	167.0 ± 8.46	176.0 ± 8.72	178.0 ± 3.74
1	191.2 ± 9.02	174.0 ± 10.77	201.0 ± 4.00 <sup>#</sup>
2	212.0 ± 11.68 <sup>#</sup>	191.0 ± 12.39	212.0 ± 4.64 <sup>#</sup>

Values are given as mean ± SEM (n=5). # = Significantly different from pre-treatment ( $p < 0.05$ ).

**Table 3.** Effect of copper sulphate on water intake (ml) of rats.

Week	I Control (Water)	II (100mg/kg)	III (200mg/kg)
Pre-treatment	45.63 ± 1.54	44.09 ± 4.84	49.66 ± 2.98
1	43.60 ± 1.50	31.40 ± 1.87 <sup>*</sup>	39.69 ± 2.45 <sup>§</sup>
2	38.89 ± 2.27	29.60 ± 2.85 <sup>*#</sup>	38.21 ± 0.24 <sup>#§</sup>

Values are given as mean ± SEM (n=5). \* = Significantly different from control. § = Significantly different from group II. # = Significantly different from pre-treatment ( $p < 0.05$ ).

### Biochemical analysis

Levels of creatinine and urea were assayed by the use of appropriate biochemical kits purchased from Randox Laboratories (Crumlin, Co. Antrim UK). The plasma creatinine was estimated by alkaline picrate method (Bonsnes and Taussky, 1945). Urea assay was carried out in the plasma according to the method of Berthelot (Fawcett and Scott, 1960). The urine concentrations of urea and creatinine were estimated in the last samples of urine collected from the rats, using the same methods that were used in the analysis of plasma. Creatinine clearance was calculated.

### Histopathological evaluation

The fixed kidney samples were dehydrated in graded alcohol and embedded in paraffin wax. They were then cut into 7-8 µm thick sections and stained with haematoxylin-eosin for photomicroscopic assessment using a Leica DM 750 Camera Microscope at 100 and 1000x magnifications.

### Statistical analysis

The results obtained were expressed as mean ± SEM. The data were analyzed using one way ANOVA followed by Tukey's multiple comparison test using GraphPad 5.03 (GraphPad Software Inc., CA, USA). The results were considered significant when  $p < 0.05$ .

## RESULTS

### Food consumption and body weight

In the first week of treatment, a significant reduction in food consumption was observed in group II when compared with the control and group III rats (Table 1). Similarly, the food consumption of group II dropped significantly during the 1st week when compared with the pre-treatment value. Group III showed a significant decrease in food consumption during the 2nd week when compared with the pre-treatment value.

Although, a significant decrease in food consumption was observed in group III during the 2nd week of treatment, the body weight of rats in this group was significantly higher during the 1st and 2nd week than that of the pre-treatment Table 2.

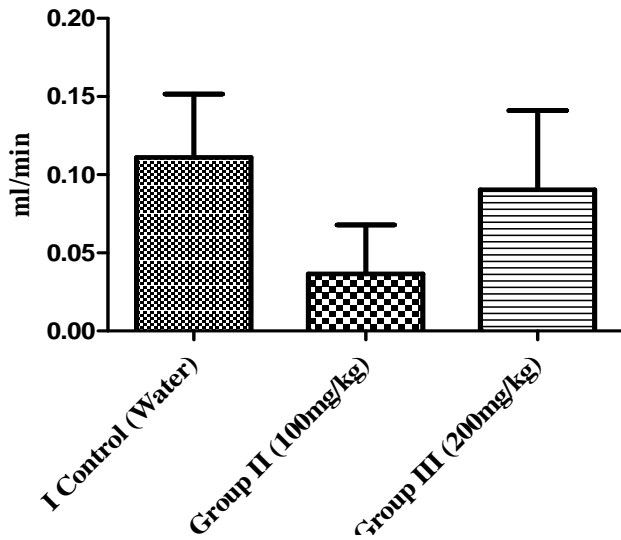
### Water intake and urinary volume

During the 1st and 2nd week, water intake fell significantly in group II when compared with the control and group III rats (Table 3). This reduction was accompanied

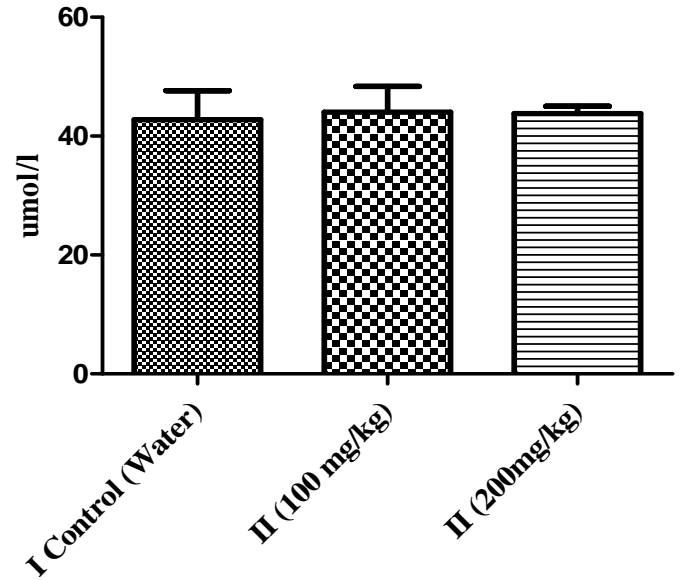
**Table 4.** Effect of copper sulphate on urine output (ml) of rats.

Week	I Control (Water)	II (100mg/kg)	III(200mg/kg)
Pre-treatment	5.34 ± 0.93	6.04 ± 1.04	10.24 ± 1.27 <sup>§</sup>
1	5.83 ± 0.59	4.01 ± 0.61	7.02 ± 0.83 <sup>§</sup>
2	5.96 ± 1.13	5.65 ± 0.99	7.53 ± 2.13

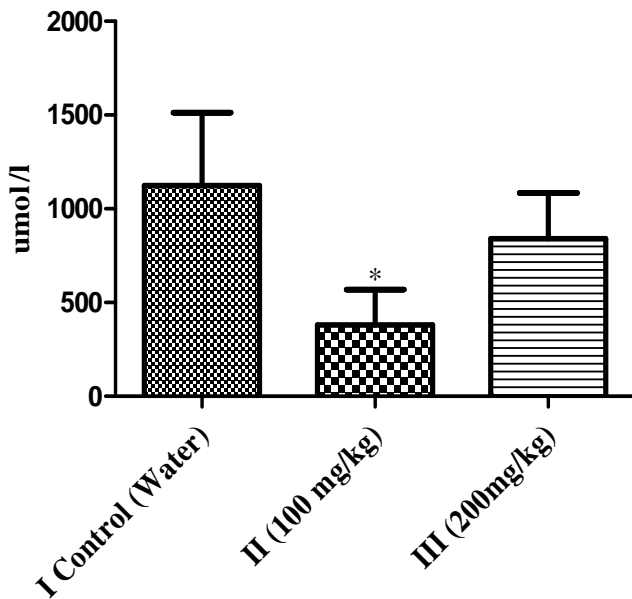
Values are given as mean ± SEM (n=5). \* = Significantly different from control. § = Significantly different from group II (p < 0.05).



**Figure 1.** Effect of copper sulphate on creatinine clearance of rats. Values are given as mean ± SEM (n=5). No significant difference was observed between groups.



**Figure 3.** Effect of copper sulphate on plasma creatinine concentration of rats. Values are given as mean ± SEM (n=5). No significant difference was observed between groups.



**Figure 2.** Effect of copper sulphate on urine creatinine concentration of rats. Values are given as mean ± SEM (n=5). \* = Significantly different from control (p < 0.05).

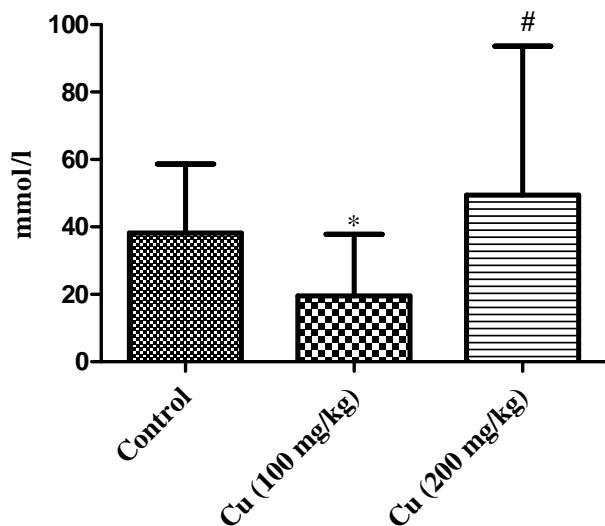
by a fall in urine volume which was not significantly different from the pre-treatment (Table 4). A significant decrease in water intake was observed in groups II and III during the 2nd week when compared with the pre-treatment. The decrease in water intake of group III was accompanied by a significant fall in urine volume during the 1st week when compared with the pre-treatment.

**Plasma creatinine, urine creatinine and creatinine clearance**

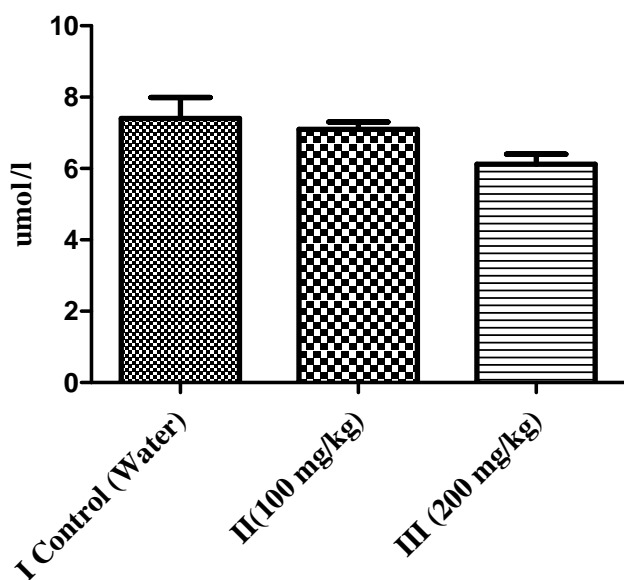
A significant reduction in urine creatinine was seen in group II when compared with the control rats (Figure 2). There was also a fall in creatinine clearance in this group of rats (Figure 1). The plasma concentration of creatinine rose slightly but it was not significantly different from that of the control rats (Figure 3).

**Urine urea and plasma urea**

The concentration of urea in the urine of group II fell



**Figure 4.** Effect of copper sulphate on urine urea concentration of rats. Values are given as mean  $\pm$  SEM (n=5). \* = Significantly different from Control. # = Significantly different from Group II ( $p < 0.05$ ).



**Figure 5.** Effect of copper sulphate on plasma urea concentration of rats. Values are given as mean  $\pm$  SEM (n=5). No significant difference was observed between groups.

significantly when compared with the control and group III rats (Figure 4). However, there was no significant difference in plasma urea concentration of group II when compared with the control and group III rats (Figure 5).

### Haematological indices

A significant reduction in red blood cell count and

**Table 5.** Effect of copper sulphate on haematological indices of Wistar rats.

	I (Control)	II (100 mg/kg)	III (200 mg/kg)
WBC count	4.08 $\pm$ 1.11	3.32 $\pm$ 0.64	4.04 $\pm$ 0.72
LYM (%)	78.46 $\pm$ 3.28	77.30 $\pm$ 3.00	76.80 $\pm$ 1.25
MON (%)	9.52 $\pm$ 0.61	10.28 $\pm$ 1.23	12.30 $\pm$ 0.39
GRAN (%)	12.02 $\pm$ 2.74	12.42 $\pm$ 1.98	10.90 $\pm$ 1.09
LYM count	3.26 $\pm$ 0.99	2.52 $\pm$ 0.41	3.12 $\pm$ 0.57
MON count	0.38 $\pm$ 0.09	0.36 $\pm$ 0.08	0.52 $\pm$ 0.10
GRAN count	0.44 $\pm$ 0.09	0.44 $\pm$ 0.17	0.40 $\pm$ 0.07
RBC count	7.43 $\pm$ 0.18	7.81 $\pm$ 0.24	6.74 $\pm$ 0.30 <sup>§</sup>
HGBg count	14.60 $\pm$ 0.21	15.06 $\pm$ 0.58	13.22 $\pm$ 0.41 <sup>§</sup>
HCT (%)	44.18 $\pm$ 1.60	46.70 $\pm$ 1.33	41.42 $\pm$ 1.27
MCV (fl)	59.66 $\pm$ 1.90	59.86 $\pm$ 0.68	61.70 $\pm$ 1.11
MCH (pg)	19.64 $\pm$ 0.31	19.22 $\pm$ 0.25	19.70 $\pm$ 0.27
MCHC (g/Dl)	33.18 $\pm$ 1.18	32.18 $\pm$ 0.37	32.02 $\pm$ 0.37
PLT/UL	507.6 $\pm$ 36.01	635 $\pm$ 79.87	519.8 $\pm$ 37.54
MPV (fl)	6.56 $\pm$ 0.12	6.74 $\pm$ 0.09	6.94 $\pm$ 0.19
PCT (%)	0.32 $\pm$ 0.03	0.42 $\pm$ 0.06	0.36 $\pm$ 0.04

Values are given as mean  $\pm$  SEM (n=5). \* = significantly different from control. § = significantly different from group II ( $p < 0.05$ ). WBC = White blood cells, LYM = lymphocyte, MON = monocyte, GRAN = granulocyte, HGB = haemoglobin, MCH = mean corpuscular haemoglobin, MCHC = mean corpuscular haemoglobin concentration, HCT = haematocrit, MCV = mean corpuscular volume, MPV = mean platelet volume, PLT = platelet count, PCT = platelet crit.

hemoglobin concentration was seen in group III when compared with the control rats (Table 5). There was also a significant decrease in red blood cell count and haemoglobin concentration in this group when compared with group II.

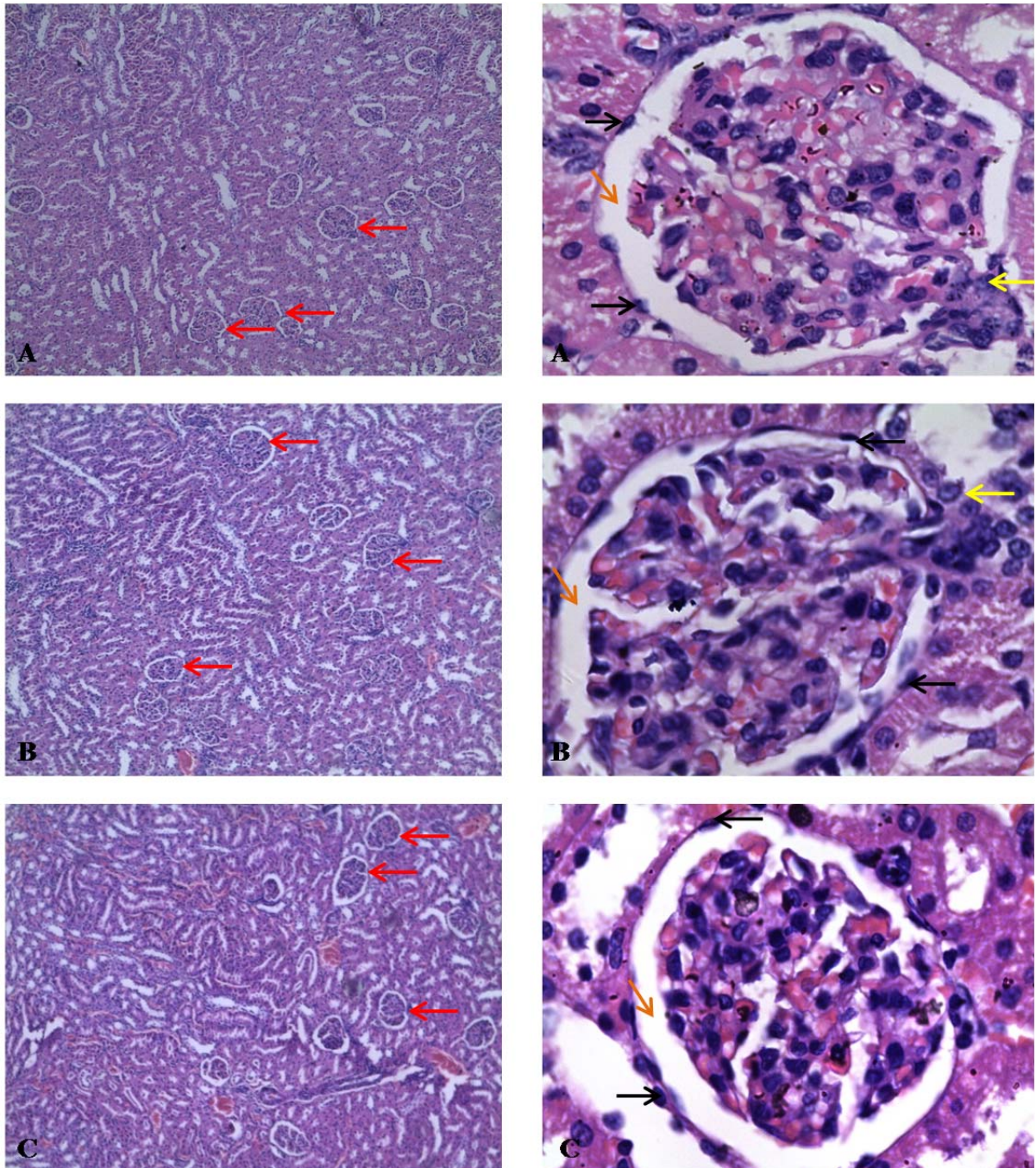
### Photomicrographs of the kidneys

Photomicrographs of the kidneys of the experimental rats show normal glomerulus with distinct and intact glomerular spaces. Macula densa and epithelial cells appear normal when compared with the control rats (Figure 6).

### DISCUSSION

This study demonstrated that oral administration of 100 and 200 mg/kg of copper sulphate for 2 weeks did not significantly alter the plasma concentration of some markers for the assessment of kidney function in the experimental rats. The most remarkable changes caused by copper sulphate were those related to food consumption and water intake. The changes were not dose dependent.

It has been suggested that one of the most consistent



**Figure 6.** Photomicrographs of the renal cortex (A; Control, B; 100 mg/kg of  $\text{CuSO}_4$ , C; 200 mg/kg of  $\text{CuSO}_4$ ) showing normal (red arrow) glomerulus. The glomerular spaces (brown arrow) are distinct and intact. Macula densa (yellow arrow) and epithelial cells (black arrow) appear normal across the groups. Magnification 100x (upper panels) and 1000x (lower panels).

clinical signs indicative of toxicity in animals administered with copper is a reduced growth rate (Haywood, 1979) which is accompanied by a fall in body weight. In the present study, a significant decrease in food consumption was observed in the experimental groups without a corresponding decrease in body weight. Water intake and

urinary volume also fell in group II when compared with the control rats. The observed increase in body weight despite the significant reduction in food consumption and water intake appeared paradoxical. However, this could have been due to an increased ability of the rats to convert the reduced food they took into body mass (Thompson et

al., 1987) or water retention which was evident as reduced urine output. This needs to be further investigated.

There are reports indicating that copper exposure is associated with renal dysfunction (Galhardi et al., 2004; Sinkovic et al., 2008). Acute renal failure due to tubular necrosis is characterized by oliguria, anuria, increased blood urea nitrogen concentration, albuminuria and hematuria (Bauer, 1975). Tubular necrosis and cellular pleomorphy were reported in rats that received supplemented diet with a copper content of 3 g/kg for up to 5 weeks (Haywood et al., 1985). In this study, the plasma concentration of urea of the experimental rats was not significantly different from the control rats. The plasma creatinine level of the treated rats rose marginally, but was not significantly different from the control rats. The urea and creatinine concentrations in the urine was reduced significantly in rats that were administered 100 mg/kg of copper sulphate compared with the control rats (Figures 2 and 4). Similar observations have been reported in animal studies by Abou-Seif et al. (2003) who found that administration of copper (II) complexes in rats caused a significant increase in superoxide dismutase activity without alteration in blood urea and creatinine levels when compared with the control rats.

Plasma urea and creatinine are the most sensitive biochemical markers used in the assessment of renal tissue damage, because urea and creatinine are excreted through the kidneys. Therefore, in cellular damage, there is retention of urea and creatinine in the blood. The decrease in creatinine clearance is an indication of tissue damage, which was supposed to have been accompanied with a significant increase in plasma concentration of creatinine. The fact that the plasma levels of urea and creatinine did not rise significantly in the experimental rats could be due to the acute nature of this study. The fall in urine creatinine is a further evidence of reduced ability of the renal tubules to extract and remove creatinine from the plasma of the experimental rats. The fall in urine excretion of urea may have resulted from diminished urea synthesis or a diminished intake of protein (Ganong et al., 2009).

The main function of red blood cells is the transportation of oxygen into tissues of the body. Any pathological condition that affects the red blood cell alters its function and this may be detrimental to the body (Agbor et al., 2005). Substances that demonstrate significant effect on red blood cell and haemoglobin would have effects on bone marrow, kidney and haemoglobin metabolism (Young and Maciejewski, 1997). A significant decrease in red blood cell and haemoglobin was observed in group III when compared with the control rats. This may have resulted from the hemolysis of red blood cells or decreased ability of the kidney to secrete erythropoietin. Erythropoietin stimulates the bone marrow to produce red blood cells. This observed change is in accordance with the finding of Savaru et al. (2007) who reported that one of the major haematological manifestations of copper sulphate poisoning is intravascular haemolysis. Glucose-6-phosphate dehydrogenase, which has a major function in maintaining

the NADPH concentration in the red blood cell, is inhibited by copper (Joshi et al., 2002). NADPH is also necessary for maintaining the level of reduced glutathione, which in turn protects the red blood cell against the haemolytic effects of oxidizing substances. The inhibition of this enzyme by copper or impaired intestinal absorption of iron (Pamila et al., 1991) could explain the reduction in haemoglobin concentration that was seen in the experimental groups. Decrease in the haemoglobin levels may impair oxygen supply to various tissues resulting in slow metabolic rate and low energy production (Ahmad et al., 1995; Atamanalp and Yanik, 2003). Intravascular hemolysis and a direct action of copper on the kidneys often lead to tubular necrosis (Iyanda et al., 2011; Matovic et al., 2010). The hem pigment released due to hemolysis and direct toxic effect of copper released from lysed red cells contributes to tubular epithelial damage of the kidney. However, the photomicrographs of the kidneys of experimental rats revealed no significant alteration in the histology of their renal tissue. This suggests that copper sulphate induced tubular necrosis could require a longer period of exposure to develop in rats.

## Conclusion

From the results of this study, it is concluded that acute copper sulphate administration to rats induced anorexia, and suppression of renal function, thereby indicating the potential toxicity of the salt if ingested for a longer period.

## Conflict of Interests

The author(s) have not declared any conflict of interests.

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