Evaluation of acute toxicity and effects of sub-acute concentrations of copper oxide nanoparticles (CuO-NPs) on hematology, selected enzymes and histopathology of liver and kidney in *Mus musculus*

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**ABSTRACT**

Industrial use of nanoparticles and their accumulation during the recent decade have created an urgent need to assess their environmental implications. The current study deals with the evaluation of acute toxicity of copper oxide nanoparticles (CuO-NPs) in the albino mice (*Mus musculus*). Lethal dose of these nanoparticles in albino mice injected via intravenous route were found to be 550 mg/kg body weight (BW). Exposure of the albino mice to sub-lethal concentrations of these nanoparticles resulted in altered hematological parameters such as a significant increase in white blood cells (WBCs), a significant decrease in red blood cells (RBCs), hemoglobin (Hb) and platelets count. NPs significantly elevated the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea and creatinine. Histopathological examination of liver and kidney showed that sub-lethal doses of CuO-NPs, in liver, led to rupture of hepatocytes, dilation of sinusoid space, hemorrhaging in hepatic tissues, and congestion of the central vein with red blood cells leading towards ultimate rupture. On the other hand, the kidney showed ruptured renal capsule, loss of urinary space, swelling in glomerulus, degeneration in podocysts, and cytoplasmic vacuolization.

**Key words:** Acute toxicity, Metal oxide nanoparticles, *Mus musculus*, Nanotechnology. Rodents.

**INTRODUCTION**

The use of nanoparticles, particularly Metal Oxide Nanoparticles (MO-NPs) has garnered much consideration and importance in the recent era owing to their variety of structures and unique properties that render them useful for numerous purposes. Different NPs are increasingly being used in material such as paints, sunscreens, ceramics, coatings, construction materials, gas sensors, luminescent oxides, rubber, UV shielding, fabrics treatment, baby powders, antistaticruff shampoos, environmental sanitation and biomedical sciences (Zhang et al., 2010). Despite the ever-growing expansion of the use of NPs in nanoindustry, there is still a lack of information and research data regarding the impact of these nanoparticles on human health and environment. Their toxic profile has not yet been properly and fully complied (Burnett and Wang, 2011).

Copper oxide nanoparticles (CuO-NPs) are one of the industrially produced commercial NPs. CuO-NPs are one of the members of group of Cu compounds and exhibit many prospective physical features, including high thermal conductivity, effects of electron correlation, and spin dynamics. These possess useful properties of producing voltage when exposed to light and properties of electrical conductivity as a result of absorbing electromagnetic radiation since CuO-NPs crystals contain a narrow band gap (Xu et al., 2012). CuO-NPs have their uses in textiles, gas sensors, photovoltaic cells, air and liquid filtration, and as anti fouling paints (Melegari et al., 2013).

CuO-NPs pose a potent threat to animals exposed to it. The primary reason of this ability is their extremely minute size, which makes them cross physiological barriers very easily and flow into the blood circulation of the host (Sampson et al., 1980). Some NPs, as small as viruses, can invade the lungs or cutaneous barriers and directly move across circulatory and lymphatic systems in living organisms; they have the potential to disrupt cellular systems and eventually cause illness (Ma et al., 2009). Moreover, NPs enter the body through ingestion, inhalation, and skin pores, reproductive and urinary tract and accumulate in vital organs such as brain, liver, or kidneys (Li et al., 2008). Research has shown NPs to change and sometimes damage cellular integrity by moving across cellular barriers to negatively interact with essential biomolecules such as DNA and protein and impart irreversible damage or pass beyond the blood-brain barrier (Afifi et al., 2016) to cause neurotoxic effects (Singh et al., 2009). Thus, it is paramount to correctly characterize their effects on mammalian models, considering the high toxicity already shown by copper ions to aquatic organisms (Khabbaz et al., 2015). The purpose of this study was to evaluate the acute toxicity of CuO in liver and kidney using the mammalian model *Mus musculus*. Mice are among the favorite animal model to be used in biological research.
due to their easy availability, small size, low cost, ease of handling, and fast reproduction rate. They are greatly regarded as valuable models of inherited human disease and share approximately 99% of their genes with humans (Begley and Ellis, 2012).

**MATERIALS AND METHODS**

**Experimental animal:** Albino mice (*Mus musculus*) were used as model animal; in total, 80 male albino mice having average age of 4 weeks and 20±5 g of body weight were used in this study. Mice were kept under standard lab conditions (temperature: 22 ± 2 °C; humidity: 60 ± 10% and light: 12 h light/dark cycle). The mice had been acclimatized for 4 weeks before the administration of CuO-NPs. Fresh pelleted feed and water was provided *ad libitum*, bedding was changed after every 3 days as described by Wang *et al.* (2006). All experiments were conducted according to the guidelines of the local animal ethics committee.

**CuO-NPs synthesis and characterization:** CuO-NPs were prepared with slight modifications of procedure described by Melegari *et al.* (2013). CuO-NPs were characterized by X-ray diffraction (XRD) while images depicting shapes and sizes of the NPs were obtained using scanning electron microscopy (SEM).

**Ecotoxicity tests:** For determination of LD50, ten different doses (350 mg/kg BW, 370 mg/kg BW, 390 mg/kg BW, 410 mg/kg BW, 430 mg/kg BW, 450 mg/kg BW, 470 mg/kg BW, 490 mg/kg BW, 510 mg/kg BW and 530 mg/kg BW) of CuO-NPs were selected and given to ten groups (each group containing 6 mice) via intravenous injections every 24 hours for 96 hours. Survival percentage was calculated for each cage at the end of the experimental time.

For sub lethal studies, two groups were made, each with ten mice (n=10). One group served as the experimental groups while the other a control. The mice of experimental group were provided with the dose half of LD50 (½ LD50) for CuO-NPs after every 24 hours for 96 hours. At the end of 96 hours period, blood was collected via cardiac puncture. One part of the blood was stored in labeled EDTA tubes for hematological studies while the other half was stored in labeled gel coated tubes for serum separation and serological analysis as described by Griffitt *et al.* (2007).

**Hematological studies:** Various hematological parameters like RBC, WBC and Platelets count was performed using improved neubauer hemocytometer at the University of Veterinary and Animal Sciences (UVAS) while hemoglobin was measured colorimetrically as described by Khabbazi *et al.* (2015).

**Serological studies:** Serum was extracted and level of alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea and creatinine levels were analyzed using Kinetic kits (Abcam) as described by Anjum *et al.* (2014).

**Histopathological examination:** Liver and kidney tissues from each of the experimental and were processed through microtomy to prepare slides for the investigation of organ damage according to the guidelines of Ma *et al.* (2009).

**Statistical analysis:** The normal distribution of data was evaluated by Kolmogorov Smirnov’s test was applied to determine the distribution of data. LD50 was calculated using probit analysis. Data are represented as mean ± S.D. One way analysis of variance (ANOVA) followed by Dunnette’s comparison tests was applied. Results with *p*<0.05 were significant. Data analysis was performed using statistical package SPSS (Version 15.0 SPSS Inc., Chicago, IL, USA).

**RESULTS AND DISCUSSION**

**Characterization of CuO-NPs:** SEM images of the synthesized CuO-NPs (Figure 1) show that the NPs have irregular morphology and their diameters ranges from 60 to 100 nm. The crystalline structures of CuO-NPs were examined by XRD showing various peaks (Figure 2).

**Ecotoxicity tests:** 96 hours LD50 value of CuO-NPs according to probit regression analysis (Table 1) was found to be 422.78 mg/kg BW.

**Hematological studies:** There was a significant difference in hematological profile of mice exposed to CuO-NPs for 96 hours (Table 2), than that of control. Statistical data indicated

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**Table 1:** Probit regression analysis and LD50 value of CuO-NPs for *M. musculus* at 96 hours post exposure

<table>
<thead>
<tr>
<th>Conc. Of CuO-NPs (mg/kg BW)</th>
<th>Number of Mice</th>
<th>Observed Response</th>
<th>Expected Response</th>
<th>LD50</th>
<th>Lower Confidence limit of LD50</th>
<th>Upper Confidence limit of LD50</th>
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<tr>
<td>530</td>
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<td>550</td>
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<td>6</td>
<td>4.694</td>
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**Table 2:** Hematological studies of mice exposed to CuO-NPs for 96 hours

<table>
<thead>
<tr>
<th>Conc. Of CuO-NPs (mg/kg BW)</th>
<th>Number of Mice</th>
<th>Observed Response</th>
<th>Expected Response</th>
<th>LD50</th>
<th>Lower Confidence limit of LD50</th>
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that WBCs count increased (p<0.05) while RBCs, Platelets and Hb count decreased significantly.

**Serological studies:** Hepatic and renal serological profile of mice exposed to CuO-NPs for 96 hours (Table 3) showed changes (p<0.05). Furthermore, statistical analysis showed that there was a significant increase in serological parameters.

**Histopathological studies:** Histopathological analysis revealed that CuO-NPs were considerably toxic to the liver and kidney tissues of the albino mice. Both organs showed the signs of tissue degradation. Even the sub-lethal doses of CuO-NPs, in liver, led to rupture of hepatocytes, dilation of sinusoid space, hemorrhaging in hepatic tissues, and congestion of the central vein with red blood cells leading towards ultimate rupture (Figure 3a). On the other hand, the kidney showed ruptured renal capsule, loss of urinary space, swelling in glomerulus, degeneration in podocytes, and cytoplasmic vacuolization (Figure 3b).

The present study indicated that acute toxicity of CuO-NPs altered normal hematological and serological profile of *M. musculus*. These findings are in agreement with the previously reported study of Griffitt et al. (2007) in zebra fish. Similar observations were reported in juvenile rainbow trout exposed to 100 µg/l CuO-NPs treatments. The reported hematological parameters are in accordance with Khabbazi et al. (2015) who observed a significant increase in the WBCs of the group treated with CuO-NPs while, RBCs, Hb and platelets count decreased significantly. The increase in WBCs count. It might be due to the oxidative stress of these nanoparticles which altered the hematopoietic pathways by an unknown mechanism (Ayaz et al., 2016) or abnormally high levels of Cu which result from CuO-NPs intake may cause a Fe-deficient status which ultimately leads to anemia (Zhang et al., 2010).

The results of liver and kidney profile enzymes clearly depict that CuO-NPs are toxic for both liver and kidneys. Similar findings were reported by Chen et al. (2006), stating that kidney and liver to be among the primary target organs of CuO-NPs. Small-sized CuO-NPs with ultrahigh reactivity interacted with and damaged the liver and kidney histology and altered the renal and hepatic serum enzyme profile. The parameters of renal and hepatic serological profile are used by biologists to predict and/or observe the exogenous response of the toxic exposure of material to kidney and liver. Serum ALT and AST concentrations are the key hepatic enzymes to evaluate liver damage while creatinine and urea depicts the functional efficacy of kidney (Sangha and Kaur 2011). The extent of degeneration in the liver of CuO-NPs-exposed mice (Figure 2a) was serious and might possibly alter the hepatic functions. Congestion of central hepatic vein and hemorrhage in hepatic tissues were also reported by Abdelhalim (2011) when rats were exposed to Au-NPs and TiO2-NPs (Ma et al., 2009). Nanoparticles get accumulated in the liver and alter the normal homeostasis of minerals resulting in swelling and rupturing of hepatocytes. This alteration in sinusoids is brought about by increasing of sinusoidal spaces related to cell death and abnormal hepatocytes. A liver sinusoid is a

### Table 2: Changes in the hematological parameters of *M. musculus* exposed to the dose half of LD50 of CuO-NPs

<table>
<thead>
<tr>
<th>Hematological Parameters</th>
<th>Groups</th>
<th>Number of mice</th>
<th>Mean</th>
<th>SD</th>
<th>SEM</th>
<th>T value</th>
<th>P value</th>
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</thead>
<tbody>
<tr>
<td>WBCs×10³/µl</td>
<td>Control</td>
<td>6</td>
<td>4.16</td>
<td>0.49</td>
<td>0.20</td>
<td>-6.364</td>
<td>0.001</td>
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<tr>
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<td>Experimental</td>
<td>6</td>
<td>6.27</td>
<td>0.64</td>
<td>0.26</td>
<td>-6.364</td>
<td>0.001</td>
</tr>
<tr>
<td>RBCs×10⁶/µl</td>
<td>Control</td>
<td>6</td>
<td>6.66</td>
<td>0.47</td>
<td>0.19</td>
<td>7.055</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>6</td>
<td>5.10</td>
<td>0.28</td>
<td>0.11</td>
<td>7.055</td>
<td>0.003</td>
</tr>
<tr>
<td>Platelets×10³/µl</td>
<td>Control</td>
<td>6</td>
<td>152.49</td>
<td>7.42</td>
<td>3.03</td>
<td>5.603</td>
<td>0.001</td>
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<tr>
<td></td>
<td>Experimental</td>
<td>6</td>
<td>129.07</td>
<td>7.04</td>
<td>2.88</td>
<td>5.603</td>
<td>0.001</td>
</tr>
<tr>
<td>Hb g/dl</td>
<td>Control</td>
<td>6</td>
<td>8.99</td>
<td>0.75</td>
<td>0.31</td>
<td>5.828</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>6</td>
<td>7.06</td>
<td>0.31</td>
<td>0.13</td>
<td>5.828</td>
<td>0.002</td>
</tr>
</tbody>
</table>

### Table 3: Changes in the serological parameters of *M. musculus* exposed to the dose half of LD50 of CuO-NPs.

<table>
<thead>
<tr>
<th>Serological Parameters</th>
<th>Groups</th>
<th>No. of mice</th>
<th>Mean</th>
<th>SD</th>
<th>SEM</th>
<th>T value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (U/l)</td>
<td>Control</td>
<td>6</td>
<td>56.83</td>
<td>6.27</td>
<td>2.56</td>
<td>-10.108</td>
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<tr>
<td></td>
<td>Experimental</td>
<td>6</td>
<td>105.67</td>
<td>10.03</td>
<td>4.09</td>
<td>-10.108</td>
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<tr>
<td>AST (U/l)</td>
<td>Control</td>
<td>6</td>
<td>156.12</td>
<td>12.84</td>
<td>5.24</td>
<td>-16.714</td>
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<tr>
<td></td>
<td>Experimental</td>
<td>6</td>
<td>306.00</td>
<td>17.81</td>
<td>7.27</td>
<td>-16.714</td>
<td>0.001</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>Control</td>
<td>6</td>
<td>21.33</td>
<td>4.18</td>
<td>1.71</td>
<td>-6.745</td>
<td>0.001</td>
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<tr>
<td></td>
<td>Experimental</td>
<td>6</td>
<td>41.67</td>
<td>6.01</td>
<td>2.49</td>
<td>-6.745</td>
<td>0.001</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>Control</td>
<td>6</td>
<td>0.33</td>
<td>0.91</td>
<td>0.37</td>
<td>-8.720</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>6</td>
<td>0.93</td>
<td>0.14</td>
<td>0.58</td>
<td>-8.720</td>
<td>0.002</td>
</tr>
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</table>

The parameters of renal and hepatic serological profile are used by biologists to predict and/or observe the exogenous response of the toxic exposure of material to kidney and liver. Serum ALT and AST concentrations are clearly depict that CuO-NPs are toxic for both liver and kidneys. Similar findings were reported by Chen et al. (2006), stating that kidney and liver to be among the primary target organs of CuO-NPs. Small-sized CuO-NPs with ultrahigh reactivity interacted with and damaged the liver and kidney histology and altered the renal and hepatic serum enzyme profile. The extent of degeneration in the liver of CuO-NPs-exposed mice (Figure 2a) was serious and might possibly alter the hepatic functions. Congestion of central hepatic vein and hemorrhage in hepatic tissues were also reported by Abdelhalim (2011) when rats were exposed to Au-NPs and TiO2-NPs (Ma et al., 2009). Nanoparticles get accumulated in the liver and alter the normal homeostasis of minerals resulting in swelling and rupturing of hepatocytes. This alteration in sinusoids is brought about by increasing of sinusoidal spaces related to cell death and abnormal hepatocytes. A liver sinusoid is a
Figure 1: Image depicting shape and size of the CuO-NPs were obtained using scanning electron microscopy (SEM)

Figure 2: Characterization of CuO-NPs by X-ray diffraction (XRD)
Figure 3a: Liver section of albino mice; control (A) and experimental (B-D) at 1000 X. The figure shows a clear difference in the liver histopathology of controlled and experimental mice. Normal central vein (CV), Hepatocytes (H) and Sinusoid (S) can be seen in the control (A), while congested central vein (cCV), ruptured hepatocytes (rH), dilated sinusoid (dS) and hemorrhaging in hepatic tissues (hH) is observed in the experimental groups (B-D).

The extent of degeneration in the kidney of CuO-NPs-exposed mice (Figure 2b) was serious and might possibly alter the renal functions. Ruptured and degenerated podocytes, due to oxidative stress, were clearly visible in the kidneys. Podocytes have a vital role to play in filtration process. Ruptured podocytes are unable to remove waste products and pose additional burden on kidney and cause further cell damage. These results are similar to those already reported in the in vitro assessment of toxicity of CuO-NPs on human podocytes (Xu et al., 2012) and TiO$_2$-NPs in mice liver (Wang et al., 2009). Swollen glomerulus, observed in experimental mice, was incapable to filter the toxic substances resulting in the accumulation of harmful products in the vicinity. Wang et al. (2009) reported the same findings and reported that toxicity of TiO$_2$-NPs resulted severe swelling of renal glomerulus in adult mice. Renal corpuscles were observed ruptured resulting in impairment of filtration by kidneys. Similar results were seen when rats were exposed to Ag-NPs (Tang et al., 2009). Vacuolization was visible in the kidney tissues of experimental animals. These changes were a direct result of imbalance and disturbances in homeostasis of kidney cells. These abnormal levels of water and ions caused vacuole formation in kidney (Ma et al. 2009).
Figure 3b: Kidney section of albino mice; control (A) and experimental (B-D) at 1000 X. The figure shows a clear difference in the kidney histopathology of controlled and experimental mice. Normal podocysts (P), glomerulus (G) and renal capsule (RC) can be seen in the control (A) while ruptured renal corpuscle (rRC), ruptured podocysts (rP), degenerated podocysts (dP), swollen glomerulus (sG) and vacuolization (V) is observed in the experimental groups (B-D).

CONCLUSION
CuO-NPs are extremely toxic and alter the normal hematological and serological parameters. Histopathological findings indicate that CuO-NPs degraded the liver and kidneys tissues; however, liver had undergone a more severe damage in comparison to kidney. In the view of range of applications of these NPs in industries and their bulk release from natural and anthropogenic sources, further advanced ecotoxicological studies of these nanoparticles are of paramount importance.

REFERENCES


