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Exploring vivo toxicity assessment of copper oxide nanoparticle in Wistar rats

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ABSTRACT

This study aims to suggest the limits of copper oxide nanoparticle uses for medicinal purposes and was performed to investigate the effect of different doses of copper oxide nanoparticle in rats. A total of 32 healthy male Wistar rats of the same age (12 weeks old) weighing 250-280 g were used. Animals were randomly divided into groups, two GNP-treated rat groups and one control group. The 100 of 200 and 400-ppm copper oxide nanoparticles was intra-peritoneally administered in rats for exposure duration of 15 days. Then, several biochemical parameters such as aspartate aminotransferase, alanine transaminase, alkaline phosphatase, creatinine, fast blood sugar, albumin, blood urea nitrogen, total protein were evaluated. Significant changes were observed ($p < 0.05$) in biochemical parameters in the 400-ppm groups. The changes were non-significant in the other groups (200,100-ppm group). In the 40 ppm group, a significant increase was also found in liver function enzymes like ALT and AST ($p < 0.05$), ALP ($p < 0.05$), creatinine, albumin, total protein, blood urea nitrogen, fast blood sugar ($p < 0.05$). This study indicates that the CuO nanoparticles in doses (<400ppm) is safe for biomedical application and has no side effects, but its high dose (≥ 400 ppm) is toxic.

Key words: Albumin, blood urea nitrogen, copper oxide nanoparticles, kidney factors

1. INTRODUCTION

Nanotechnology is one of the most rapidly growing science with a wide range of applications, especially in medicine, information technology, sensor development, catalysis, and biological sciences. Copper oxide (CuO) is used in wood preservation and antimicrobial textiles. Other studies have shown that nanoparticles (NP) of some metal oxides can be toxic to human cells. Most studies that investigated the metal oxide NP toxicity have focused on mammalian cells, primarily on transformed cancer cell lines; however, NPs effects o Besides, the diameter of NPs may affect the level of their arrival into cells and thus their toxicity. Recently, the increased toxicity of nanoparticles due to their tiny physical dimensions has been widely recognized. The wide scale use of copper oxide nanoparticles (CuONPs) due to their unique properties and important applications in magnetic, thermal, electrical, sensor devices and cosmetics makes human beings more prone to the exposure of CuONPs and its potential adverse effects. Exposure to such nanoparticles is mainly through skin and inhalation. However CuoNPs were found to be highly toxic compared to other methal oxide nano-materials due to their small size, Nps may cross biological barriers to reach different organs and according to their size and surface properties, accumulation of metal NPs was previously observed in all the different organs *in vivo*. In general, most studies have excluded the toxicity of CuNPs (1-2 nm in diameter) via direct association with DNA strands. There are few toxicological reports of copper oxide nanoparticles in animal model, which is the preferred system for toxicological evaluation of a novel agent and should be used to characterize the toxicity of copper oxide nanoparticles. This investigation was therefore aimed to study the effect of copper nanoparticles (50nm) on kidney and liver markers in rat.

2. MATERIALS AND METHODS

32 adult male rats of Wistar strain, weighing 250-280g were used for this study. They were housed individually in stainless steel mesh-bottomed cages and were acclimatized before the starting of the experiments at suitable conditions of temperature and light for a period of four weeks. The environmental conditions were set at a temperature of 22-24^0 c relative humidity of 55± 5% and a 12 h light/dark cycle. Institutional Animal Ethical Clearance (IAEC) carried out this study according to the guidelines approval. The rats were randomly assigned to four groups that in each group were 8 animals. The first (control) group received 0.5cc saline and the second one received 0.5cc copper Oxide Nanoparticles at 400-ppm concentration and the third one received 0.5cc copper Oxide Nanoparticles at 200-ppm concentration and the fourth group received 0.5cc copper oxide nanoparticles at 100ppm concentration for 15 days interaperitoneally. The rats did not show any symptoms of toxicity such as modification in fur color, weight loss and any symptoms in the morphology and behavior. Once finished the animals were anesthetized with ether and killed by utnzy. The amount of 8 ml of their blood was collected approximately by cardiac puncture into Lithium Heparin bottles. The blood sample were centrifuged at 3000xg for 15 minutes in order to measure the concentration of ALP, AST, ALT and the Cr, FBS, ALB, TP,BUN factors were given to the Spectrophotometer and the biochemical kit. All data were stored in SPSS for operating system of Microsoft .Co (version 19). Group comparisons were done using the analysis of variance (ANOVA) test. Dunnett assessed significant differences between them. All data were expressed as mean ± standard error of mean (SEM). P-values less than 0.05 were considered significant.

2.1. Copper oxide nanoparticles synthesis procedure

In order to synthesize CuO nanoparticles with Iron impurity by Sol-Gel method, deionized water as well as Ethanol (C_2H_5OH, Merck, >99.9%) in molar ratio of 1:1 as solvent, Copper nitrate [$Cu(NO_3)_2.3H_2O$] and Ferric nitrate [$Fe(NO_3)_2.6H_2O$] as initiators, Citric acid and Ethylene glycol as the factors to generate complex and polymer, respectively. The solution was stirred with magnetic stirrer at room temperature for 1 hour. The color of solution is blue without supplementation of Iron nitrate; as the Iron nitrate is added gradually a green clear solution is obtained. Oil bath was utilized for indirect and homogeneous heating. After refluxing in thermal range for 90-110˚C for about 4 hours a homogenous solution was acquired and after direct heating at 120˚C for 7 hours with the evaporation of additives a dried gel was made from green gel against IR lamp which the final powder containing nanoparticles generated after grinding operation following to placing it inside the oven at 160˚C, eventually.

2.2. Preparation of nanoparticles suspension

In order to prepare nanoparticles stock solution, 10g of nanoparticles were transformed to suspension in 1 Litre sterilized medium for their appropriate dispersion, the Ultrasonic device (PARSONIC 7500s, Pars Nahand ENGG.Co.IRAN) was used for 30 minutes. Nanoparticles suspension was provided to prevent error.

3. RESULTS AND DISCUSSION

3.1. X-Ray diffraction of CuO nanoparticles

The XRD pattern Fig. 2 for CuO nanoparticles, the diffraction peaks are absorbed at 2θ values. The prominent peaks have been utilized to estimate the grain size of sample with the help of Scherrer equation $\boldsymbol{D = K\lambda/(\beta \cos \theta)}$ where K is constant(0.9), λ is the wavelength(λ = 1.5418 A°) (Cu Kα), β is the full width at the half-maximum of the line and θ is the diffraction angle. The grain size estimated using the relative intensity peak for CuO nanoparticles was found to be 50 nm and increase in sharpness of XRD peaks indicates that particles are in crystalline nature. All different peaks in Figure 1 are related to CuO nanoparticles and are matched to Joint Committee for Powder Diffraction Studies.

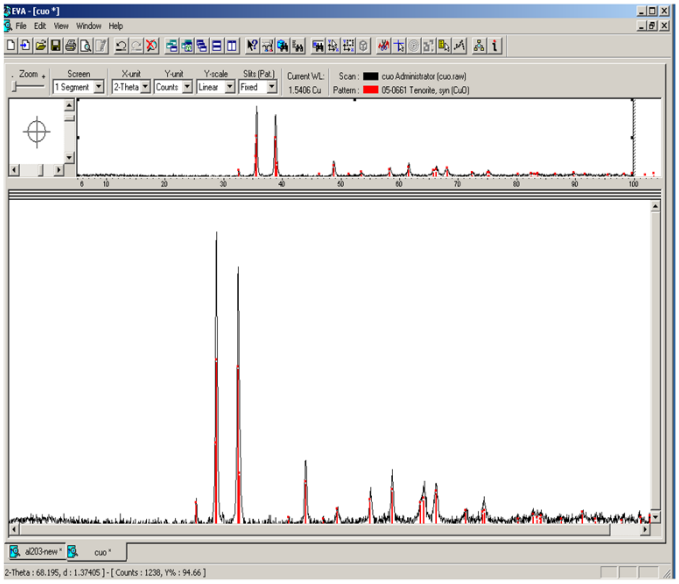

Figure 1. XRD pattern for CUO nanoparticles

3.2. Effect of CuO NPs over the Kidney factors

The effects of different concentrations of copper oxide nanoparticles on the factors were studied for 15 days and level serum changes were shown in experimental groups compared to control groups. These changes included kidney factor effect, liver enzymes. 400-ppm copper oxide nanoparticles consumption was leaded to significantly ($p<0.05$) rise FBS level (Table 1) in comparison with normal group. Non-significant difference was observed in these enzymes level between fed groups with 200 and 100-ppm copper oxide nanoparticles with normal group.

Table 1. Serum concentrations of FBS in experimental groups were treated by different doses of copper oxide nanoparticles. Table show mean values (± SD, n=8; P < 0.05). *: Significant difference with other groups

FBS	N	Mean	Std. Deviation	Std. Error	P value
control	8	88.7500	22.32071	7.89156	
100ppm	8	96.2500	23.26094	8.22398	
200ppm	8	111.2500	11.25992	3.98098	
400ppm	8	163.1250	24.91951	8.81038	P=0.00***

As shown in the Table 2 creatinine level significantly ($p<0.05$) enhanced with used high concentration of copper oxide nanoparticles (400ppm) as compared to normal group. Using two, other concentration of copper oxide nanoparticles (100 and 200ppm) had no significant effect on creatinine level in comparison with normal group.

Table 2. Serum concentrations of Cu in experimental groups were treated by different doses of copper oxide nanoparticles. Table show mean values (± SD, n=8; P < 0.05). *: Significant difference with other groups

Creatinine	N	Mean	Std. Deviation	Std. Error	P value
control	8	0.7250	0.14880	0.05261	
100ppm	8	0.7875	0.12464	0.04407	
200ppm	8	0.8125	0.14577	0.05154	
400ppm	8	0.9375	0.05175	0.01830	P=0.005**

As shown in the Table 3 total protein level significantly ($p<0.05$) enhanced with used of high concentration of copper oxide nanoparticles (400ppm) as compared to normal group. Using two, other concentration of copper oxide nanoparticles (100 and 200ppm) had no significant effect on TP level in comparison with normal group.

Table 3. Serum concentrations of TP in experimental groups were treated by different doses of copper oxide nanoparticles. Table show mean values (± SD, n=8; P < 0.05). *: Significant difference with other groups

TP	N	Mean	Std. Deviation	Std. Error	P value
control	8	6.6000	0.72703	0.25704	
100ppm	8	6.8375	0.79989	0.28280	
200ppm	8	7.0000	0.72506	0.25635	
400ppm	8	7.6250	0.74402	0.26305	P=.028*

As shown in the Table 4 albumin level significantly ($p<0.05$) enhanced with use of high concentration of copper oxide nanoparticles (400ppm) as compared to normal group. Using two, other concentration of copper oxide nanoparticles (100 and 200ppm) had no significant effect on alb level in comparison with normal group.

Table 4. Serum concentrations of alb in experimental groups were treated by different doses of copper oxide nanoparticles. Table show mean values (± SD, n=8; P < 0.05). *: Significant difference with other groups.

alb	N	Mean	Std. Deviation	Std. Error	P value
control	8	4.1375	0.34615	0.12238	
100ppm	8	4.3250	0.34538	0.12211	
200ppm	8	4.5000	0.41057	0.14516	
400ppm	8	4.9125	0.56426	0.19950	P=.003**

As shown in the Table 5 blood urea nitrogen level significantly ($p<0.05$) enhanced with use of high concentration of copper oxide nanoparticles (400ppm) as compared to normal group. Using two, other concentration of copper oxide nanoparticles (100 and 200ppm) had no significant effect on BUN level in comparison with normal group.

Table 5. Serum concentrations of BUN in experimental groups were treated by different doses of copper oxide nanoparticles. Table show mean values (± SD, n=8; P < 0.05). *: Significant difference with other groups

BUN	N	Mean	Std. Deviation	Std. Error	P value
control	8	16.7500	3.37004	1.19149	
100ppm	8	17.3750	2.92465	1.03402	
200ppm	8	18.1250	2.90012	1.02535	
400ppm	8	20.7500	3.37004	1.19149	P=.044*

3.3. Effect of CuO NPs over the liver enzymes

The results in Table 6 showed that activity of ALT enzyme increased in all groups. This increase compare to the control group in fourth group that received 400ppm nanoparticles is significant from the statistical point($p<0.05$).

Table 6. Serum concentrations of ALT in experimental groups were treated by different doses of copper oxide nanoparticles. Table show mean values (± SD, n=8; P < 0.05). *: Significant difference with other groups

ALT	N	Mean	Std. Deviation	Std. Error	P value
control	8	60.0000	17.32051	6.12372	
100ppm	8	61.0000	18.05547	6.38357	
200ppm	8	62.8750	17.42279	6.15989	
400ppm	8	86.2500	12.74755	4.50694	P=.010**

The results in Table 7 showed that activity of ALP enzyme increased in all groups. This increase compares to the control group in fourth group that received 400ppm nanoparticles is significant from the statistical point($p<0.05$).

Table 7. Serum concentrations of ALP in experimental groups were treated by different doses of copper oxide nanoparticles. Table show mean values (± SD, n=8; P < 0.05). *: Significant difference with other groups

ALP	N	Mean	Std. Deviation	Std. Error	P value

control	8	39.3750	10.50085	3.71261	
100ppm	8	40.8750	10.66955	3.77226	
200ppm	8	41.6250	10.92752	3.86346	
400ppm	8	52.2500	8.68085	3.06914	P=.046*

The results in Table 8 showed that activity of AST enzyme increased in all groups. This increase compares to the control group in fourth group that received 400-ppm nanoparticles is significant from the statistical point($p<0.05$).

Table 8. Serum concentrations of AST in experimental groups were treated by different doses of copper oxide nanoparticles. Table show mean values (± SD, n=8; P < 0.05). *: Significant difference with other groups

AST	N	Mean	Std. Deviation	Std. Error	P value
control	8	174.2500	94.72630	33.49080	
100ppm	8	203.2500	40.20572	14.21487	
200ppm	8	208.7500	39.79860	14.07093	
400ppm	8	274.2500	42.31092	14.95917	P=.006**

When the liver is injured these enzymes are spilled in to the blood stream. ALT, AST and ALP are in liver cells in normal conditions when the cell is damaged and they go into the serum. In study was done by Abdelhalim MA demonstrates that the increase in the enzymes AST and the decrease in ALP are smaller gold nanoparticles (10 nm) size-dependent while the decrease in the enzymes ALT are bigger GNPs (50 nm) size-dependent. The levels of creatinine values indicated no significant changes with the administration of 10 and 50 nm GNPs for exposure compared with the control. The administration of 10 and 50 nm GNPs for short exposure duration of 3 days induced only significant variations with some liver enzymes while kidney showed no significant variations. In a study done by Tiwari DK silver nanoparticle (AgNP) uses for medicinal purpose was performed to explore the effect of various doses of silver nanoparticle in rats. Four different doses of AgNP (4, 10, 20, and 40 mg/kg) were injected intravenously. A significant increase was also found in liver function enzymes like ALT and AST, ALP. Like viruses, some nanoparticles can penetrate lung or dermal (skin) barriers and enter the circulatory and lymphatic systems of humans and animals, reaching most bodily tissues and organs, and potentially disrupting cellular processes and causing disease. Researches made by Gholam Ali Kojouri *et al*. suggest that in using selenium nanoparticles the nanoparticles exhibit increases in cr levels and BUN after the treatment and the results we obtain were alike with the results of this study in applying copper oxide nanoparticles. Researches made by Shailendra Giri *et al.* in using Cerium nanoparticles showed that nanoparticles exhibit increases in level cr and BUN after the treatment but it led to the decrease in the alb serum level so, we obtained similar results in this study in applying copper oxide nanoparticles but in regard with my results, the level of alb serum shows an increase. Researches made by Sangiliy *et al*. in using AuNPs showed that AuNPs expose caused an increase in FBS in male mice. This study showed the toxicity of AuNPs in kidney tissue, kidney section shows normal glomerular tubes and renal cortex and gold treatment kidney show no pathological changes in the animals as well as the increase of creatinine in their serum.

4. CONCLUSION

This study indicates that the CuO nanoparticles in doses (<400ppm) is safe for biomedical application and has no side effects, but its high dose (≥ 400 ppm) is toxic and the use of CuONPs in different doses do not show identical results for all states.

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AUTHORS CONTRIBUTION

This work was carried out in collaboration between all authors.

CONFLICT OF INTEREST

The authors declared no potential conflicts of interests with respect to the authorship and/or publication of this article.

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