

Original Research

Acute effect of nano-copper on liver tissue and function in rat

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Abstract

Objective(s): This paper reports on the toxicity of CuO NPs on hepatic enzymes and liver and lung histology.

Materials and Methods: To assess the toxicity of copper nanoparticles (10-15 nm) in vivo, pathological examinations and blood biochemical indexes including serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) at various time points (2, 7 and 14 days) were studied. Thirty two Wistar rats were randomly divided into four groups. Treatment groups (group 1, 2, 3) received CuO NP solution containing 5, 10 and 100 mg/kg, respectively. Control group received 0.5 mL of normal saline via ip injection for 7 consecutive days. After 14 days, the tissue of liver and lung were collected and investigated for their histological problems.

Results: The histology of the hepatic tissues showed vasculature in central veins and portal triad vessels in all three treatment groups. Histology of lungs showed air sac wall thickening and increased fibrous tissue in all three groups. Biochemical results of the hepatic enzymes showed that the SGOT levels in groups 1 and 2 were significantly higher than the control group two days after the intervention.

Conclusion: Results of this study indicated that all concentration of copper nanoparticles [with 10-15 nm diameters, spherical shape, purity of 99.9%, mineral in nature, and wet synthesis method in liquid phase (alternation)] induce toxicity and changes of histopathological changes in liver and lung tissues of rats. It is evident that these nanoparticles cannot be used for human purposes because of their toxicity.

Keywords: Copper nanoparticles, Fibrosis, Liver enzymes (Hepatic)

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Introduction

In recent years, the use of nanoparticles in human activities has increased. Therefore, study of the biological effects of different nanoparticles and nanocomposite materials, specially their effects on human and animal organs need special attention. The primary concern is the toxicity of nanoparticles in humans and the potential risk of nanoparticles and their related products on human health. Copper nanoparticles are widely synthesized and used as metal catalyst, heat transfer fluids in machine tools, semiconductors, and even in antibacterial medications (1, 2). Copper nanoparticles have antibacterial activity in vitro (3). Copper nanoparticles are one of the first engineered nanoparticles which are used in industrial applications; thus, concerns about engineered nanoparticles being released into the environment and the consequences of their various effects on human health have increased. It is indicated that copper nanoparticles are dispersed in animal tissues and organs, causing particular structural changes. Increase of copper nanoparticles dose to level around its toxicity threshold has led to dystrophy or tissue necrosis. Kim et al. investigated the pulmonary effects of inhaled copper nanoparticles in mice in 2011 (4). The results showed that copper nanoparticles produce a stronger inflammatory response, increase in total cell recruitment and neutrophils for the lungs, and increased LDH activity in the bronchus compared to iron oxide, titanium dioxide, and silver (4). Cytotoxicity and DNA damage were also reported in A549 type II lung epithelial cells from all metal oxide particles tested (CuO, TiO₂, ZnO, Fe₂O₃, and Fe₃O₄) at dose of 40 and 80 µg (5). One of the most discussed mechanisms, in addition to the health effects induced by metal oxide nanoparticles, is their ability to cause oxidative stress (6-9). Researchers believe that this performance is the most important mechanism of the toxicity caused by nanoparticles. It was

recently reported that copper nanoparticles, compared to other metal oxide nanoparticles, are highly toxic in vitro conditions (10, 11). However, our data on in vivo toxicity is still not enough. Reason of the difference in distribution, penetration, and tissue damage of nanoparticles in different studies might be as a result of different synthesis methods, leading to different size, shape and other physical and chemical properties of nanoparticles. Therefore, the interaction and impact of nanoparticles on animal cells and tissues will vary (12). In the present study, the effects of intraperitoneal injection of different doses (10, 100, and 300 mg/kg) of copper nanoparticles with 10-15 nm diameters, spherical shape, purity of 99.9%, mineral in nature, and wet synthesis method in liquid phase on liver, lungs, and hepatic enzyme functions on rats were studied.

Materials and Methods

CCuo particle specifications

Colloidal copper nanoparticles were provided by Tehran Neutrino Company. It had a 10-15 nm diameter, with spherical shape and purity of 99.9%, mineral in nature, and wet synthesis method in liquid phase. TEM electron microscope image of copper nanoparticles is shown below (Figure 1).

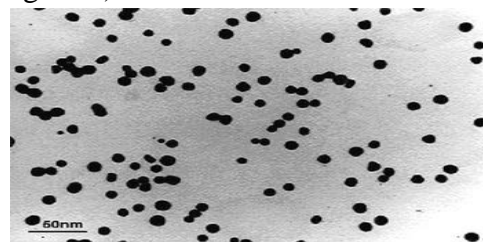


Figure 1. TEM of nanoparticles, 10-15 nm diameter, spherical shape, 99.9% degree of purity, mineral nature, and wet synthesis method in the liquid phase (alteration).

Animal treatment

Twenty two male Wistar rat (purchased from the Animal Center of Shahrekord University), weighing 225± 25 g were used in the experiments. They were acclimated in the controlled environment

(temperature: 22 ± 1 °C; humidity: $60\pm 10\%$ and light: 12 h light/dark cycle) with free access to water and a food. All animal experiments were performed in compliance with the local ethics committee.

Animals were randomly divided into groups, three copper nanoparticle-treated groups and one control group. Group 1, 2 and 3 received 0.5 ml of solution containing 5, 10, 100 mg/kg CuO nanoparticle via ip injection for 7 successive days, respectively. The control group received 0.5 ml of normal saline with the same procedure.

Evaluation of biochemical parameters

Several biochemical parameters such as serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) were evaluated at various time points (2, 7 and 14 days). After 14 days, the tissue of liver and lung were collected and investigated.

Statistical analysis

All data were analyzed by using the statistical package for social sciences (SPSS v.19) software and were summarized and expressed as mean ($\text{mean}\pm\text{S.E}$). Statistical analysis of data was performed by Multivariate Analyses of Variance models, in which serum values at 2, 7 and 14 day after intervention were used as dependent variables.

Using Wilk's lambda or Roy largest Root for total, cumulative differences between groups, p-value of each dependent variable were reported in last row of tables and the Tukey was used for paired comparisons. p-values less than 0.05 were considered significant.

Results

A. Histology of liver

The histological photomicrographs of the liver sections are shown in Figures 2, 3, and 4, respectively.

No histopathological sign was observed in the livers of control animals (Figure 5).

B. Histology of lung

The histological photomicrographs of the lung sections are shown in Figures 6, 7, and 8, respectively.

No histo-pathological sign was observed in the lungs of control animals (Figure 9).

Biochemical results

According to MANOVA model and test statistics of $P = 0.04$, and Roy largest root = 0.432 mean, SGOT levels were different between groups. Based on Dunnett's posttest, SGOT levels two days after intervention in group receiving 10 mg/kg ($p = 0.026$) and 100 mg/kg ($p = 0.041$) were significantly higher than the control group.

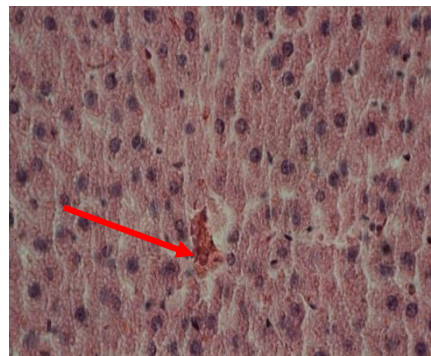


Figure 2. Treatment group 1 receiving 10 mg/kg CuO nanoparticles: Except hyperemia in the central venous, other pathological changes were not seen ($\times 40$).

the day 14 of the intervention between nanoparticles of Cu 300 mg/kg and Cu 10 mg/kg a significant difference was observed ($p = 0.016$) (figure 10).

14 days after the intervention a significant difference in SGPT levels between groups receiving 300 mg/kg and 10 mg/kg of CuO nanoparticles ($p=0.016$) (Figure 10).

Based on Tukey's HSD posttest, mean SGPT enzyme level in group receiving 10 mg/kg of CuO nanoparticles was significantly higher than the one receiving 100 mg/kg ($p=0.035$) two days after the intervention (Figure 11).

Effect of nano-copper on function and tissue liver of rat

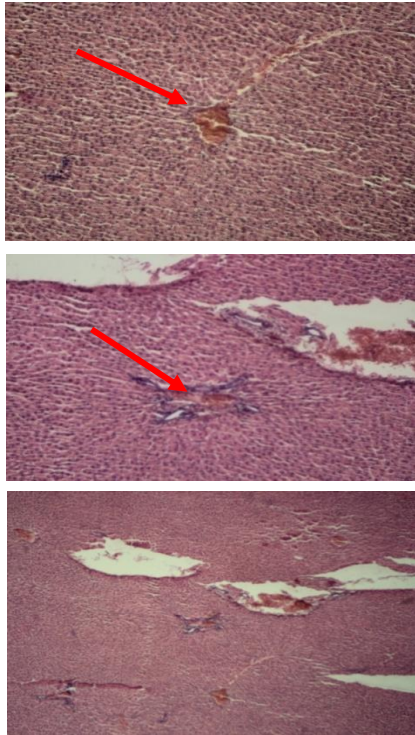


Figure 3. Treatment group 2 receiving 100 mg/kg CuO nanoparticles: Vasculature in the central venous and portal triad vessels and the disappearance of hexagonal liver lobules are observed (other parts were normal) ($\times 40$).

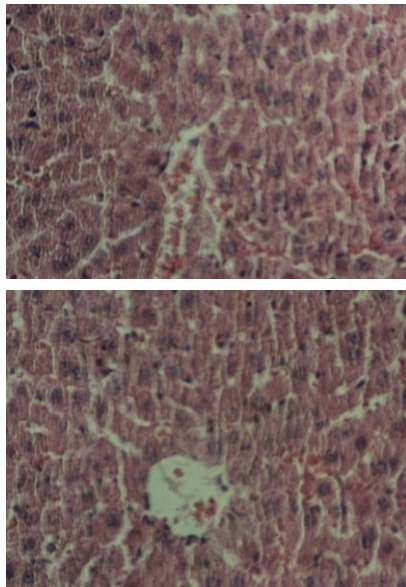


Figure 4. Treatment group 3 receiving 300 mg/kg CuO nanoparticles: Except hyperemia in some central veins, other pathological changes were not seen ($\times 40$).

Discussion

In this study, the toxicity of copper nanoparticles in vivo was investigated by

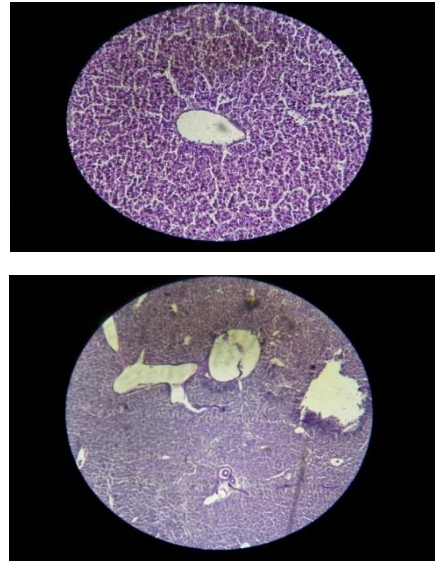


Figure 5. Light micrographs of sections in the liver of control group ($\times 40$).

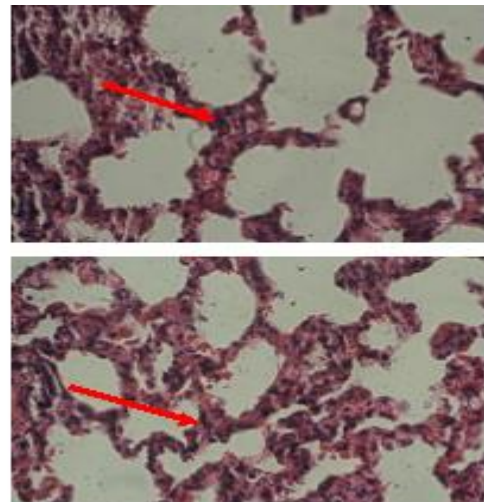


Figure 6. Treatment group 1 receiving 10 mg/kg CuO nanoparticles: Increase in the fibrous tissue between the air sacs, and vascular hyperemia are the only notable points. Other parts were normal ($\times 40$).

evaluating the histological changes and hepatic enzymes levels. The histology of the hepatic tissues showed vasculature in central veins and portal triad vessels, and the disappearance of hexagonal liver lobules in all three treatment groups receiving different doses of CuO nanoparticles.

Results of biochemical analysis on the hepatic enzymes showed that the mean SGOT enzymes, two days after intervention, in the group receiving 10 mg/kg and 100 mg/kg CuO nanoparticles

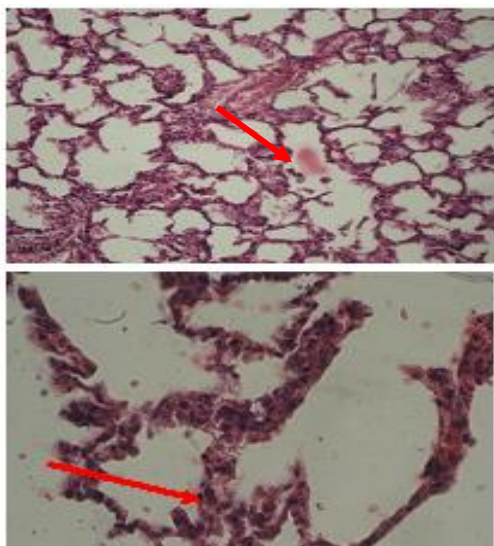


Figure 7. Treatment group 2 receiving 100 mg/kg CuO nanoparticles: Air sac wall thickening and increased fibrous tissues are the only notable points. Other parts were normal. (× 40).

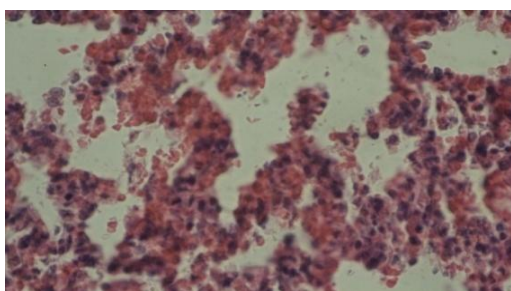


Figure 8. Treatment group 3, 300 mg/kg: Air sac wall thickening and increased fibrous tissues are the only notable points. Other parts were normal. (× 40).

The histology of the lungs also showed air sac wall thickening and increased fibrous tissue in all three groups.

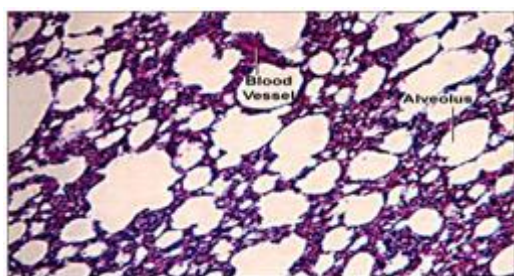


Figure 9. Light micrographs of sections in the lung of control group (× 40).

were significantly higher than those of the control group. On day 14 after the the intervention, a significant difference was observed between groups receiving 300 and 10 mg/kg of CuO nanoparticles.

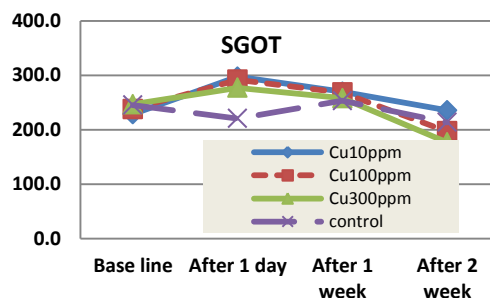


Figure 10. Comparison between the levels of serum glutamate oxaloacetate transaminase (SGOT) in all four groups.

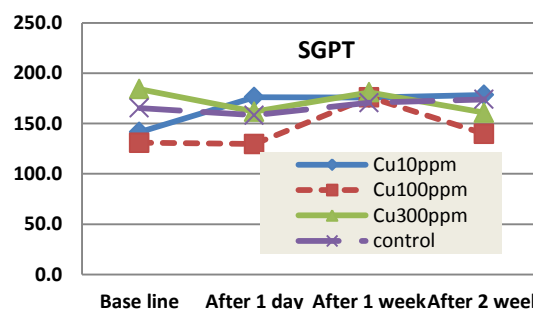


Figure 11. Comparison of the levels of serum glutamate pyruvate transaminase (SGPT) in all four groups.

Moreover, SGPT level was significantly higher in group receiving 10 mg/kg than the one receiving 100 mg/kg.

Copper nanoparticles are one of the industrially produced nanoparticles and are commercially available. Recently copper nanoparticles are used as additives to oils, polymers/plastics, metallic coatings, inks (13). Copper nanoparticles, similar to other metal nanoparticles, enter the environment and human body through different ways (such as effluent, spillage during shipping, and handling). Recent researches on the activity of copper nanoparticles after subcutaneous injection showed that regulatory effects of nanoparticles in organisms depend on the dosage used (14).

The study by Sizova et al. showed that 3 hours after the injection of 2 mg/kg copper nanoparticles into the muscle, these nanoparticles could be observed in vascular areas of periportal hepatocytes and in the cytoplasm of Kupffer liver cells of the treated rats. However, they disappeared within 3 days after treatment.

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In periportal hepatocytes, hydropic degeneration symptoms could be seen but after 7 days these symptoms could no longer be observed. One week after a repeated treatment of copper nanoparticle injection into the muscle, nanoparticles were mainly observed in periportal hepatic vascular segments. One day after the second nanoparticle treatment, signs of hydropic degeneration were visible in periportal hepatic vascular areas. They also found that copper nanoparticle hepatotoxicity and nephrotoxicity appeared in 6 mg/kg dosage. However, apoptosis could be observed in periportal hepatic and kidney tubule epithelium after 3 days and 3 hours, respectively by 3 injections of copper nanoparticles (15).

Results of the study by Chena et al. indicated that copper nanoparticles (23.5 nm) induced grave toxicological effects and acute injuries on the kidney, liver, and spleen of experimental mice (16).

Lei et al. studied the biochemical compounds in urine, serum, and tissue extracts of liver, and kidney of rats treated with 50, 100, and 200 mg/kg/d of copper nanoparticles for 5 days. Biochemical and histological analysis of the liver and kidney of all the rats were performed simultaneously. The results showed that the effects produced by copper nanoparticles at dosage of 50 or 100 mg/kg/d were lower than the higher dose (200 mg/kg/d). Copper nanoparticles caused severe hepatotoxicity and nephrotoxicity by at 200 mg/kg/d for 5 days. This mainly reflected in hepatic necrosis and renal proximal tubules. Obvious changes in aqueous extracts of the liver include increased lactate and creatine associated with decreased levels of taurine (17).

It has been reported that the toxicity of nanoparticles depends on the oxidative stress damage to DNA (18). However, other mechanisms must also be considered. For example, damage of cell membranes and organelles would potentially facilitate the transfer of toxic

factors. Genotoxic and allergenic activity are also possible (19). Studies of Fahm et al. (20) indicated that in comparison with normal cells, in cells exposed to copper nanoparticles, level of catalase and glutathione reductase activity decreased and activity of glutathione peroxidase increased. Increased activity of glutathione suggests that copper nanoparticles not only produce free radical, but also they stop the cell antioxidant defense.

Conclusion

The findings of the present study showed that all concentrations of copper nanoparticles induce toxicity and changes in histopathology of liver and lung tissues of rats. Therefore, they cannot be used by humans due to their toxicity.

Nano-copper exposure increased the production of reactive oxygen species, one of the most frequently reported nanoparticles-associated toxicities. Signal transduction mechanism studies showed that nano-copper exposure disturbed mitochondrial membrane potential and subsequently helped releasing cytochrome c from mitochondria to cytosol. Nano copper can trigger both intrinsic and extrinsic apoptotic pathways in oxidative stress (20).

Changes in structural and physicochemical properties of nanoparticles (NP) can lead to changes in biological activities including ROS generation. Oxidative stress induced by NP is as a result of cellular factors such as composition, size, particle surface and presence of metals, while cellular responses such as mitochondrial respiration, NP-cell interaction, and immune cell activation are responsible for ROS-mediated damage.

NP-induced oxidative stress responses are torch bearers for further pathophysiological effects including genotoxicity, inflammation, and fibrosis as demonstrated by activation of associated cell signaling pathways (21).

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References

1. Aruoja V, Dubourguier HC, Kasemets K, Kahru A. Toxicity of nanoparticles of CuO, ZnO and TiO₂ to microalgae *Pseudokirchneriella subcapitata*. *Sci Total Environ*. 2009; 407(4): 1461-1468.
2. Wang H, Huang Y, Tan Z, Hu X. Fabrication and characterization of copper nanoparticle thin-films and the electrocatalytic behavior. *Anal Chim Acta*. 2004; 526(1): 13-17.
3. Ruparelia JP, Chatterjee AK, Duttagupta, SP, Mukherji S. Strain specificity in antimicrobial activity of silver and copper nanoparticles. *Acta Biomater*. 4(3) :707-716.
4. Kim JS, Adamcakova-Dodd A, O'Shaughnessy PT, Grassian VH, Thorne PS. Effects of copper nanoparticle exposure on host defense in a murine pulmonary infection model. *Part Fibre Toxicol*. 2011; 8:29. doi: 10.1186/1743-8977-8-29.
5. Karlsson HL, Cronholm P, Gustafsson J, Möller L. Copper oxide nanoparticles are highly toxic: a comparison between metal oxide nanoparticles and carbon nanotubes. *Chem Res Toxicol*. 2008; 21(9): 1726-1732.
6. Jones CF, Grainger DW. *In vitro* assessments of nanomaterial toxicity. *Adv Drug Deliv Rev*. 2009; 1: 438-456.
7. Landsiedel R, Kapp MD, Schulz M, Wiench K, Oesch F. Genotoxicity investigations on nanomaterials: methods, preparation and characterization of test material, potential artifacts and limitations--many questions, some answers. *Mutat Res*. 2009; 681(2-3): 241-258.
8. Møller P, Jacobsen NR, Folkmann JK, Danielsen PH, Mikkelsen L, Hemmingsen JG, et al. Role of oxidative damage in toxicity of particulates. *Free Radic Res*. 2010; 44(1): 1-46.
9. Oberdörster G, Stone V, Donaldson K. Toxicology of nanoparticles: a historical perspective. *Nanotoxicology*. 2007; 1(1): 2-25.
10. Karlsson HL, Cronholm P, Gustafsson J, Möller L. Copper oxide nanoparticles are highly toxic: a comparison between metal oxide nanoparticles and carbon nanotubes. *Chem Res Toxicol*. 2008; 21(9): 1726-1732.
11. Karlsson HL, Gustafsson J, Cronholm P, Möller L. Size-dependent toxicity of metal oxide particles--a comparison between nano- and micrometer size. *Toxicol Lett*. 2009; 188(2): 112-118.
12. Chaves SB, Lacava LM, Lacava ZGM. Light microscopy and magnetic resonance characterization of a DSMA-coated magnetic fluid in mice. *IEEE Transactions on magnetic*. 2002; 38(5): 3231-3233.
13. Liu G, Li X, Qin B, Xing D, Guo Y, Fan R. Investigation of the mending effect and mechanism of copper nanoparticles on a tribologically stressed surface. *Tribol Lett*. 2004; 17(4): 961-966.
14. N. N. Glushchenko, I. P. Olkhovskaya, T. V. Pleteneva, L. D. Fatkullina, Yu. A. Ershov and Yu. I. Fedorov. The Biological Effect of Superfine Metal Powders. *Izvestiya AN*. 1989; 3: 415-418.
15. Sizova EA, Miroshnikov SA, Polyakova VS, Natalia Gluschenko, Anatoly Skalny. Copper nanoparticles as modulators of apoptosis and structural changes in tissues. *J Biomater Nanobiotechnol*. 2012; 3: 97-104.
16. Chen Z, Meng H, Xing G, Chen C, Zhao Y, Jia G, et al. Acute toxicological effects of copper nanoparticles *in vivo*. *Toxicol Lett*. 2006; 163(2): 109-120.
17. Lei R, Wu C, Yang B, Ma H, Shi C, Wang Q, et al. Integrated metabolomic analysis of the nano-sized copper particle-induced hepatotoxicity and nephrotoxicity in rats: a rapid *in vivo* screening method for nanotoxicity. *Toxicol Appl Pharmacol*. 2008; 232(2): 292-301.
18. Murray AR, Kisin E, Leonard SS, Young SH, Kommineni C, Kagan VE, et al. Oxidative stress and inflammatory response in dermal toxicity of single-walled carbon nanotubes. *Toxicology*. 2009; 257(3):161-171.
19. G. G. Onishchenko. The Concept of Toxicological Studies, Estimated Risk Methodology, Methods of Identification and Quantification of Nanomaterials. Resolution 79, Registered in the Russian Ministry of Justice, Registration. 2007; 10528.
20. Fahmy B, Cormier SA. Copper oxide nanoparticles induce oxidative stress and

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- cytotoxicity in airway epithelial cells. *Toxicol In Vitro*. 2009; 23(7): 1365-1371.
21. Sarkar A, Das J, Manna P, Sil PC. Nano-copper induces oxidative stress and apoptosis in kidney via both extrinsic and intrinsic pathways. *Toxicology*. 2011; 18; 290(2-3): 208-217.
22. Ju-Nam Y, Lead JR. Manufactured nanoparticles: an overview of their chemistry, interactions and potential environmental implications. *Sci Total Environ*. 2008; 400(1-3): 396-414.