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Research Article

Hepatotoxicity Induced By Copper Oxide and Zinc Oxide Nanoparticles and Their Mixtures in Male Albino Rats

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Abstract: **Background:** Nanotechnology is a fast growing field that provides for the development of materials that have new dimensions, novel properties, and a broader array of applications. Various scientific groups are keen about this technology and are devoting themselves to the development of more, new, and better nanomaterials. In the near future, expectations are that no field will be left untouched by the magical benefits available through application of nanotechnology. **Objectives:** The aim of the present study was to evaluate the hepatic toxicity induced by nano-CuO and Zn-O mixture in male rats. **Materials and Methods:** Twenty adult male rats were grouped randomly into four groups (n=5 each group). Group I (control): Rats were injected with saline intraperitoneally and at a dose of 1.0 ml/kg b.w. for 28 days. Group II (ZnONPs): Rats were administrated orally with ZnONPs (10 mg/kg/day) for 28 days. Group III (CuONPs): Rats were injected with CuONPs (0.5 mg/kg/day, in saline; intraperitoneally) for 28 days. Group IV (ZnONPs + CuONPs): Rats were given orally ZnONPs (10 mg/kg/day) follwed by injected with CuONPs (0.5 mg/kg/day, in saline; intraperitoneally), for 28 days. At the end of the experimental period, rats were anesthetized using light ether. Blood and liver tissue samples were taken and prepared for biochemical and histological measurements. **Results:** Serum total protein, albumin, and globulin concentrations in rats treated with CuONPs, ZnONPs and their mixture were significantly lower compared to the control group. On the other hand, significant increase in total bilirubin levels in rats treated with CuONPs, ZnONPs and their mixture treated groups compared to the control group. Pronounced increase in total bilirubin due treatments of rats with nanoparticles mixture compared to the individual treatemnts of each nanoparticles tested. The ALT, AST and AlP values in the CuONPs rats group were significantly higher compared to the control group. These changes were also observed in rats treated with CuONPs and ZnONPs mixture. On the other hand ZnONPs by itself increased ALT and AlP levels, while induce no change in AST compared to the control group. There was significant reduction in the GGT levels in rats treated with CuONPs and ZnONPs individually while a significant rise in serm GGT when animals treated these nanoparticls mixture compared treatemnts of each nanoparticles tested. Histopathological abnormalities were observed in liver of rats treated with CuO-NPs, ZnO-NPs, and CuO-NPs + ZnO-NPs. All treated groups showed vacuolated hepatocytes containing pale stained cytoplasm and darkly stained nuclei, necrotic hepatocytes, vascular congestion, and inflammatory cells in the portal area. **Conclusion:** It can be concluded that CuONPs, ZnONPs, and their mixture were induced physiological and morphological changes in hepatic tissues. Also, these results demonstrate that metal oxide nanoparticles induce a range of biological responses that vary from cytotoxic and can only be properly understood by using a tiered test strategy to study other aspects of nanoparticle toxicity. Toxicological studies must be performed before nano-particles application specially nano-oxide nano-particles. Caution should be taken in nano-particles use in worke place, preparations as well as while handling.

Keywords: Copper oxide nanoparticles, Zinc oxide nanoparticles, CuO& ZnO mixture, NPs, Hepatic toxicity, Hepatic Histopathology.

1. INTRODUCTION

In recent decades, environmental biology has received immense attention because pollution emerged as a serious and challenging side effect of the increased developmental activities. (Akinyeye, A. J., & Okorie, T. G. 2012; Goldin, Q., & Athalye, R. P. 2012; Mishra, P.

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C., & Dash, A. K. 2012) The release of both organic and inorganic hazardous substances is causing degradation of natural resources, making them unsuitable for use. Heavy metals are capable of causing many patho-physiological altrations in the living organisms (Nwajei, G. E. *et al*., 2012; Nwajei, G.E. *et al*., 2012).

Rapid developmental activities have made heavy metals ubiquitous contaminants of the environment. Heavy metals and their possible effects on living world have increasingly become a prime focus of the environmental biology. Excessive presence of essential metals as well as minute presence of non-essential metals can cause many patho-physiological changes in the living organisms via generation of reactive species (Manoj, K., & Padhy, P. K. 2013).

Although essential metals like zinc, copper, iron, and manganese are natural part of bio-molecules, and, thus, are needed for normal physiological and biochemical functions in the body. On the other hand, non-essential metals like mercury, lead, and cadmium are toxic even at minute concentrations. These metals are widely spread in our environment and easy entrance into the living organisms. Heavy metals can produce patho-physiological changes in our bodies by reactive oxygen species (ROS), which induce oxidative stress. The accumulation of ROS like hydroxyl radical, hydrogen peroxide, and singlet oxygen disturbs the oxidant-antioxidant balance bringing about a change called oxidative stress. These active species react with bio-molecules like proteins, DNA, and lipids impairing their functional properties. (Hippeli, S., & Elstner, E. F. 1999) Involvement of ROS in metal induced cell death is widely reported (Bi, Y., Chen, W. *et al*., 2009).

Nanoparticles (NPs) are defined as particles with sizes between 1 and 100 nm that show properties not found in bulk samples of the same materials. (Auffan, M. *et al*., 2009) NPs are of two main types, engineered and non-engineered. Engineered NPs are made from many different materials like phospholipids, dextran, chitosan, lactic acid, carbon, polymers, silica, and various metals. (De Jong, W. H., & Borm, P. J. 2008) They can be designed in different shapes including tubes, solid spheres, rods, hollows and complex strands (Provenzale, J. M., & Silva, G. A. 2009).

Nanotechnology is considered to be the next industrial revolution. (Gerber, C., & Lang, H.P. 2006) Humans have always been exposed to tiny particles via dust storms, volcanic ash, and other natural processes, and that our bodily systems are well adapted to protect us from these potentially harmful intruders. The reticuloendothelial system in particular actively neutralizes and eliminates foreign matter in the body, including viruses and non-biological particles. Technological advancement has changed the character of particulate pollution, increasing the proportion of nanometer-sized particles and expanding the variety of chemical compositions. Recent epidemiological studies have shown a strong correlation between particulate air pollution levels, respiratory and cardiovascular diseases, various cancers, and mortality. Adverse effects of nanoparticles on human health depend on individual factors such as existing disease and genetics, as well as exposure, and nanoparticle chemistry, shape, size, agglomeration state, and electromagnetic properties (Buzea, C. *et al*., 2007).

The manufacture and use of metal oxide nanoparticles is continuously expanding. It becomes increasingly important to investigate and identify their possible toxicological effects and to identify which particles pose the greatest harm to human health (Fahmy, B., & Cormier, S. A. 2009).

Copper oxide nanoparticles (CuO NPs) are of great interest in nanoscience and nanotechnology because of their broad industrial and commercial applications. Therefore, toxicity of CuO NPs needs to be thoroughly understood. Akhtar *et al.*, (2012) investigated the cytotoxicity and oxidative stress induced by CuO NPs in human lung epithelial cells. CuONPs are increasingly used as catalysts, heat transfer fluids, microelectronics, gas sensor, and cosmetics. (Chang, H. *et al*., 2005; Zhou, K. *et al*., 2006) There are some studies reporting the toxicity of CuONPs in microalgae, bacteria, yeast, protozoan, and crustaceans. (Kasemets, K. *et al*., 2009; Kahru, A., & Dubourguier, H. C. 2010) However, there are few studies evaluating the toxicity of CuONPs in mammalian cells. There are a few informations concerning the impact of CuONPs on the environment and human health (Sizova, E. *et al*., 2011).

Zinc oxide nano-particles are one of the most commonly used nanomaterials, with commercial and industrial applications, including personal skin and hair care products, pigments, sunscreens, coatings, paints, and ceramic products (Brar, S. K. *et al*., 2010; Blinova, I. *et al*., 2010; Dechsakulthorn, F. *et al*., 2010; Fan, Z., & Lu, J. G. 2005).

2. OBJECTIVES

Presently, there is only limited knowledge concerning the toxicological effects of NPs. However, it is now known that the toxic behavior of NPs differ from their bulk counterparts. Even NPs that have the same chemical composition differ in their toxicological properties; the differences in toxicity depend upon size, shape, and surface covering. Hence, before NPs are commercially used it is most important that they be subjected to appropriate toxicity evaluation. The aim of the present study was to evaluate the hepatic toxicity induced by nano-CuO and Zn-O mixture in male rats.

3. MATERIAL AND METHODS

3.1. Chemicals

Copper oxide and Zinc oxide as nanoparticles with an average size of 6 and 51 nm, respectively, were a gift from Dr. Amina El-Trass. Synthesis, characterization, optical properties and interaction with amino acids of CuO nanoparticles to confirm the negative surface of CuO nanoparticles were performed by El-Trass *et al*., (2012).

3.2. Animals and Housing

Twenty healthy male Wistar Albino rats weighing 150 ± 10 g, were obtained from the Animal Breeding House of the National Research Centre (NRC), Dokki, Cairo, Egypt. The rats were allowed to acclimatize for a week before starting the experiments. Rats were maintained under temperature-controlled conditions (25 ^{o}C), and a normal photoperiod of 12 h of darkness and 12 h of light. They were fed with standard food and had free access to water. Animals were randomly divided into 4 groups of five rats each, with one group assigned to be an untreated control. The housing and management of the animals and the experimental protocols were conducted as stipulated in the Guide for Care and Use of Laboratory Animals. (NRC, Seventh Revised Edition. 1996)

3.3. Experimental Protocol

Twenty adult male rats were grouped randomly into four groups (n=5 each group). Group I (control): Rats were injected with saline intraperitoneally and at a dose of 1.0 ml/kg b.w. for 28 days. Group II (ZnONPs): Rats were administrated orally with ZnONPs (10 mg/kg/day) for 28 days. Group III (CuONPs): Rats were injected with CuONPs (0.5 mg/kg/day, in saline; intraperitoneally) for 28 days. (Liao, M.Y., & Liu, H.G. 2012) Group IV (ZnONPs + CuONPs): Rats were given orally ZnONPs (10 mg/kg/day) follwed by CuONPs (0.5 mg/kg/day, in saline; intraperitoneally), for 28 days.

At the end of the experimental period, rats were anesthetized using light ether. Blood samples were taken from the vena cava of rat heart within 1 min after sacrifation. Tubes were used to compile blood drawn from the heart directly; the blood was collected in glass tubes for coagulation and serum formation, blood was allowed to set for 30 min at 4^{o}C to clot, then centrifuged for 5 minutes at 1000 x g. Packed cells were discarded and the supernatant serum samples were decanted and stored into capped sterile poly-ethelene tubes at -20^{o}C until used (within 24 hours). The abdominal cavity of each rat was opened where the liver was excised.

3.4. BIOCHEMICAL PARAMETERS IN RAT SERUM AND TISSUES

3.4.1. Determination of Serum Total Protein, Albumin, Globulin, and Total Bilirubin

Protein was determined by colorimetric determination of total protein according to the method described by Lowery *et al*., (1951). Albumin was determined according to the method described by Doumas *et al*., (1971). The described method provides a simple and direct procedure for the quantitative determination of serum globulins. The method should have wide application in both the human and veterinary medical fields, because of its easy adaptability to automation in the clinical laboratory, and because of the ease with which the manual method may be performed in the clinician's office. (Mishra, P. C., & Dash, A. K. 2012) The total bilirubin in serum was determined using a commercially available kit (Bilirub-kit 1.03358.001 Bilirubin, Merckotest, France). Total bilirubin concentration in the sample was calculated which is equal = $\Delta A \times 10.5$ mg/dl (Jendrassik, L., & Grof, P. 1983).

3.4.2. Determination of Serum ALT, AST, ALP, And ɣ-GT Activities

ALT was determined according to the method described by Bergmeyer *et al*., (Bergmeyer, H.U. *et al*., 1986). AST was determined according to the method described by Bergmeyer (Rosalki, S. B. *et al*., 1993). ALP was determined according to the method described by Rosalki *et al*., (Tietz, D.) using a commercial diagnostic kit (Spectrum, Hannover, Germany). ɣ-GGT was measured according to the method of Tietz (Drury, R. & Wallington, E. 1980) using commercial kits obtained from Bio ADWIC, Egypt**.**

3.3. Histopathological Analysis

Dissected liver was immediately fixed into 10% formaldehyde saline. Tissues were processed by embedding in paraffin. Sections were cut by rotatory microtome and mounted on glass slides. The sections were stained by conventional Hematoxylin & Eosin (H&E) stain. The sections were examined by light microscope (Nemmar, A. *et al*., 2002).

3.6. Statistical Analysis

Values obtained as mean ± SEM were subjected to one-way analysis of variance (ANOVA) followed by Tukey test using GraphPad Prism version 4.0 for windows from GraphPad Software, San Diego, California, USA). Values of $P < .05$ were considered significant.

4. RESULTS AND DISCUSSION

Nanoparticles (NPs) were found to reach the systemic circulation after inhalation, ingestion or intravenous injection. They are known to disseminate to several organs such as liver, spleen, kidneys, brain or heart. (Oberdörster, G. *et al*., 2005; De Jong, W. H. *et al*., 2008; Jain, T. K. *et al*., 2008; Klein, S. *et al*., 2007) Such translocation depends on the physicochemical properties of NPs, and their migration to distant sites is an important issue with regard to their toxicity.

4.1. Effect of Cuo and Zno Nano-Particles on Rat Serum Total Protein, Albumin, Globulin, and Total Bilirubin Concentrations.

Serum total protein, also called plasma total protein or total protein, is a biochemical test for measuring the total amount of protein in blood plasma or serum. This test is often done to diagnose nutritional problems, kidney disease or liver disease. If total protein is abnormal, further tests must be done to identify the specific problem. The elevation in protein than normal may be due to: Chronic inflammation or infection, including HIV and hepatitis B or C Multiple myeloma Waldenstrom's disease. Lower-than-normal levels may be due to: agammaglobulinemia bleeding (hemorrhage), burns (extensive), Glomerulonephritis, liver disease, malabsorption, malnutrition, nephrotic syndrome, protein-losing enteropathy (Zantop, D.W. 1997).

Serum proteins divided into two groups, albumin and globulin. Proteins act as transport substances for hormones, vitamins, minerals, lipids and other materials. In addition proteins help balance the osmotic pressure of the blood tissue. Most of the circulating cholesterol is carried in birds by high-density lipoprotein cholesterol (α-2globulin fraction) and LDL (β-globulin fraction) (Ncibi, S. *et al*., 2008). These lipoproteins became the principal cholesterol transport and carried about 40 to 44% of the total serum proteins.

Serum total protein, albumin and globulin concentration significantly reduced in animal groups received CuO, ZnO NPs and their mixture compared to control group (Tables .1 and Figures 1-3).

The study did record a relatively lower total protein, albumin and globulin concentration in the nanoparticles treated groups, did suggest some level of hepatocellular injury which has been demonstrated following metals oxides exposure. The use of albumin as antioxidant in scavenging to metals oxide nanoparticles reactive oxygen species may have contributed to its apparent reduction in the intoxicated groups. Normally, the reduction of albumin level indicates a liver disease. This reduction could be attributed to changes in the protein and free amino acid metabolism and their synthesis in the liver (Bhatnagar, P., & Jain, N. 1986).

Bilirubin concentration had been used to evaluate chemically induced hepatic injury. (Hoet, P. H. *et al*., 2004) In the present study, serum bilirubin was increased in rats treated with CuO and ZnO NPs and their mixture compared to control group (Table .1 and Figure .4).

The increase in the level of billirubin in the serum might be attributed to the pathological changes such as necrosis of hepatocytes which causes an increase in the permeability of the hepatic cell membrane (Hoet, P. H. *et al*., 2004).

Table (1). Effects of treatment of rats with zinc oxide, and copper oxide nanoparticles, and their mixture on serum total protein, albumin, globulin, and total bilirubin concentrations

Groups / Parameters	Groups			
	Control	CuONP	ZnONP	CuO + ZnONP
	Mean±SE	Mean± SE	Mean± SE	Mean± SE
Serum total protein (mg/dl)	7.544±0.288 [bcd]	6.848±0.208 [a]	6.664±0.358 [a]	6.574±0.229 [a]
Serum albumin (mg/dl)	4.198 ± 0.143 [bcd]	3.570 ± 0.311 [a]	3.860 ± 0.107 [ad]	3.612 ± 0.047 [ac]
Serum globulin (mg/dl)	3.688 ± 0.324 [bcd]	2.736 ± 0.351 [a]	2.804 ± 0.216 [a]	2.908 ± 0.531 [a]
Serum total bilirubin (mg/dl)	0.256 ± 0.016 [bcd]	0.328 ± 0.052 [ad]	0.366 ± 0.046 [ad]	0.388 ± 0.067 [abc]

Significance at $P < 0.05$. [a] Comparison of control and other groups; [b] Comparison of CuONP and other groups; [c] Comparison of ZnONP and other groups; [d] Comparison of CuO+ZnONP and other groups

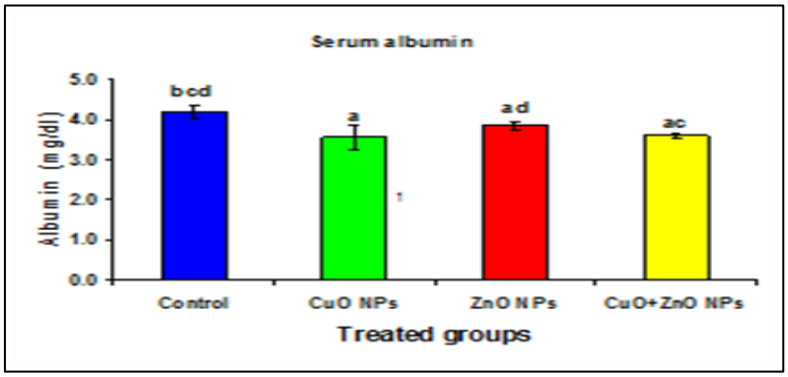

Figure 1: Serum total protein (mg/dl) of rat treated with zinc oxide (ZnO) and copper oxide (CuO) nanoparticles (NPs). Significance at P > 0.05. a Comparison of control and other groups; b Comparison of CuO NPs and other groups; c Comparison of ZnO NPs and other groups; d Comparison of CuO+ZnONPs and other groups.

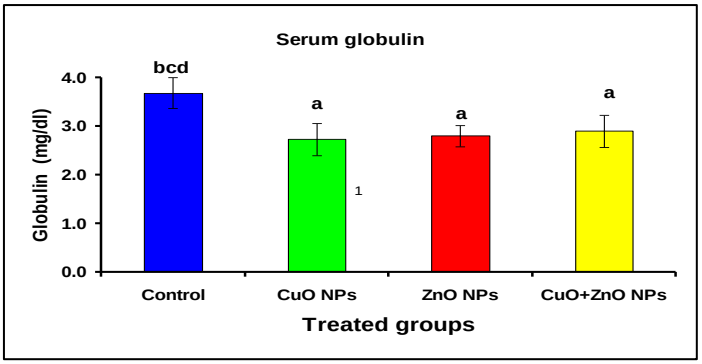

Figure 2: Serum albumin (mg/dl) of rat treated with zinc oxide (ZnO) and copper oxide (CuO) nanoparticles (NPs). Significance at P > 0.05. a Comparison of control and other groups; b Comparison of CuO NPs and other groups; c Comparison of ZnO NPs and other groups; d Comparison of CuO+ZnONPs and other groups

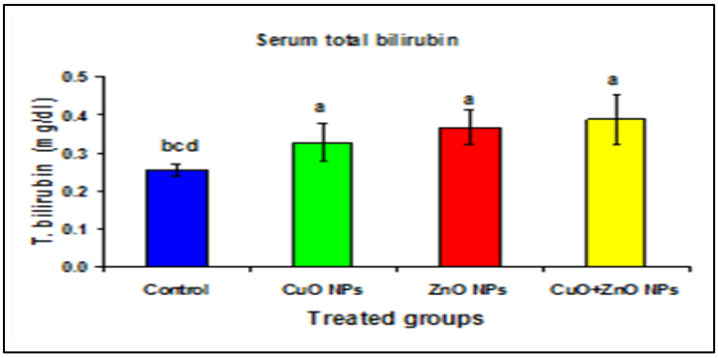

Figure 3: Serum globulin (mg/dl) of rat treated with zinc oxide (ZnO) and copper oxide (CuO) nanoparticles (NPs). Significance at P > 0.05. Significance at P > 0.05. a Comparison of control and other groups; b Comparison of CuO NPs and other groups; c Comparison of ZnO NPs and other groups; d Comparison of CuO+ZnONPs and other groups

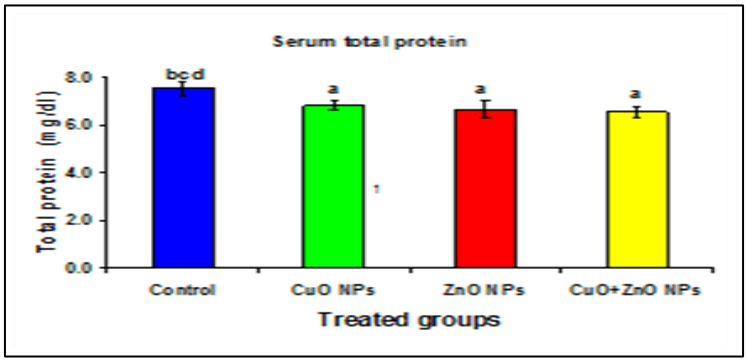

Figure 4: Serum total bilirubin (mg/dl) of rat treated with zinc oxide (ZnO) and copper oxide (CuO) nanoparticles (NPs). Significance at P > 0.05. a Comparison of control and other groups; b Comparison of CuO NPs and other groups; c Comparison of ZnO NPs and other groups; d Comparison of CuO+ZnONPs and other groups

4.2. Effect of Cuo and Zno Nano-Particles On Serum ALT, AST, ALP, And Ɣ-GT Activities.

The AST and AlP values in the CuO, ZnO NPs and their mixture groups were significantly higher compared to control ($P < 0.05$). CuO NPs and CuO and ZnO NPs mixture induced serum (Tables 2; Figures 5-7).

The cellular damage and oxidative stress of nanoparticles in the liver cells were related to the particle size and chemical compositions of nanoparticles. Nanoparticles that enter the rat liver induced oxidative stress locally (Wang, S. *et al*., 2008). The increased level of hepatic enzymes (ALT and AST) indicates liver damage or injury as supported by the work of Wang *et al*., (Chen, J. *et al*., 2009); Chen. *et al*., (2007). Most nanoparticles tend to accumulate in the liver. (Zhou, K. *et al*., 2006; Sadauskas, E. *et al*., 2007; Jeon, J. M. *et al*., 2013) They have been shown to be retained by the liver leading to tissue injury in mice (Chen, J. *et al*., 2009).

The damages of liver function occurred by nanosized-CuO and ZnO and their mixture as evidenced by the increased activities of ALT, AST, and ALP. Hepatic enzymes increase during liver dysfunction

indicating severe inflammation or liver injury (Chen, J. *et al*., 2009; Selim, K. K. *et al*., 2007). It has been speculated that part of the ROS generation might be due to the catalytic properties of nanosized-CuO. The overproduction of ROS would break down the balance of the oxidative/antioxidative system in the liver, resulting in the lipid peroxidation via ROS and MDA production and the hepatocyte apoptosis, which may be closely related to the reduction of antioxidative enzymes (Brown, D. M. *et al*., 2001). In fact, the reactive surface of ultra-small particles can result in the direct generation of harmful oxyradicals (ROS): these can cause cell injury by attacking DNA, proteins and membranes (Cheng, X. *et al*., 2004; Moore, M. N. 2006). Furthermore, the ability of these particles to penetrate the body and cells provides potential routes for the delivery of nanoparticle-associated toxic pollutants to sites where they would not normally go (Vroon, D.H., & Israili, Z. 1990). It is worth knowing that medicinal applications of nanoparticles benefit the same property to deliver drugs to diseased cells in order to improve the bioavailability of a drug; but biodistribution of some nanoparticles may not be known exactly, so they may accumulate in the body over time, leading to potential dangers.

An increment is observed in the activity of ɣ-GT in serum of rats treated with CuO and ZnO NPs. These changes were markedly pronounced in the nanoparticles mixture compared to control group. Although, there are reductions in the activity of ɣ-GT noted in CuO and ZnO NPs individually, while pronounced elevation in serum GGT when nanopaticles administered as a mixture (Table 2 and Figure 8).

Gama-glutamyl transferase catalyzes the transfer of ɣ- glutamyl group from **a** ɣ-glutamyl peptide to an amino acid or another peptide. This enzyme is widely used as a biomarker in preneoplastic lesions of the liver during chemical carcinogenesis. (Jia, X. *et al*., 2002; Mansour, S. A., & Mossa, A. T. H. 2011) Our results demonstrated that nanoparticles may affect liver metabolism and the leakage of certain intracellular enzymes, suggesting damage in hepatocytes (Adams, S. M. 2002). The interaction between nanoparticles may induce more deletrious effects on cell damage compared to exposure as individual.

Table (2). Effects of treatment of rats with zinc oxide, and copper oxide nanoparticles, and their mixture on serum ALT, AST and ALP activities

Groups	Groups			
Parameters	Control	CuONP	ZnONP	CuO + ZnONP
	Mean±SE	Mean± SE	Mean± SE	Mean± SE
Serum ALT activity (U/L)	50.6 ± 7.53 [bd]	87.2 ± 8.49 [acd]	50.0 ± 3.29 [ad]	61.4 ± 5.54 [ab]
Serum AST activity (U/L)	65.40 ± 4.32 [bcd]	118.20 ± 24.90 [a]	95.20 ± 12.53 [a]	100.00 ± 15.51 [a]
Serum ALP activity (U/L)	150.2 ± 12.94 [bcd]	191.8 ± 16.72 [ad]	190.8 ± 11.67 [ad]	233.0 ± 35.65 [abc]
Serum ɣ-GT activity (U/L)	4.406± 0.31[bcd]	2.738± 0.54[ad]	3.598± 0.61[ad]	7.940± 0.88[abc]

Significance at $P < 0.05$. [a] Comparison of control and other groups; [b] Comparison of CuONP and other groups; [c] Comparison of ZnONP and other groups; [d] Comparison of CuO+ZnONP and other groups

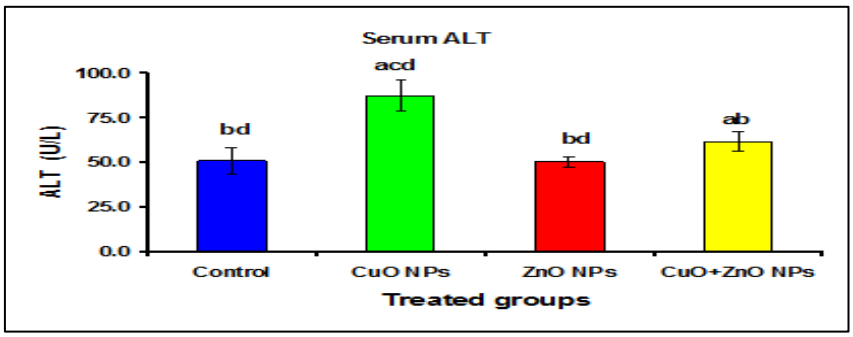

Figure 5: Serum ALT (U/L) of rat treated with zinc oxide (ZnO) and copper oxide (CuO) nanoparticles (NPs). Significance at P > 0.05. a Comparison of control and other groups; b Comparison of CuO NPs and other groups; c Comparison of ZnO NPs and other groups; d Comparison of CuO+ZnONPs and other groups.

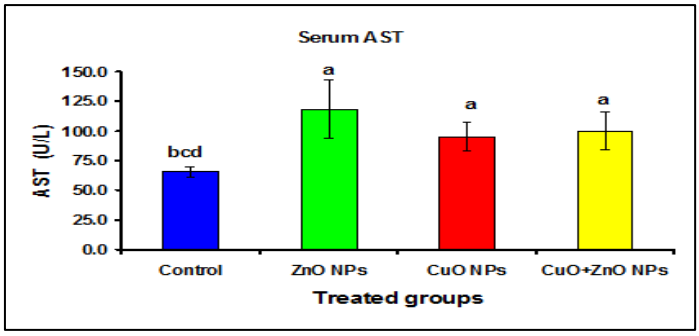

Figure 6: Serum AST (U/L) of rat treated with zinc oxide (ZnO) and copper oxide (CuO) nanoparticles (NPs). Significance at P > 0.05. Significance at P > 0.05. a Comparison of control and other groups; b Comparison of CuO NPs and other groups; c Comparison of ZnO NPs and other groups; d Comparison of CuO+ZnONPs and other groups.

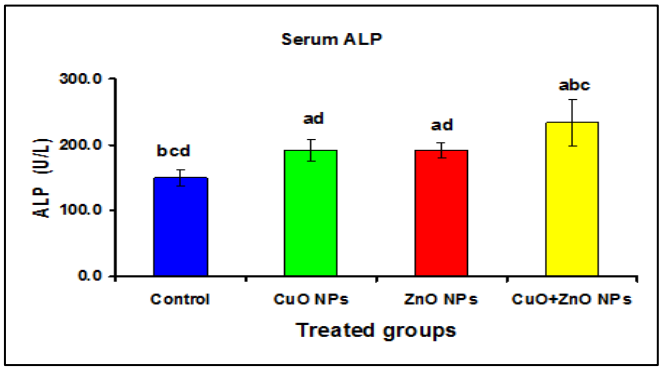

Table 7: Serum ALP (U/L) of rat treated with zinc oxide (ZnO) and copper oxide (CuO) nanoparticles (NPs). Significance at P > 0.05. Significance at P > 0.05. a Comparison of control and other groups; b Comparison of CuO NPs and other groups; c Comparison of ZnO NPs and other groups; d Comparison of CuO+ZnONPs and other groups.

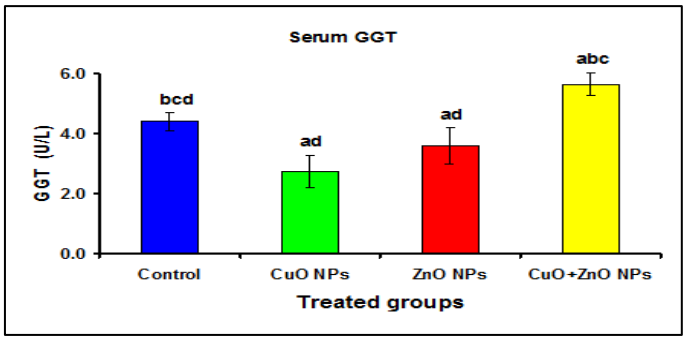

Figure 8: Serum GGT (U/L) of rat treated with zinc oxide (ZnO) and copper oxide (CuO) nanoparticles (NPs). Significance at P > 0.05. a Comparison of control and other groups; b Comparison of CuO NPs and other groups; c Comparison of ZnO NPs and other groups; d Comparison of CuO+ZnONPs and other groups

4.4. Effects of Copper Oxide Nanoparticles (Cuo Nps) and Zinc Oxide Nano-Particles (Zno Nps) On Rats Liver Histology.

Microscopic examination of organs to evaluate the toxic effects of contaminants has been used as a biomarker to evaluate the toxicity of various pollutants (Mela, M. *et al.*, 2007; An, L. *et al.*, 2012). Histopathology studies of target organs along with the studies of oxidative stress would give the complete risk assessment and toxic potential of copper and zinc oxide nanoparticles (CuO NPs & ZnO-NPs). In the present study, histopathological abnormalities were observed in liver of rats treated with CuO-NPs, ZnO-NPs, and CuO-NPs + ZnO-NPs.

Figures (9 -12) reveal that CuO-NPs, ZnO-NPs, and CuO-NPs + ZnO-NPs treatment induced histological changes in the liver. Control group presented normal hepatic structure, characterized by anastomosing cords of hepatocytes radiating from central vein separated by blood sinusoids and the hepatocytes contain central pale stained nuclei. All treated groups showed vacuolated hepatocytes containing pale stained cytoplasm and darkly stained

nuclei, necrotic hepatocytes, vascular congestion, and inflammatory cells in the portal area.

Data on internal organ pathologies from CuO-NPs, and ZnO-NPs are generally lacking. Nano-CuO – induced histological changes in hippocampus, liver and kidney of rats. (Loginova, N. V. 2014; Chuang, H. C. *et al*., 2014) ZnO-NPs produced an inflammatory cytological profile in the heart and lung of rats (Pasupuleti, S. *et al*., 2012; Esmaeillou, M. *et al*., 2013), in liver, pancreas, and stomach (Esmaeillou, M. *et al*., 2013) and pathological changes in mice liver such as hepatocytes necrosis[63], and in mice kidney as glomeruli segmentation, hydropic degeneration of the epithelium, necrosis of the tubular epithelial cells, and swelling in the epithelium of proximal tubules (Bogiswariy, S. *et al*., 2008).

Liver of the different treated groups showed distorted hepatocytes with cytoplasmic vacuolation. It has been reported that toxicant exposed liver show vacuolation because of the excessive accumulation of fat in the cytoplasm (Sun, J. *et al*., 2011).

Also, CuO, ZnO, and MgO NPs produced the cytotoxicity at the concentration-dependent and time-dependent manner, and elicited the permeability and inflammation response in human cardiac microvascular endothelial cells. These results demonstrated that cytotoxicity, permeability, and inflammation in vascular endothelial cells following exposure to metal oxide nanoparticles depended on particle composition, concentration, and exposure time.

The histopathological abnormalities observed in the present study, in liver of rats treated with ZnO-NPs, CuO-NPs, and CuO-NPs + ZnO-NPs could be explained on basis of the reported increase in lipid peroxidation, and decreased level of reduced glutathione. Furthermore, these histological changes were in line with the reported changes in the liver (AST, ALT & alkaline phosphatase) in the present study.

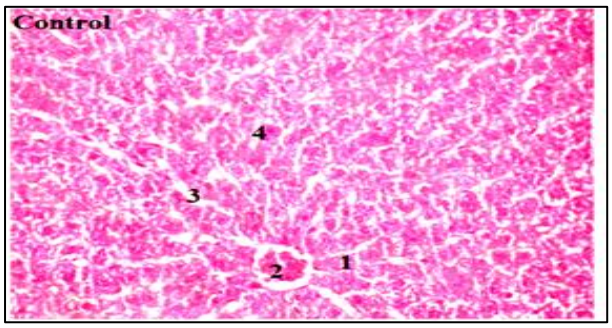

Figure 9: Photomicrograph of section of liver of control group showing anastomosing cords of hepatocytes (1) radiating from central vein (2) separated by blood sinusoids (3) and the hepatocytes contain central pale stained nuclei (4) (H&E X 400).

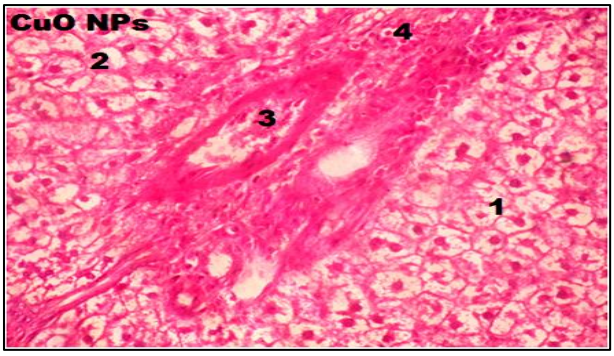

Figure 10: Photomicrograph of section of liver of CuO NPs treated group showing vacuolated hepatocytes containing pale stained cytoplasm and darkly stained nuclei (1), necrotic hepatocytes (2), vascular congestion (3). The portal area contains inflammatory cells (4) (H&E X 400).

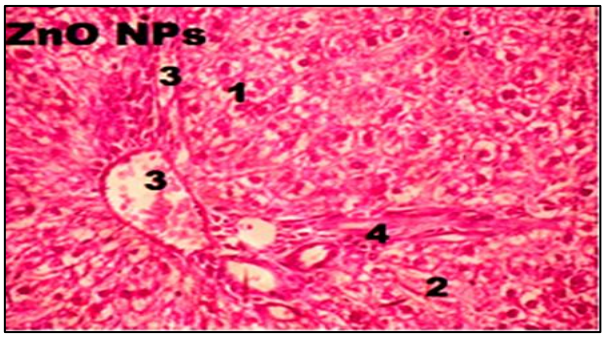

Figure 11: Photomicrograph of section of liver of ZnO NPs- treated group showing vacuolated hepatocytes containing pale stained cytoplasm and darkly stained nuclei (1), necrotic hepatocytes (2), vascular congestion (3). The portal area contains inflammatory cells (4) (H&E X 400).

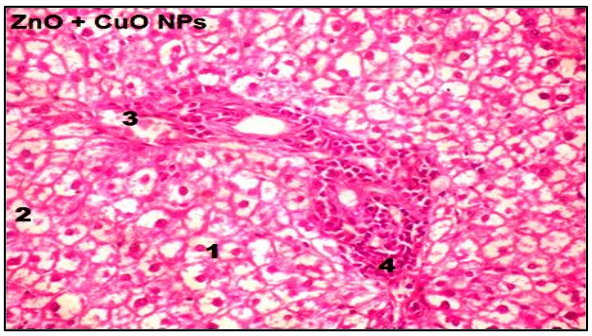

Figure 12: Photomicrograph of section of liver of ZnO + CuO NPs treated group showing vacuolated hepatocytes containing pale stained cytoplasm and darkly stained nuclei (1), necrotic hepatocytes (2), vascular congestion (3), The portal area contains inflammatory cells (4) (H&E X 400).

5. CONCLUSION

It can be concluded that CuONPs, ZnONPs, and their mixture produce cell damage and alter liver physiology. These results demonstrate that metal oxide nanoparticles induce a range of biological responses that vary from cytotoxic and can only be properly

understood by using a tiered test strategy to study other aspects of nanoparticle toxicity. Toxicological studies must be performed before nano-particles application specially nano-oxide nano-particles. Caution should be taken in nano-particles use in worke place, preparations as well as while handling.

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