EAS Journal of Pharmacy and Pharmacology
Abbreviated Key Title: EAS J Pharm Pharmacol
ISSN 2663-0990 (Print) & 2663-6719 (Online)
Published By East African Scholars Publisher, Kenya
Volume-1 | Issue-6 | Nov-Dec-2019 |

OPEN ACCESS

Research Article

Effects of Copper Oxide and/or Zinc Oxide Nanoparticles on Oxidative Damage and Antioxidant Defense System in Male Albino Rats

Rabia A. M. Yahya[1], Azab Elsayed Azab[2*], Karema El.M.Shkal[3]

[1]Pharmacology Department, Faculty of Medicine, Sabratha University, Libya
[2]Physiology Department, Faculty of Medicine, Sabratha University, Libya

*Corresponding Author
Azab Elsayed Azab

Abstract: **Background:** Oxidative stress is one of several mechanisms leading to nanotoxicity. Some nano-metal oxides can enhance ROS generation, inducing oxidative stress, DNA damage, and unregulated cell signaling, and eventually leading to changes in cell motility, apoptosis, and even carcinogenesis. The level of ROS generation by engineered nanomaterials is dependent on the chemical nature of the nanoparticles. Antioxidants play an important role in preventing, or in most cases, limiting the damage caused by ROS. **Objectives:** The aim of the present study was to evaluate the effects of copper oxide and/or zinc oxide nanoparticles on oxidative damage and antioxidant defense system in male albino 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) followed 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 measurements. **Results:** Catalase enzyme activity was decreased in the serum of rats treated with CuONPs and/or ZnONPs compared to control group. Mixture of CuONPs and ZnONPs antagonized each other and induced less effects on catalse changes compared with treatemnts of each nanoparticles tested. Microsomal protein, b_5 and P_{450} were decreased in rats treated with CuONPs and/or ZnONPs treated groups compared to the control group. NADPH cytochrome C reductase activity was increased in the liver of rats due to individual exposed to CuONPs or ZnONPs, while a significant reduction occurred in liver NADPH when rats treated with these nanopaticles as a mixture. Glutathione S transferase activity was increased in the liver of rats treated with CuONPs and/or ZnONPs compared to control group. Mixture of CuONPs and ZnONPs antagonized each other and induced less effects on GST changes compared with treatments of each nanoparticles tested. Lipid peroxidation marker (TBARS) was increased in the liver of rats treated with CuONPs, ZnONPs and their mixture compared to control group. Pronounced increase in TBARS due treatments of rats with nanoparticles mixture compared to the individual treatemnts of each nanoparticles tested. Liver GSH concentration was decreased in rats treated with CuONPs, ZnONPs and their mixture treated groups compared to the control group. **Conclusion:** It can be concluded that Copper oxide and zinc oxide nano-particle produce lipid peroxidation and affects on non enzymatic and enzymatic antioxidant. 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. Caution should be taken in nano-particles use in work place, preparations as well as while handling.

Keywords: Copper oxide nanoparticles, Zinc oxide nanoparticles, CuO& ZnO mixture, Oxidative stress, Non enzymatic antioxidant, Enzymatic antioxidant.

1. INTRODUCTION

Metal oxide nanoparticles are well known to generate oxidative stress and deregulate normal cellular activities, which subsequently leads to cellular toxicity.

Quick Response Code

Journal homepage:
http://www.easpublisher.com/easjpp/

Article History
Received: 29.10.2019
Accepted: 08.11.2019
Published: 26.11.2019

DOI: 10.36349/easjpp.2019.v01i06.002

Hence, oxidative stress has been considered as one of the primary causes of nanotoxicity and has been reported to use as bio-indicator to evaluate the toxic effects of nanoparticles (Libralato, G. *et al*., 2017).

Generation of ROS induced by nanomaterials, directly or indirectly, plays a vital role in genotoxicity. Oxidative DNA damage is associated with biological mechanisms involving mutagenesis, carcinogenesis, and aging-related diseases in humans. Oxidative stress is one of several mechanisms leading to nanotoxicity. Some nano-metal oxides can enhance ROS generation, inducing oxidative stress, DNA damage, and unregulated cell signaling, and eventually leading to changes in cell motility, apoptosis, and even carcinogenesis. Therefore, it is imperative that the mechanisms by which nanomaterials mediate and/or promote these adverse events be understood. DNA is a critical cellular target of ROS. Oxidative DNA damage involves base and sugar lesions, DNA-protein-crosslinks, single- and double-strand breaks, and the formation of abasic sites (Valko, M. *et al*., 2006). Highly reactive radicals, such as hydroxyl radicals, can damage DNA quickly in the vicinity; whereas the less-reactive ROS may interact with DNA at a distance. The level of ROS generation by engineered nanomaterials is dependent on the chemical nature of the nanoparticles (Gonzalez, L. *et al*., 2008). Compared to their bulk-size counterparts, engineered nanomaterials possess a small size, high specific surface area, and high surface reactivity, leading to the production of higher levels of ROS, and resulting in cytotoxicity and genotoxicity (Oberdörster, G. *et al*., 10994). A variety of nanomaterials has been found to induce toxicity mediated by ROS in many biological systems, such as human erythrocytes and skin fibroblasts (Li, X. *et al*., 2011).

Antioxidants play an important role in preventing, or in most cases, limiting the damage caused by ROS. The hydroxyl radical possesses the highest one-electron reduction potential of all the physiologically relevant ROS, and is extremely reactive with almost every type of biomolecule, including proteins and nucleic acids (Halliwell, B. 1989; Evans, M. D. *et al*., 2004; Lubec, G. 1996). There is no known enzymatic reaction that can scavenge the hydroxyl radical in vivo. The only known defense against hydroxyl radicals comes from antioxidants. Antioxidants are essentially reducing agents; they participate in redox reactions by donating electrons or hydrogen atoms. Within limitations, this action allows cells to function normally and avoid the consequences of oxidation of structural and other vital components.

2. OBJECTIVES

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 effects of copper oxide and/or zinc oxide nanoparticles on oxidative damage and antioxidant defense system in male albino 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*., (2002).

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 (Wu, D., & Cederbaum, A. I. 2003).

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. (Jorquera, F. *et al*., 1996) 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. Determination of Catalase Activity:

Caltalese was determined according to Goth (Goth, L. 1991).

3.5. Determination of Lipid Peroxidation as Thiobarbituric Acid Reactive Substances (TBARS)

TBARS are expressed in terms of malondialdehyde (MDA) equivalents using the molar absorbtivity of 149000 M^{-1} cm^{-1} (Slater, T. F., & Sawyer, B. C. 1971.

3.6. Determination of Glutathione

Reduced glutathione was estimated by the method Moron *et al*., (1979).

3.7. LIVER MICROSOMES:

3.7.1. Preparation of Liver Microsomes

At the end of the treatment, rats were fasted 24 h prior to being sacrificed. The abdominal cavity opened immediately and liver was removed, washed with cold 0.1 M phosphate buffer, pH 7.4, weighed and chilled on ice. All the following procedures were carried out in cold condition. A 33% (W/V) crude homogenate was prepared in 0.1 M phosphate buffer, pH 7.4 by homogenization with a Teflon pestle, using 5 strokes. The crude homogenate was then centrifuged at 11,000 xg for 20 min at 4°C to remove the intact cells, nuclei and mitochondria. The supernatant solution was subsequantly centrifuged at 105000 xg for 60 min at 4^oC to sediment the microsomal pellet. The pellet was re-suspended in 0.1 M phosphate buffer, pH 7.4, kept in ice bath and used as the enzyme source.

3.7.2. Liver Microsomal Assays

Liver microsomal cytochrome b_5 and P_{450} were determined according to Omura and Sato, (Omura, T., & Sato, R. 1964). The activity of microsomal NADPH-cytochrome-C reductase was assayed according to the method of Williams and Kamin (Williams, C. H., & Kamin, H. 1962). Glutathione S-transferase activity was assayed according to the method of Habig *et al*., (1974).

3.8. 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

4.1. Effect of Cuo and Zno Nano-Particles on Rat Serum Catalase Enzyme Activity

The risk of potential human exposure to mixed nanomaterials in consumer, occupational, and medicinal settings is increasing as nanomaterials enter both the workplace and the marketplace. In this study, we investigated the toxicity of mixed engineered CuO and ZnO nanoparticls on serum catalase.

Catalase activity was found to decrease significantly ($p < 0.05$) in the serum of the CuO and ZnO nanoparticls treated group, when compared to the normal control group. Also, significant increase ($p < 0.05$) is noticed in the group treated with the CuO and ZnO nanoparticles mixture compared to CuO and/or ZnO nanoparticles group reflecting antagonistic effects of these nanoparticles when treated together to rats (Table 1 and Figure 1).

Jomova and Valko (2011) reported that detailed studies in the past two decades have shown that redox active metals like Fe, Cu, Cr, Co and other metals undergo redox cycling reactions and possess the ability to produce reactive radicals such as superoxide anion radical and nitric oxide in biological systems. Disruption of metal ion homeostasis may lead to oxidative stress, a state where increased formation of ROS overwhelms body antioxidant protection and subsequently induces DNA damage, lipid peroxidation, protein modification and other effects, all symptomatic for numerous diseases, involving cancer, cardiovascular disease, diabetes, atherosclerosis, neurological disorders**.**

Free radical oxidative stress has been implicated in the pathogenesis of a wide variety of clinical disorders, resulting usually from deficient natural antioxidant defenses. Potential antioxidant therapy, therefore, should include either natural free-radical scavenging antioxidant enzymes or agents which are capable of augmenting the activity of these enzymes. Reactive oxygen species (ROS) has received considerable attention in the recent past because of its role in several pathological conditions including cancer, diabetes, arthritis, aging, and atherosclerosis. ROS produced *in vivo* O_2^-, hydrogen peroxide (H_2O_2), and hypochlorous acid (HOCl), and H_2O_2 can interact in the presence of transition metal ions to yield a highly reactive oxidizing species, the hydroxy radical (Shinmoto, H. *et al*., 1992). If human disease is believed to be due to the imbalance between oxidative stress and antioxidative defense, it is possible to limit oxidative tissue damage and hence prevent disease progression by antioxidant defense supplements.

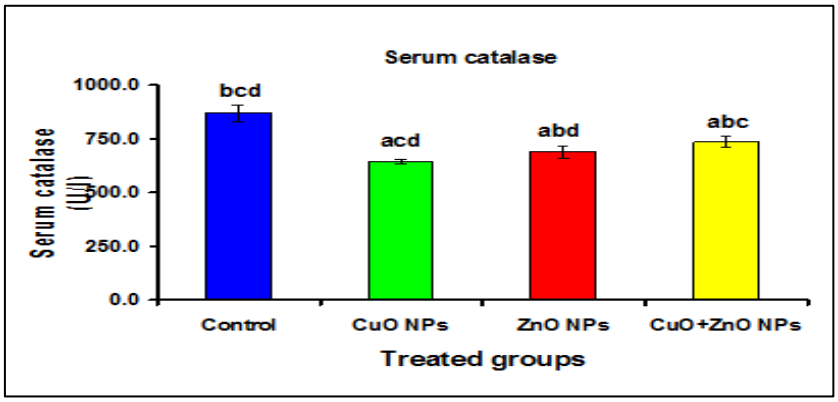
Catalase is one of the most active enzymes and its levels change first following induction of oxidative stress. The level of inhibition observed in the activities of these enzymes on exposure with CuO and ZnO individually and/or in combination, confirming the view that this metals produce oxidative stress. Several reports are available showing alteration in the activities of

antioxidant enzymes following metals oxide exposure (Bi, Y. *et al*., 2009; Pompella, A. *et al*., 2009). The decrease in enzymatic antioxidants may results in increase free radicals and enhancing the disease progression to non-target organisms.

Table (1). Effects of treatment of rats with zinc oxide and/or copper oxide nanoparticles on serum catalase and Liver microsomal protein, b_5, and P_{450}

Groups	Groups			
Parameters	Control	CuONP	ZnONP	CuO + ZnONP
	Mean±SE	Mean± SE	Mean± SE	Mean± SE
Serum catalase (U/L)	870.4± 38.9 [bcd]	644.0± 9.78 [acd]	689.2± 26.8 [abd]	737.1± 26.0 [abc]
Liver microsomal protein (mg protein/g liver)	1.35 ± 0.12 [bcd]	0.60 ± 0.05 [a]	0.61 ± 0.12 [ad]	0.70 ± 0.07 [ac]
Liver microsomal b_5 (nmole/mg protein)	2.53 ± 0.26 [bcd]	1.73 ± 0.23 [ad]	1.63 ± 0.28 [ad]	2.02 ± 0.13 [ac]
Liver microsomal P_{450} (nmole/mg protein)	1.81 ± 0.08 [bcd]	1.26 ± 0.12 [ad]	1.30 ± 0.12 [ad]	1.58 ± 0.08 [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



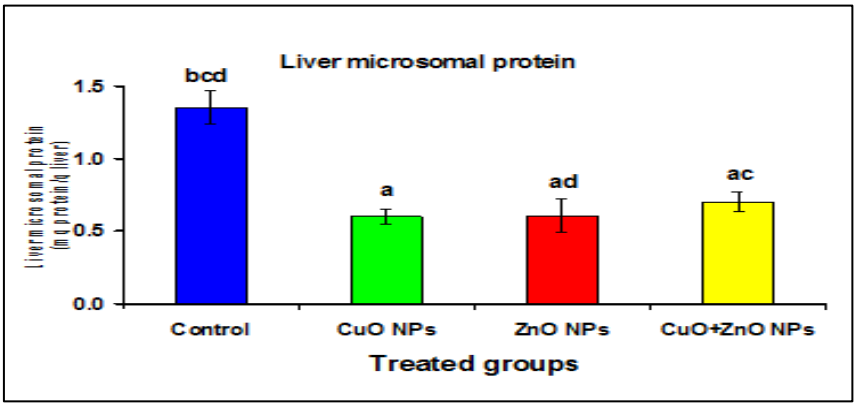


Figure 1: Serum catalase (U/l) of of rat treated with zinc oxide (ZnO) and copper oxide (CuO) nanoparticles (NPs). Significance at P > 0.05. a Comparison of control and other groups; b Comparison of CuO NPs and other groups; c Comparison of ZnO NPs and other groups; d Comparison of CuO+ZnONPs and other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

Figure 2: Liver microsomal protein (mg protein/g liver) 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. Liver Microsomal Protein, B_5 And P_{450} Of Rat Treated With Cuo And Zno Nano-Particles

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 (Nemmar, A. *et al*., 2002; Oberdörster, G. *et al*., 2005; De Jong, W. H. etg al., 2008; Jain, T. K. *et al*., 2008).

Copper oxide nanoparticles (CuO NPs) are heavily utilized in semiconductor devices, gas sensor, batteries, solar energy converter, microelectronics and heat transfer fluids. It has been reported that liver is one of the target organs for nanoparticles after they gain entry into the body through any of the possible routes.

Recent studies have shown cytotoxic response of CuO NPs in liver cells (Siddiqui, M. A. *et al*., 2013).

A protein being involved in the architecture and also in the physiology of the cell seems to occupy a key role in the cell metabolism (Yeragi, S. G. *et al*., 2003). Data revealed a significant decrease ($p<0.05$) in microsomal protein, b_5 and P_{450} in rats received CuO, ZnO NPs and their mixtures compared to the control (Tables 1, Figure.3, 4). The reduction in protein levels in rats treated with nanoparticles indicates an acceleration of protein anabolism during metal oxids nanopaticls intoxication. Inhibition of cytochrome P_{450} system was found to be effective in protecting the liver against the toxicity of a wide variety of toxic agents (Jorquera, F. *et al*., 1996). CuO and ZnO NPs were found to decrease the hepatic content of cytochrome P_{450}, which might protect the liver against the toxicity of nanoparticles in the present study. The mechanism of cytochrome P_{450} inhibition caused by nanoparticles might be due to interaction of its active component, with one or more of the seven cysteinyl residues of cytochrome P_{450} hemoprotein (Kwak, M. *et al*., 1994; Volans, G. N.)

The establishment of verifiably safe nanotechnology requires the development of assessment tools to identify hazardous nanomaterial properties that could be modified to improve nanomaterial safety. While there is a lot of debate of what constitutes appropriate safety screening methods, one approach is to use the assessment of cellular injury pathways to collect knowledge about hazardous material properties that could lead to harm to humans and the environment (George, S. *et al*., 2009).

Wang *et al*., (2012) investigated the toxicity of CuO nanoparticles (NPs) to human lung epithelial (A549) cells. CuO NPs (10-100 mg/L) had significant toxicity to A549 cells, whereas CuO bulk particles (BPs) showed much lower toxicity (24 h IC_{50}, 58 and 15 mg/L for CuO BPs and NPs, respectively). Transmission electron microscopic analysis demonstrated CuO NP entry into A549 cells and organelles, including lysosomes, mitochondria, and nucleus. Endocytosis was the primary pathway of CuO NPs uptake. CuO NPs (15 mg/L) induced mitochondrial depolarization, possibly mediated by reactive oxygen species (ROS) generation.

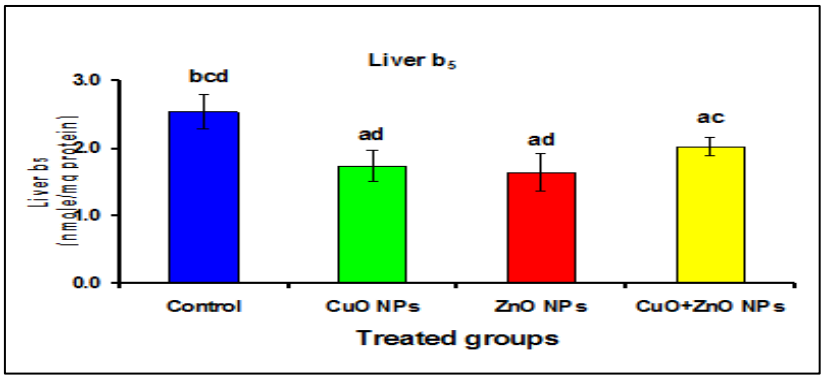


Figure 3: Liver microsomal b5 (nmole/mg protein) of rat treated with zinc oxide (ZnO) and copper oxide (CuO) nanoparticles (NPs). Significance at $P > 0.05$. Significance at $P > 0.05$. a Comparison of control and other groups; b Comparison of CuO NPs and other groups; c Comparison of ZnO NPs and other groups; d Comparison of CuO+ZnONPs and other groups

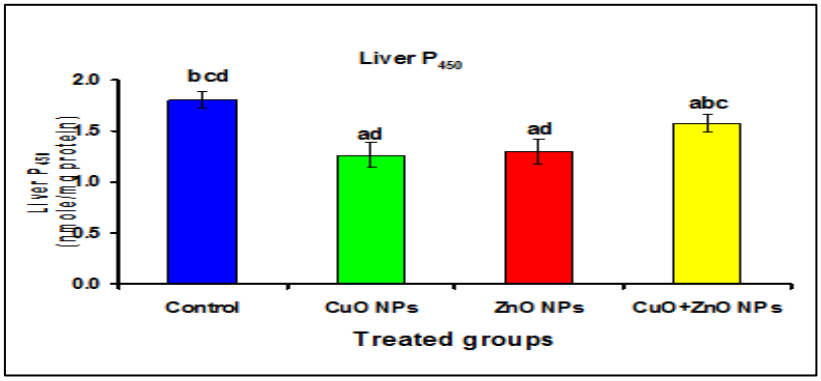


Figure 4: Liver microsomal P450 (nmole/mg protein) of rat treated with zinc oxide (ZnO) and copper oxide (CuO) nanoparticles (NPs). Significance at $P > 0.05$. a Comparison of control and other groups; b Comparison of CuO NPs and other groups; c Comparison of ZnO NPs and other groups; d Comparison of CuO+ZnONPs and other groups

4.3. Effects of copper oxide nanoparticles (CuO NPs) and zinc oxide nano-particles (ZnO NPs) on rats liver NADPH cytochrome C reductase and glutathione S treansferase (GST) activities

Intracellular CuO NPs first generate ROS, which subsequently induces the expression of p38 and p53 and ultimately causes DNA damage (Comet assay). They confirmed for the first time that the primary cytotoxic response is oxidative stress rather than DNA damage. A fraction of the CuO NPs was exported to the extracellular environment. Centrifugal ultrafiltration tubes were successfully employed to determine the dissolved Cu^{2+} from CuO NPs in the cell medium. Dissolved Cu^{2+} ions contributed less than half of the total toxicity caused by CuO NPs, including ROS generation and DNA damage. The study provided useful data for understanding transport and toxicity of metal oxide NPs in human cells (2012).

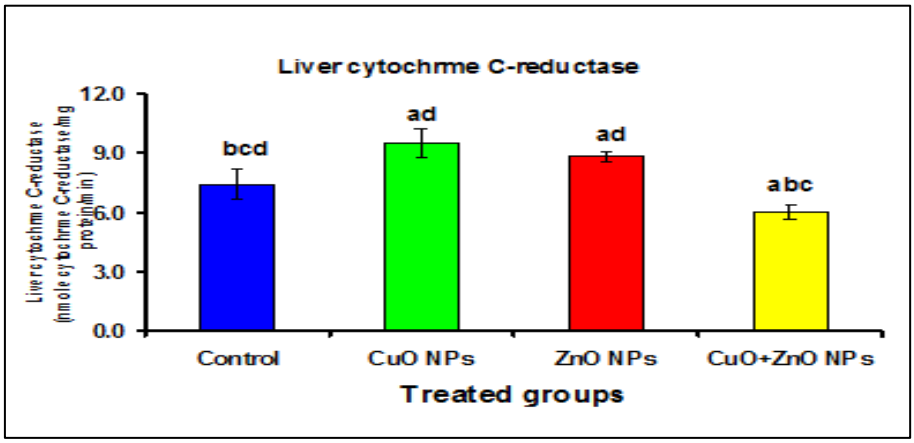
NADPH cytochrome C-reductase activity is a component of the microsomal mixed-function oxidase system which catalyses hydroxylation reaction, and this process is of a prime importance in the metabolism of lipids, drugs and other foreign compounds (Vermilion, J. L. *et al*., 1981). The rate-limiting step in the activation and detoxification of toxic compounds is dependant on the rate of reduction of cytochrome P_{450} substrate complex, which in turn is dependant on the activation and turn over rates of NADPH cytochrome C-reductase, cytochrome b_5 and on the total cytochrome P_{450} content (Dalvi, R. R. 1992). Data revealed a significant increase ($p<0.05$) in NADPH cytochrome C-reductase activity in rats treated with CuO and ZnO NPs individually, while this elevation was decreased when animals given the mixture of of tesed NPs compared to control (Tables 2, Figures 5, 6). The induction of NADPH cytochrome C-reductase activity in CuO and ZnO NPs groups could be one of the defense mechanism to increase the rate of reduction of cytochrome P_{450} substrate complex (Sheweita, S. A. *et al*., 2001).

In the present study, treatement rats with NPs revealed a significant hepatic damage as observed from the elevation of hepatospecific enzyme activities, as well as, severe alteration in different liver parameters. These findings demonstrate that, *in vivo* acute administration of NPs augments lipid peroxidation, and modulates the activities of drug-metabolizing enzymes in rat liver, suggesting that ROS may be involved in the toxic effects of the metal oxides NPs through inhibition of cytochrome P_{450} and b_5.

Table (2). Effects of treatment of rats with zinc oxide and/or copper oxide nanoparticles on serum catalase and Liver microsomal protein, b5, and P450

Groups	Groups			
Parameters	Control	CuONP	ZnONP	CuO + ZnONP
	Mean±SE	Mean± SE	Mean± SE	Mean± SE
Liver microsomal cytochrome C-reductase (nmole cytochrme C-reductase/mg protein/min)	7. 40± 0.74 ^{bcd}	9.52 ± 0.72 ^{ad}	8.81 ± 0.25 ^{ad}	6.01 ± 0.33 ^{abc}
Liver GST (U/mg protein)	33.22 ± 1.32 ^{bcd}	81.96 ± 6.31 ^{acd}	64.26 ± 2.71 ^{abd}	52.76 ± 3.29 ^{abc}
Liver TBARS (µmole/g tissue)	1.20 ± 0.13 ^{bcd}	3.47 ± 0.24 ^{ad}	3.22 ± 0.12 ^{abd}	3.87 ± 0.27 ^{abc}
Liver GSH (mg/g tissue)	42.10 ± 1.57 ^{bcd}	21.92 ± 1.01 ^{acd}	24.91 ± 1.86 ^{ab}	24.11 ± 0.68 ^{ab}

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



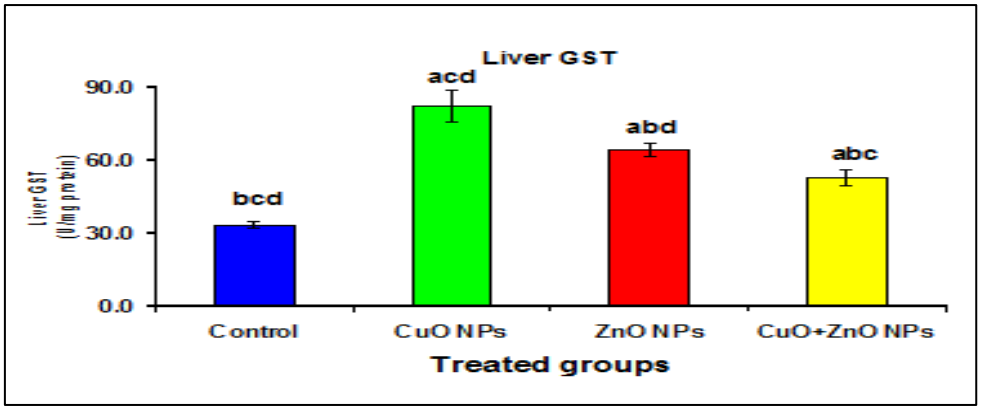


Figure 5: Liver microsomal cytochrome C-reductase (nmole cytochrpme C-reductase/mg protein/min) of rat treated with zinc oxide (ZnO) and copper oxide (CuO) nanoparticles (NPs). Significance at P > 0.05. Significance at P > 0.05. a Comparison of control and other groups; b Comparison of CuO NPs and other groups; c Comparison of ZnO NPs and other groups; d Comparison of CuO+ZnONPs and other groups

Figure 6: Liver GST (U/mg protein) 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

4.3. Effects of copper oxide nanoparticles (CuO NPs) and zinc oxide nano-particles (ZnO NPs) on rats liver thiobarbituric acid reactive substances (TBARS).

Zinc oxide (ZnO) is being used worldwide in consumer products and industrial applications. As humans are being directly exposed to ZnO nanoparticles (NPs) through different routes, it is likely that the NPs would gain access to the liver (Sharma, V. *et al*., 2011). Therefore, the present study investigated the cytotoxic potential of ZnO nanoparticles in rats liver TBARS.

Oxidative stress induces lipid peroxidation that can be quantified by TBARS measure [36]. Data revealed a significant increase ($p<0.05$) in liver homogenates TBARS concentration in rats treated with CuO and ZnO NPs individually, while this elevation was more pronounced when animals given the mixture of of tesed NPs compared to control (Table 2, Figure 7).

Jomova and Valko (Jomova, K., & Valko, M. 2011) reported that detailed studies in the past two decades have shown that redox active metal like Cu and other metals undergo redox cycling reactions and possess the ability to produce reactive radicals such as superoxide anion radical and nitric oxide in biological systems. Disruption of metal ion homeostasis may lead to oxidative stress, a state where increased formation of ROS overwhelms body antioxidant protection and subsequently induces DNA damage, lipid peroxidation, protein modification and other effects, all symptomatic for numerous diseases, involving cancer, cardiovascular disease, diabetes, atherosclerosis, neurological disorders (Alzheimer's disease, Parkinson's disease), chronic inflammation and others. The underlying mechanism of action for all these metals involves formation of the superoxide radical, hydroxyl radical (mainly via Fenton reaction) and other ROS, finally producing mutagenic and carcinogenic malondialdehyde (MDA), 4-hydroxynonenal (HNE) and other exocyclic DNA adducts. CuONP may induce serum TBARS due to free radical produced from the metal leads to lipid peroxidation in the present study. Also, supporting the present observation CuO NPs were also found to induce oxidative stress in a concentration-dependent manner, which was indicated by induction of ROS and lipid peroxidation along with glutathione depletion (Akhtar, M. J. *et al*., 2016).

Chusuei *et al*., (2013) indicated that cytotoxicity is a function of particle surface charge, the relative number of available surface binding sites, and metal ion dissolution from NPs. These findings provide a physicochemical basis for both risk assessment and the design of safer nanomaterials (Chusuei, C. C. *et al*., 2013).

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

Zinc oxide nanoparticles (ZnO NPs) are one of the most abundantly used nanomaterials in consumer products and biomedical applications. As a result, human exposure to these NPs is highly frequent and they have become an issue of concern to public health (Valdiglesias, V. *et al*., 2013). Although toxicity of ZnO NPs has been extensively studied and they have been shown to affect many different cell types and animal systems, there is a significant lack of toxicological data for ZnO NPs on animals' immune system.

Alteration in total GSH (tGSH) level content in cells can be considered as an indication of adaptive response of the cell to oxidative damage. As shown in Table 2 and Figure 8, CuO NP, ZnO NP and their mixture significantly decreased the tGSH level compared with control values ($p < 0.05$) in liver homogenate. Intracellular tGSH was greatly reduced indicating functional damage to liver cells. CuO-nano toxicity is predominantly mediated by intracellular uptake and subsequent release of copper ions (Cronholm, P. *et al*., 2013).

The results showed time dependent significant generation of oxidative stress in the liver. This was evident by an increased Malondialdehyde (MDA), the end product of lipid peroxidation and decreased GSH level in the liver of rat treated with CuO NPs when compared to the liver of control rat. These findings were coincided with Long *et al*., (2007), Ma *et al*., (2010) who stated that another nanopaticles; TiO_2NP have more biological activities to produce ROS. The results of the present study revealed depletion in GSH level of the liver of CuONPs rat. Pompella *et al*., (2003) stated that GSH is an endogenous, peptidal, antioxidant, which prevents damage to the cellular components by ROS and peroxides. In addition to working as a direct free-radical scavenger, GSH also functions as a substrate for GPx and GST. Glutathione-S-transferase (GST) plays a critical role in defending the organism against reactive electrophiles by removing them through conjugation with GSH (Pompella, A. *et al*., 2007). In addition to the functions of GSH itself, the GSH/GSSG redox couple acts to maintain the redox environment of the cell. This observation may have important implications in nanocopper-induced nephrotoxicity, because the glutathione production pathway can be considered as a first-line defense against oxidative stress, a common toxicological mechanism leading to cell death.

Down regulation of the pathways related to the oxidative stress response, including glutathione metabolism and antioxidant genes, may also be related to the mechanism of nanocopper-induced renal injury. Glutathione is the most abundant antioxidant in cells, where it is formed predominantly in two redox forms: reduced (GSH) and oxidized (GSSG). Glutathione and glutathione-associated metabolism provide the major line of defense for protecting cells from oxidative and other forms of stress (Hayes, J. D., & McLELLAN, L. I. 1999).

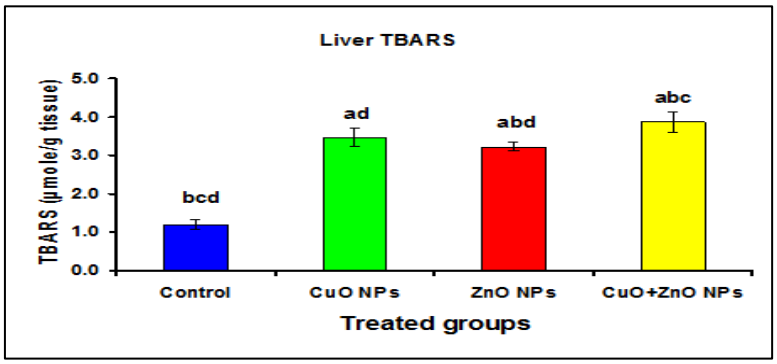


Figure 7: Liver TBARS (µmole/g tissue) of rat treated with zinc oxide (ZnO) and copper oxide (CuO) nanoparticles (NPs). Significance at P > 0.05. Significance at P > 0.05. a Comparison of control and other groups; b Comparison of CuO NPs and other groups; c Comparison of ZnO NPs and other groups; d Comparison of CuO+ZnONPs and other groups

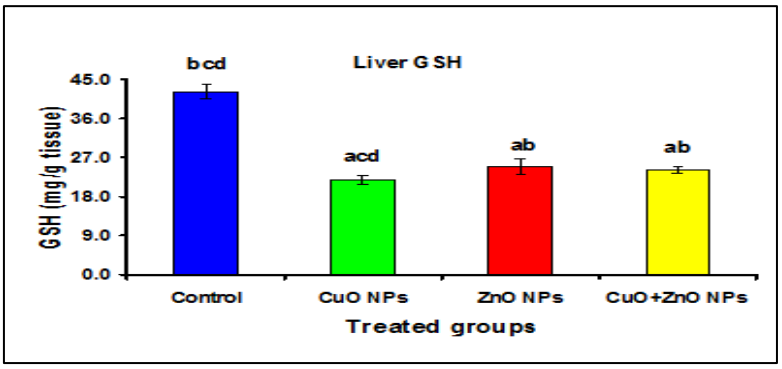


Figure 8: Liver GSH (mg/g tissue) of rat treated with zinc oxide (ZnO) and copper oxide (CuO) nanoparticles (NPs). Significance at P > 0.05. a Comparison of control and other groups; b Comparison of CuO NPs and other groups; c Comparison of ZnO NPs and other groups; d Comparison of CuO+ZnONPs and other groups

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.

REFERENCES

1. Libralato, G., Galdiero, E., Falanga, A., Carotenuto, R., De Alteriis, E., & Guida, M. (2017). Toxicity effects of functionalized quantum dots, gold and polystyrene nanoparticles on target aquatic biological models: a review. Molecules, 22(9), 1439.
2. Valko, M., Rhodes, C., Moncol, J., Izakovic, M. M., & Mazur, M. (2006). Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chemico-biological interactions, 160(1), 1-40.
3. Gonzalez, L., Lison, D., & Kirsch-Volders, M. (2008). Genotoxicity of engineered nanomaterials: a critical review. Nanotoxicology, 2(4), 252-273.
4. Oberdörster, G., Ferin, J., & Lehnert, B. E. (1994). Correlation between particle size, in vivo particle persistence, and lung injury. Environmental health perspectives, 102(suppl 5), 173-179.
5. Li, X., Lu, X., Xu, H., Zhu, Z., Yin, H., Qian, X., ... & Liu, B. (2011). Paclitaxel/tetrandrine coloaded nanoparticles effectively promote the apoptosis of gastric cancer cells based on “oxidation therapy”. Molecular pharmaceutics, 9(2), 222-229.
6. Halliwell, B. (1989). The chemistry of oxygen radicals and other oxygen-derived species. Free radicals in biology and medicine, 29-32.
7. Evans, M. D., Dizdaroglu, M., & Cooke, M. S. (2004). Oxidative DNA damage and disease: induction, repair and significance. Mutation Research/Reviews in Mutation Research, 567(1), 1-61.
8. Lubec, G. (1996). The hydroxyl radical: from chemistry to human disease. Journal of investigative medicine: the official publication of the American Federation for Clinical Research, 44(6), 324.
9. Fang, Y.Z., Yang, S., & Wu, G. (2002). Regulation of Physiological Systems by Nutrients, Free Radicals, Antioxidants and Nutrition, Nutrition, 18 (10), 872– 879.
10. Wu, D., & Cederbaum, A. I. (2003). Alcohol, oxidative stress, and free radical damage. Alcohol Research and Health, 27, 277-284.
11. Goth, L. (1991). A simple method for determination of serum catalase activity and revision of reference range. Clinica chimica acta, 196(2-3), 143-151.
12. Slater, T. F., & Sawyer, B. C. (1971). The stimulatory effects of carbon tetrachloride and other halogenoalkanes on peroxidative reactions in rat liver fractions in vitro. General features of the systems used. Biochemical Journal, 123(5), 805-814.
13. Moron, M. S., Depierre, J. W., & Mannervik, B. (1979). Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochimica et Biophysica Acta (BBA)-General Subjects, 582(1), 67-78.
14. Omura, T., & Sato, R. (1964). The carbon monoxide-binding pigment of liver microsomes I. Evidence for its hemoprotein nature. Journal of Biological Chemistry, 239(7), 2370-2378.
15. Williams, C. H., & Kamin, H. (1962). Microsomal triphosphopyridine nucleotide-cytochrome c reductase of liver. Journal of Biological Chemistry, 237(2), 587-595.
16. Habig, W., Pabst, M. J., & Jakoby, W. B. (1974). The first enzymatic step in mercapturic acid formation. Glutathione-S-transferase. J Biol Chem, 249, 7130-7139.
17. Jomova, K., & Valko, M. (2011). Advances in metal-induced oxidative stress and human disease. Toxicology, 283(2-3), 65-87.
18. Shinmoto, H., DOSAKO, S. I., & Nakajima, I. (1992). Anti-oxidant activity of bovine lactoferrin on iron/ascorbate induced lipid peroxidation. Bioscience, biotechnology, and biochemistry, 56(12), 2079-2080.
19. Bi, Y., Chen, W., Zhang, W., Zhou, Q., Yun, L., & Xing, D. (2009). Production of reactive oxygen species, impairment of photosynthetic function and dynamic changes in mitochondria are early events in cadmium-induced cell death in Arabidopsis thaliana. Biology of the Cell, 101(11), 629-643.
20. Pompella, A., Visvikis, A., Paolicchi, A., De Tata, V., & Casini, A. F. (2003). The changing faces of glutathione, a cellular protagonist. Biochemical pharmacology, 66(8), 1499-1503.
21. Nemmar, A., Hoet, P. M., Vanquickenborne, B., Dinsdale, D., Thomeer, M., Hoylaerts, M. F., ... & Nemery, B. (2002). Passage of inhaled particles into the blood circulation in humans. Circulation, 105(4), 411-414.
22. Oberdörster, G., Oberdörster, E., & Oberdörster, J. (2005). Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environmental health perspectives, 113(7), 823-839.
23. De Jong, W. H., Hagens, W. I., Krystek, P., Burger, M. C., Sips, A. J., & Geertsma, R. E. (2008). Particle size-dependent organ distribution of gold nanoparticles after intravenous administration. Biomaterials, 29(12), 1912-1919.

24. Jain, T. K., Reddy, M. K., Morales, M. A., Leslie-Pelecky, D. L., & Labhasetwar, V. (2008). Biodistribution, clearance, and biocompatibility of iron oxide magnetic nanoparticles in rats. Molecular pharmaceutics, 5(2), 316-327.
25. Siddiqui, M. A., Alhadlaq, H. A., Ahmad, J., Al-Khedhairy, A. A., Musarrat, J., & Ahamed, M. (2013). Copper oxide nanoparticles induced mitochondria mediated apoptosis in human hepatocarcinoma cells. PloS one, 8(8), e69534.
26. Yeragi, S. G., Rana, A. M., & Koli, V. A. (2003). Effect of pesticides on protein metabolism of mud skipper Boleophthalmus dussumieri. Journal of Ecotoxicology & Environmental Monitoring, 13(3), 211-214.
27. Jorquera, F., Culebras, J. M., & González-Gallego, J. (1996). Influence of nutrition on liver oxidative metabolism. Nutrition, 12(6), 442-447.
28. Kwak, M., Kim, S., Kwak, J., Novak, R., & Kim, N. (1994). inhibition of P450 2E1 expression by organosulfur compounds allylsulfide, allylmercaptan and allylmethylsulfide in rats. Biochem. Pharmacol., 47: 531-539.
29. Reactive intermediates and interaction with biological systems. In: Volans, G. N., Sims, J., Sullivan, F. and Turner (Eds.), Basic Science in Toxicology
30. George, S., Pokhrel, S., Xia, T., Gilbert, B., Ji, Z., Schowalter, M., ... & Nel, A. E. (2009). Use of a rapid cytotoxicity screening approach to engineer a safer zinc oxide nanoparticle through iron doping. ACS nano, 4(1), 15-29.
31. Wang, Z., Li, N., Zhao, J., White, J. C., Qu, P., & Xing, B. (2012). CuO nanoparticle interaction with human epithelial cells: cellular uptake, location, export, and genotoxicity. Chemical research in toxicology, 25(7), 1512-1521.
32. Vermilion, J. L., Ballou, D. P., Massey, V., & Coon, M. J. (1981). Separate roles for FMN and FAD in catalysis by liver microsomal NADPH-cytochrome P-450 reductase. Journal of Biological Chemistry, 256(1), 266-277.
33. Dalvi, R. R. (1992). Alterations in hepatic phase I and phase II biotransformation enzymes by garlic oil in rats. Toxicology letters, 60(3), 299-305.
34. Sheweita, S. A., El-Gabar, M. A., & Bastawy, M. (2001). Carbon tetrachloride changes the activity of cytochrome P450 system in the liver of male rats: role of antioxidants. Toxicology, 169(2), 83-92.
35. Sharma, V., Anderson, D., & Dhawan, A. (2011). Zinc oxide nanoparticles induce oxidative stress and genotoxicity in human liver cells (HepG2). Journal of Biomedical Nanotechnology, 7(1), 98-99.
36. Mauriz, J. L., Matilla, B., Culebras, J. M., Gonzalez, P., & González-Gallego, J. (2001). Dietary glycine inhibits activation of nuclear factor kappa B and prevents liver injury in hemorrhagic shock in the rat. Free Radical Biology and Medicine, 31(10), 1236-1244.
37. Akhtar, M. J., Kumar, S., Alhadlaq, H. A., Alrokayan, S. A., Abu-Salah, K. M., & Ahamed, M. (2016). Dose-dependent genotoxicity of copper oxide nanoparticles stimulated by reactive oxygen species in human lung epithelial cells. Toxicology and industrial health, 32(5), 809-821.
38. Chusuei, C. C., Wu, C. H., Mallavarapu, S., Hou, F. Y. S., Hsu, C. M., Winiarz, J. G., ... & Huang, Y. W. (2013). Cytotoxicity in the age of nano: the role of fourth period transition metal oxide nanoparticle physicochemical properties. Chemico-biological interactions, 206(2), 319-326.
39. Valdiglesias, V., Costa, C., Kiliç, G., Costa, S., Pásaro, E., Laffon, B., & Teixeira, J. P. (2013). Neuronal cytotoxicity and genotoxicity induced by zinc oxide nanoparticles. Environment international, 55, 92-100.
40. Cronholm, P., Karlsson, H. L., Hedberg, J., Lowe, T. A., Winnberg, L., Elihn, K., ... & Möller, L. (2013). Intracellular uptake and toxicity of Ag and CuO nanoparticles: a comparison between nanoparticles and their corresponding metal ions. Small, 9(7), 970-982.
41. Long, T.C., Tajuba, J., Sama, P., Saleh, N., Swartz, C., Parker, J., Hester, S., Lowry, G.V., & Veronesi, B. (2007). Nano-TiO2 stimulates ROS in brain microglia and damages neurons in vitro. Environ. Health Perspect, 115: 1631-1637.
42. Ma, L., Liu, J., Li, N., Wang, J., Duan, Y., Yan, J., ... & Hong, F. (2010). Oxidative stress in the brain of mice caused by translocated nanoparticulate TiO2 delivered to the abdominal cavity. Biomaterials, 31(1), 99-105.
43. Maher, P. (2005). The effects of stress and aging on glutathione metabolism. Ageing research reviews, 4(2), 288-314.
44. Hayes, J. D., & McLELLAN, L. I. (1999). Glutathione and glutathione-dependent enzymes represent a co-ordinately regulated defence against oxidative stress. Free radical research, 31(4), 273-300.