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Dyslipidemia Induced by Copper Oxide and/or Zinc Oxide Nanoparticles in Male Albino Rats

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ABSTRACT

Background: *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. The toxicological investigations of nanoparticles are highly recommended because of the increasing use in various industrial and consumer products.*

Objectives: *The aim of the present study was to evaluate the effects of copper oxide and/or zinc oxide nanoparticles on serum lipids profile 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 samples were taken and prepared for biochemical measurements.*

Results: *Serum chloesterol and triglycerides were decreased due to exposure of rats to CuONPs, ZnONPs and their mixture compared to control group. Also, a reduction in serum high density lipoprotein was observed compared to control groups, while serum low density lipoprotein was increased in rats treated with CuONPs, ZnONPs and their mixture. Conclusion: It can be concluded that exposure of rats to copper oxide and zinc oxide nano-particle produced different patterns of dyslipidemia. 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 and ZnO nanoparticles mixture, Lipid profile, Dyslipidemia.*

INTRODUCTION

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 [1, 2].

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. 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 [3].

Various scientific groups are keen about this technology and are devoting themselves to the development of more, new, and better nano-materials. In the near future, expectations are that no field will be left untouched by the

magical benefits available through application of nanotechnology [2].

Evaluation of nonmaterial's interaction with blood ingredients is a part of preclinical risk assessment of newly-synthesized materials, especially for nano-sized pharmaceuticals which are intravenously administrated. Nanoparticles could induce oxidative stress that eventually leads to cell toxicity and hemolysis of RBCs at certain doses [4].

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 nano-materials is dependent on the chemical nature of the nanoparticles [5].

Engineered nanoparticles are developed for various applications in industrial, electrical, agricultural, pharmaceutical and medical fields due to their unique properties. Nanoparticles such as TiO_2 and ZnO are widely used in cosmetics for UV protection. The toxicological investigations of ZnO NPs are highly recommended because of the increasing use in various industrial and consumer products. The toxic potential of ZnO NPs was assumed to be caused by the release of free Zn+ ions in the medium. Exposure to ZnO NPs significantly affected cellular viability in a dose-dependent manner. Formation of reactive oxygen species (ROS) was found to be the mechanism of cellular toxicity. The release of Zn^+ ions from the nanoparticles, due to the instability of ZnO NPs in the acidic compartment of lysosomes, also increases the ROS generation. In addition to increased ROS production, damage of lysosomal membrane and the activation of executioner caspase-3 and caspase-7 were observed, which eventually ends in apoptosis [6].

Copper oxide nanoparticles (CuO NPs) are increasingly used in various applications. Cell viability was reduced by CuO NPs and degree of reduction was dose dependent. CuO NPs were also found to induce oxidative stress in dose-dependent manner indicated by depletion of glutathione and induction of lipid peroxidation, catalase and superoxide dismutase [7].

Objectives

The aim of the present study was to evaluate the effects of copper oxide and/or zinc oxide nanoparticles on serum cholesterol, triglycerides, high density lipoprotein (HDL), and low density lipoprotein (LDL) concentrations in male albino rats.

Material and Methods

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*., [8].

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°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 [9].

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. 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°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°C until used (within 24 hours).

Biochemical Parameters in Rat Serum

Determination of Cholesterol

Cholesterol was determined after enzymatic hydrolysis and oxidation according to the method described by Richmond [10].

Determination of Triglycerides

Triglycerides were determined according to the method described by Carr *et al.* [11].

LDL-Cholesterol

LDL Cholesterol test results are based on a reading of light reflected off a test strip that has changed color after blood is applied. The intensity of the color is directly proportional to the concentration of LDL cholesterol in the sample. The analyzer converts this reading into a LDL cholesterol result and displays it. This test, which selectively measures LDL cholesterol, is an enzymatic colorimetric test based on the "Trinder Method" for the determination of cholesterol. In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase to cholesterol-4-en-one and hydrogen peroxide. In the presence of peroxidase, hydrogen peroxide reacts with 4-aminoantipyrine and N, Ndisubstituted aniline to form a blue dye [12].

HDL-Cholesterol

Phossphotungstic acid and magnesium ions selectively precipitating all lipoproteins except the HDL fraction-cholesterol present in the supernatant can be determined by the same method used for total cholesterol [13].

Statistical Analysis

Values obtained as mean ± SEM were subjected to one-way analysis of variance (ANOVA) followed by Tukey test using GraphPad Prism version 5.0 for windows from GraphPad Software, San Diego, California, USA). Values of $P < .05$ were considered significant.

Results and Discussion

Cholesterol is a lipid found in the cell membranes of all animal tissues, and it is transported in the blood plasma of all animals. Cholesterol is also a sterol (a combination steroid and alcohol). Although insignificant decrease found in blood cholesterol belonged to animals groups intoxicated by CuO and ZnO NPs individually when compared to control group (Table 1 and Figure 1).

Triglycerides, as major components of very low density lipoprotein and chylomicrons, play an important role in metabolism as energy sources and transporters of dietary fat. They contain more than twice energy as carbohydrates and proteins. There are a significant reduction in serum triglycerides concentration is appeared in rats treated with CuO and ZnO NPs and their mixture compared to control group (Table 1 and Figure 2). The present study has also demonstrated a relatively lower triglycerides serum level in the CuO and ZnO NPs treated groups and this observation agreed with the idea that relative hypotriglyceridemia in the CuO and ZnO NPs group may have resulted from liver damage or hypolipoproteinemia, causing reduced interaction of triglycerides serum with the lipoproteins, especially VLDL. The CuO and ZnO NPs evoked hypotriglyceridemia may have been due to impaired fatty acids synthesis, enhanced catabolism of VLDL, activation of Lecithin: Cholesterol Acyltransferase (LCAT) and tissue lipases [14], inhibition of acetyl-CoA carboxylase [15], and production of triglycerides precursors such acetyl-CoA and glycerol phosphate.

Table1. *Effects of treatment of rats with zinc oxide and/or copper oxide nanoparticles on serum cholesterol, triglycerides, HDL, and LDL*

Groups	Groups			
Parameters	Control	CuONP	ZnONP	CuO + ZnONP
	Mean±SE	Mean± SE	Mean± SE	Mean± SE
Serum Cholesterol (mg/dl)	80.80 ± 3.71 [bcd]	64.00 ± 3.29 [a]	65.40 ± 8.01 [a]	62.00 ± 5.18 [a]
Serum Triglycerides (mg/dl)	24.42 ± 1.42 [bcd]	43.56 ± 7.85 [ad]	40.88 ± 5.93 [a]	34.26 ± 3.49 [ab]
Serum HDL (mg/dl)	25.46± 3.49[bcd]	18.56± 1.83[ac]	14.76± 0.74[abd]	19.72± 2.27[ac]
Serum LDL (mg/dl)	24.42 ± 1.42 [bcd]	43.56 ± 7.85 [ad]	40.88 ± 5.93 [a]	34.26 ± 3.49 [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

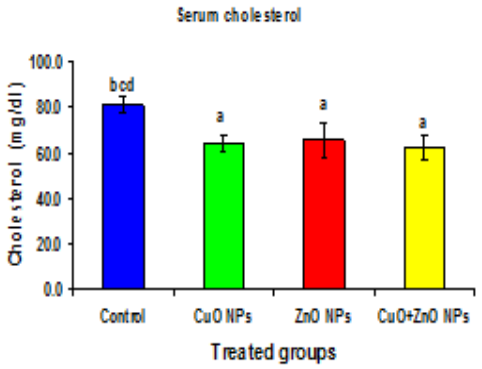

Figure1. ***Serum Cholesterol of rat treated with zinc oxide (ZnO) and copper oxide (CuO) nanoparticles (NPs).*** *Significance at P < 0.05. Significance at P < 0.05. [a] Comparison of control and other groups; [b] Comparison of CuO NPs and other groups; [c] Comparison of ZnO NPs and other groups; [d] Comparison of CuO+ZnONPs and other groups*

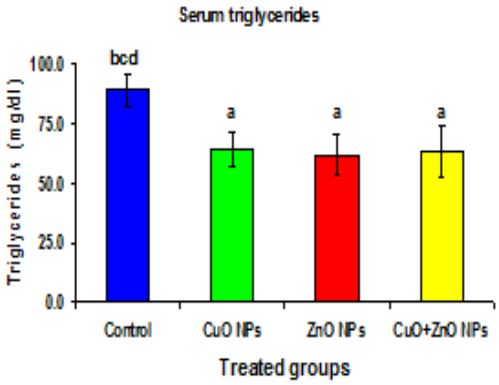

Figure2. ***Serum Triglycerides of rat treated with zinc oxide (ZnO) and copper oxide (CuO) nanoparticles (NPs)****. Significance at P < 0.05. a Comparison of control and other groups; b Comparison of CuO NPs and other groups; c Comparison of ZnO NPs and other groups; [d] Comparison of CuO+ZnONPs and other groups*

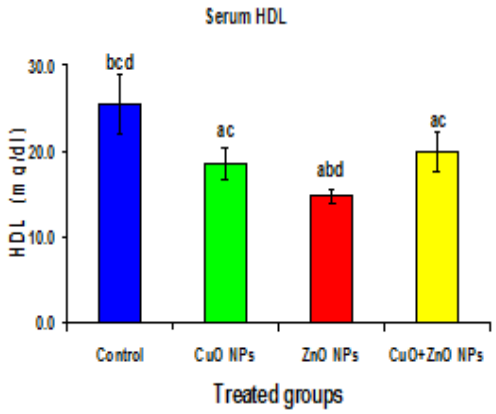

Figure3. ***Serum HDL of rat treated with zinc oxide (ZnO) and copper oxide (CuO) nanoparticles (NPs).*** *Significance at P < 0.05. Significance at P < 0.05. [a] Comparison of control and other groups; [b] Comparison of CuO NPs and other groups; [c] Comparison of ZnO NPs and other groups; [d] Comparison of CuO+ZnONPs and other groups*

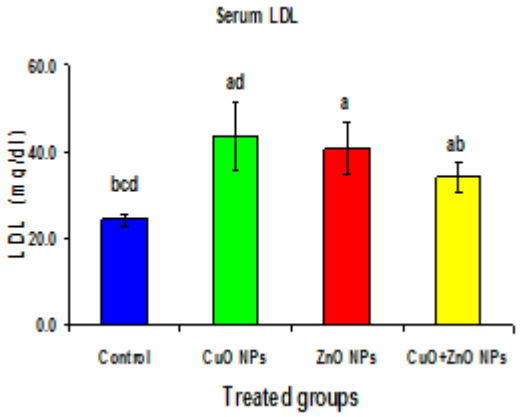

Figure4. ***Serum LDL of rat treated with zinc oxide (ZnO) and copper oxide (CuO) nanoparticles (NPs)****. Significance at P < 0.05. a Comparison of control and other groups; b Comparison of CuO NPs and other groups; c Comparison of ZnO NPs and other groups; [d] Comparison of CuO+ZnONPs and other groups*

Most of the circulating cholesterol is carried in birds by high-density lipoprotein cholesterol (α-2globulin fraction) and LDL (β-globulin fraction) [16]. These lipoproteins became the principal cholesterol transport and carried about 40 to 44% of the total serum proteins [17]. High-density lipoproteins (HDL) form a class of lipoproteins, varying somewhat in their size (8–11 nm in diameter), that carry fatty acids and cholesterol from the body's tissues to the liver. About thirty percent of blood cholesterol is carried by HDL, it is hypothesized that HDL can remove cholesterol from atheroma within arteries and transport it back to the liver for excretion or re-utilization—which is the main reason why HDL-bound cholesterol is sometimes called good cholesterol. A high level of good cholestrol seems to protect against cardiovascular diseases, and low HDL cholesterol levels increase the risk for heart disease [18].

HDL, which is mainly synthesized in the liver and intestinal cells plays an important role in cholesterol efflux from tissues and carries it back to the liver for removal as bile acids [19]. HDL concentrations in rats treated with CuO, ZnO NPs and their mixture significantly less than control group (Table 1 and Figure 3).

It has been established that the elevated serum or plasma HDL levels have anti-atherogenic effect [20], whereas the reduced levels are associated with an increased risk for coronary artery disease. High level of HDL may compete with LDL receptor sites on arterial smooth muscle cells and thus partially inhibit uptake and degradation of LDL. Also, HDL could protect LDL against oxidation *in vivo* because the lipids in HDL are preferentially oxidized

before those in LDL [18]. The decrease in HDL due to CuO and ZnO NPs in the present study may leads to increase in LDL because it hasn't a sufficient protection.

Treatment of rats with CuO, ZnO NPs and their mixture induced elevation in LDL serum levels compared to control group (Table 1 and Figure 4). Several lines of evidence suggest that oxidative modification of plasma LDL plays a major role in the pathogenesis of atherosclerosis [21]. The increased LDL may be due to hypercholestrolemia, which suppresses the formation of new LDL receptors thereby decreasing the cellular intake of cholesterol in the form of LDL. Although hypercholesterolemia is universally accepted as a major risk factor for atherosclerosis, but at any given serum cholesterol concentration, there is variability in the occurrence of cardiovascular events, as it has been shown that the oxidative modification of LDL may be a crucially important step in the development of atherosclerotic plaque [22].

LDL transport cholesterol to the arteries where it is retained by arterial proteoglycans, which initiate the formation of plaques. LDL oxidation is thought to be the first step of atherogenesis, followed by foam cell, fatty streakand plaque formation [23]. It has been hypothesized that LDL can be transported through endothelial tight junctions and/or endothelial transcytosis from the lumen into the intima [24], in which blood antioxidants are unlikely to be available and therefore prone to undergo atherogenic oxidative changes [23].

Conclusion

It can be concluded that exposure of rats to copper oxide and zinc oxide nano-particle produced different patterns of dyslipidemia. Caution should be taken in nano-particles use in work place, preparations as well as while handling.

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