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

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ABSTRACT

Background: *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.*

Objectives: *The aim of the present study was to evaluate the hematological changes induced by copper oxide and/or zinc oxide nanoparticles 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 for hematological evaluation.*

Results: *Significant increase in WBC in the CuONPs and/or ZnONPs treated groups compared to the control group. Pronounced increase in WBC due treatments of rats with nanoparticles mixture compared to the individual treatments of each nanoparticles tested. The RBCs, Hb and HCT counts and concentration were significantly lower in the CuONPs, ZnONPs and their mixture treated groups compared to the control group. Significant increase in MCV and decrease in MCH values while no determine changes were observed in MCHC values in rats treated with CuONPs, ZnONPs and their mixture compared to the control group. Significant decrease in blood platelet levels in rats treated with CuONPs, ZnONPs and their mixture treated groups compared to the control group. Pronounced increase in platelet due treatments of rats with nanoparticles mixture compared to the individual treatments of each nanoparticles tested.*

Conclusion: *It can be concluded that Copper oxide and/or zinc oxide nano-particles induced changes in hematological parameters. 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 workplace, preparations as well as while handling.*

Keywords: *Copper oxide nanoparticles, Zinc oxide nanoparticles, Hematotoxicity, Hematological changes, CBC, RBCs, WBCs, Platelets*

INTRODUCTION

Nanomaterials are defined as a new class of materials having unique physical and chemical properties. There is great interest in applying nanomaterials in different fields of medicine and industry. The potential benefit of nanomaterials in medicinal devices or drug delivery proposes new methods in diagnosis, medication, and treatment of complicated diseases such as cancers [1].

Metal oxide nanoparticles are often used as industrial catalysts and elevated levels of these

particles have been clearly demonstrated at sites surrounding factories. The manufacture and use of metal oxide nanoparticles is continuously expanding due to their wide applications and unique physicochemical properties. It becomes increasingly important to investigate and identify their possible toxicological effects and to identify which particles pose the greatest harm to human health [2].

Copper oxide nanoparticles (CuONPs) are increasingly used in various applications such as catalysts, gas sensor, heat transfer fluids, microelectronics and cosmetics [3, 4]. Sizova *et al*. [5] reported that despite an increasing application of copper nanoparticles, there is a serious lack of information concerning their impact on human health and the environment.

Zinc oxide nanoparticles (nano-ZnO) are one of the most commonly used nonmaterial's, with industrial and commercial applications, including personal skin and hair care products, sunscreens, pigments, coatings, ceramic products, and paints [6-9].

Decreasing size results in increasing NPs' specific surface area, which promotes not only the accumulation of NPs, but also an increase of reactivity and enhanced interactions between NPs and biomolecules. Because of their tiny size, NPs can cross the small intestine by persorption and further distribute into the blood, brain, kidney, and liver [10-12]. The release of both inorganic and organic hazardous substances is causing degradation of natural resources, making them unsuitable for use. Heavy metals form one such group of materials that are capable of causing many patho-physiological conditions in living organisms [13, 14]. An increasing body of evidence shows that excessive presence of essential metals as well as minute presence of non-essential metals can cause many patho-physiological complications in the living organisms via generation of reactive species [15].

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. The red blood cells (RBCs) are susceptible to oxidative stress damage. Nanoparticles could induce oxidative stress that eventually leads to cell toxicity and hemolysis of RBCs at certain doses [1].

Several studies have shown that the unique structure and size of the nanoparticles could lead to oxidative injury and cell toxicity in different cell cultures and also in blood cells in vivo and in vitro [1, 16-19]. Smaller nanosized particles caused thrombolytic and granulocyte activation and hemolysis and induced inflammation and hemolysis in human blood samples [12, 18].

OBJECTIVES

The objective of this study was to evaluate the hematological changes induced by CuO and/or Zn-O nano-particles in male rats.

MATERIALS AND METHODS

Chemicals

Copper oxide and Zinc oxide as nanoparticles with an average size of 6 and 51 nm, respectively, is 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*., [20].

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 [21].

Experimental Protocol

Adult male 20 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 [22].

Group IV (ZnONPs + CuONPs): Rats were given orally ZnONPs (10 mg/kg/day) followed 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; 1 ml were collected on sodium heparin for hematological studies.

Hematological Investigation

One milliliters of blood collected into heparinized sample bottles were analyzed for hematological parameters; white blood cells (WBCs), red blood cells (RBCs) hemoglobin (Hb), mean cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), hematocrit (HCT) and total platelets count using an automatic hematological assay analyzer, Advia 60 Hematology system (Bayer Diagnostics Europe Ltd, Ireland).

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.

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 [23-26]. Such translocation depends on the physicochemical properties of NPs, and their migration to distant sites is an important issue with regard to their toxicity.

Effects of Copper Oxide Nanoparticles (CuO NPs) and Zinc Oxide Nano-Particles (ZnO NPs) and Their Mixture on Rats Hemoglobin (Hb) and Red Blood Cells (RBCs) Concentrations

Data obtained as a result of comparing haematological parameters in control and treated groups are shown in Tables 1, 2. Compared to the control rats, those after treatment with CuO NPs, ZnO NPs and their mixture had significantly lower red blood cell count (RBC) ($p<0.05$) and haemoglobin (Hb) (Table 1 & Figures 1,2).

The low Hb shows that chronic CuO NPs and ZnO NPs administration causes anemia. This agreed with earlier findings [27]. He attributed the anemia to the ability of copper to reduce serum iron concentration, thereby compromising the synthesis of Hb. The anemia may also be related to interference with Hb synthesis and shortening of RBC lifespan [27]. Copper ions are able to generate oxidative stress [28]. Although, oral administration of copper oxide nanoparticles induces hepatotoxicty and nephrotxicity in exposed rats [29], it also produce lipid peroxidation. The increased lipoperoxidation in the CuO NPs group, reflected by significant TBARS concentration, may have caused increased vulnerability of the RBC to destruction, but may directly destroy the erythrocytes thereby leading to anemia. MDA is a major oxidation product of peroxidized polyunsaturated fatty acids (PUFA's), and increased MDA content is an important indicator of lipid peroxidation [30].

It is predicted that high concentration of CuO NPs and ZnO NPs nanoparticles, reduced the number of blood cells due to inhibition of cell activity, antimiotic properties and also stimulation of oxidative stress in cells, reduction of cellular antioxidants and Increasing of involvement cells in the immune processes. In 1989 Machiedo *et al* demonstrated that free radicals that produced from nanoparticles can be main cause destruction of red blood cells [31]. Whatever nanoparticles diameters were smaller; the increase they influence on cell and intracellular molecular mechanisms [32, 33].

Effects of Copper and Zinc Oxide Nanoparticles (CuO, ZnO NPs) and Their Mixture on Rats Blood Hematocrit, MCV, MCH, and MCHC Indices

The field of nanotechnology is one of the most active research areas in modern materials science [34-36]. The challenge that nanomaterials pose to environmental health is that they are not one material [36]. Compared to the control rats, those after treatment with CuO NPs, ZnO NPs and their mixture had significantly lower haematocrit percentage (Ht), and mean corpuscular haemoglobin (MCH). However CuO NPs, ZnO NPs and their mixture induced increase in mean corpuscular volume (MCV) compared to control while unchenge in mean corpuscular haemoglobin concentration (MCHC) was oserved (Table .1, Figures 3-6).

Investigated results showed that a decrease of overall mean blood percent hematocrit was seen in CuO NPs, ZnO NPs and their mixture treated group. This percentage of blood made up of RBCs is a frequently measured number and is called the hematocrit**.** The reduction in hematocrite induced by CuONPs, ZnO NPs and their mixture due to the decstruction in RBCs cells and by the fact that the number decreased also the blood percent of hematocrit decreased.

The increased MCV may reflect the presence of immature RBCs in the peripheral blood, perhaps arising from the body compensatory mechanism to cater for the CuO NPs, ZnO NPs and their mixture -induced deficit in RBC concentration. It is difficult to generalize about them because, similar to polymers, they represent a very broad class of systems.

Table1. *Effects of treatment of rats with zinc oxide or copper oxide nanoparticles, and their mixture on RBCs Indices*

Groups	Groups			
Parameters	**Control**	**CuONP**	**ZnONP**	**CuO + ZnONP**
	Mean±SE	Mean± SE	Mean± SE	Mean± SE
RBCs Count (x10^6/µl)	7.22 ± 0.13 ^{bcd}	6.59 ± 0.17 ^{a}	6.52 ± 0.13 ^{a}	6.62 ± 0.19 ^{a}
Hb (g/dl)	13.86 ± 0.31 ^{bcd}	12.06 ± 1.03 ^{a}	12.56 ± 0.65 ^{a}	13.00 ± 0.31 ^{a}
Haematocrit %	43.24 ± 0.75 ^{bcd}	36.34 ± 2.87 ^{a}	38.04 ± 1.15 ^{a}	38.52 ± 0.93 ^{a}
MCV (µ^3)	54.12± 2.96^{bcd}	59.72± 1.24^{a}	61.30± 2.27^{a}	59.86± 1.34^{a}
MCH (pg)	20.26±0.26 ^{bcd}	17.98 ± 1.00 ^{a}	18.98 ± 0.69 ^{a}	19.56 ± 0.56 ^{a}
MCHC (g/dl)	33.78±0.36 ^{bcd}	33.52±0.60 ^{a}	33.56±0.42 ^{a}	33.58±0.55 ^{a}

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*

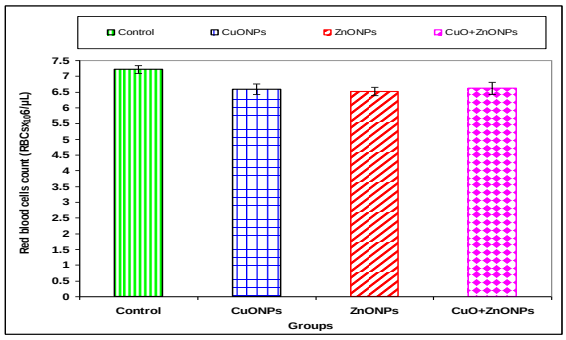

Figure1. *Effect of treatment of rat with zinc oxide (ZnO) and/or copper oxide (CuO) nanoparticles (NPs) on RBCs count (x10^6/µL).*

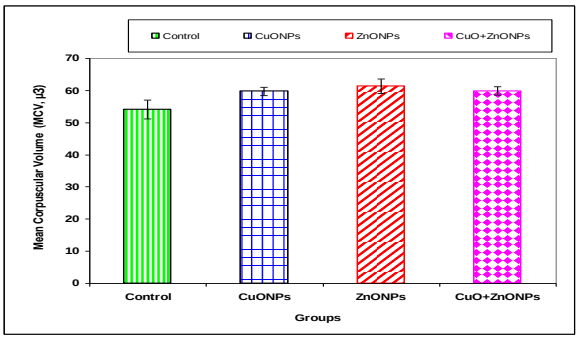

Figure4. *Effect of treatment of rat with zinc oxide (ZnO) and/or copper oxide (CuO) nanoparticles (NPs) on Mean corpuscular volume (MCV, µ^3)*

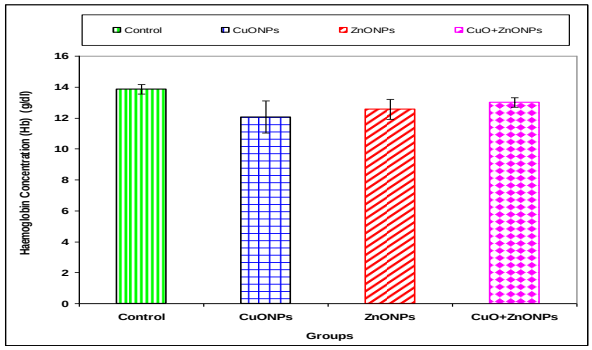

Figure2. *Effect of treatment of rat with zinc oxide (ZnO) and/or copper oxide (CuO) nanoparticles (NPs) on Haemoglobin Concentration (Hb, g/dl*

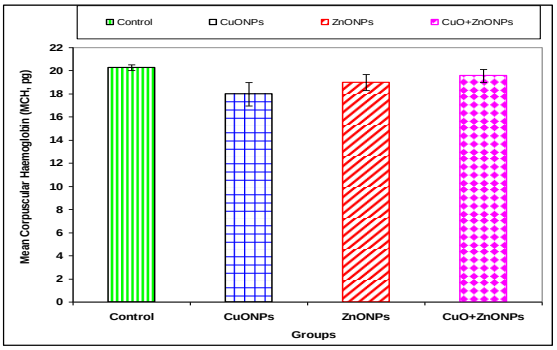

Figure5. *Effect of treatment of rat with zinc oxide (ZnO) and/or copper oxide (CuO) nanoparticles (NPs) on mean corpuscular haemoglobin concentration (MCH, pg)*

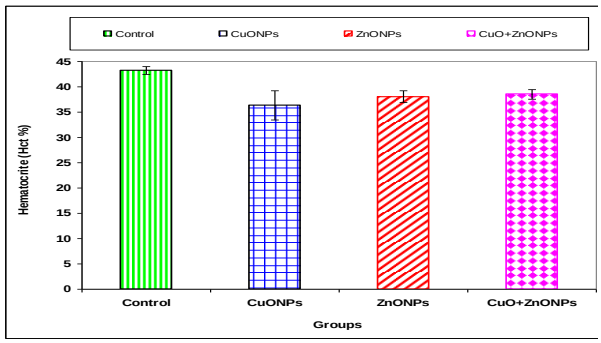

Figure3. *Effect of treatment of rat with zinc oxide (ZnO) and/or copper oxide (CuO) nanoparticles (NPs) on Hematocrit (%)*

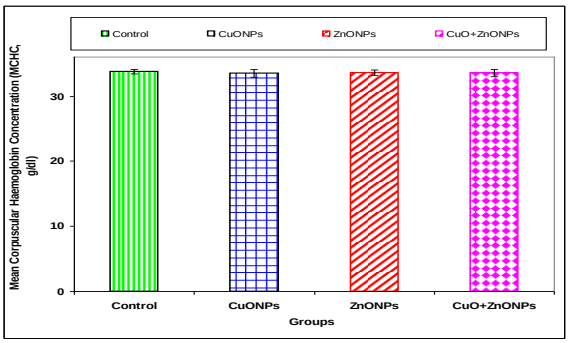

Figure6. *Effect of treatment of rat with zinc oxide (ZnO) and/or copper oxide (CuO) nanoparticles (NPs) on mean corpuscular haemoglobin concentration (MCHC, g/dl)*

Effects of Copper Oxide Nanoparticles (Cuo Nps) and Zinc Oxide Nano-Particles (Zno Nps) on Rats White Blood Cells and Platelets Count

The average number of white blood cells (WBC) from the specified time period after treatment with CuO NPs, ZnO NPs, and their mixtures was measured. The number of WBC was significantly higher in rats treated with CuO NPs, ZnO NPs, and their mixtures compared to either the control ($P < 0.05$) group (Table 2 and Figure 7).

The number of white blood cells was increased due to CuO NPs administration to rats compared to control group. The fourth group, treated with nanoparticles mixture shows pronounced elevation in WBC. Immune response of rats to CuO nanoparticles is increasing the number of phagocytic white blood cells against these nanoparticles. Due to the importance of white cells in defending the body and the important role of hepatocytes in detoxification, any changes done in building and the number can cause very large physiological changes in the human body. However, many applications of nanoparticles in the whole world and especially in our country required precise and comprehensive studies about the effects of nanoparticles on blood cells. As noted at high concentrations of nanoparticles, ZnO and CuO nanoparticles can enter into lymphatic system; then inflammation occurs in lymph nodes. The Induced inflammation in the lymph nodes helps to increase the number of white cells, but after special period, the activity of these glands become weakened and atrophy of lymph nodes make them irreversible.

Compared to the control rats, those after treatment with CuO NPs, ZnO NPs and their mixture had significantly lower platelets count (Table 2 and Figure 8).

The nanoparticle charge plays an essential role in their uptake by platelets and their influence on blood clot formation. The significant decrease in platelet count in the CuONPs, ZnO NPs and their mixture group shows that sub acute exposure to the NPs caused thrombocytopenia. Thrombocytopenia may be related to CuONPs, ZnO NPs and their mixture-induced oxidative damage to the platelet membranes. The challenge that nanomaterials pose to environmental health is that they are not one material [36]. It is difficult to generalize about them because, similar to polymers, they represent a very broad class of systems. Over a third of the atoms in a nanoparticle are at the surface, and these are extremely reactive systems, which in some cases can generate oxygen radicals; Because of the size of nanostructures, it is possible to manipulate the surface interface to allow for interactions with biological systems [37]. Until now it was thought that blood clots can be formed due to three main causes: when the blood flow is obstructed or slowed down, when the vascular endothelial cells are damaged, or due to the blood chemistry. However, it seems possible, in the view of findings that nanoparticles may act as nucleating centers for blood clots [38-40].

Table2. *Effects of treatment of rats with zinc oxide or copper oxide nanoparticles, and their mixture on WBCs and Platelets Count*

Groups	**Groups**			
Parameters	**Control**	**CuONP**	**ZnONP**	**CuO + ZnONP**
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
WBCs Count ($x10^3/\mu l$)	9.48± 0.70 ^{bcd}	12.22± 1.49 ^{ad}	12.76± 0.46 ^{ad}	19.44± 2.12 ^{abc}
Platelets Count ($x10^3/\mu l$	727.80±51.81 ^{bcd}	551.40±33.43^{a}	523.00±46.76^{a}	494.0±29.97^{a}

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

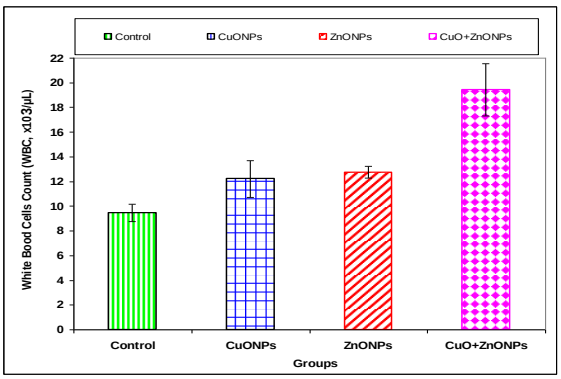

Figure7. *Effect of treatment of rat with zinc oxide (ZnO) and/or copper oxide (CuO) nanoparticles (NPs) on WBCs count ($x10^3/uL$).*

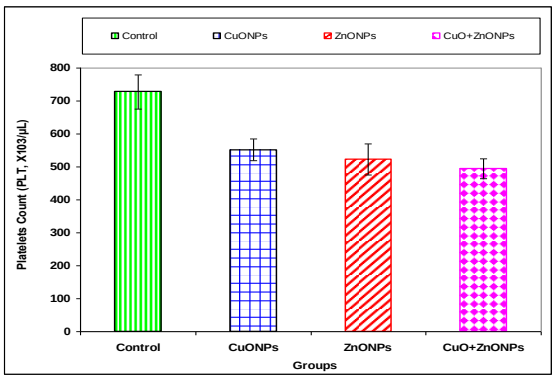

Figure8. *Effect of treatment of rat with zinc oxide (ZnO) and/or copper oxide (CuO) nanoparticles (NPs) on Platelets count ($x10^3/uL$).*

Conclusion

It can be concluded that Copper oxide and/or zinc oxide nano-particles induced changes in hematological parameters. 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 nanoparticles toxicity. Caution should be taken in nano-particles use in work place, preparations as well as while handling.

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