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ORIGINAL ARTICLE

The Toxicity Effect of Cerium Oxide Nanoparticles on Blood Cells of Male Rat

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ABSTRACT
In this work, we investigated the toxicity effect of cerium oxide nanoparticles on blood cells of male Rat. The cerium oxide nanoparticles synthesized and morphological properties of it such as UV-Vis spectrum characterization and Microscopic characterization (SEM&TEM) were investigated. The hematological studies were performed on 40 male rats that divided into five octet groups. After treatment rats with different concentrations of cerium oxide nanoparticles, the results showed that a decrease of overall mean Hematocrit and mean number of Red blood cells (RBC) was seen in all experimental groups; but the white blood cells (WBC) compare to control group, had only a linear increase from first group to third group and in the fourth group, namely the concentrations of 200ppm cerium oxide nanoparticles, the white blood cells was severely reduced and even reached lower than of control group.
Keywords: *ceo2 nanoparticles, blood cells, toxicity effect, Rat*

INTRODUCTION

Nanoscience is one of the most important research and development frontiers in modern science. The use of nanoparticles (NPs) materials offers many advantages due to their unique size and physical properties [1]. Because of the widespread applications of magnetic nanoparticles (MNPs), in biomedical, biotechnology, engineering, material science and environmental areas [2-4], much attention has been paid to the preparation of different kinds of MNPs. The synthesis of uniform-sized (or monodisperse, with a relative standard deviation of < 5%) nanocrystals is of key importance because the properties of these nanocrystals depend strongly on their dimensions [5-6]. From the fundamental scientific viewpoint, the synthesis of uniform-sized nanocrystals with controllable sizes is very important to characterize the size-dependent physical properties of nanocrystals [7-9]. Ceria (CeO_2) is a cubic fluorite-type structured ceramic material that does not show any known crystallographic change from room temperature up to its melting point (2700°C) [10]. In recent years, nanocrystalline cerium oxide (CeO_2) particles have been extensively studied owing to their potential uses in many applications, such as UV absorbents and filters, gas sensors, electrolytes in the fuel cell technology, water-gas shift catalysts, polishers for chemical mechanical planarization (CMP), ceramic pigments, etc[11-14]. Most of the applications require the use of non-agglomerated nanoparticles, as aggregated nanoparticles lead to inhomogeneous mixing and poor sinter ability. Moreover of above good application of these nanoparticles, they have toxicity effect on physiological systems of animals [15]. The study of the toxicity of nanomaterials toxicity on living cells and within the context of environmental air pollution is a very large research field [16-18]. The same properties that make nanoparticles useful in a variety of applications can potentially make them toxic and harmful to the environment. The potential toxicity of nanomaterials has been recognized, and reviews and perspectives are available [19]. Nevertheless, a better understanding of the risks associated with specific nanomaterials may reduce environmental damage or adverse health effects [20-21]. Nanoparticles might enter the body by a variety of different routes and this makes the assessment of the risks in relation to any material difficult [22]. As will be seen, particles may enter the body by one route but be widely disseminated to various organs and tissues. The most significant method of exposure to nanoparticles is by inhalation, though ingestion for example with food, or application to the skin, either deliberately or inadvertently, is other means of access to the body [23]. The information available from previous studies is insufficient to determine a possible mechanism of action by which nanoparticles might be causing hematological alterations. Alterations in prothrombin and coagulation times could be secondary to liver dysfunction, but more information is necessary to confirm this hypothesis. There are three main types of cells in the

blood (Figure 1): red cells in charge of oxygen transport; white cells responsible for fighting infections; and platelets that help prevent bleeding by forming blood clots [24]. The uptake of nanoparticles by each type of blood cells is essentially different. Nanoparticle uptake by red blood cells (that do not have phagocytic abilities, due to the lack of phagocytic receptors) is entirely dictated by size [25], while the nanoparticle charge or material type plays little importance [26].

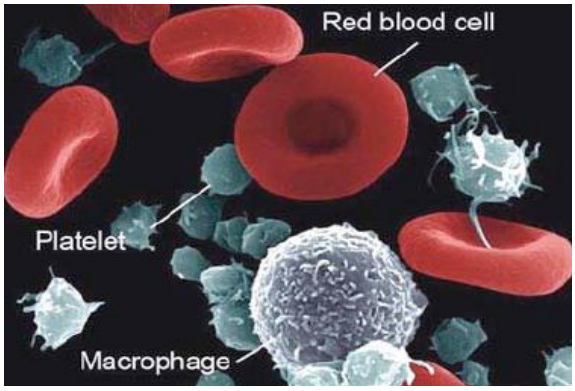

Figure 1. Microscopic image of Blood cells (*Platelets, red, and white blood cells).*

The nanoparticle charge plays an essential role in their uptake by platelets and their influence on blood clot formation [27]. Uncharged polystyrene particles do not have an effect on blood clots formation. Negatively charged nanoparticles significantly inhibit thrombi formation, while positively charge nanoparticles enhance platelet aggregation and thrombosis [28]. The interaction between platelets and positively charged particles seems to be due to the net negative charge that platelets carry on their surface [29]. The positively charged nanoparticles interact with negatively charged platelets and reduce their surface charge, making them more prone to aggregation. 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 recent findings that nanoparticles may act as nucleating centers for blood clots [30-32]. It is important to note that pulmonary instillation of large nanoparticles (400 nm) caused pulmonary inflammation of similar intensity to that caused by 60 nm particles, but did not lead to peripheral thrombosis [33]. The fact that the larger particles failed to produce a thrombotic effect suggests that pulmonary inflammation itself is insufficient to cause peripheral thrombosis [34], and that thrombi formation occur via direct activation of platelets [35-36]. Here we investigated toxicity effect of ceo_2 nanoparticles on rat blood cells and seem this case of study is very important in physiological study of human health.

MATERIALS AND METHOD

Reagents

The biologic material used for the experiment consists in whole Rat blood freshly withdrawn in the presence of heparin. All other chemicals used were of reagent grade and were from standard commercial sources.

Apparatus for study of cerium oxide nanoparticles and Synthesis method

The obtained nanoparticles were measured and recorded using a TU-1901 double-beam UV–visible spectrophotometer were dispersed in toluene solution. The morphologies and particle sizes of the samples were characterized by JEM-200CX transmission electron microscopy (TEM) working at 200 kV. The procedure uses an aqueous solvent, cerium chloride as the precursor material, and ammonium hydroxide as the reducing agent. Cerium chloride is a better material to use in biological applications because leftover chlorine should not harm a biological system, as it is likely to already have chlorine in its environment. All water used was deionized. The ceria nanoparticles were produced by introducing a metal salt, cerium chloride (99.9%, Merck), into an aqueous environment. The salt breaks down and produces Ce^{3+} and Cl^- ions in the water. The solution was stirred and kept in a water bath that held at 60° Celsius during the initial synthesis stage. Ammonium hydroxide (30%, Merck) was then added and cerium oxide nanoparticles form according to the following reaction:

$NH_3 + H_2O \rightarrow NH_4^+ + OH^-$ Reaction 1

$CeCl_3 \rightarrow 3Cl^- + Ce4^+$ Reaction 2

$Ce4^+ + 4OH^- \rightarrow Ce(OH)_4$ Reaction 3

$Ce(OH)_4 \rightarrow CeO_2 + 2H_2O$ Reaction 4

For every 20 mL of solvent, 0.25 g of $CeCl_3$ and 0.8 mL ammonium hydroxide was used, where the pH of the resulting solution was approximately 10.5. After two hours, the heat was turned off and the solution was left to spin for another 22 hours at room temperature. The 22-hour stirring stage breaks down the large nanorods, which form in the initial reaction, into smaller nanoparticles. After the stirring stage was finished, the solution was centrifuged, rinsed with deionized water, and sonicated. When the ammonium hydroxide was added and the reaction was initiated, the solution immediately turned light purple and slowly faded to an opaque white over the course of the heating stage. The final color of the ceria was white, with a light yellowish tint that can be seen at high ceria concentrations. Lab-made ceria solutions that were centrifuged once were the particles used in the experiments presented in this report. Assuming full reactivity of the reactants, the concentration after the first wash can be calculated based on the amount of cerium chloride used. An assumption was made that the differences between ceria washed three times versus ceria washed once were small and unlikely to affect experimentation results.

Investigation of Rats and hematology method

These experimental studies were performed on 40 male rats. The animals were purchased from Pasteur Institute of Tehran; and to prepare condition, they were kept for a month in the animal's room. Animals were kept in proper laboratory and temperature conditions in enough room light (12 h light and 12 h dark). The average weight of animals were (250±15 g) that divided into five octet groups. These groups included a control group that received a rate of 1 ml physiological saline, until the shock effect of injection in treatment and control groups been equal; The second group, 1 ml of cerium oxide nanoparticles was received with 25ppm concentration; The third group, 1 ml of cerium oxide nanoparticles was received with 50ppm concentration; The fourth group, 1 ml of cerium oxide nanoparticles was received with 100ppm concentration and the fifth group, 1 ml of cerium oxide nanoparticles was received with 200ppm concentration. These injections were continued for a week. The method of injection was Intraperitoneal in all groups. After mentioned treatment, the blood sampling was done of rats. The blood sampling was done from the corner of the eye lids of animals by using of Capillary tube. Then in the next step, hematocrit, white and red blood cells were taken. The number of blood cells was determined with a cell counter device (model em – 251, Microteknik, India). After data collection, statistical analysis was done with using of SAS software and also Tukey Dunnett and T tests were done. The $p<0/05$ was considered as a significant Index and results display as Mean±SD.

RESULTS

UV-Vis spectrum characterization of cerium oxide nanoparticles

The UV–visible absorption spectra of cerium oxide nanoparticles are shown in Fig. 2 although the wavelength of our spectrometer is limited by the light source, the absorption band of the cerium oxide nanoparticles have been shows an increase of wavelength due to the quantum confinement of the excitons present in the sample compare with bulk cerium oxide particles. This optical phenomenon indicates that these nanoparticles show the quantum size effect.

Microscopic characterization of cerium oxide nanoparticles

The Morphology of the cerium oxide nanoparticles was investigated by using of transmission electron microscopy (TEM), in Fig.3 was shown the TEM image of the synthesized cerium oxide nanoparticles. The assembly was attached with a computer software programming to analyze the mean size of the particles in sample. The part (a) of figure 3 indicated that size of cerium oxide nanoparticles in 50 nanometer scale and part (b) of figure 3 indicated that size of cerium oxide nanoparticles in 15 nanometer scales.

Hematological Results

Investigated results showed that a decrease of overall mean Hematocrit and mean number of Red blood cells was seen in all experimental groups. Study of results showed that the general reductions in all experimental groups in mean hematocrit and mean number of red blood cells are seen. This decrease compare to the control group in the fourth group that received 200ppm nanoparticles is significant from the statistical point ($p<0/05$). The reduction rate of blood percent

hematocrit in rats that treated with different concentrations of cerium oxide nanoparticles can be seen in Figure 4. And notable point is that there is a direct relationship between Dosage of nanoparticles and reducing the percentage of blood hematocrit.

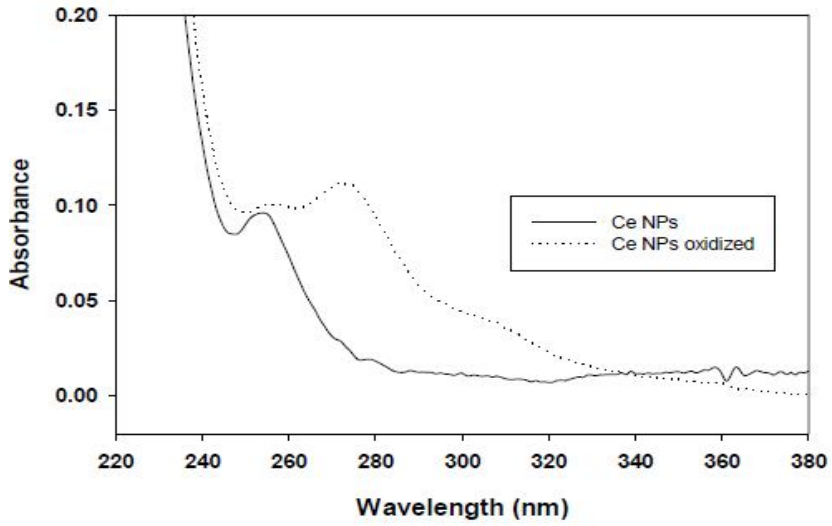

Figure 2. UV-Vis Absorption spectra for ceo_2 nanoparticles and bulk ceo_2 particles

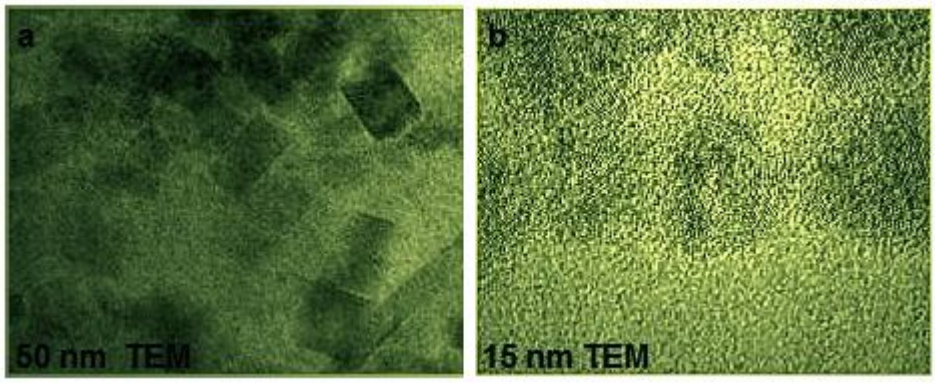

Figure 3. TEM analyses of ceo_2 nanoparticles (a. in 50 nm size and (b. in 15 nm size.

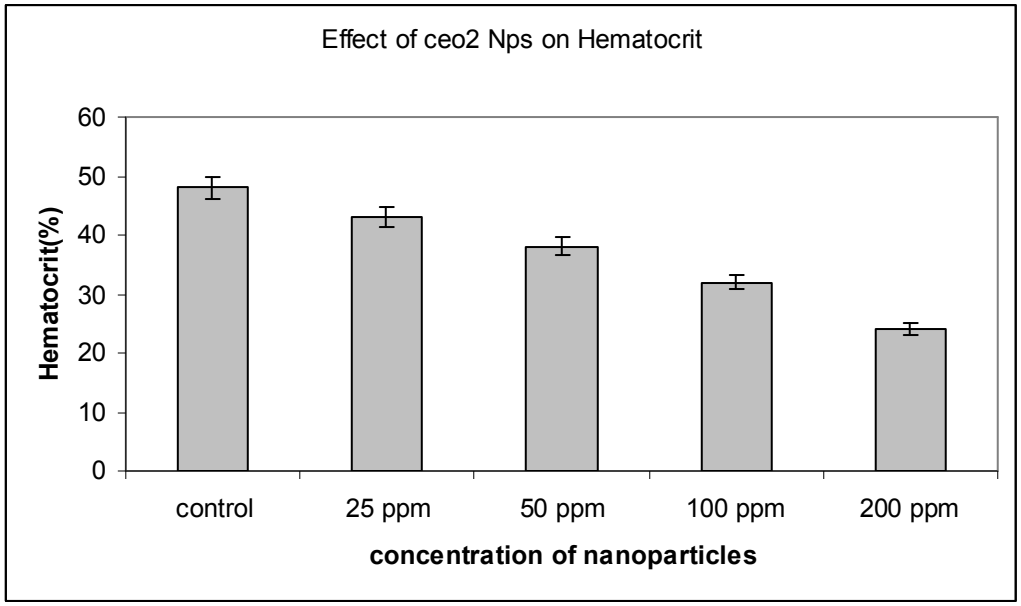

Figure 4. Effect of different concentration of ceo_2 nanoparticles on hematocrit **.**

Figure 5 shows the effect of different cerium oxide nanoparticles on the number of red blood cells (RBC), and reduction and a linear relationship between increase of dose and reduce the number of red blood cells can be seen; And it is clear that the greatest reduction in the number of red blood cells occurred at the highest dose (200PPM) of cerium oxide nanoparticles.

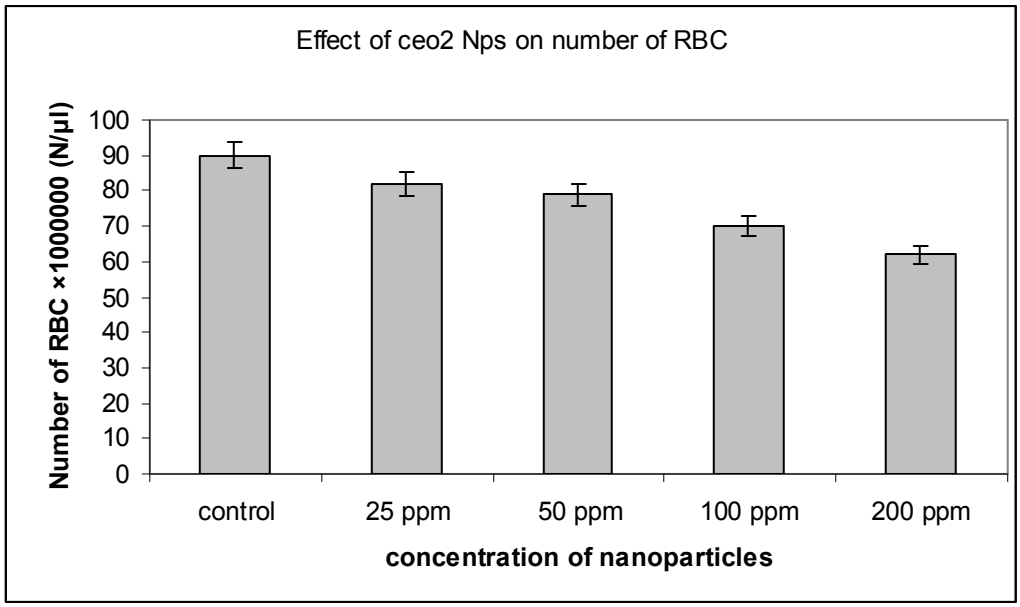

Figure 5. Effect of different concentration of ceo_2 nanoparticles on RBC.

In the next study, the average number of white blood cells (WBC) from the specified time period after treatment with different concentrations of cerium oxide nanoparticles was measured. The number of white blood cells compare to control group, had a linear increase from first group to third group. But in the fourth group, namely the concentrations of 200ppm cerium oxide nanoparticles, the white blood cells was severely reduced and even reached lower than of control group. These results proved that the increase dosage effect of Cerium oxide nanoparticles on number of white blood cells was non-linear.the results are shown in figure 6.

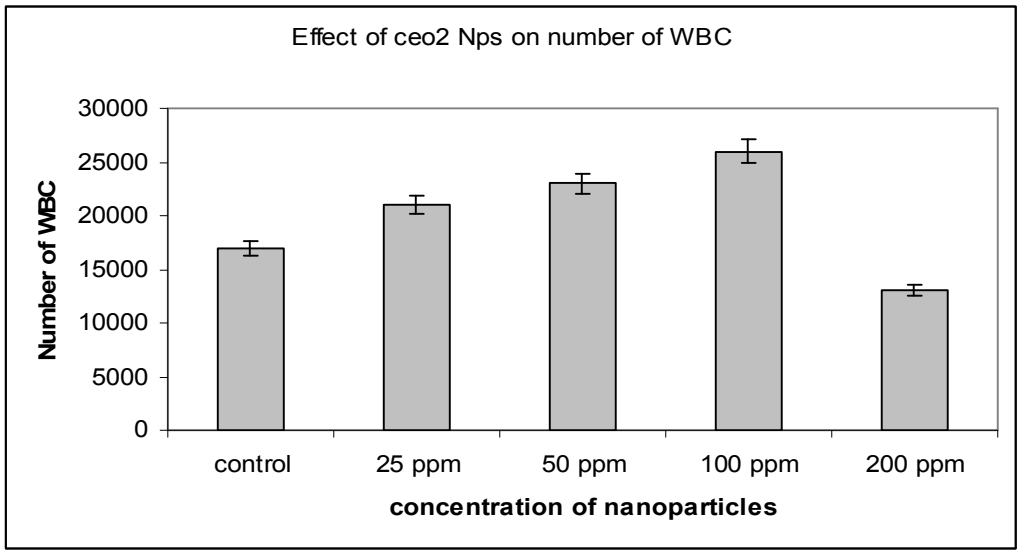

Figure 6. Effect of different concentration of ceo_2 nanoparticles on WBC.

DISCUSSION

The field of nanotechnology is one of the most active research areas in modern materials science [1-4]. The challenge that nanomaterials pose to environmental health is that they are not one material [16]. 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]. With the correct coating, particles below 50 nm can translocate them into cells relatively easily and are able to interact with channels, enzymes, and other cellular proteins [38]. The scientist's typical view of toxicology, which is driven by the composition of an inorganic particle, may have to be modified for nanoscale materials, because surface characteristics are going to affect different dimensions of environmental and health effects[16-18]. It is predicted that High concentration of cerium oxide 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 [39]. Susan et al in 2009 showed that with diameter changes of nanoparticles, their distribution in tissues and their effect will be different [40]. Whatever nanoparticles diameters were smaller; the increase they influence on cell and intracellular molecular mechanisms [41-42]. Immune response of rats to cerium oxide 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 of they 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, cerium oxide nanoparticles can enter into lymphatic system; then inflammation occurs in lymph nodes. The Induced inflammation in the lymph nodes helps to Increasing the number of white cells, but after special period, the activity of these glands become weakened and atrophy of lymph nodes make them irreversible. In this study, we also recognized that in group 4 with 200ppm concentration of cerium oxide nanoparticles, the number of WBC was reduced that Cleary confirm above statements. The main result of this study is that nanoparticles have high toxicity effect on biological and physiological systems and we should have more attention to control unwanted effect of them. In this study all blood cells of rats infected with cerium oxide nanoparticles, which change them physiological parameters. We hope these studies can be introducing a way for prevent and be aware of toxicity effect of nanoparticles in human health.

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