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## The toxicity effect of cerium oxide nanoparticles on ALT, AST and ALP enzymes in male Rat

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### ABSTRACT

Like nanoorganisms (viruses), nanoparticles are able to enter cells and interact with cellular structures. Human exposure to toxic nanoparticles can be reduced through identifying creation-exposure pathways of toxins. In this work, we investigated the toxicity effect of cerium oxide nanoparticles on ALT, ALP and AST enzymes of male Rat. The cerium oxide nanoparticles synthesized and morphological properties of it such as UV-Vis spectrum characterization and transmission electron microscopy (TEM) were investigated. The enzymology studies were performed on 40 male rats that divided into five octet groups. The results showed that with increasing concentration of nanoparticles also increased levels of these three enzymes. And found linear equation between concentration of nanoparticles and levels of these three enzymes.

**Keywords:** ceo<sub>2</sub> nanoparticles, ALT, ALP and AST enzymes, toxicity effect, Rat

### INTRODUCTION

Nano has been and continues to be one of the most hyped areas in science and technology today [1-2]. It is a collective realization that interesting chemistry and physics occurs in the previously unexplored hinterland between the truly molecular (the traditional realm of chemistry and even physics) and bulk matter (the traditional realm of engineers) [3]. Nanomaterials go by a variety of names (nanoparticles, nanocrystals, nanocrystallites, and colloidal quantum dots). Often in the literature these terms are used interchangeably and in some cases can result in confusion. Here we briefly describe one (our) suggested naming convention for small (spherical) nanometer-sized materials made of metals and semiconductors. In particular, we will use the term “nanoparticle” (NP) as a generic description for either spherical metal or semiconductor particles with nanometer-sized diameters [4-5]. However, more often than not, we will use it to refer to metal particles. The name “nanocrystal” (NC) or “nanocrystallite” is often used in conjunction with semiconductor particles and as such will be reserved exclusively for these materials [6]. Ceria (CeO<sub>2</sub>) 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) [7]. In recent years, nanocrystalline cerium oxide (CeO<sub>2</sub>) 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[8-11]. 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 [12]. 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 [13-15]. The same properties that make nanoparticles useful in a variety of applications can potentially make them toxic and harmful to the environment. In general, the toxicological data specific to nanoparticles remains insufficient due to the small number of studies, the short exposure period, the different composition of the nanoparticles tested (diameter, length and agglomeration), and the often-unusual exposure route in the work environment, among other factors. Additional studies (absorption, translocation to other tissues or organs, biopersistence, carcinogenicity, etc.) are necessary to assess the risk associated with inhalation exposure and percutaneous exposure of workers. Four separate liver enzymes are included on most routine laboratory tests [16]. They are- aspartate aminotransferase (AST or SGOT) and alanine aminotransferase (ALT or SGPT), which are known together as transaminases; and alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT), which are known together as cholestatic liver enzymes. Elevations of these enzymes can indicate the presence of liver disease. Aspartate aminotransferase (AST or SGOT) is an exception to the rule that aminotransferases transfer amino groups to  $\alpha$ -Ketoglutarate to form glutamate [17-19]. Since during amino acid catabolism, aspartate aminotransferase transfers amino groups from glutamate to oxaloacetate, to form  $\alpha$ -Ketoglutarate and aspartate respectively, aspartate is then used as a source of nitrogen in the urea cycle [20]. Alanine aminotransferase (ALT or SGPT), catalyzes the transfer of the amino group of alanine to  $\alpha$ -ketoglutarate, resulting in the formation of pyruvate and glutamate [21-22]. Here we investigated toxicity effect of cerium oxide nanoparticles on ALT, ALP and AST enzymes in male Rat and seem this results can be use for increase health of human against toxicity effect of nanoparticles.

## MATERIALS AND METHOD

### 2.1. Reagents

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

### 2.2. Apparatus for study of cerium oxide nanoparticles and Synthesis method

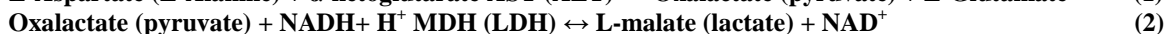
The obtained nanoparticles were measured and recorded using a TU-1901 double-beam UV-visible spectrophotometer were dispersed in toluene solution. The morphologies and particle sizes of the samples were characterized by JEM-200CX transmission electron microscopy (TEM) working at 200 kV. The procedure 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  $\text{Ce}^{3+}$  and  $\text{Cl}^-$  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:



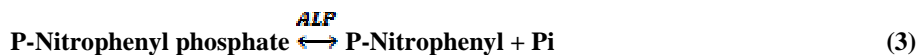
For every 20 mL of solvent, 0.25 g of  $\text{CeCl}_3$  and 0.8 mL ammonium hydroxide was used, where the pH of the resulting solution was approximately 10.5. After two hours, the heat was turned off and the solution was left to spin for another 22 hours at room temperature. The 22-hour stirring stage 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.

**2.3. Investigation of Rats and Enzymology 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. For measurement of ALT, ALP and AST enzymes, some of taken blood for 15 minutes Centrifuged With 3000 rpm to separate serum from clot. After separation the serum from clot by using of sampler, Samples until the enzymatic measurements were frozen and kept at -20 °c. then by using of enzymatic kits from biochemistry CO and by suggested method of International Federation of Clinical Chemistry (IFCC), Enzymatic assays were performed. In the measurement of activity of AST and ALT, activity of both enzymes indicates reduction of Nicotine amide adenine dinucleotide (NADH) in equations 1&2:



Activity of alkaline phosphatase (ALP) with standard method of *IFCC* is a reflex of conversion of P-Nitrophenyl phosphate to P-Nitrophenyl that shown in equation 3:



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****3.1. 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.

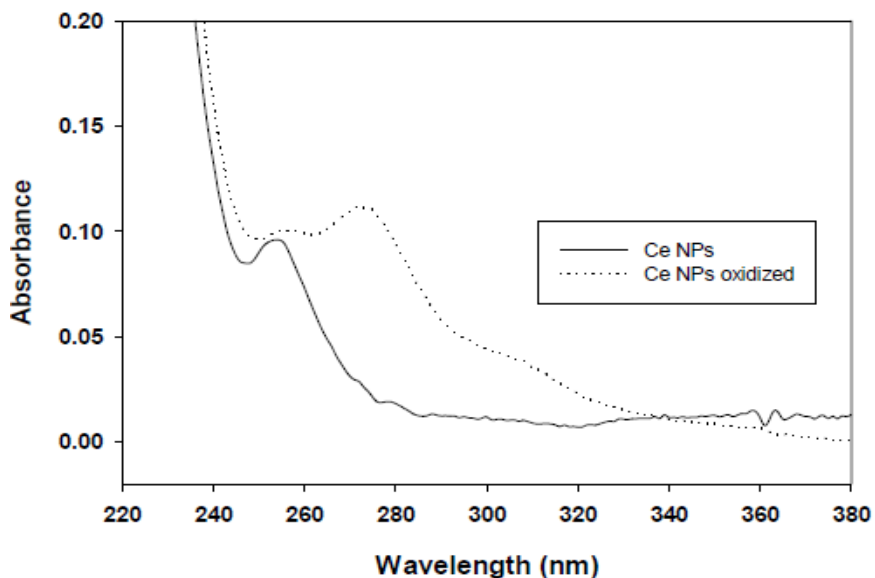


Figure 1. UV-Vis Absorption spectra for  $\text{CeO}_2$  nanoparticles and bulk  $\text{CeO}_2$  particles

### 3.2. 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.

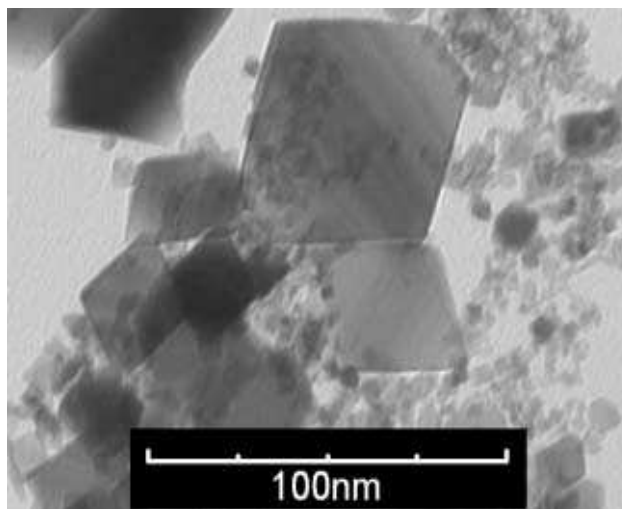


Figure 2. TEM analyses of  $\text{CeO}_2$  nanoparticles (scale bar was 100 nm).

The results showed that activity of ALT enzyme Increased in all groups. This increase compare to the control group in the third and fourth groups that received 100ppm and 200ppm nanoparticles respectively is significant from the statistical point ( $p < 0/05$ ).

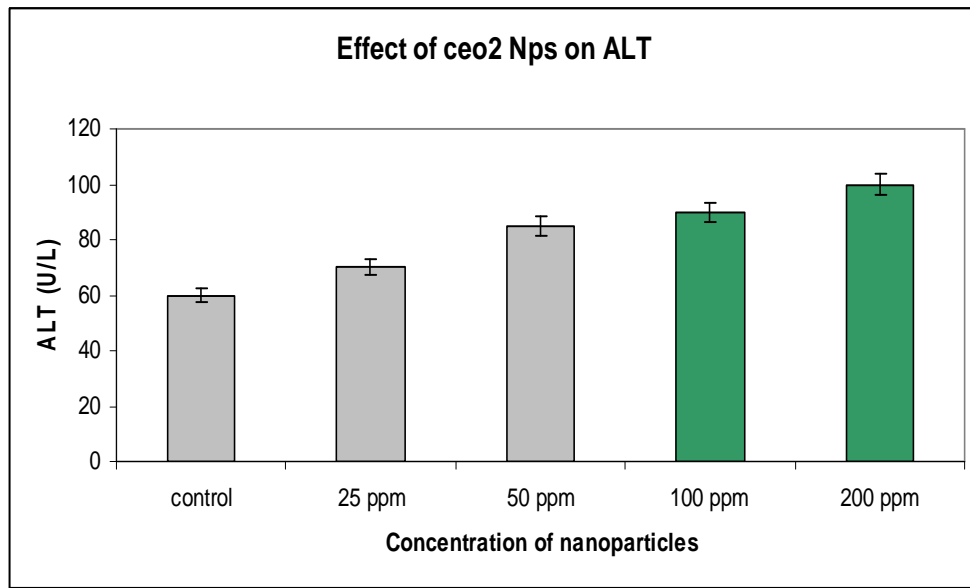


Figure 3. Effect of different concentration of ceo<sub>2</sub> nanoparticles on ALT.

The results showed that activity of AST enzyme Increased in all groups. This increase compare to the control group in the fourth group that received 200ppm nanoparticles is significant from the statistical point ( $p < 0/05$ ).

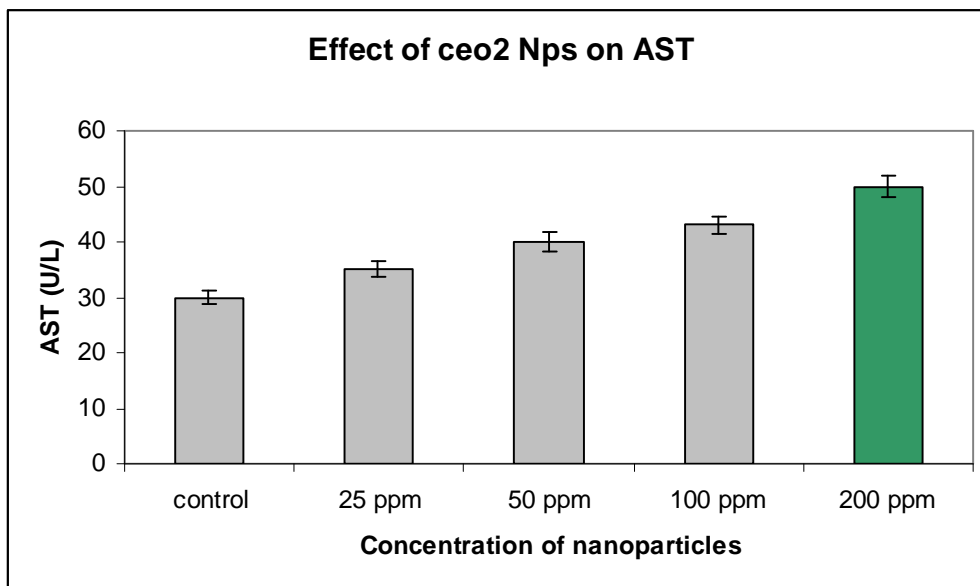


Figure 4. Effect of different concentration of ceo<sub>2</sub> nanoparticles on AST.

The results showed that activity of ALP enzyme Increased in all groups. This increase compare to the control group in the second, third and fourth groups that received 50ppm, 100ppm and 200ppm nanoparticles respectively is significant from the statistical point ( $p < 0/05$ ).

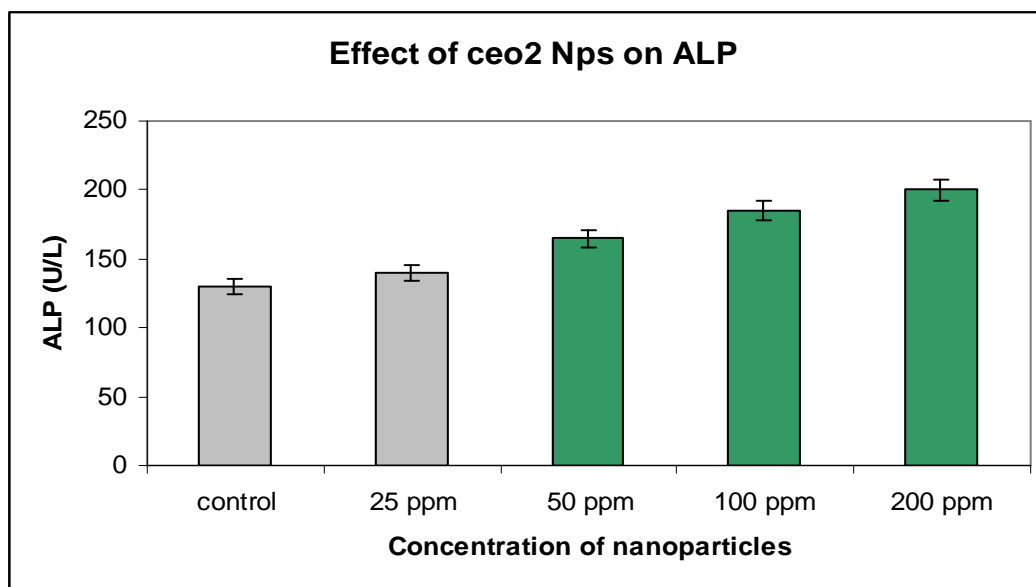


Figure 5. Effect of different concentration of ceo<sub>2</sub> nanoparticles on ALP.

#### DISCUSSION

Very small particles, so-called nanoparticles, have the ability to enter, translocate within, and damage living organisms [1-2]. This ability, results primarily from their small size, which allows them to penetrate physiological barriers, and travel within the circulatory systems of a host [23]. While natural processes have produced nanoparticles for eons, modern science has recently learned how to synthesize a bewildering array of artificial materials with structure that is engineered at the atomic scale [1-5]. The smallest particles contain tens or hundreds of atoms, with dimensions at the scale of nanometers - hence nanoparticles. They are comparable in size to viruses, where the smallest have dimensions of tens of nanometers (for example, a human immunodeficiency virus, or HIV, particle is 100 nm in diameter), and which in the emerging science of nanotechnology might be called 'Nano-organisms' [24]. Like viruses, some nanoparticles can penetrate lung or dermal (skin) barriers and enter the circulatory and lymphatic systems of humans and animals, reaching most bodily tissues and organs, and potentially disrupting cellular processes and causing disease [25-26]. The toxicity of each of these materials depends greatly, however, upon the particular arrangement of its many atoms. Due to their small size, nanoparticles can translocate from these entry portals into the circulatory and lymphatic systems, and ultimately to body tissues and organs [27]. Some nanoparticles, depending on their composition and size, can produce irreversible damage to cells by oxidative stress or/and organelle injury [28-29]. Here we investigated toxicity effect of cerium oxide nanoparticles on ALT, ALP and AST enzymes in male Rat. Understanding the specific mechanisms of nanoparticles and its interaction Require very extensive research in this field. When the nanoparticles are accumulated in a tissue, may be absorbed into the cells or not to be absorbed. If these particles are absorbed, the finally replacement in cell lysosomes or cell cytoplasm will depend on the characteristics of nanoparticles. If the nanoparticles are located in the cytoplasm, the presence of some coarse grain material can cause direct damage or cell death is caused by this interactions. In this study, to evaluate the toxicity effect of nanomaterials on the rat's liver, the ALT, ALP and AST were measured. That with increasing concentration of nanoparticles also increased levels of these three enzymes. And found linear equation between concentration of nanoparticles and levels of these three enzymes. ALT and AST were located in cell and ALP was located in cell membrane. In effect the loss of liver cells, these enzymes are released in the blood. Therefore, increases of these enzymes are a sign of liver cells damage. ALT and AST indicate Status of liver cells. ALP further demonstrates the performance and biliary Hungarian injuries, especially Hungarian extrahepatic. We conclude that the development of nanotechnology and the study of nanotoxicology have increased our awareness of environmental particulate pollution generated from natural and anthropogenic sources, and hope that this new awareness will lead to significant reductions in human exposure to these potentially toxic materials. With increased knowledge, and ongoing study, we are more likely to find cures for diseases associated with nanoparticle exposure, as we will understand their causes and mechanisms. We foresee a future with better-informed, and hopefully more

cautious manipulation of engineered nanomaterials, as well as the development of laws and policies for safely managing all aspects of nanomaterial manufacturing, industrial and commercial use, and recycling.

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