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Investigation toxicity properties of zinc oxide nanoparticles on liver enzymes in male rat

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ABSTRACT

The toxicity Properties of Zinc Oxide Nanoparticles on Liver Enzymes (ALT, ALP and AST) in Male Rat was investigated. Characterization and morphological properties of Zinc Oxide nanoparticles was performed by means of X-ray diffraction (XRD) transmission electron microscopy (TEM), Scanning electron microscopy (SEM) and UV–visible (UV-VIS) spectrophotometer. Experimental studies were performed on 40 male rats that used for treatment with ZnO Nps. The results showed that activity of ALT enzyme Increased and 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); 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) and also activity of ALP enzyme Increased in all groups (figure.6). 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).

Keywords: Toxicity Properties, ZnO Nps, Liver Enzymes, Male Rat

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INTRODUCTION

Nanotoxicology is an emerging field, with a small number of peer-reviewed studies published to date. It is often suggested by nano proponents that we do not yet know enough about the behavior of nanoparticles to determine whether they pose enhanced risks to human health [1-3]. However, researchers suggest clearly that nanoparticles have a greater risk of toxicity than larger particles. This body of evidence has been sufficient for the world's oldest scientific organization to warn that we should not continue to release products containing nanomaterials until we have vastly improved requirements for safety testing [4-5]. 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 [6-7]. 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; in fact Nanotoxicology is a field of study centered on trying to understand how nanomaterials may affect cellular function and their degree of toxicity. Because of their small size (1-100 nanometers), nanomaterials oftentimes exhibit unusual physical, chemical, and biological properties [8]. Although not well understood, it is thought that these

properties may be related to the increased surface area to volume, chemical composition, shape, surface structure, surface charge, aggregation and solubility. 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 [9]. 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 α-Ketoglutarate to form glutamate [10-11]. Since during amino acid catabolism, aspartate aminotransferase transfers amino groups from glutamate to oxaloacetate, to form α-Ketoglutarate and aspartate respectively, aspartate is then used as a source of nitrogen in the urea cycle [12]. Alanine aminotransferase (ALT or SGPT), catalyzes the transfer of the amino group of alanine to α-ketoglutarate, resulting in the formation of pyruvate and glutamate [13-14]. Here we investigated toxicity effect of zinc oxide nanoparticles on ALT, ALP and AST enzymes in male Rat and seem this results can be used for increase health of human against toxicity effect of nanoparticles.

MATERIALS AND METHODS

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 such as Merck and sigma.

2.2. Preparation of Zno nanoparticles

To prepare of ZnO NPs, in a typical experiment, a 0.45M aqueous solution of zinc nitrate (Zn $(NO3)_2 \cdot 4H_2O$) and 0.9M aqueous solution of sodium hydroxide (NaOH) were prepared in distilled water. Then, the beaker containing NaOH solution was heated at the temperature of about 55.C. The Zn $(NO_3)_2$ solutions were added drop wise (slowly for 1 h) to the above-heated solution under high-speed stirring. The beaker was sealed at this condition for 2 hour. The precipitated ZnO NPs were cleaned with deionized water and ethanol then dried in air atmosphere at about 60.C.

2.3. Investigation study methods of zinc oxide nanoparticles

The phase characterization of nanoparticles was performed by means of X-ray diffraction (XRD) using a D/Max-RA diffractometer with CuKα radiation. The morphologies and particle sizes of the samples were characterized by JEM-200CX transmission electron microscopy (TEM) working at 200 kV and Scanning electron microscopy (SEM) images were obtained with a ZIESS EM 902A scanning electron microscope. Samples were measured and recorded using a TU-1901 double-beam UV–visible spectrophotometer.

2.4. Investigation of Rats and Enzymology method

These experimental studies were performed on 40 male rats that used for treatment with ZnO Nps. 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 ten octet groups. These groups included a control group that received 1 ml of rats physiological saline, until the shock effect of injection in treatment and control groups been equal; The second group was received 1 ml of Zinc oxide nanoparticles with 25ppm concentration; The third group was received 1 ml of Zinc oxide nanoparticles with 50ppm concentration; The fourth group was received 1 ml of Zinc oxide nanoparticles with 100ppm concentration and the fifth group was received 1 ml of Zinc oxide nanoparticles with 200ppm concentration; These injections were continued for a week. The method of injection was Intra peritoneal 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 ^{o}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:

L-Aspartate (L-Alanine) + α-ketoglutarate AST (ALT) ↔ Oxalactate (pyruvate) + L-Glutamate (1)

Oxalactate (pyruvate) + NADH+ H^+ MDH (LDH) ↔ L-malate (lactate) + NAD^+ (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:

P-Nitrophenyl phosphate $\xrightarrow{ALP}$ P-Nitrophenyl + Pi (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. X-Ray diffraction of Zno nanoparticles

The x-ray diffraction data were recorded by using Cu Kα radiation (1.5406 A^o). The intensity data were collected over a 2θ range of 20-80^o. The average grain size of the samples was estimated with the help of Scherrer equation using the diffraction intensity of (101) peak. x-ray diffraction studies confirmed that the synthesized materials were ZnO with wurtzite phase and all the diffraction peaks agreed with the reported JCPDS data and no characteristic peaks were observed other than ZnO. The mean grain size (D) of the particles was determined from the XRD line broadening measurement using Scherer equation[15]:

$$D=0.89\lambda / (\beta Cos\theta) \quad (1)$$

Where λ is the wavelength (Cu Kα), β is the full width at the half- maximum (FWHM) of the ZnO (101) line and θ is the diffraction angle. A definite line broadening of the diffraction peaks is an indication that the synthesized materials are in nanometer range. The lattice parameters calculated were also in agreement with the reported values. The reaction temperature greatly influences the particle morphology of as-prepared ZnO powders. Figure 1 (a &b) shows the XRD patterns of ZnO nanoparticles.

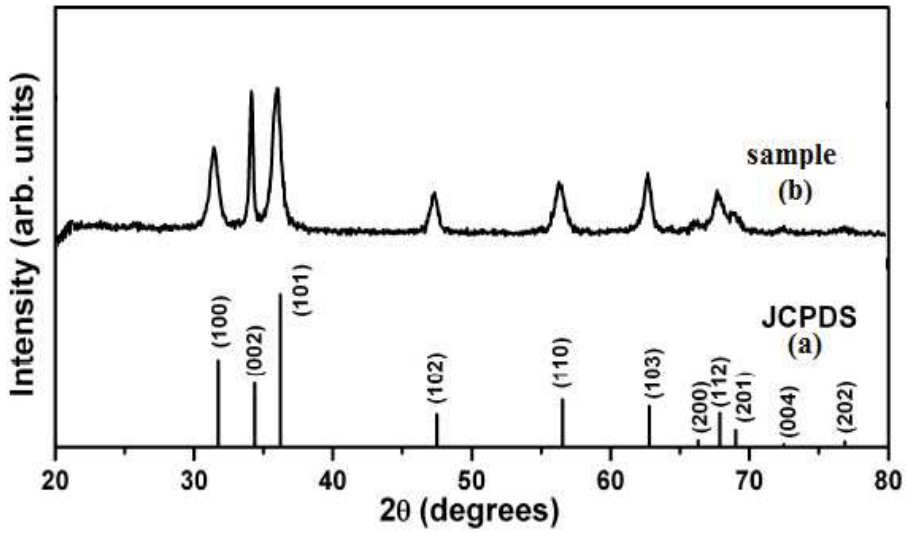

Figure. 1. XRD patterns of ZnO nanoparticles. (a) Indicate standard XRD pattern and (b) indicate sample XRD pattern

3.2. UV–visible absorption spectra for ZnO nanoparticles

The UV–visible absorption spectra of ZnO nanoparticles are shown in Figure. 2 although the wavelength of our spectrometer is limited by the light source, the absorption band of the ZnO nanoparticles have been shows a blue shift due to the quantum confinement of the exciting present in the sample compare with bulk ZnO particles. This optical phenomenon indicates that these nanoparticles show the quantum size effect [16].

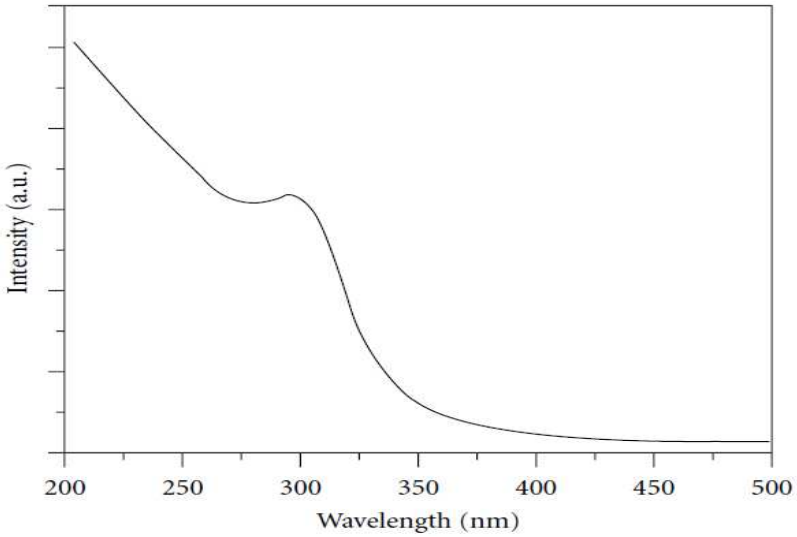

Figure 2. UV-Vis absorption spectra for ZnO nanoparticles

3.3. Electron microscopic investigation of Zno nanoparticles

Morphology of the sample was investigated using SEM and TEM. Parts (a) and (b) of Figure 3 show the typical SEM and TEM images of the sample respectively. The SEM image was captured in 10-nanometer scale bar size of Zno nanoparticles and the TEM image was captured in 20 nanometer scale bar sizes of Zno nanoparticles.

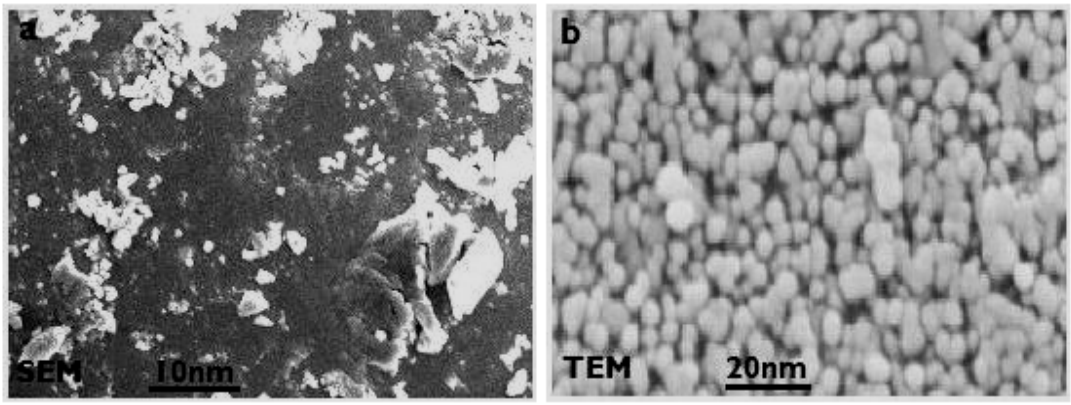

Figure 3. (a) SEM image and (b) TEM image, of ZnO NPs

3.4. Toxicological Results

The results showed that activity of ALT enzyme Increased in all groups (figure.4). 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 ($p<0/05$).

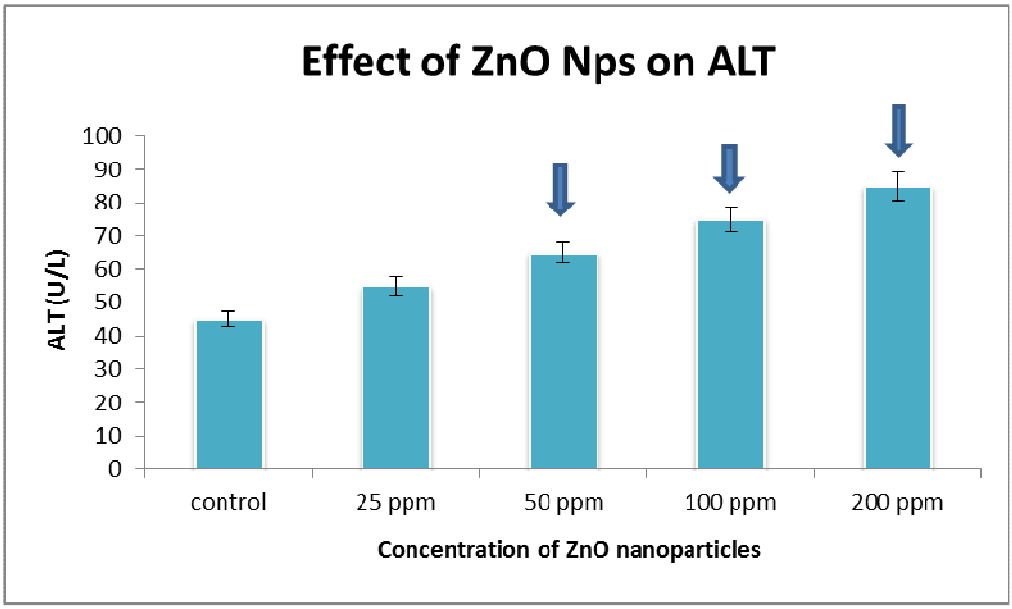

Figure 4. Effect of different concentrations of ZnO nanoparticles on ALT enzyme

The results showed that activity of AST enzyme Increased in all groups (figure.5). This increase compare to the control group in the fourth group that received 200ppm nanoparticles is significant from the statistical point ($p<0/05$).

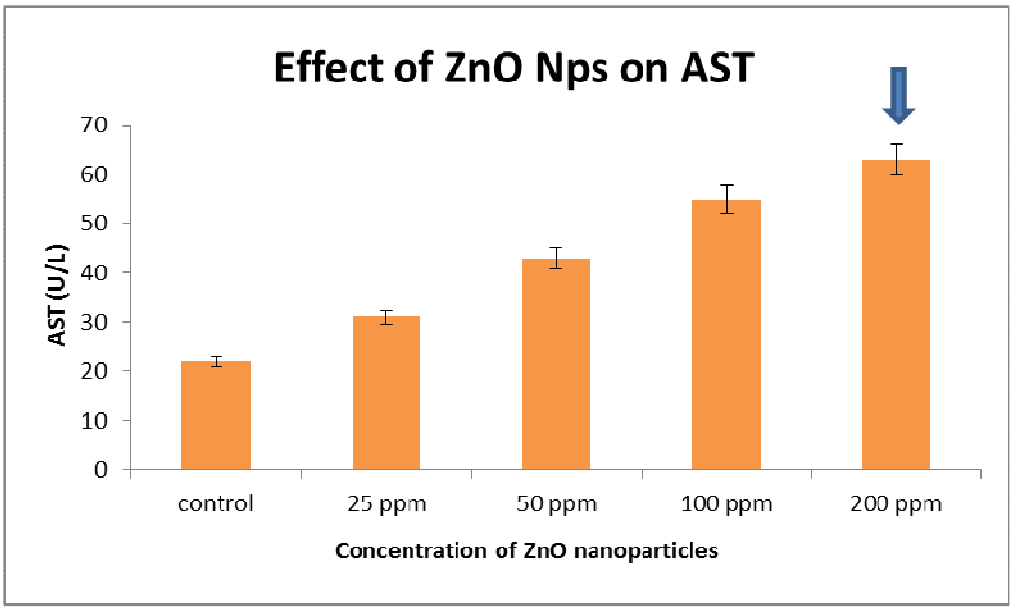

Figure 5. Effect of different concentrations of ZnO nanoparticles on AST enzyme

The results showed that activity of ALP enzyme Increased in all groups (figure.6). 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 ($p<0/05$).

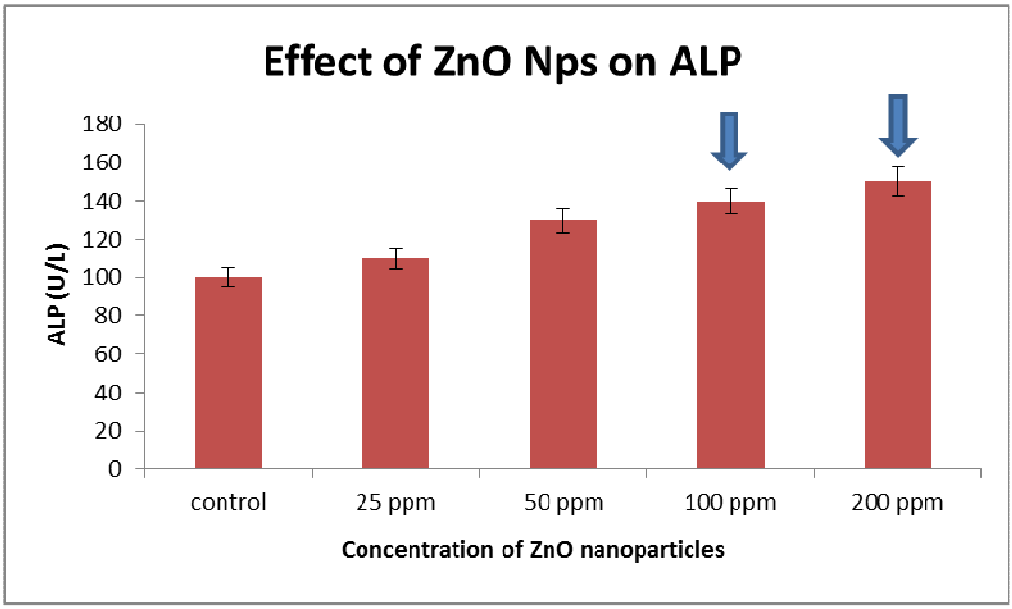

Figure 6. Effect of different concentrations of ZnO nanoparticles on ALP enzyme

DISCUSSION

Very small particles, so-called nanoparticles, have the ability to enter, translocate within, and damage living organisms [17-18]. This ability, results primarily from their small size, which allows them to penetrate physiological barriers, and travel within the circulatory systems of a host [19]. 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 [20-21]. 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 'Nanoorganisms' [22]. 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 [23-25]. 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 [26]. Some nanoparticles, depending on their composition and size, can produce irreversible damage to cells by oxidative stress or/and organelle injury [27-28]. Here we investigated toxicity effect of Zinc 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 extra hepatic. 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.

CONCLUSION

The current knowledge of the toxic effects of nanoparticles is relatively limited. Nonetheless, the available data indicate that some insoluble nanoparticles can pass through the different protective barriers, be distributed in the body and accumulate in several organs. In this study results I saw that zinc oxide nanoparticles had toxicity on liver enzymes and will lead to harmful effects on body metabolism, according to my results, I recommend to all researchers that used of nanomaterials in their researches, have notice to these toxicity effects of nanoparticles.

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