وزارة التعليم العالي والبحث العلمي
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[Study The Effect of Metal Oxide Nanoparticle on Antioxidant Enzyme]

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Abstract:

zinc oxide nanoparticle (ZnO NPs) have been widely used in various commercial products. Application of ZnO NPs is expected to apply to cancer diagnosis and therapy, used as drug delivery carriers. This research was carried out to investigate the effect of zinc oxide nanoparticles (ZONPs) on the antioxidant enzyme activity(SOD) of the serum of humans. Ten healthy volunteers male was included in this study. All healthy volunteers were non-smokers and free of deseases. SOD was measured and ZNONPs was prepared. In the present study, SOD was measured before and after addition of(0.003,0.005 and 0.007 w/v) concentrations of ZNONPs into serum of volunteers . Results revealed that ZONPs had decreased significantly ($P<0.05$) serum super oxide dismutase(SOD) enzymes activity. In conclusion, current study indicated that zinc oxide nanoparticle acted as oxidative stress when addition into the serum of human . However, it is very clear from had improved oxidant state and their effect on the several serum enzymes activity.

The aim of the work :

The aim of this paper therefore is to characterize the effect of a ZNO NPs concentrations on antioxidant enzyme activity(SOD) of the serum of humans .

Introduction:

The application of nanoparticles (NPs) in daily life has increased and studies have assessed the biological effects of nano-composite materials and different types of NPs, particularly on the structure and function of organs. The toxicity of NPs and products related to them are important biological mechanisms that can endanger human health. These mechanisms and the effects of engineered and natural NPs depend on factors such as surface functionalization, aggregation, size, crystallinity, and composition (1).

Metal oxides play a very important role in many areas of chemistry, physics and materials science (2). They are important industrial materials widely used as additives in cosmetics, pharmaceuticals, and food colorants. Skin is usually exposed heavily to solid nanoparticles through the application of lotions or

creams containing nano-TiO2 or ZnO as a sunscreem component or fibrous materials coated with nanoscale substances for water or stain repellent properties. Since the manufacture and use of nanoparticles are increasing, humans are more likely to be exposed occupationally or via consumer products and the environment. There are destructive effects of NPs are inflammation, genetic damage, oxidative stress, inhibition of cell division, and Death cell.

Several groups have examined the uptake and toxicity of metal oxide nanoparticles . The metal elements are able to form a large diversity of oxide compounds.(1) In technological applications, oxides are used in the fabrication of microelectronic circuits, sensors،piezoelectric devices, fuel cells, coatings for the passivation of surfaces against corrosion and as catalysts.

Oxide nanoparticles can exhibit unique physical and chemical properties due to their limited size and a high density of corner or edge surface sites. The similar size of nanoparticles and biomolecules such as proteins and polynucleic acids provide a wide utilization of these materials in biology and medicine (3). Moreover, because of their ultra-small size, they can easily cross through biological membranes and therefore can be highly absorbed by the digestive system. Such characteristics have been eventuated in the utilization of nanoparticles via oral root. Although impressive from the perspective of material science, the novel properties of nanoparticles could lead to adverse biological effects with the potential to create toxicity (4). The body possesses organs and tissues to help break down and eliminate toxins we may otherwise accumulate from our environment or even from natural metabolic processes. Without this detoxification ability, the health impacts would be severe. Over time, chronic and/or excessive exposure to environmental toxins can, unfortunately, exceed our body's ability to metabolize and eliminate them. A buildup of toxins, including chemicals and heavy metals in the body, can lead to detrimental health effects. Reactive Oxygen Species (ROS) are produced during normal cellular function. ROS include hydroxyl radicals, superoxide anion, hydrogen peroxide and nitric oxide. They are very transient species due to their high chemical reactivity that leads to lipid peroxidation and oxidation of DNA and proteins. Under normal conditions, antioxidant systems of the cell minimize the perturbations caused by ROS. When ROS generation is increased to an extent

that overcomes the cellular antioxidants, the result is oxidative stress. Antioxidants are substances that delay or prevent the oxidation of cellular oxidizable substrates.(5)

The body has a powerful mechanism using substances known as antioxidants . Antioxidants can be either an enzyme such as Superoxide Dismutases (SOD), Catalases (CAT), Glutathionle Peroxidases (GPx), Glutatione Reductases (GRx) and Glutathione Transferases (GST) or, a vitamin like beta-carotene, vitamin E (vit. E) and vitamin C (vit.C).

The antioxidant enzymes are several enzyme systems that catalyze reactions to neutralize free radicals and reactive oxygen species. These form the body's endogenous defence mechanisms to help protect against free radical-induced cell damage. Antioxidants are the first line of defense against damage from free radicals and maintain optimal health and well-being. The need for antioxidants is becoming more important with increased exposure to free radicals. In fact, there are a growing number of studies which indicate toxicity of nanoparticles by various mechanisms including generation of reactive oxygen species (ROS) in particular (6).Among many kinds of nanoparticles is zinc oxide nanoparticles (ZnO) .

Material and Method :

The study included 10 healthy volunteers in Hilla city , all of them were males . Aged between (20) and (30) years. All healthy volunteers were non-smokers and free of deseases. About 5 ml of the blood was drawn from vein of healthy volunteers. The 5ml of whole blood samples were allowed to clot, and then centrifuged in (3000 r.p.m for 10 minute) to be used serum samples immediately for measuring. The obtained sera immediately use in determining SODand were frozen at -20 C° until analysis .

SOD was measured according to the method of Winterboun et al (7). Nanoparticle (ZnO) were prepared and studied the morphology and size by sol gel(8) . (ZnO) was weighed as: 0.003, 0.005 and 0.007 mg then dissolved in 100 ml distilled water yielding stock solutions of (0.003, 0.005 and 0.007 w/v) concentrations had been prepared for the determination of the perturbation occurs within the serum at room temperature using vortex to ensure good

distribution. This process was repeated three times to avoid aggregation then mixing with serum.

Take blood sample from persons and extract the serum from it ,then inject the sample with 0.003,0.005, and 0.007 mg/ml of ZnO and read the absorbance of SOD before and after addition of ZnO into the serum by UV-visible spectrophotometer after injection .

Biostatistical analysis:

Statistical data analysis was done using Statistical Package for Social Sciences (SPSS version 18). All data were presented as mean ± Standard Deviation (SD) and the comparison was made. The level of significance was fixed at level P< 0.05.

Results and Discussion

The motivation of the work was to understand the effect the perturbation caused by the ZnO NP on the antioxidant enzymes, which are away from the interacting site. To achieve this we have performed long simulations on sufficiently concentrations of ZnO NPs systems. Results related to the effect of different levels of ZnONPs on serum antioxidant enzyme(SOD) and they were presented in Table 1. The analyzed data for serum of healthy volunteers show a significant decrease in SOD activity at (P<0.05) when compared with activity of SOD before the addition of ZnO NPs. The activity of SOD before addition of ZnONPs was ١.٨٣±0.191(U/ml) . Also the results in the Table (1) showed that the activity of SOD was decreased with increased levels of ZnONPs at (P<0.05).

Table 1: The effect of ZNO NPs on antioxidant enzyme SOD

Levels of ZONPs(mg/ml)	SOD (U/ml) after the addition M ± SD	SOD (U/ml) before the addition M ± SD
0.003	1.161*+0.025	١.٨٣±0.191
0.005	١.٥٤ *±0.041	
0.007	٠.8*±0.022	

*** p<0.05**

In previous years, several kinds of nanoparticles have been proposed as drug delivery system and some of them were evaluated for their

possible curative effects (9). Regarding increasing risk of human exposure to these nanoparticles, there is an urgent need to investigate their harmful effects. In this study, we found that ZnO NP exposure results in ROS production, leading to decrease SOD. The solubility of ZnO NPs is the pivotal factor in causing cytotoxicity in vitro. It has been previously reported that the dissolution of metal oxide NPs greatly influences its toxicity in vitro (10). Several studies have also shown that the toxicity of ZnO NPs is due to the dissolution of the ZnO NPs, forming Zn^{2+} ions (11) . When the oxidative stress exceeds the antioxidant ability of the cell, oxidative damage on critical biomolecules occurs, leading to cell death.(12) Importantly, in this study we have demonstrated that ROS are one of the direct causes of ZnO NP-mediated cell death in both in vitro and in vivo models.

Several previous studies *in vivo* have shown the toxicity of ZnO NPs. The present study investigated the distribution and accumulation

of ZnO NPs in serum . The present study indicated that ZnO NPs were mainly accumulated in the serum after injection. The toxicity of ZnO NPs have been shown to be related to oxidative stress, lipid peroxidation, cell membrane leakage, and oxidative DNA damage *in vitro* (13).

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