



ORIGINAL ARTICLE

Histopathological Effects of ZnO Nanoparticles on Liver and Heart Tissues in Wistar Rats

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ABSTRACT

Considering the development of nanotechnology and extensive use of nano-materials are in different fields of industry, it is necessary to investigate their destructive effects on biological systems. Zinc oxide (ZnO) is used in the production of different dyes, cosmetics, ceramics, photocatalysts, water and sewage treatment and a lot of other products. In the present study, of different concentration of ZnO nanoparticles examined on heart and liver tissues of wistar rats. Rats were divided into four experimental groups including a healthy control group and gavaged daily for 14 days. Ascending concentrations of Nano-ZnO in groups one to three were from 100, 200 and 400mg/kg respectively. Blood samples were collected at day zero before the first gavage and day 14 after day 14. Liver and heart organs were dissected and fixed in 10% formalin for pathological investigations. Increasing the concentration of Nano-ZnO induced two folds elevation of liver enzymes in plasma. Mild to moderate apoptosis were observed in groups one and two. Moderate to severe portal hepatitis and apoptosis appeared in the liver tissue of in groups two and three. Congested blood vessels were observed in both liver and heart tissues from all groups from mild to severe except the control group. In some cases focal necrotic hepatocytes and abnormality in fat distribution along with degeneration heart myofibrils accompanied with dense nuclei were distinguished. Results of the present study proved the toxicity of ZnO nanoparticles on the living organisms. So, further studies are recommended to predict ZnO toxicity.

Keywords: Nano-ZnO , ZnO toxicity, Liver and Heart Histopathology

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INTRODUCTION

Nanoparticles are important scientific tools that have been recruited in various biotechnological, pharmacological applications. They have two particular properties including their large surface area that dominates the contributions made by the small bulk of the material and their quantum effects [1]. These factors affect the chemical reactivity of materials, as well as their mechanical, optical, electric, and magnetic properties. The fraction of the atoms at the surface in nanoparticles is increased compared to microparticles or bulk. Compared to microparticles, nanoparticles have a very large surface area and high particle number per unit mass. Increased surface area to volume ratio of the particle size reduction occurs gradually, Dominated by the behavior of atoms in the particle surface to the inner atoms activity. While chemical reactivity generally increases with decreasing particle size, surface coatings and other modifications can have complicating effects, even reducing reactivity with decreasing particle size in some instances [1-2]. Once the particles are small enough, they can start to behave quantum mechanics and have new properties [2]. Dioxide nanoparticles have unique characteristics that can produce a variety of colors, cosmetic, ceramics, construction of photocatalytic water and wastewater treatment, filtration of gases and many other industrial applications [3]. For instance, zinc oxide particles have been found to have superior UV blocking properties compared to its bulk substitute. This is one of the reasons why it is often used in the preparation of sunscreen lotions. Hypotheses have been raised about the possible damage to a threatening Nanotechnology that will slow growth and development. In order to fulfill the needs of the toxicology of nonmaterial, a very important role in the development of safe and sustainable nanotechnology is required [4-5]. Although there is currently little information available regarding the toxicological effects of nanomaterials, the physical and chemical properties of nanomaterials are expected to interact with biological components of this material to induce significant effects on the behavior and properties of macromolecules, cells and body [6]. Due to the large variety of nanomaterials in comparison

to other materials - a common chemical features are plentiful and unique [7-8]. Physical properties (such as particle size, morphology, and solubility coefficient) and chemical properties (composition and chemical structure of nanoparticle coating) are important and to change any of these properties is expected depending on the type and amount of biological.

Zinc oxide (ZnO) is one of the most commonly utilized materials in diverse industrial fields such as dyes, paints, pigments, metallurgy additives, rubber, alloys, ceramics, chemical fibers, electronics, catalyst, medical diagnosis, sunscreens, cosmetics, personal care products, and food additives [9–10]. The wide range of applications of ZnO is attributed to their unique characteristics, including semiconducting, electrical, optical, catalytic, magnetic, antimicrobial and ultraviolet light absorption properties [10–11]. Recently, rapid advances in nanotechnology have contributed to manufacture and control of engineered nanoparticles, which are generally defined as particles in the size range of 1–100 nm in one dimension. Along with extensive application of ZnO nanoparticles in the industrial field, it is conceivable that the human body may be intentionally or unintentionally exposed to nanoparticles via several possible routes, including oral ingestion, inhalation, intravenous injection, and dermal penetration. Among these, uptake of nanoparticles by the gastrointestinal tract is one of the most important routes.

The aim of this study was to investigate the destructive effects of ZnO nanoparticles with a size of 15 nm on the heart and liver of Wistar rats.

MATERIALS AND METHODS

ZnO particles 15 nm in size were purchased from American Elements (Los Angeles, CA), for oral administration of ZnO nanoparticles with a negative surface charge, the nanoparticles were suspended in 20 mM HEPES buffer containing 1% sodium citrate and then dispersed by vortexing for one minute. The final pH of the suspension was 7.3, and 20% of the surface-modified ZnO nanoparticles were used as a stock solution. Before administration, the suspension was vortexed for 10 seconds and then diluted with distilled water.

Twenty male adult Wistar rats aged 8 weeks old and weighed 200 to 250 g was purchased from Pasteur Institute of Iran's animal care and production facility. Rats were divided to four groups of 5 rats receiving 100, 200 and 400 mg ZnO/kg and a control group treated with saline, respectively. All groups were placed in a Controlled environment with 22°C, 60% humidity, 12 h light and darkness, and free access to food and water. All experiments with animals were performed according to the standard protocols recommended by the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes committee. It was also approved by the Ethical Committee of Pasteur Institute of Iran, where the work was performed in. Suspension of ZnO nanoparticles was administered to rats (1 ml every day) by gavage for 14 days, with different doses such as 100, 200 and 400 mg/kg body weight.

Blood samples were collected every week (three time points of analysis in total). Using a biochemical auto analyzer (VITALAB, Merck, and The Netherlands), serum biochemical analysis was carried out to determine the serum level of total aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH).

Animals were monitored if they were ill or died during the experiment, and at day 15 were anaesthetized by ketamin hydrochloride 70mg/kg and Xylazine 10mg/kg via intraperitoneal injection. Blood samples were collected in heparinized tubes followed by the collection of Heart and the liver. The collected tissues were washed with saline and fixed in 10% buffered formalin for histopathological investigations. Tissue samples were embedded in paraffin and 5 micron sections were prepared and stained with hematoxylin and eosin (H and E) for histopathology evaluation. Injuries were examined microscopically by an invert microscope for evidence of cellular damages.

Statistical analysis

Statistical differences between the control and treated cells were examined with the aid of one-way ANOVA test followed by Tukey's test, using SPSS 12.0KO (SPSS Inc., Chicago, IL, USA).

RESULTS

During the study period, treatment with ZnO nanoparticles for 2 weeks did not cause any adverse effects on growth because no statistically significant differences in the body weight gain were observed between the ZnO-treated and control mice (data was not shown). Further, no abnormal clinical signs and behaviors were detected in both the control and treated groups (data not shown).

In the liver tissue, the trends in alteration of ALS concentrations were similar to those of ALT and ALP activity (Fig. 1A-C). ZnO administration increased all three enzymes in the rats' liver significantly in a dose dependent manner as compared to the control group (Figs. 1A-C). Exposure to 400 mg/L of ZnO NPs for 14 days generally caused the highest degree of increase of these enzymes in experimental animals. In

the mean time, the level of LDH was elevated significantly as a result of ZnO administration (Fig 2). Interestingly, the blood glucose level was also increased significantly in all groups of ZnO treated animals, suggesting the defective effects of ZnO on pancreatic cells. Other, blood components such as uric acid, albumin calcium and total protein remained at normal level (Table 2).

Comparison between ALT and ALP in group 3 of animals showed that by elevation of ALT concentration, the level of ALP was increased as well. This was in accordance with the enzymes localization within the cells. ALT is localized in the cytoplasm, whereas ALP is located under the cytoplasmic membrane. It is suggested that by severity of cell destruction, more ALT as well ALP will be released in the blood stream.

Histopathological effects

Oral administration of ZnO induced significant pathological effects on the main organs such as liver and heart. Mild hyaline degeneration of the heart muscle tissue demonstrated damages associated with severe anemia and high condensation of nuclei (Fig 3, Magnification X400) along with focal hemorrhages and bleeding.

In the liver, nuclei of some hepatocytes showed degrees of chromatin condensation and fragmentation as a result of apoptosis (Table1). Some degree of hemorrhages and focal necrosis was apparent as well. Lymphocyte infiltration was also present dominantly within the liver tissue of group 3 treated with oral administration of ZnO at the dose of 400 mg/kg of body weight. (Fig 4A, B; Magnification X100).

DISCUSSION

Zinc (Zn) is a component of many enzymes (e.g., alcohol dehydrogenase, matrix metalloproteinase) and transcription factors (e.g., zinc finger protein transcription factors). Disruption of cellular Zn homeostasis in *in vitro* systems has been linked to loss of viability, oxidative stress, and mitochondrial dysfunction [12]. Similarly, Deng et al concluded from their study on ZnO NP exposure of neural stem cells that toxicity results from dissolved Zn²⁺ in the culture medium or inside cells [13]. Furthermore, Müller *et al* showed that pH-triggered intracellular release of ionic Zn²⁺ is responsible for the toxicity of ZnO nanowires [14]. The toxic effects of ZnO NPs are due to their solubility, resulting in increased intracellular [Zn²⁺]. This results in cytotoxicity, oxidative stress, and mitochondrial dysfunction.

Retention of metal oxide nanoparticles in the environment and food chain is high and continuous exposure to them may affect human health [15]. Nanoparticles are expected to increase inflammation in the lymph nodes, the cells involved in the inflammatory reaction and therefore an increase in G 1 phase lymphocytes transport to S phase is cell division [16, 17].

It has been reported Nanoparticles with high activity is likely to influence their target tissues and its penetration into tissue may form a thick coating of nanoparticles that cannot be absorbed by phagocytes and entered the lymph flow and accumulated in lymph nodes [10]. Identifying the specific mechanisms of interaction of nanoparticles requires extensive research in this field.

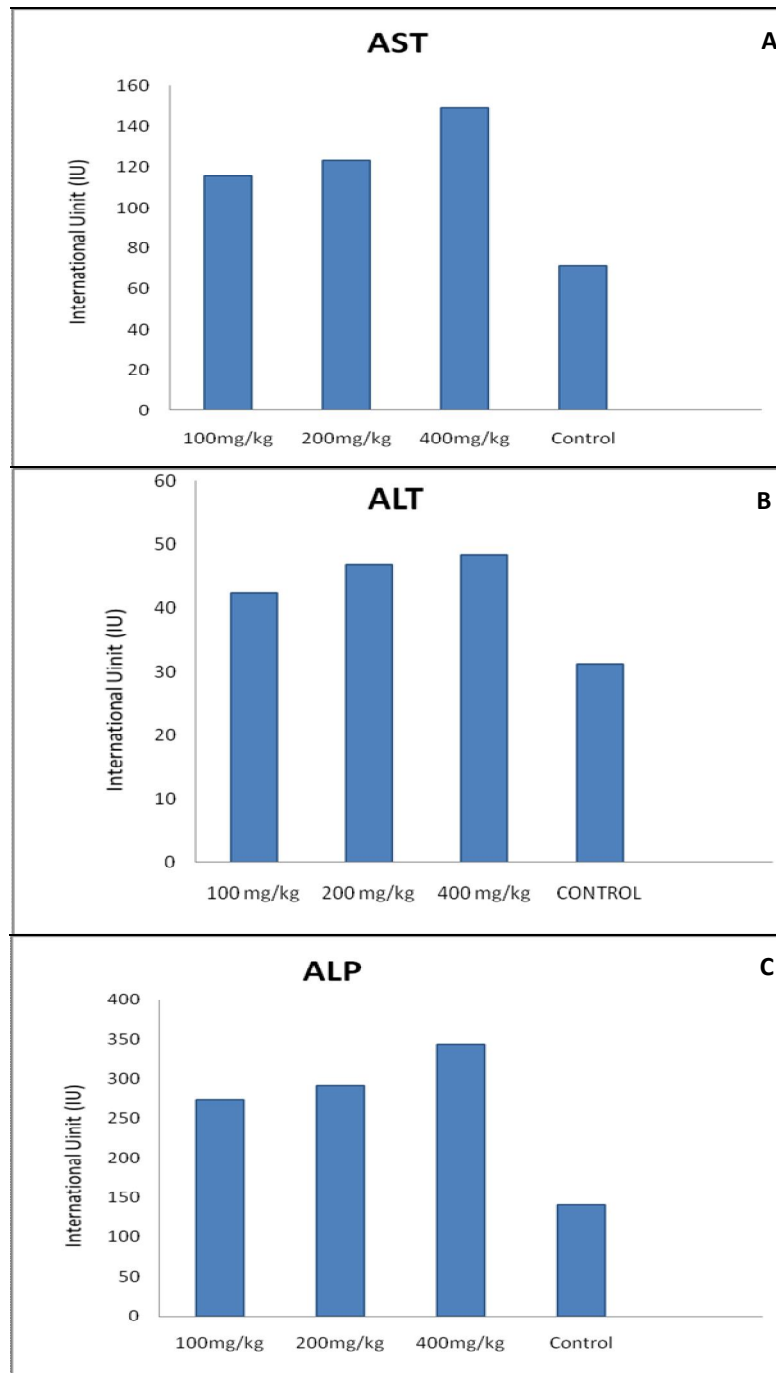
Various cell types (representing, among others, airways, skin, intestines, and the immune system) are affected *in vitro* by ZnO NPs. Most studies suggest that these effects are caused by Zn²⁺ that results from NP dissolution outside the cell. One study suggests that NPs are taken up by the cell, after which dissolution takes place inside the cell. The *in vitro* results indicate that induction of oxidative stress is the most important or most likely mechanism underlying ZnO NP toxicity. After systemic exposure pathological effects may occur. Additional studies are needed regarding potential neurological responses after systemic ZnO NP exposure. So far, genotoxicity was only observed in *in vitro* studies, while *in vivo* studies were negative. Additional studies are needed before definitive conclusions on the genotoxicity of ZnO NPs can be made. Following uptake, ZnO nanoparticles dissolved completely generating intracellular Zn (2+) complexed by molecular ligands. These results confirm a model for ZnO nanoparticle toxicity that can cause direct injury or cell death.

Table1. Percentage rate of histopathological findings in test groups

	Liver congestion			Heart congestion		
	Mild	moderate	High	High	Mild	moderate
group1	60	40	-	20	60	20
group2	-	80	20	20	40	40
Group3	-	60	40	-	60	40

Table2. Alteration of liver enzymes and serum glucose, creatinine, uric acid, albumin, calcium and protein concentrations.

	AST	ALT	ALP	Glucose	Creatinine	Uric Acid	Albumin	Ca	TOTAL PROTEIN
GROUP1	115	42.4	271.4	195.8	0.9	1.1	4.0	12.45	7.5
GROUP2	123.6	48.4	292	195.8	0.9	1.1	4.0	12.45	7.5
GROUP3	149	48	343	214	0.66	1.24	4.82	12.15	6.88
Control	62.8	31.3	140.2	117.6	0.66	1.22	4.58	10.74	7.3

**Figure1.** Liver enzymes concentration in the test and control groups.

A) Comparison of AST enzyme concentration. B) Comparison of ALT enzyme concentration. C) Comparison of ALP enzyme concentration.

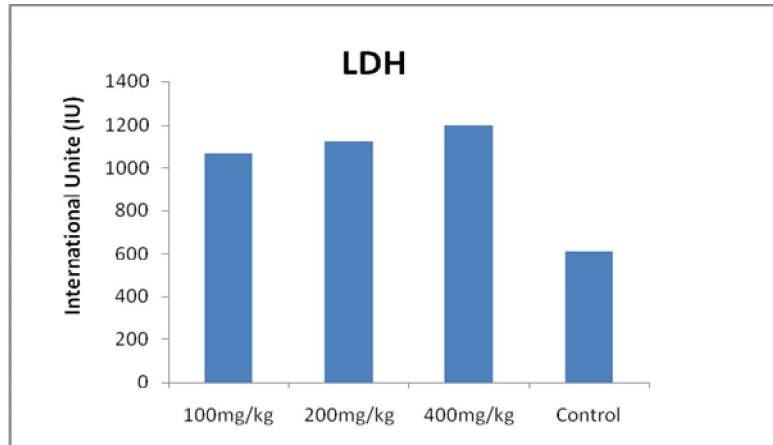


Figure2. Elevation of LDH concentration after ZnO administration. All number are based on international units (IU).

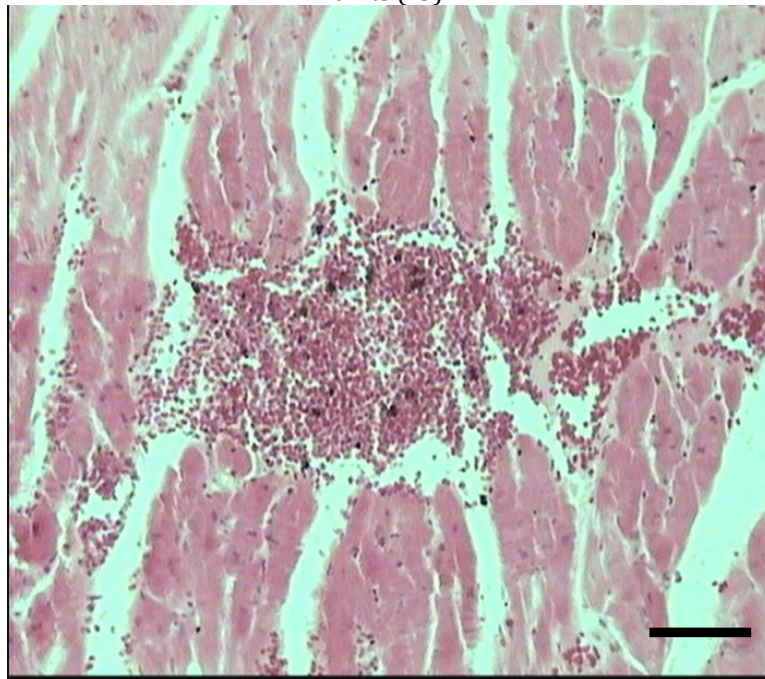


Figure3. Hyaline degeneration of muscles along with mild nuclear condensation and severe congestion. The scale bar represents 100 μ m.

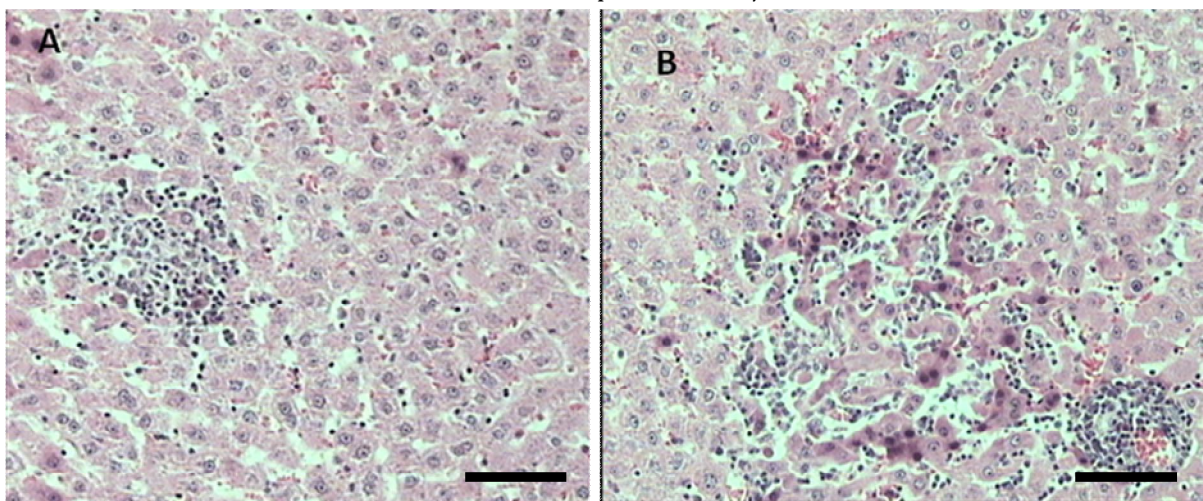


Figure4. Lymphocytes infiltration into the liver tissue. A) Multifocal necrotic and lymphatic portal hepatitis. B) Hyperemia along with lymphocytes infiltration and necrosis in liver tissue. The scale bar represents 100 μ m.

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