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Research Paper:

Chronic Zinc Oxide Nanoparticles Exposure Produces Hepatic and Pancreatic Impairment in Female Rats

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ABSTRACT

Background: Zinc Oxide (ZnO) nanoparticles are used for various industrial and domestic purposes and its release into the environment leads to the adverse effects among humans. This study aimed to evaluate the effect of rat exposure to ZnO nanoparticles on the histopathology of the liver and pancreas tissues, and serum oxidative stress parameters.

Methods: Eighty female adult Wistar rats were divided into eight experimental, control and sham groups. They received ZnO nanoparticles at 4, 8, 25, 50, 100, or 200 mg/kg, or normal saline intraperitoneally for 30 days twice a week. Then, the blood samples of the rats were collected by heart puncture for biochemical analyses, and then sacrificed. Finally, the liver and pancreas tissues were harvested for histopathological examinations.

Results: Significant amounts of nanoparticles were accumulated in the liver and pancreas of the rats, causing tissue and cellular damages. The ZnO nanoparticles reduced the levels of serum triglyceride, glucose, cholesterol, albumin, and increased the bilirubin and liver enzymes, such as ALT, AST, ALP, amylase and lipase at high doses. In addition, the evidence of histopathological lesions, hyperemia, inflammatory cell infiltration, and necrosis were noted in the liver and pancreas tissue slides upon microscopic examinations. Finally, the body and liver weights decreased in the rat groups receiving ZnO nanoparticle dose dependently.

Conclusion: ZnO nanoparticles had toxic effects on the liver and pancreas, leading to destructive tissue and cellular changes in the rats.

Keywords: Histopathology; Zinc oxide nanoparticles; Liver; Pancreas impairments; Chronic toxic exposure

Introduction

Zinc Oxide (ZnO) nanoparticles are used for industrial and cosmetic purposes [1]. The study of nanomaterials toxicity on living cells and environment is a vital and needed research field. Nanoparticle products offer advantages to the users and economy; however, these products cause health threats to the researchers, workers, and consumers. Zinc Oxide (ZnO) nanoparticles are used for industrial and cosmetic purposes [1]. The toxicity of nanoparticles on living cells within the background of environmental air pollution has attracted much attention. Although nanotechnology benefits the users and economy, exposure of workers, consumers and researchers to the potentially dangerous materials can lead to harmful health effects [2]. However, little information is available about the toxicological effects of nanoparticles, and their physical

and chemical attributes are believed to interact with biological ingredients of this issue in order to affect considerably on the treatment and attributes of macromolecules, cells, and body [3].

Nanoparticles power affect the toxicological action of materials in organisms by considering the exclusive physicochemical confidants containing small size effect, great special surface area, very high biological area reactivity, and so forth [4]. Histopathological changes along with the information on the body burden of heavy metals provide useful information about the efficacy of heavy metals on the situation of living organisms, and the level of zinc available in tissues depends on age, genus, and physiological condition of exclusive, and homeostatic mechanisms which certify the alignment of the concentration of these metals about the metabolic optimum [5]. Therefore, the use of nanoparticles in biomedicine fields such as cancer diagnosis and treatment imports straight human exposure. Further, use of ZnO nanoparticle suspensions through the digestive tract of mice can lead to diarrhea, vomiting, hard liver and renal damage, anemia, and other physiological irregularities [6]. Further, zinc is necessary for the biosynthesis of fatty acids and takes part in both inflammatory and immune systems. Furthermore, it is involved in the metabolism of vitamin A [1]. The production, growth and utilization of ZnO nanoparticles raise the possibility of disposal in professional and environmental contexts, which has been highlighted in several studies investigating the toxicity of ZnO nanoparticles in various biological systems such as bacteria [7].

The toxicity of these metals is primarily related to the body's responses to oxidative stress without the enzymatic systems within the body, which causes the neurotoxicity, hepatotoxicity, and nephrotoxicity in humans and animals [8, 9]. Lead (Pb) exclusion has a dose-response relationship with the changes in antioxidant enzyme activities including Superoxide Dismutase (SOD) and Glutathione-S-Transferase (GST) [10]. Acute exposure to cadmium leads to the elevation of the levels of serum liver enzymes such Asaspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), and Alkaline Phosphatase (Alp) [11]. Some epidemiological studies represented the relationshipbetween chronic arsenic exposure and unusual liver function, hepatomegaly, hepatoportal sclerosis, ascites, liver fibrosis and cirrhosis. The liver is an essential organ which plays a major role in the metabolism and detoxification of biological materials. In addition, most of the materials attracted by the intestine pass first through the liver where toxins and heavy metals may be accumulated [12]. Zn is considered as a nutrient in the body, while high concentration of Zn may lead to oxidative damage to lipids and proteins [13]. Reactive Oxygen Species (ROS) are produced in oxidative stress and high intracellular ROS levels can result in peroxiding membrane lipids. Malondialdehyde (MDA) is the end product of polyunsaturated fatty acid peroxidation and considered as a biological marker for determining the amount of oxidative damage [14].

Considering the above issues, the present study aimed to investigate the effect of nano ZnO on the oxidative stress status among adult female Wistar rats by measuring MDA as an index of lipid peroxidation; liver and pancreas enzymes, as a function of the liver and pancreas conditions, and atomic absorption of the liver and histopathological changes in the liver and pancreas tissue.

Materials and Methods

Preparation of nanoparticles: ZnO nanoparticle solutions with the sizes of 10-30 nm were purchased from Iranian Nanopishgaman Company (Mashhad, Iran). This material was diluted in normal saline to prepare the solutions of 4, 8, 25, 50, 100, and 200 mg. These solutions were homogenized before injection by sonicator (Misonix Sonicator, 55 W). ZnO NPs used for animal exposure were prepared in normal saline by sonication at 4°C (10times, 30 sec. every 2 min). The NPs were vortexed 5 times for 10s before using to break down agglomerates and ensure a uniform suspension.

Animals: First, 80 female Wistar adult rats with 180-220g weight were purchased from Pasteur institute in northern Iran. The rats were kept in well-ventilated rooms with the temperature of 23±2 ˚C and relative humidity of 60±10%with a 12 h light/dark cycle. The rats were fed with commercial pellet diet and water ad libitum. The body weights of the rats were recorded before and after the administration time.

Experimental design: Animals were randomly assigned into eight groups, each including ten animals. Control group animals (A) were fed with normal food and water without any additive material or drug in the diet. The animals were received intraperitoneally twice a week for four weeks. Sham group (B) was given 10% normal saline body weight. Finally, 4, 8, 25, 50, 100 or 200mg/kg of ZnO nanoparticle was given to group 1 through group 6, respectively. The experimental design was approved by the Animal Ethics Committee of Islamic Azad University, Babol branch, Babol, Iran.

Biochemical analysis: Blood samples were taken directly from the heart's left ventricle after anesthesia with intraperitoneal injection of xylazine and ketamine (20 and 60 mg/kg body weight, respectively, (Alfasan,

Woerden, Netherlands) and opening the chest. The collected blood samples were kept at room temperature for 30 minutes and centrifuged at 5000 rpm for 15 min to separate the sera. In addition, the sera were used for estimating the blood biochemical parameter, including Total Cholesterol, Triglyceride (TG), Glucose, Albumin, liver marker enzymes such as AST, ALT, and ALP, pancreatic enzymes including Amylase and Lipase by appropriative Pars Azmoon kit (Pars Azmoon Co., Tehran, Iran), and an automatic biochemical analyzer (Hitachi 902).

Histopathological examination: The rat was weighted and sacrificed after being anesthetized for analyzingthe biochemical factors. The tissues from the liver and pancreas were obtained as small pieces, fixed in 10% buffered neutral formalin, and cut into 5µm thick sections and mounted on glass slides after processing the tissue embedded in paraffin blocks. The sections were stained with Hematoxylin and Eosin (H&E) and observed by optical microscopy(Olympus CX21) [15].

Atomic absorption: The liver organ was dried and put in oven at 150˚C to reach a constant weight. The samples were homogenized and grinded to fine powder. One gram of the tissue samples was isolated and added in a clean test tube. For chemical digestion, 5 ml of nitric acid-perchloric acid (10:4) was added in tubes. Then, the mixture of the test tubes was plugged with cotton and left overnight at the room temperature. After sample digestion, it was heated at 100°C in water bath for about 2 hours for completely transparent solution. The contents of the tubes were cooled, filtered through filter paper, transferred to 25ml volumetric flasks, and made up to mark with 1% nitric acid. The digests were stored in polyethylene bottle for analysis. Finally, Atomic Absorption Spectrometer Model Buck-210 VGP was used for measuring atomic absorption rate [16].

Measurement of MDA: The content of MDA as a lipid peroxidation index was determined spectrophotometrically in 10% homogenates of liver and pancreas tissues (prepared in 0.9% NaCl) according to manufacturer's protocol(MDA assay kit; ZellBio, Germany) [17].

Statistical analysis: All results were performed as Mean±SEM for the animal weight and ANOVA was used for biochemical factor. Normality and homogeneity of the data were confirmed before ANOVA analysis. In all analyses, the P-value of <0.05 was considered as statistically significant. Duncan analysis is commonly used as a post hoc test.

Results

Body and organ weights: The results of this study indicated that ZnO-NP treatments in 4 mg/kg dose group increased body weight compared to the control, while weight

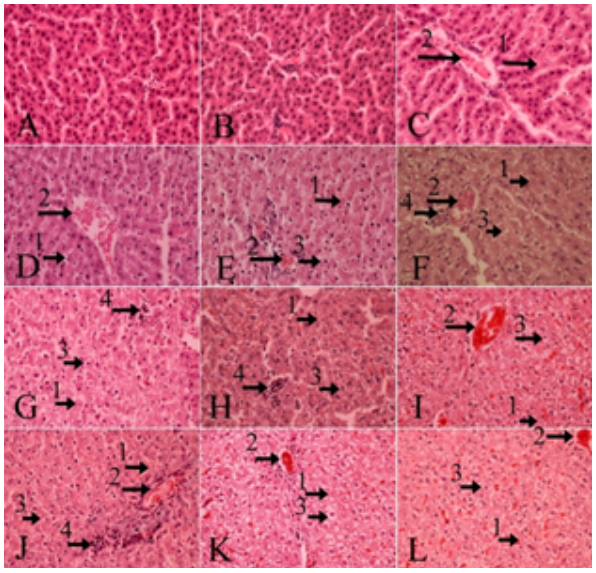

Figure 1. Histopathological examination of rats' liver tissue between groups

A & B: Control group and Sham group, Normal liver tissue; C: 4 mg/kg Zno nps, Necrosis (arrow 1), Hyperemia (arrow 2); D: 8 mg/kg Zno nps, Necrosis (arrow 1), Hyperemia (arrow 2); E: 25 mg/kg Zno nps, Necrosis (arrow 1), Hyperemia (arrow 2), Vacuolar degeneration (arrow 3); F & G: 50 mg/kg Zno nps, Necrosis (arrow 1), Hyperemia (arrow 2), Vacuolar degeneration (arrow 3), Inflammatory cell infiltration (arrow 4); H & I: 100 mg/kg Zno nps, Necrosis (arrow 1), Vacuolar degeneration (arrow 2), Inflammatory cell infiltration (arrow 3), Hyperemia (arrow 4); J, K & L: 200 mg/kg Zno nps, Necrosis (arrow 1), Vacuolar degeneration (arrow 2), Hyperemia (arrow 3), Inflammatory cell infiltration (arrow 4); H & E: Staining, X40 magnifications.

Table 1. Body and liver weights of the rats exposed to ZnO NPs

Groups	Mean±SE		
	Initial Body Weight	Final Body Weight	Liver Weight (g)
Control	210±3.87	242.7±3.52 ^{c}	9.99±.22^{c}
Sham	208±2.99	240.8±2.48 ^{c}	9.83±.3^{c}
4 mg/kg	207.2±2.11	256.8±6.03^{d}	9.86±.2^{c}
8 mg/kg	208.4±2.54	241.3±5.51^{c}	10.01±.31^{c}
25 mg/kg	208.8±2.86	240.6±4.84^{c}	9.39±.29^{b,c}
50 mg/kg	216.4±1.69	233.8±4.24^{b,c}	9.06±.23^{b}
100 mg/kg	211.9±1.27	221.9±2.88^{a,b}	8.05±.23^{a}
200 mg/kg	206±2.26	211.4±2.89^{a}	7.58±.19^{a}

Different letters a, b and c, etc to show statistically significant differences between groups

Table 2. Pathological alterations of the rats' liver and pancreas after exposure to ZnO nanoparticles

	Liver					Pancreas					
Group	Hyperemia	Necrosis	Vacuolar Degeneration	Eosinophil Ifiltration	Lymphocyte Infiltration	Hyperemia	Eosinophil Infiltration	Lymphocyte Infiltration	Necrosis	Exocrine Ductal hyperplasia	Fibrosis
Control	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
Sham	+	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
4 mg/kg	+	+	NC	NC	NC	NC	NC	NC	NC	NC	NC
8 mg/kg	++	++	NC	NC	NC	+	NC	NC	NC	NC	NC
25 mg/kg	++	++	+	NC	NC	++	NC	+	+	++	++
50 mg/kg	++	++	+	NC	+	++	++	++	++	++	++
100 mg/kg	++	+++	++	NC	+	++	+++	++	+++	++	+++
200 mg/kg	++	+++	+++	+	++	++	+++	++	+++	NC	+++

Histopathological grading symbol: NC, no change; $^{+}$ Mild changes; $^{++}$ Moderate changes; and $^{+++}$ Severe changes

loss was dose-related. In addition a significant difference is observed between 200 mg/kg and the control group (Table 1). In addition, liver weight increased in 8 mg/ kg group, and subsequently reduced by increasing the dose of ZnO-NP. In fact, there is a significant difference between 200 mg/kg group compared to the control (Table 1).

Histopathological examination: No macroscopic and microscopic lesion was observed in liver and pancreas tissue in the control group (Figure 1). Histopathological finding was graded based on (NC), (+), (++) and (+++) indicating no change, mild, moderate, and sever changes, respectively. The histopathology of the liver and pancreas tissue in control and sham group showed a normal tissue. Histopathological findings in liver tissue included necrosis, hyperemia, and infiltration in eosinophil, lymphocyte, and vacuolar degeneration (Figure 1 & Table 2). In addition, as shown in Figure 2 and Table 2, infiltration of eosino-

Table 3. Biochemical assay of serum and MDA level in the pancreas of rats exposed to ZnO NPs

Groups	Mean±SE			
	Glucose	Amylase	Lipase	MDA of Pancreas (nmol/mg)
control	106.2±4.22^{e}	1750.4±79.79^{b,c,d}	18.5±0.39^{b}	6.7±0.18^{a}
Sham	100.2±4.21^{d,e}	1735±55.16^{b,c}	18.68±0.53^{b,c}	6.93±0.22^{a}
4 mg/kg	88.95±3.39^{c,d}	1902.5±81.99^{c,d,e}	20.9±0.62^{c,e}	16.05±0.32^{c}
8 mg/kg	87.25±3.28^{c}	1964.6±54.18^{d,e}	21.4±0.7^{e}	26.9±0.4^{e}
25 mg/kg	69±6.06^{b}	2014.9±39.46^{e}	24.4±0.81^{f}	41.83±0.25^{f}
50 mg/kg	61.1±4.97^{a,b}	2271.03±76.68^{f}	25.1±0.97^{f}	43.21±0.21^{g}
100 mg/kg	59.5±3.36^{a,b}	1559.35±108.63^{b}	19.1±1.05^{b,c}	23.21±0.24^{d}
200 mg/kg	51.75±3.66^{a}	1315.4±73.01^{a}	15.70±0.7^{a}	13.36±0.93^{b}

Superscript letters a, b, c, d, e & f denote statistically significant differences among the groups;

Table 4. Biochemical of serum assay, MDA level and, atomic absorption in the liver rats exposed to ZnO NPs

Groups	Mean±SE				
	Cholesterol	Triglyceride	Bilirubin	Albumin	Total Protein
control	63.4±2.88^{c}	196.5±2.01^{e}	0.27±0.01^{a}	3.65±0.03^{e}	7.5±0.06^{c}
Sham	62±2.39^{c}	187.8±1.75^{e}	0.28±0.01^{a,b}	3.63±0.03^{d,e}	7.48±0.06^{c}
4 mg/kg	60.4±2.28^{c}	137.8±6.14^{d}	0.3±0.01^{a,b,c}	3.62±0.03^{d,e}	7.46±0.13^{c}
8 mg/kg	59.5±2.82^{c}	124.5±5.57^{c}	0.31±0.01^{b,c}	3.6±0.03^{d,e}	7.35±0.1^{c}
25 mg/kg	59.4±1.89^{c}	105 ±4.40^{b}	0.31±0.01^{c}	3.52±0.05^{d}	7.32±0.14^{c}
50 mg/kg	58.5±1.41^{c}	94.6±2.81^{b}	0.32±0.01 ^{c}	3.4±0.05^{c}	7.2±0.15^{c}
100 mg/kg	49±0.92^{b}	77±2.39^{a}	0.32±0.011 ^{c}	3.25±0.05^{b}	6.1±0.06^{b}
200 mg/kg	36.5±0.86^{a}	74±1.69^{a}	0.53±0.02^{d}	3.1±0.03^{a}	5.43±0.14^{a}

Groups	AST	ALT	ALP	Atomic Absorption	MDA of Liver nmol/mg
control	190±13.69^{a,b}	42.5±0.7^{a}	281±10.74^{a}	0.3933±0.01^{a}	5.99±0.17^{a}
Sham	189.75±17.88^{a,b}	42.5±1.98^{a}	309±18.33^{a}	0.4333±0.01^{a}	6.32±0.18^{a}
4 mg/kg	197.66±16.93^{b}	48.88±1.93^{a,b}	324.66±37.43^{a}	0.4550±0.01^{a}	31.84±1.12^{c}
8 mg/kg	217.6±25.63^{b}	54.4±3.52^{a,b}	335.2±18.61^{a}	0.5000±0.01^{a}	94.78±4.03^{f}
25 mg/kg	233±22.20^{b}	60.4±2.55^{b}	379.6±28.2^{a}	0.6510±0.06^{b}	135.77±3.8^{e}
50 mg/kg	297.6±19.7^{c}	77.6±9.1^{c}	390.75±13.89^{a}	0.9533±0.07^{c}	79.38±2.03^{d}
100 mg/kg	326±20.34^{c}	103.3±38.85^{d}	897.43±73.24^{b}	1.1700±0.06^{d}	46.79±1.44^{g}
200 mg/kg	137.1±9.89^{a}	43.4±2.83^{a}	1071.25±97.84^{c}	1.3311±0.07^{e}	21.93±1.2^{b}

phil, lymphocyte and neutrophil, necrosis, hyperemia, exocrine ductal hyperplasia and fibrosis are considered as the predominant histopathological findings in pancreas tissue. Further, histopathological lesions in the liver and pancreas increased in a dose-dependent manner.

Serum biochemical parameters: Tables 3 and 4 indicate the changes of biochemical parameters in the serum of rats after intraperitoneal injection of ZnO nanoparticle suspension. As shown, exposure to ZnO nanoparticle high dose led to a decrease in TG, where the 200 mg/kg dose group

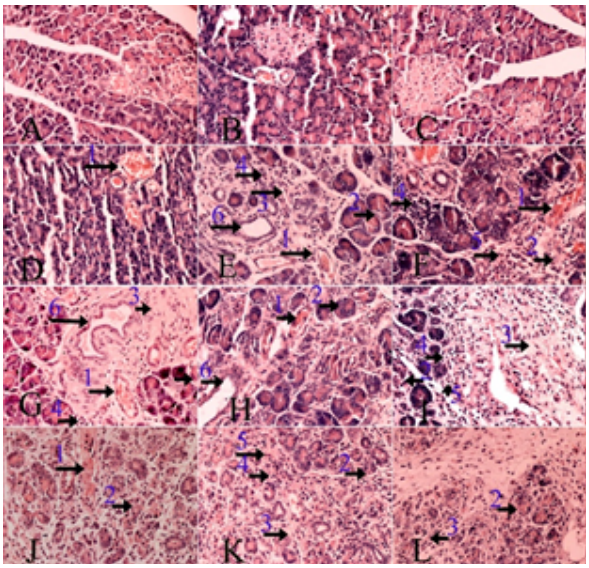

Figure 2. Histopathological examination of rats' pancreas tissue between groups

A, B, C: Control group, Sham group and 4 mg/kg Zno nps, Normal pancreas tissue; D: 8 mg/kg Zno nps, Hyperemia (arrow 1); E: 25 mg/kg Zno nps, Hyperemia (arrow 1), Necrosis (arrow 2), Fibrosis (arrow 3), Inflammatory cell infiltration (arrow 4), Exocrine ductal hyperplasia (arrow 5); F, G: 50 mg/kg Zno nps, Hyperemia (arrow 1), Necrosis (arrow 2), Inflammatory cell infiltration (lymphocyte arrow 3, Eosinophil arrow 4), Fibrosis (arrow 5), Exocrine ductal hyperplasia (arrow 6), H, I:100 mg/kg Zno nps, Hyperemia (arrow 1), Necrosis (arrow 2), exocrine ductal hyperplasia (arrow 3), Fibrosis (arrow 4), inflammatory cell infiltration (lymphocyte arrow 5, Eosinophil arrow 6); J, K, L: 200 mg/kg Zno nps, Necrosis (arrow 1), Hyperemia (arrow 2), inflammatory cell infiltration (lymphocyte arrow 3, Eosinophil arrow 4), Fibrosis (arrow 5); H & E staining, X40 magnifications.

reported the lowest level. There is a significant difference between 100 and 200 mg/kg dose groups and other groups (P<0.05). The serum cholesterol levels, High-Density Lipoprotein Cholesterol (HDL), Total Protein (TP) and albumin decreased in the rats exposed to the low and high doses compared to the control group. In addition, a significant decrease occurred in the 200 mg/kg dose group compared to other groups (P<0.05). However, the content of Bilirubin increased in ZnO nanoparticles exposed groups. The dose-dependent increases were significantly observed in 200 mg/kg treated group (P<0.05).

The effects of the varying concentrations of ZnO nanoparticles on liver enzymes are presented in Table 4. As shown, treatment with ZnO nanoparticles increased ALT and AST activity to 100 mg/kg dose group. Thus, the highest level enzymes were observed in 100 mg/kg concentration, while 200 mg/kg dose of ZnO nanoparticles led to severe reduction of enzyme concentration indicating a significant difference between control and sham groups (P>0.05). It seems that the decreases occurred due to hepatocytes' scattered necrosis, which is consistent with our histopathological results.

The average blood glucose levels in 100 and 200 mg/kg experimental groups were significantly lower than the control group. Further, no significant difference was observed between control, sham, 4 and 8 mg/kg experimental groups, while no significant difference was reported with other groups (P<0.05). Average blood amylase and lipase levels increased dose-dependently to 50 mg/kg, while it reduced in 100, 200 mg/kg treated group. In fact, there is a significant difference between 200 mg/kg groups and other groups (P<0.05).

Malondialdehyde levels: MDA levels increased dose-dependently in liver of 50 mg/kg experimental group. Then, the level reduced due to an increase in the extent of damage in liver and pancreas tissue. Finally, MDA levels were significantly higher in experimental groups compared to the control group (P<0.05) (Tables 3 & 4).

Zinc Concentration in liver: Zinc concentration in liver tissue was determined by atomic absorption spectrometry. High liver zinc concentrations were observed in 200 mg/kg experimental groups. In other words, as shown in Table 4, a significant difference is observed between 200 mg/kg experimental groups and other groups (P<0.05).

Discussion

During the recent years, zinc toxicity has been fully evaluated in different studies. The presence of this metal in food canned or stored in galvanized containers can lead to long-

term toxicity and carcinogenicity. In addition, zinc concentration may increase in water flows through galvanized, copper, or plastic tubes [18]. The exposure of ZnO fumes commonly occurs in welders and smelters. Air pollutants can be diffused in different distances based on the size and diameter of the particles [19]. Destructive effects on different organs and systems can be caused by short- and long-term exposure of heavy metals such as zinc. Thus, damage effects on liver and pancreas are possible due to the inhaled pollutant particles pass into the systemic circulation [20]. ZnO-NP treatment at 4mg/kg dose caused an increase in body weight compared to the control group, while weight loss was dose-dependent (Table 1). The findings are in agreement with the previous reports demonstrating that heavy metal toxicity often accompanies weight loss [21]. Further, the weight of the liver increased in the 8 mg/ kg group, which subsequently reduced by increasing the dose of ZnO-NP. Some studies indicated the toxic effects of heavy metals on liver tissue. The liver is the target organ for the toxicity of heavy metals which can cause some changes in relative liver weight. Adaptation to metal toxicity depends on their removal, metabolism, and excretion [22].

The serum biochemical levels including Lactate Dehydrogenase (LDH), ALT, ALP, TP, total cholesterol, TG, and plasma glucose concentration were assayed by an automatic biochemical analyzer. The treatment with ZnO in rats led to a significant increase in serum creatinine, BUN, Bilirubin, and Phosphorus levels, and a significant decrease in glucose, HDL, TG, cholesterol, TP, and uric acid levels. A significant decrease in serum TP and albumin levels in exposed animals are related to the changes in protein synthesis and metabolism [23]. Zhu et al. [24] studied toxic responses in oral administration for rat exposed to a mixture of five toxic elements, and found that the total cholesterol, HDL, and TG reduced in the high dose group compared to the control group. In another study, the mice of both sexes in the 30,000 ppm group indicated a slight to moderate decrease in TP, glucose, and cholesterol [25]. Amara et al. [26] reported that glucose concentration significantly decreased in the rats injected with Zno nanoparticles. Dudley et al. [27] concluded that the acute exposure of cadmium by IV injection led to a decrease in plasma glucose concentration, which remained low. In addition, the results in the present study indicated a reduction in glucose levels. It seems that detectable significant structural changes can be used as the ways for preventing and treating diabetes with respect to the hypoglycemia at dose of 4 mg/kg and absence of lesions.

In this study, an increase occurred in the liver enzymes, such as ALP, ALT, and AST. ALT and AST levels measured in the blood can be considered as sensitive indicators for hepatocellular attrition. Therefore, a significant increase in these enzyme activities in all rats exposed to ZnO nanoparticles supports the hepatotoxic effect of these nanoparticles compared to the control group. In addition, the toxicity effect of ZnO nanoparticles on the liver enzymes was considered in this study. In another study, an increase occurred in liver enzymes such as AST following ZnO nanoparticles IV injection in 5 and 10 mg/kg doses in Wistar Han rats after 7 days. Further, this metal led to a fivefold increase in AST after 24 hours at the higher dose of 20 mg/kg [28]. Further, Fazilati [2] indicated that ZnO nanoparticles after Intra peritoneal injection in male Wistar result in increasing AST, ALT, and ALP enzymes in all groups compared to the control group. Hosseini et al. [29] concluded that the levels of hepatopancreatic enzymes increased significantly due to treatment with AlCl3. Brzoska et al. [30] reported that serum levels of ALT and AST increased in the rats exposed to water containing cadmium and ethanol, compared to the control group. The result in the present study is consistent with some other findings which indicated the toxicity effect of heavy metals on the liver enzymes. In the histopathological examination, the liver in moderate and high doses treated with ZnO nps indicated necrosis, congestion, and inflammatory infiltration in the hepatocytes.

Zhu et al. [24] reported that swollen hepatocytes and fatty degeneration, as well as scattered spotty necrosis can occur followed by subchronicoral exposure to toxic elements, such as cadmium chloride, potassium dichromate, mercuric chloride, sodium arsenite, and lead acetate. Some studies indicated that liver is considered as an organ, which can be damaged by Cr (VI) and some histopathological changes such as parenchymal degeneration, steatosis of hepatocytes, and necrosis were observed. In another study, Jadhav et al. [31] showed that subchronic exposure via drinking water to a mixture of eight water-contaminating metals such as cadmium chloride, lead acetate, and nickel chloridelead to some complications such as vascular engorgement, erythrocyte extravasation, and mononuclear cell infiltration in male rat liver. Additionally, the degeneration and necrosis were observed in the liver. Further, oral administrations of ZnO at the dose of 400 mg/kg inbody weight lead to some degree of hemorrhages and focal necrosis, as well as lymphocyte infiltration in the liver [3].

Histopathological results indicated dose-dependent increases in lesions with different doses of ZnO nanoparticles injection including hyperemia, inflammatory cell infiltration, necrosis, exocrine ductal hyperplasia, and fibrosis. Aughey et al. [32] indicated that chronic exposure to oral zinc supplementation results in enlarging pancreatic islets and replacing pancreatic acinar tissue by connective tissue compared to the control mice. Pancreatic histopathological lesions of the same features included regressive changes of

the exocrine glandular tissue in both mice and rats as observed by Maita et al. [25]. The results confirmed that zinc is ingested into the body accumulates in the liver and pancreas to high concentrations and excreted into the pancreatic fluid with bile. An increase in MDA levels was observed in Zno-induced experimental groups compared to the control group, which indicated an increase in lipid peroxidation. Aykin-Burns et al. [33] reported that lead exposure can cause MDA high levels on liver and brain of young and old lead-exposed rats compared to the control group. In another study, the use of cadmium increased MDA in some tissues such as liver, kidney, brain, and testes [34]. The results are in line with some previous studies where MDA levels increased in mercury, chromium, or silver treated mice results in lipid peroxidation [35].

Based on the results of the present study, lipid peroxidation is considered one of the molecular mechanisms in cell injury in poisoning chronic ZnO nanoparticles. The nanoparticles were accumulated in the target tissues. The ZnO concentrations were determined by atomic absorption spectrophotometric method. The results indicated a higher concentration of ZnO in the liver tissues in high-dose groups compared to the control group. Finally, a significant accumulation of nanoparticles in the liver and pancreas tissues led to cellular injury after its injection. Future studies are recommended to reduce the effects of ZnO nanoparticles.

Conclusions

Heavy metals are important in many respects such as manufacturing important products of human use, although the bio-toxic effects are potentially life-threatening because of probabilistic environmental pollution. The results confirmed that ZnO nanoparticles have toxic effects on the liver and pancreas tissue in the animal model used in this study.

Ethical Considerations

Compliance with ethical guidelines

The laboratory programs were approved by the Animal Ethics Committee of Islamic Azad University, Babol Branch, Babol, Iran.

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Author's contributions

Project design, pathological evaluation, data analysis, and editing the article: Seyed Mohammad Hosseini; Project administration and original draft preparation: Reza Amani and Amir Hossein Moshrefi; Care and maintenance of laboratory animals, laboratory examination and review of article: Seyed Vahid Razavimehr, Mohammad Hasan Aghajanikhah and Zahra Sokouti; Read and approved the final manuscript: All authors.

Conflict of interest

The authors declare no conflict of interest with any entity in conducting this research.

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