



TOXICOLOGICAL EFFECT OF EXPOSURE TO DIFFERENT DOSES OF ZINC OXIDE NANOPARTICLES ON BRAIN AND HEART STRUCTURES OF MALE WISTAR RATS

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Summary

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Nanotechnology is rapidly developing in the fields of industry, medicine and nutrition. The aim of this study was to evaluate the effect of zinc oxide nanoparticles (ZnO Nps) toxicity on rats' heart and brain. Eighty Wistar male rats were allotted into eight groups: control group, sham group receiving 0.9% normal saline and six treatment groups receiving ZnO Nps (4, 8, 25, 50, 100 and 200 mg/kg) intraperitoneally twice a week over 28 days. For behavioural evaluation, shuttle box and Y-maze tests were done. The heart and brain structures were obtained for bioaccumulation, histopathological examination and biochemical analysis. Histopathologic lesions in the heart structures of 200 mg/kg ZnO Nps group included necrosis, hyperaemia, and vacuolar degeneration. In brain structures, changes included necrosis, gliosis and spongiform change. Serum levels of creatine phosphokinase (CPK) in the treated groups showed an increase compared to the control group. The accumulation of nanoparticles has also shown a dose-dependent increase in the heart and brain. Moreover, there was a significant difference between the control group and the 200 mg/kg group ($P < 0.05$). The mean acquisition of the passive avoidance test showed a significant decrease in the 200 mg/kg group compared to the control group ($P < 0.05$). The alternation behaviour test differed significantly between the 100 and 200 mg/kg groups with other groups ($P < 0.05$). The results indicated that zinc nanoparticles at doses more than 25 mg/kg were related to heart and brain toxicity in the form of increased bioaccumulation, malondialdehyde (MDA), histopathological lesions and CPK and decrease in behaviour index, glutathione peroxidase (GPx), superoxide dismutase (SOD) and ferric reducing antioxidant power (FRAP).

Key words: brain, heart, nanoparticle, rat, zinc oxide

INTRODUCTION

Zinc (Zn) is known as the second most common trace metal after iron in mammalian structures and as an essential element for the growth, development, and synthesis of DNA; safety and other important processes of cells. Nanoparticles refer to matters with size less than one to hundred nanometers. In recent years, the use of nanoparticles has been significantly increased in biotechnology, medicine, pharmacy, food, and chemical industries (Echegoyen & Nerin 2013; Durán *et al.*, 2015; Young *et al.*, 2016).

The increasing application of nanoparticles in products is likely to increase the workers and consumers' exposure to these materials. Due to their small size, there are concerns that unwanted nanoparticles may pass the barriers in the human body. Several studies conducted on rodents indicated that nanoparticles can pass through the lung, intestine, skin, and placenta depending on their application route, time, concentration, and properties (Braakhuis *et al.*, 2015).

Nanoparticles are transmitted through contaminated drinking water, breathing polluted air, and consumption of food sources affected by contaminated soils. Contamination with metallic trace elements such as zinc generate risks to human health.

Zinc is a component of essential enzymes and proteins that play important roles in the structure and function of membrane, hormone activities, enzymatic reactions, duplication, translation of genetic material, and protection against free radicals. Zinc is also effective on preventing cancer, boosting the immune system, and improving the male's fertility; however, its excess values in the environment and body may have some adverse effects

on body structures (Garcia *et al.*, 2005; Heinlaan *et al.*, 2008).

Oxidative damage results from an imbalance between oxidants and antioxidants including oxidative changes in cellular macromolecules, cell death (apoptosis or necrosis), and structure destruction (Lykkesfeldt & Svendsen, 2007).

Reduced zinc levels were reported to induce hippocampus lesions causing spatial memory deficit in adult rats (Golub *et al.*, 1995). It was also found that the acute use of nano particles of zinc oxide causes the destruction of memory in the Single-Trial Passive Avoidance learning model (Valipour Chahardahcharic *et al.*, 2014).

The oxide form is the most common compound of zinc (Heinlaan *et al.*, 2008, Garcia *et al.*, 2005). Human beings and other organisms may be exposed to this element orally, through inhalation, intravenous injection, and skin penetration. Moreover, zinc oxide has the highest concentration of zinc as well as a high absorption in body (Diaz *et al.*, 2001; Hotz *et al.*, 2005).

Given the available information on the toxic effects of nano-materials as well as their physical and chemical properties in having interactions with biological components, their significant inductive effects on the behaviour and properties of body molecules and cells have been proven. The occurrence of oxidative stress, lipid peroxidation, cell membrane leakage, oxidative damage of DNA, the increased intracellular calcium, and even anti-proliferative activity by ZnO have also been proven in cell lines (Huang *et al.*, 2010).

There is a need for further studies on this important element due to its abundance in nature and foods and its numerous ways to enter the body, and on the

other hand, the vulnerability of brain and heart structures, Therefore, the aim of this study was to evaluate the effect of zinc oxide nanoparticles toxicity on rats' heart and brain.

MATERIALS AND METHODS

Preparation of nanoparticle solution

Roughly spherical creamy white crystals of ZnO Nps were prepared in dimensions of 10 to 30 nm, with a purity of 99% from Nanosany Company. To prepare solutions of 4, 8, 25, 50, 100, and 200 mg/kg, nanoparticles were dissolved in 0.5 mL of 0.9% saline solution and then homogenised using a sonicator before injection (Misonix Sonicator, 55 W).

Experimental groups

The present experimental study included 80 adult male Wistar rats weighing 200 ± 20 g and aged between 10 and 12 weeks. The animals were obtained from the reproduction center of the Pasteur Institute, north of Iran. Thereafter, the test animals were kept under controlled conditions at 20 °C, humidity $55 \pm 5\%$, 12-hour light cycle, in terms of the ethical standards of working with laboratory animals. The animals received water and food throughout the experiment with no restrictions.

Animals were randomly divided into eight groups including control group, sham group as well as 6 experimental groups, each containing 10 animals. In the control group, the animals received only normal water and food without any drug or medication. The animals of the sham group received intraperitoneal injection of 0.5 mL of 0.9% normal saline solution per kg of body weight twice a week. The first, second, third, fourth, and fifth experimental groups received intraperitoneally 0.5

mL of solutions at concentrations of 4, 8, 25, 50, 100, and 200 mg of ZnO nanoparticle per kg of body weight.

Animal research ethics

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

Y-maze task

This test was performed using Y-Maze apparatus after 28 days. The apparatus has three perpendicular arms, each marked with an alphabetical letter of A, B or C. Testing was performed in silence, and rats (with no previous knowledge of maze) were placed at the end of an arm, where they could freely enter into other arms. Therefore, changes in animals' entry to arms were recorded at 8 minutes. However, the condition for animal entering into one arm was the full entry of trunk and the beginning of tail. In fact, the spontaneous alternation behaviour, referring to the spatial memory, means the alternate entry to all three arms that is considered in a triple mode. The amount of animal spatial memory was presented in alternation percents that can be measured according to the following equation (Nobakht *et al.*, 2011): Behavioural Alternation Percent = $100 \times (\text{total number of moves}) / (\text{correct moves})$.

Single-trial passive avoidance test

This test was performed using the Shuttle-Box apparatus (BPT Co., Tehran, Iran). This apparatus has two dark and light rooms of the same size, connected by a small central guillotine door. In this apparatus, when rats are in the dark room, electric shock is given through a lattice metal floor. All animals were adapted to apparatus during the first two days and

after 5 minutes of staying in a dark room. On the third day, the rats entered the light room, and the guillotine door was opened after 2 minutes. Due to the lack of willingness to light, rats entered the room, and then the door was closed. Next, an electric shock of 1 mA was given to rats for 2 seconds from the room floor. By passing 24 hours from the replication of test, entry of rats into the light room, and opening the door between two rooms, the latency to step through the dark compartment (600 s maximum) was measured as record of this test (Nobakht *et al.*, 2011).

Blood sampling and creatinine phosphokinase assay

The animals were weighed at the end of the 28th day and were intraperitoneally anesthetised by injection of ketamine (60 mg/body weight) and xylazine (20 mg/body weight). Blood samples were then taken by direct blood sampling from the rats' heart via the left ventricle using a 19–21 gauge needle. Blood was withdrawn slowly to prevent the heart from collapsing. To evaluate serum levels of CPK, the obtained samples were centrifuged at 5000 rpm for 15 minutes. Thereafter, sera were collected for enzyme measurements in an autoanalyzer (Hitachi 902, Japan) using the exclusive kits of Pars Azmun Company (Iran) in terms of the recommended method of the International Federation of Clinical Chemistry (IFCC).

Zinc bioaccumulation in the brain and heart

In order to determine the bioaccumulation of zinc, brain and heart structures were completely dried and then placed in the oven at 150 °C to reach a constant weight. A gram of the separated tissue samples was removed, and placed in a clean test

tube. For chemical digestion, 5 mL of nitric acid-perchloric acid was added to the test tube at a ratio between 4 and 10. The test tubes were closed with cotton wool and kept for a night at room temperature. The digested samples were heated in a water bath for 20 minutes at 100 °C. After the addition of 1 ML of hydrogen peroxide into each tube, they were let to cool and remained overnight at room temperature to prevent the extra foam. On the next day, the samples were reheated for an hour at 100 °C and then cooled at room temperature. The cooled contents of the tubes were filtered through a filter paper. The remaining solution was transferred to a volumetric flask and diluted to 25 mL. Atomic absorption spectrophotometry (Buck-210VGP model, USA) was used to measure the bioaccumulation of zinc (Ajayi *et al.*, 2012).

Tissue samples and oxidative stress indices assays

Homogeneous mixtures were prepared by combining 1 g of either brain or heart structures with 10 ML of phosphate buffer using an Ultra Turrax homogenizer (IKAT18 model, USA). Afterwards, this mixture was centrifuged using a refrigerated centrifuge at 5000 rpm for 20 minutes. The resulted solution was collected and stored frozen at –80 °C to measure oxidative stress indices.

The activity of glutathione peroxidase (GPx) was examined using ELISA reader (Stat fax-2100 Model) at 412 nm with ZellBio Kit (ZellBio GmbH Ulm, Deutschland, Germany).

The activity of superoxide dismutase (SOD) was determined colorimetrically on ELISA reader (Stat fax-2100 Model) at 420 nm using ZellBio kit.

Tissue levels of MDA were measured in terms of the protocol of Zell Bio com-

pany (ZellBio GmbH Ulm Deutschland, Germany) based on the reaction of thiobarbituric acid (TBA) at a boiling temperature. In this test, MDA or quasi MDA matters reacted with thiobarbituric acid, producing pink coloured compound with a maximum light absorption at 532 nm. The absorption was measured on Pharmacia LKB Novaspec II spectrometer apparatus (Biochrom Ltd., Cambridge, UK).

Ferric reducing antioxidant power (FRAP) of samples was assayed on the basis of ferric tripyridyltriazine complex reduced by sample antioxidants to ferrous form coloured in dark blue. The intensity of this colour was measured at a spectrophotometer at 593 nm and it had a direct relationship with the total antioxidant reducing power of sample. In this test, a series of iron sulfate solutions was used as a standard (Benzie & Strain, 1999).

Histopathological examination

After the autopsy of the animals, their brain and heart tissues were removed, put in pathological sampling containers containing 10% buffered formalin for 24 hours, and then transferred to the pathology laboratory. Tissue passage, prepara-

tion of paraffin blocks, and preparation of 5 micrometer sections using a rotary microtome (Leica, RM2235, Germany) were also performed. Coronal brain sections were prepared at -2.5 mm to -4.5 mm from bregma. All sections were stained with haematoxylin-eosin (Sigma, England) and examined using a light microscope (CX31- OLYMPUS, Japan). The severity of lesions was recorded as no lesion (-), mild lesions (+), moderate lesions (++), and severe lesions (+++) (Hosseini *et al.*, 2018).

Data analysis

SPSS22 was used to compare data between the treatment and control groups. Data analysis was done using the one-way analysis of variance (ANOVA), and Duncan's *post hoc* test to indicate the level of significant differences. Data were recorded as mean $\pm$ standard deviations.

RESULTS

Y-maze task

The mean behavioural change (Fig. 1) was 95.60% for the control group and the lowest value was 41.40% in the group ex-

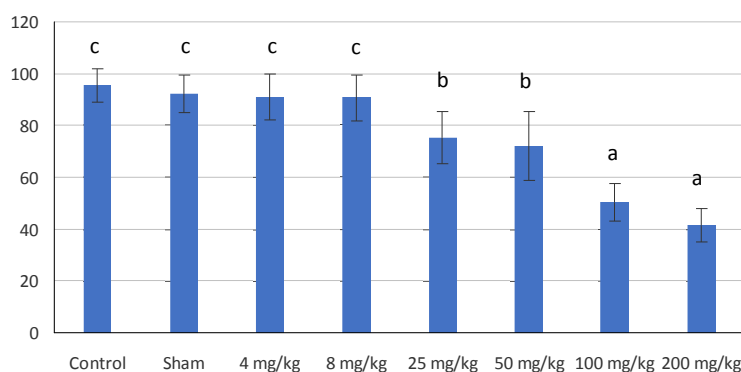


Fig. 1. Alternation behaviour percentage (mean $\pm$ SD) of control rats, sham-treated rats and rats exposed to different doses of zinc oxide nanoparticles. Significant difference between the groups ($P < 0.05$; one-way ANOVA followed by Duncan's test) is shown with different letters (a, b, and c).

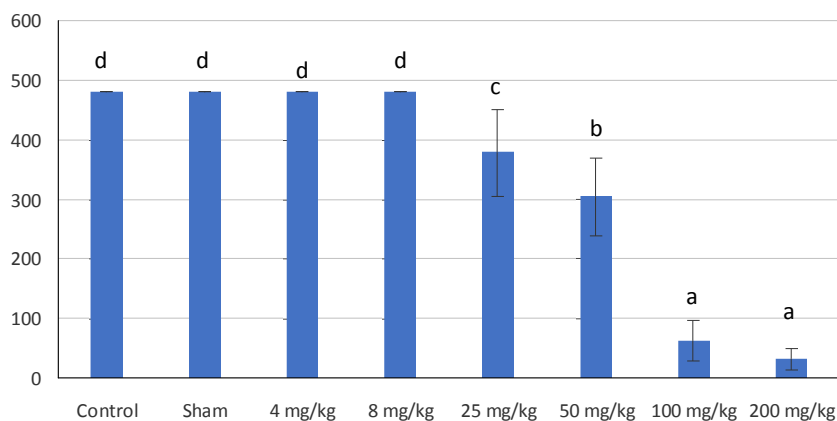


Fig. 2. Delayed response (seconds, mean $\pm$ SD) in control rats, sham-treated rats and rats exposed to different doses of zinc oxide nanoparticles. Significant difference between the groups ($P < 0.05$; one-way ANOVA followed by Duncan's test) is shown with different letters (a, b, c and d).

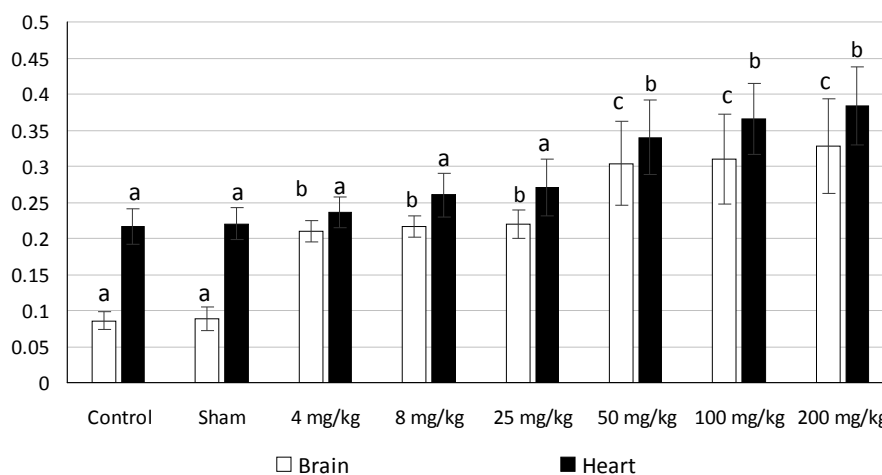


Fig. 3. Changes in bioaccumulation of zinc (ppm, mean $\pm$ SD) in control rats, sham-treated rats and rats exposed to different doses of zinc oxide nanoparticles. Significant difference between the groups ($P < 0.05$; one-way ANOVA followed by Duncan's test) is shown with different letters (a, b, and c).

posed to 200 mg/kg ZnO Nps. The behavioural change differed significantly between the 200 mg/kg group and other groups, except the 100 mg/kg group ($P < 0.05$).

Single-trial passive avoidance test

The mean delay time of entry in the dark room was 480 seconds in the control

group and 31.60 s in the 200 mg/kg group ($P < 0.05$) (Fig. 2). Notably, it seemed that memory was affected by concentrations higher than 25 mg/kg of this nanoparticle.

Blood and tissue biochemical analyses

Bio-accumulation values of ZnO nanoparticles in tissues of brain and heart were

Table 1. Evaluation of serum creatine phosphokinase (CPK) and oxidative stress indices in heart and brain structures in rats exposed to different doses of zinc oxide nanoparticles (mean±SD; n=5)

Groups	Serum CPK, U/L	MDA, nmol/mg		GPX, U/mg		SOD, U/mg		FRAP, µmol/g	
		heart	brain	heart	brain	heart	brain	heart	brain
Control	430±189 ^a	1.02±0.06 ^a	0.36±0.08 ^a	1.64±0.16 ^c	1.26±0.12 ^c	0.62±0.04 ^c	0.52±0.06 ^c	1.94±0.09 ^c	2.06±0.15 ^c
Sham	512±178 ^{a,b}	1.03±0.05 ^a	0.37±0.07 ^a	1.56±0.15 ^{b,c}	1.22±0.12 ^{b,c}	0.61±0.05 ^c	0.52±0.07 ^c	1.96±0.11 ^c	2.08±0.22 ^c
4 mg/kg	750±258 ^{a,b,c}	1.04±0.05 ^a	0.38±0.07 ^a	1.60±0.10 ^{b,c}	1.22±0.14 ^{b,c}	0.62±0.07 ^c	0.53±0.08 ^c	2.00±0.14 ^c	2.10±0.22 ^c
8 mg/kg	857±240 ^{b,c}	1.06±0.06 ^a	0.39±0.07 ^a	1.52±0.11 ^{b,c}	1.20±0.13 ^{b,c}	0.61±0.05 ^c	0.53±0.06 ^c	1.97±0.12 ^c	2.09±0.16 ^c
25 mg/kg	977±266 ^c	1.08±0.06 ^a	0.40±0.06 ^a	1.49±0.18 ^{b,c}	1.16±0.10 ^{b,c}	0.59±0.04 ^c	0.50±0.06 ^{b,c}	1.66±0.24 ^b	1.76±0.36 ^c
50 mg/kg	1429±328 ^d	1.16±0.20 ^{a,b}	0.41±0.07 ^a	1.45±0.17 ^{b,c}	1.45±0.13 ^{b,c}	0.55±0.06 ^{b,c}	0.48±0.06 ^{b,c}	1.52±0.18 ^b	1.40±0.35 ^b
100 mg/kg	1435±341 ^d	1.30±0.27 ^b	0.45±0.07 ^a	1.39±0.19 ^b	1.39±0.11 ^{a,b}	0.51±0.06 ^b	0.43±0.05 ^{a,b}	1.48±0.13 ^b	1.20±0.39 ^{a,b}
200 mg/kg	1526±344 ^d	1.50±0.20 ^c	0.68±0.12 ^b	1.16±0.14 ^a	1.16±0.10 ^a	0.40±0.06 ^a	0.37±0.06 ^a	1.18±0.19 ^a	0.98±0.22 ^a

Significant difference between the groups are shown with different letters (a, b, c and d) (P< 0.05; one-way ANOVA followed by Duncan's test)

significantly higher in the groups exposed to 50, 100, and 200 mg/kg compared to the other groups ($P<0.05$) (Fig. 3).

Measurement of biochemical values of serum CPK in animals exposed to doses of 50, 100, and 200 mg/kg showed a significant difference with the other groups ($P<0.05$) (Table 1).

GPx levels were minimum in heart and brain tissues in the group exposed to 200 mg/kg. In the heart tissue, there was a significant difference between 200 mg/kg group vs all other groups ($P<0.05$). In the brain tissue, there was no significant difference between the groups exposed to 100 and 200 mg/kg, but 200 mg/kg group showed significant differences vs the other groups ($P<0.05$) (Table 1).

The SOD levels in brain and heart tissues were the lowest in the 200 mg/kg group. There was no significant difference between the groups of 100 and 200 mg/kg in the brain tissue, but brain SOD in 200 mg/kg group of was statistically significantly different compared to the other

groups ($P<0.05$). In the heart tissue, the 200 mg/kg group showed significant differences with the other groups ($P<0.05$) (Table 1).

With respect to MDA levels in heart and brain tissues, the highest level was seen in the group exposed to 200 mg/kg ZnO NPs with significant differences vs all other groups ($P<0.05$) (Table 1).

Evaluation of antioxidant power levels in heart and brain tissues indicated the minimum level in the 200 mg/kg group. The difference between the 200 mg/kg group and the other groups in the heart tissue was statistically significant ($P<0.05$). In the brain tissue, there was a significant difference between the 200 mg/kg group and the other groups, except the 100 mg/kg group ($P<0.05$) (Table 1).

Histopathological analysis

Histopathologic data obtained from cerebral cortex tissue, demonstrated lesions such as necrosis, gliosis, and spongiform change after the use of different doses of

Table 2. Evaluation of histopathologic changes in rats exposed to different doses of zinc oxide nanoparticles

Brain cortex	Hippocampus	Brain cortex	Hippocampus	Brain cortex	Hippocampus	Tissue	Heart		
							Vacuolar degeneration	Hyperaemia	Necrosis
Spongy		Gliosis		Necrosis					
–	–	–	–	–	–	Control	–	–	–
–	–	–	–	–	–	Sham	–	–	–
–	–	–	–	–	–	mg/kg 4	–	–	–
–	–	–	–	–	–	mg/kg 8	–	+	–
–	–	–	–	+	+	mg/kg 25	–	++	+
–	–	+	+	++	++	mg/kg 50	–	++	++
+	–	+	+	+++	+++	100 mg/kg	–	++	++
+	–	+	+	+++	+++	200 mg/kg	–	+++	++

The histopathologic changes were scored as followed: no lesion (–), mild lesion (+), moderate lesion (++), and severe lesion (+++).

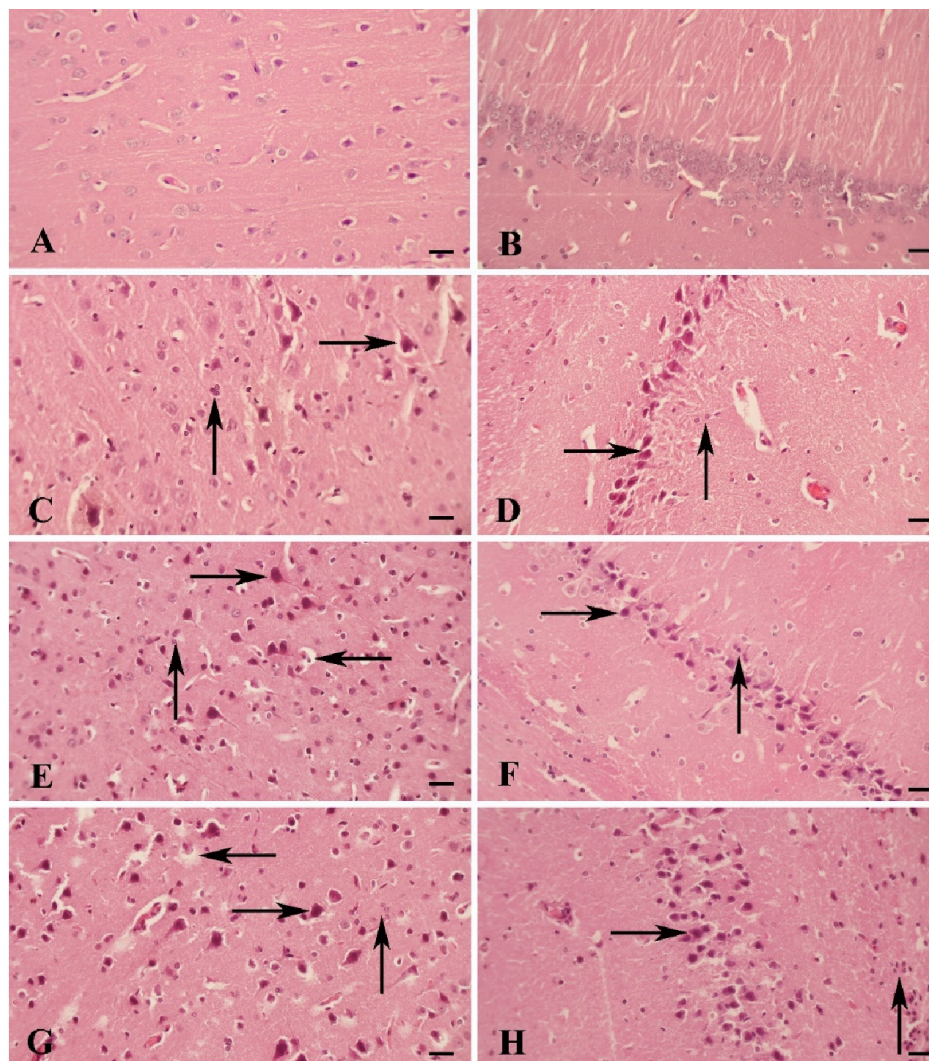


Fig. 4. Brain cortex tissue and hippocampus. A, B: control group, normal tissue, C, D: rats exposed to 50 mg/kg ZnO; E, F: rats exposed to 100 mg/kg ZnO; G, H: rats exposed to 200 mg/kg ZnO; neuronal necrosis (right arrow), gliosis (upward arrow), spongiform change (left arrow). H&E staining (bar=100 μ m).

ZnO nanoparticles. Lesions such as necrosis and gliosis were also observed in the histopathologic examination of hippocampus brain tissue. Accordingly, the highest percentage of lesions occurred in the groups exposed to 100 and 200 mg/kg ZnO Nps (Table 2, Fig. 4).

In the heart tissue, some lesions such as necrosis, hyperaemia, and vacuolar degeneration were seen after the use of different doses of ZnO Nps with the highest percentage of lesions in the group exposed to 200 mg/kg (Table 2, Fig. 5).

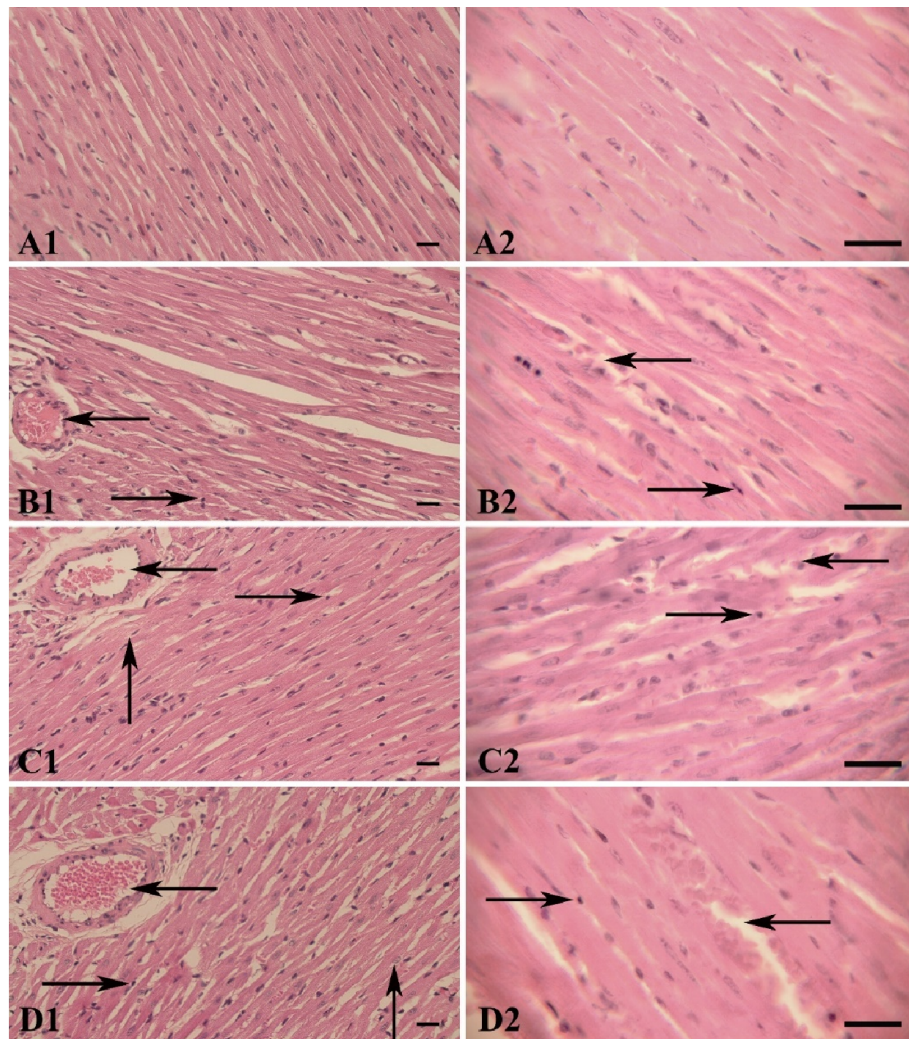


Fig. 5. Heart tissue. A1, A2: control group, normal tissue, B1, B2: rats exposed to 50 mg/kg ZnO; C1, C2: rats exposed to 100 mg/kg ZnO; D1, D2: rats exposed to 200 mg/kg ZnO; cardiac cells necrosis (right arrow), hyperaemia (left arrow), vacuolar degeneration (upward arrow). H&E staining (bar = 100 μ m).

DISCUSSION

The results of the present study indicated that compared to the control group, the administration of ZnO nanoparticles caused changes in the concentration of oxidative stress indices, in a way that an in-

crease in the amounts of these nanoparticles enhanced the concentrations of MDA which consequently led to the reduced levels of GPx, SOD, and FRAP.

Nanoparticles induce oxidative stress responses and cause cellular damage by entering into the mitochondria and causing physical damages that led to oxidative

stress and can increase inflammation by activation of related genes (Nguyen *et al.*, 2007; Durán *et al.*, 2015). Additionally, inflammatory cells such as macrophages and neutrophils activated phagocytosis of nanoparticles. Afterwards, this may lead to the production of free radicals of oxygen and nitrogen. However, other metal nanoparticles (iron, copper, chromium, and vanadium), can directly produce oxygen free radicals (Badkoobeh *et al.*, 2013, Espanani *et al.*, 2015).

The results of the studies conducted in this regard indicated that the exposure to ZnO nanoparticles can cause gene damage resulting from the lipid peroxidation and oxidative stress in epidermal cells (Sharma *et al.*, 2009). Furthermore, some researchers have found that Zn^{2+} released from ZnO plays a major role in the nanotoxicity (Ji *et al.*, 2011).

A study stated that nanoparticles can cause a significant reduction in enzymatic activities of GPx and catalase (Hao & Chen, 2012). The results of another experimental study indicated that the production of free radicals of oxygen significantly increased at concentrations of 50 and 100 mg/L of ZnO Nps (Zhao *et al.*, 2013). Moreover, ZnO Nps in the culture medium resulted in the production of oxygen free radicals followed by oxidative damage, inflammation and cell death (Hanley *et al.*, 2009). Correspondingly, the results of the above-mentioned studies were consistent with those of the present study.

Zinc is known as an essential element in the body metabolism that plays roles in the release of folate and its transmission from the cell membrane. Notably, high absorption of zinc is associated with the headache, nausea, spasm, and abdominal and digestive pains (Esmaili sari, 2002). Several studies conducted on species of

Atatürk Lake of Turkey and Manzala of Egypt in 2010 and 2011, respectively, indicated that zinc had the highest frequency of metal accumulation in skin and muscle tissues (Dadalhi *et al.*, 2008).

It was shown that the effects of ZnO nanoparticles on the liver and pancreas tissue leads to accumulation of ZnO nanoparticles and histopathological lesions including hyperaemia, inflammatory cell infiltration (Hosseini *et al.*, 2020). In a study performed on the spleen tissue and sedimentation of ZnO nanoparticles in spleens of mice, the highest absorption was observed at high doses (Kasra *et al.*, 2016). In our study, the amounts of ZnO nanoparticles were significantly higher in heart and brain structures of rats receiving higher doses compared to the other groups, which indicates a higher accumulation of heavy metals at the above-mentioned doses, in line with above cited studies.

The histopathologic examination showed some lesions such as the necrosis, gliosis, and spongiform change after exposure to different doses of ZnO nanoparticles in the brain tissue. In addition, necrosis, hyperaemia, and vacuolar degeneration were observed in the heart tissue. Naghsh *et al.* (2013) examined the toxicity of silver nanoparticles on heart tissues of Wistar rats and reported tissue changes and start of the apoptosis.

Seok *et al.* (2013) examined the effects of ZnO nanoparticles on pancreas as well as biological resistance of nanoparticles and possible solutions; their results indicated that the effects of ZnO nanoparticles were toxic for the pancreas causing pancreatitis, and the increased biological resistance against ZnO nanoparticles in the body. It seemed that the accumulation of heavy metals in brain and heart tissues was along with pathological lesions, the

result of which was consistent with those of the present study.

In a study by Hosseini *et al.* (2018) some lesions such as necrosis, hyperaemia, hyaline casts, inflammatory cell infiltration, glomerular proliferation, and fibrosis were observed during the intraperitoneal injection of different doses of ZnO nanoparticles into renal tissue; and most lesions occurred in the 200 mg/kg group (Hosseini *et al.*, 2018) in agreement with our results.

In another study performed several years ago, the major histological changes were seen in hearts and kidneys of rats after two intraperitoneal injections of various concentrations of copper oxide nanoparticle. These findings indicated the passage of nanoparticles through various cell membranes, as well as their entry into the blood stream and eventually into the heart and kidneys (Seyedalipour *et al.*, 2015). These results were consistent with ours in terms of the effect of metal nanoparticles on the heart tissue.

The increased serum CPK levels may be due to a damage to muscles including the heart muscle. Other researchers (Choi *et al.*, 2015), examined the toxicity of ZnO nanoparticles and observed that the intravenous administration of ZnO increased the activity of CPK enzyme. Correspondingly, this result was consistent with ours.

The short-term memory and avoidance behaviour decrease parallel to increasing doses of ZnO nanoparticles over 25 mg/kg suggested that high doses of nanoparticles can cause a negative effect on neural tissues. It was previously indicated that the acute administration of ZnO nanoparticles undermined the process of memory in Wistar rats (Valipour *et al.*, 2014) in line with our results.

CONCLUSION

Zinc plays important roles in the structure and function of membranes, hormone activities, enzymatic reactions, and protection against free radicals; however, its excess values in the environment and body may have some adverse effects on tissues. The results indicate that zinc nanoparticles at doses over 25 mg/kg were related to heart and brain toxicities manifested with increased bioaccumulation, tissue MDA, histopathological lesions and serum CPK as well as decreased behaviour index, tissue GPx, SOD and FRAP.

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