ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Toxic Effect of Zinc Oxide Nanoparticles on Some Organs in Experimental Male Wistar Rats

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Manuscript Info

Manuscript History:

Received: 12 February 2014
Final Accepted: 15 March 2014
Published Online: April 2014

Key words:

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Abstract

Considering nanotechnology development and their extensive use in different fields of industry, it is necessary to investigate their destructive effects on biological systems. Zinc oxide (ZnO) nanoparticles is an essential heavy metal and have been used as a source of zinc, in food industry and is applied in cosmetic products, but its accumulation in the tissues causes a hazard toxic effect. In present study, the effect of three different concentrations of ZnO nanoparticles was examined on male Wistar rats by daily intramuscular injection for 10 days. 24 male wistar rats were divided into four groups including a negative control group. At the end of experiment (day 11), rats were scarified and spleen, testis and heart tissues were dissected and fixed in 10% formline for histopathological investigations. Histopathological examinations showed severe damage and histological alterations in all tissues specimens. Results of present study proved ZnO nanoparticles toxicity and concluded that ZnO nanoparticles dose is one of critical factor influencing on their toxic effect. It is important to predict their toxicity potential on human. So, further studies are recommended to predict ZnO nanoparticles toxicity

INTRODUCTION

Nanomaterials have gained increasing attention because of their novel properties, including a large specific surface area and high reaction activity [1]. Due to rapid development of nanotechnology, nanomaterials with various shapes, doses and diameters, have been prepared and used in many industrial, agricultural and cosmetics products [2]. The fraction of atoms at nanoparticles surface is increased compared to microparticles or bulk. Compared to microparticles, nanoparticles have a very large surface area and high particle number per unit mass [3].

Several studies have reported that nano-sized particles always showed more serious toxicity than bulks [4] and suggested that their size and dose were one of the key factors influencing nanoparticles toxic effects. ZnO (zinc oxide) nanoparticles have been used as a source of zinc as it is considered one of the most important essential heavy metals in food industry, but if it accumulated inside the tissue, it will cause many hazard toxic effects [5]. Because of their small size (1-100 nanometers), nanoparticles oftentimes exhibit unusual physical, chemical and biological properties [6]. Nanoparticles dose is directly correlated to many essential properties, such as their effects on the interactions between nanomaterials and cell biomolecules that subsequently affect nanotoxicological behaviors of nanoparticles in vivo [9].

ZnO nanoparticles has been also widely applied to cosmetics such as personal care and sunscreen products [7]. Thus, nano-sized ZnO has recently attracted much attention in order to enhance the uptake of zinc. It is now generally accepted that nanoparticles efficiently penetrate the cell membrane compared to micro-sized particles.

However, few researches were performed to demonstrate the effects of ZnO nanoparticles with different doses on cellular uptake, which may provide critical information on their toxicity potential [8].

AIM OF THE WORK

ZnO usage in many food industrial, agricultural and cosmetics products opens the field to study the dose effect of ZnO nanoparticles as it considered one of critical factors influencing on ZnO nanoparticles toxic side effect. So, in this study, we investigated the effect of ZnO nanoparticles on some organs tissue (spleen, testis and heart) with three different doses (100mg/kg, 200mg/kg and 400mg/kg).

EXPERIMENTAL METHOD

Materials: Zinc acetate $Zn(CH_3COO)_2$, 2-propanol and sodium hydroxide were used in ZnO nanoparticles synthesis and were obtained from King abdullah institute for nanotechnology-King Saud University-KSA.

Animals: The experiments were carried out on 24 male Wistar rats which ageing about 6 weeks old, weighed about 100 to 150 g and were obtained from Faculty of Pharmacy-King Saud University-KSA. During the experiment, animals were housed in clean properly ventilated cages under constant controlled climatic conditions: temperature (25°C: 27°C) and lighting conditions (12 h light/12 h dark). Rat food and filtered tap water were provided to all animals. They were acclimatized to their environment at least two weeks before starting the experiment. All rats were monitored daily for any abnormal behavior and possible appearance of symptoms. The breeding and experimental part of the study was done in Faculty of Medicine and Applied Medical Sciences -Northern Borders University-KSA.

Group Design: 24 male Wistar rats were randomly divided into four groups. The group two, three and four were intramuscular injected with 2 mL ZnO nanoparticle/day for 10 days.

Group 1 (n=6): rats receiving saline intramuscular injection only for 10 days.

Group 2 (n=6): rats, received intramuscular injection for 10 days of low dose (100 mg ZnO nanoparticles /kg).

Group 3 (n=6): rats, received intramuscular injection for 10 days of middle dose (200 mg ZnO nanoparticles/kg).

Group 4 (n=6): rats, received intramuscular injection for 10 days of high dose (400 mg ZnO nanoparticles /kg).

Methods: 1.Synthesis of ZnO nanoparticles: ZnO nanoparticles were prepared by precipitation of ZnO nanoparticles by adding drop wise of 0.6 M NaOH solution to 0.2 M $Zn(CH_3COO)_2$ dissolved in 2-propanol solution with heating (35:37 Cº) [10]. ZnO nanoparticles synthesis was done in Faculty of Medicine and Applied Medical Sciences-Northern Borders University-KSA. The overall reaction for ZnO nanoparticles synthesis from Zn(II) acetate can be written as:

$$Zn(CH_3COO)_2 + 2NaOH \rightarrow ZnO + 2Na(CH_3COO)_2 + H_2O.$$

2. Characterization of Zinc Oxide Nanoparticles: purity and size of ZnO nanoparticles were examined by and X-ray diffractometer [11]. The morphology of ZnO nanoparticles were examined by transmission electron microscopy TEM [12]. ZnO nanoparticles characterization was done in King abdullah institute for nanotechnology-Riyadh-KSA.

3.Dosage Preparation: prepared ZnO nanoparticles were suspended in normal saline buffer, then dispersed by vortexing for one minute to prepare a stock solution with a concentration of 500 mg ZnO nanoparticles/mL as the final suspension pH was 7.3. The required doses of ZnO nanoparticles were then prepared with concentrations (100, 200 and 400 mg/mL normal saline). ZnO nanoparticles dosage preparation was done in faculty of medicine and applied medical sciences-Northern Borders University-KSA.

4.Histopathological studies: by the end of the experiment (day 11), rats were anaesthetized by ether and sacrificed. Spleen, testis and heart were extracted. The specimens were fixed, washed, dehydrated, cleared and embedded in paraffin wax. Specimens with 5 μm sections were prepared for light microscopic examination by applying hematoxylin and eosin stain (H&E) method [13]. Histopathological studies was done in faculty of medicine and applied medical sciences-Northern Borders University-KSA.

RESULTS

1. Characterization of ZnO nanoparticles

X-ray diffraction studies confirmed that the synthesized materials were ZnO nanoparticles by comparing their phase to wurtzite ZnO phase (Figure 1) and all detected diffraction peaks of ZnO nanoparticles sample agreed with the reported diffraction peaks of ZnO by The Joint Comission for Powder Diffraction Standards (JCPDS) data and no other characteristic peaks were observed in the analyzed sample (b) other than specific characteristic peaks of ZnO according to JCPDS (a) (Figure 2). X-ray diffraction data were recorded by using Cu Kα radiation (1.5406 Aº). The intensity data were collected over a 2θ range of 20-80º (Figure 1,2). The average grain size of the samples was estimated with the help of Scherrer equation. The mean grain size (D) of the particles was determined from the XRD

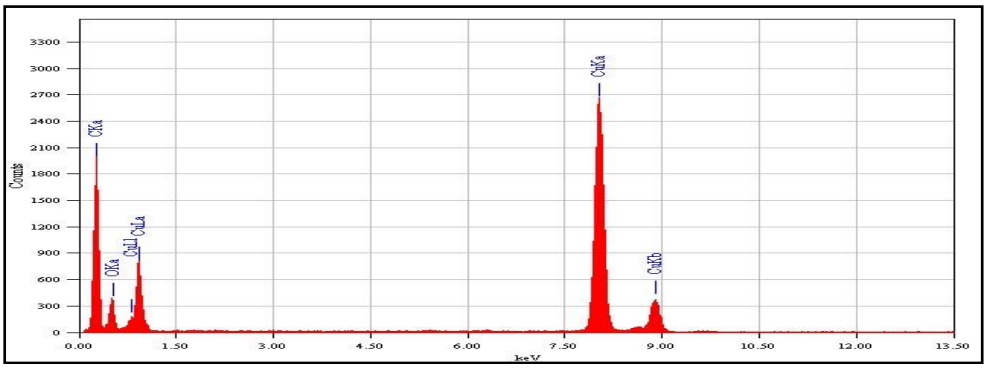
line by using Scherer equation [11]: {D=0.89 λ / (β Cos θ)}, Where λ is wavelength (Cu Kα), β is full width at the half-maximum (FWHM) of ZnO nanoparticles line and θ is the diffraction angle. Morphology of Zno nanoparticles sample was investigated using TEM. ZnO nanoparticles sample appeared as aggregated hexagonal shape particles (Figure 3). The measured particle size has mean diameter with 20 nm (Figure 1, 3).

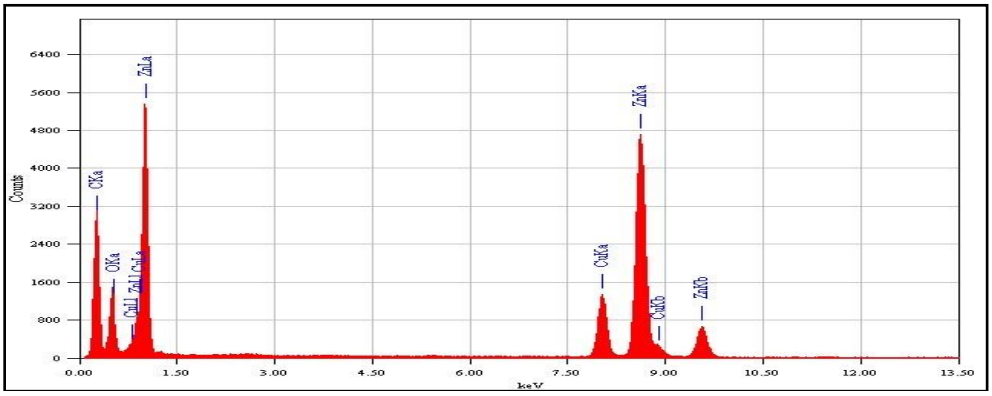
2. Histopathological studies

2.i. Spleen: sections from treated groups showed prominent red pulp at expense of white pulp/lymphoid follicular areas due to increasing fibroblasts numbers and interstitial fibrosis. Besides these areas of fibrosis there were haemosiderin pigments and calcium deposits (Figure 4). These histopathological alterations appeared lightly, moderately and severely after daily intramuscular injection with 100 mg ZnO nanoparticles/kg, 200 mg ZnO nanoparticles/kg and 400 mg ZnO nanoparticles/kg respectively (Figure 4).

2.ii.Testis: sections from treated groups showed testicular atrophy and decline in spermatogenesis process (Figure 5). There was more thickening of both interstitum (hyalinization) and seminiferous tubules basement membrane after intramuscular injection by 200 mg ZnO nanoparticles/kg daily and 400 mg ZnO nanoparticles/kg daily. Also, there were degenerated seminiferous tubules after intramuscular injection 400 mg ZnO nanoparticles/kg daily (Figure 5).

2.iii.Heart: Heart muscle fibres specimens of treated groups showed presence of fragmented muscle fibres. Endomysium gradually was increased with dose increasing. Also, splitted muscle fibres were clearly demonstrated with marked variation in size of muscle fibres. Many muscle fibres are terminated with pointed end muscle (angulated muscle fibres). Darkening and prepherlization of nuclei (undergoing atrophy) was noticed, but at case of high dose 400 mg ZnO nanoparticles/kg daily, it was observed loss of normal muscle fibre arrangement accompanied by focal areas of deep myolysis, thinning and shorting in muscle fibres (Figure 6).





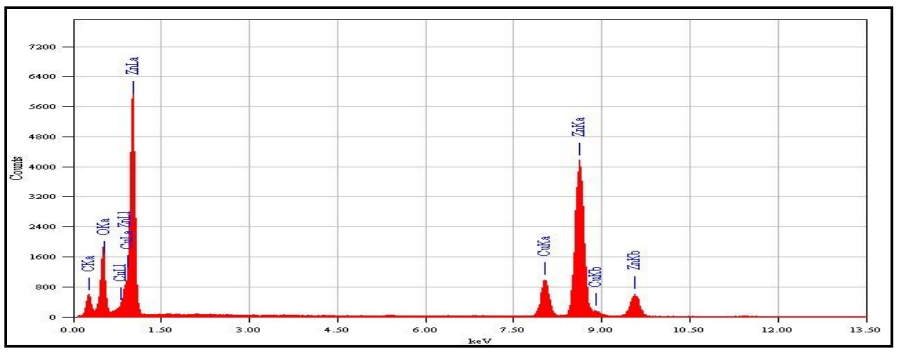


Figure 1: X-ray diffraction patterns of synthesized ZnO nanoparticles.

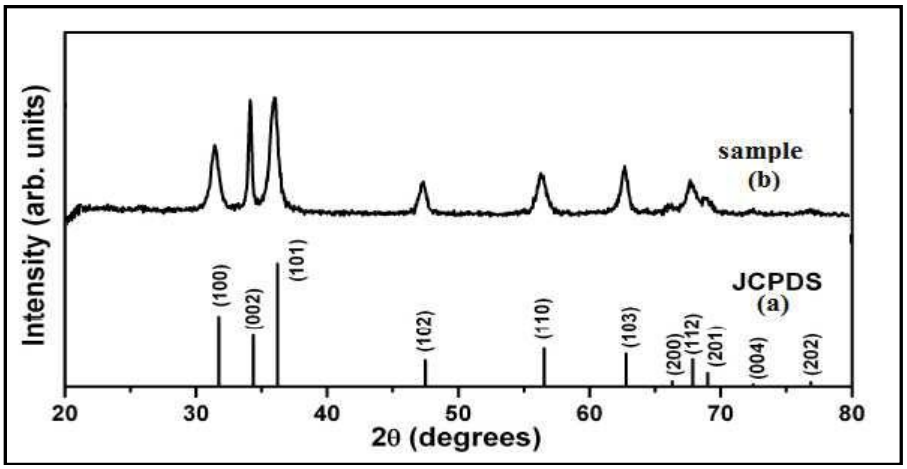


Figure 2: XRD patterns of ZnO nanoparticles. (a) Indicate standard XRD pattern according to JCPDS and (b) indicate XRD pattern of ZnO nanoparticles sample.

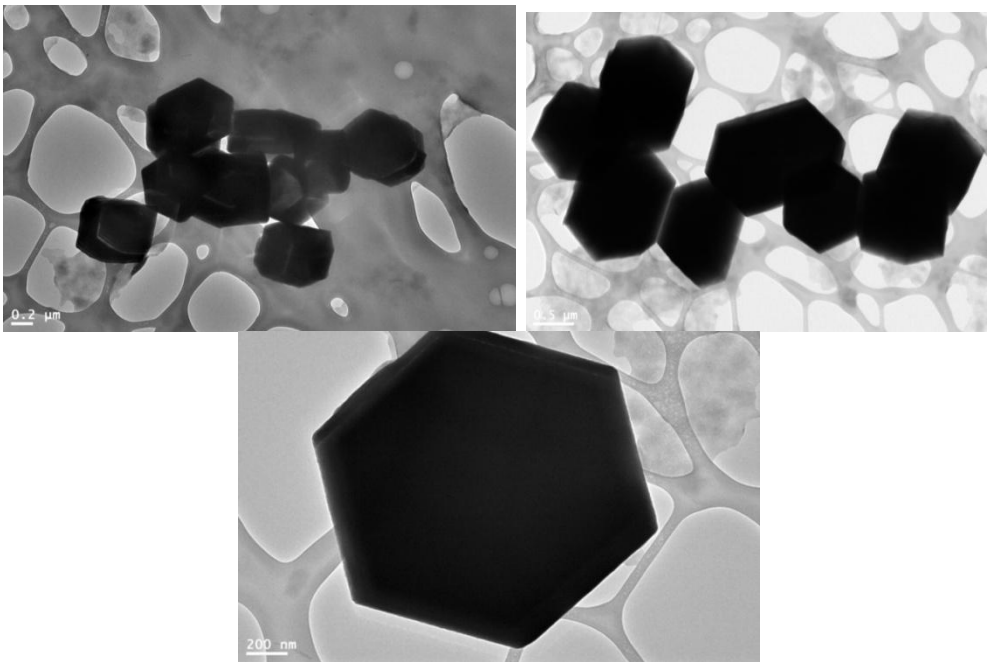


Figure 3: TEM images of ZnO nanoparticles sample. TEM images were captured in 0.2, 0.5 and 200 nanometer scale bar respectively.

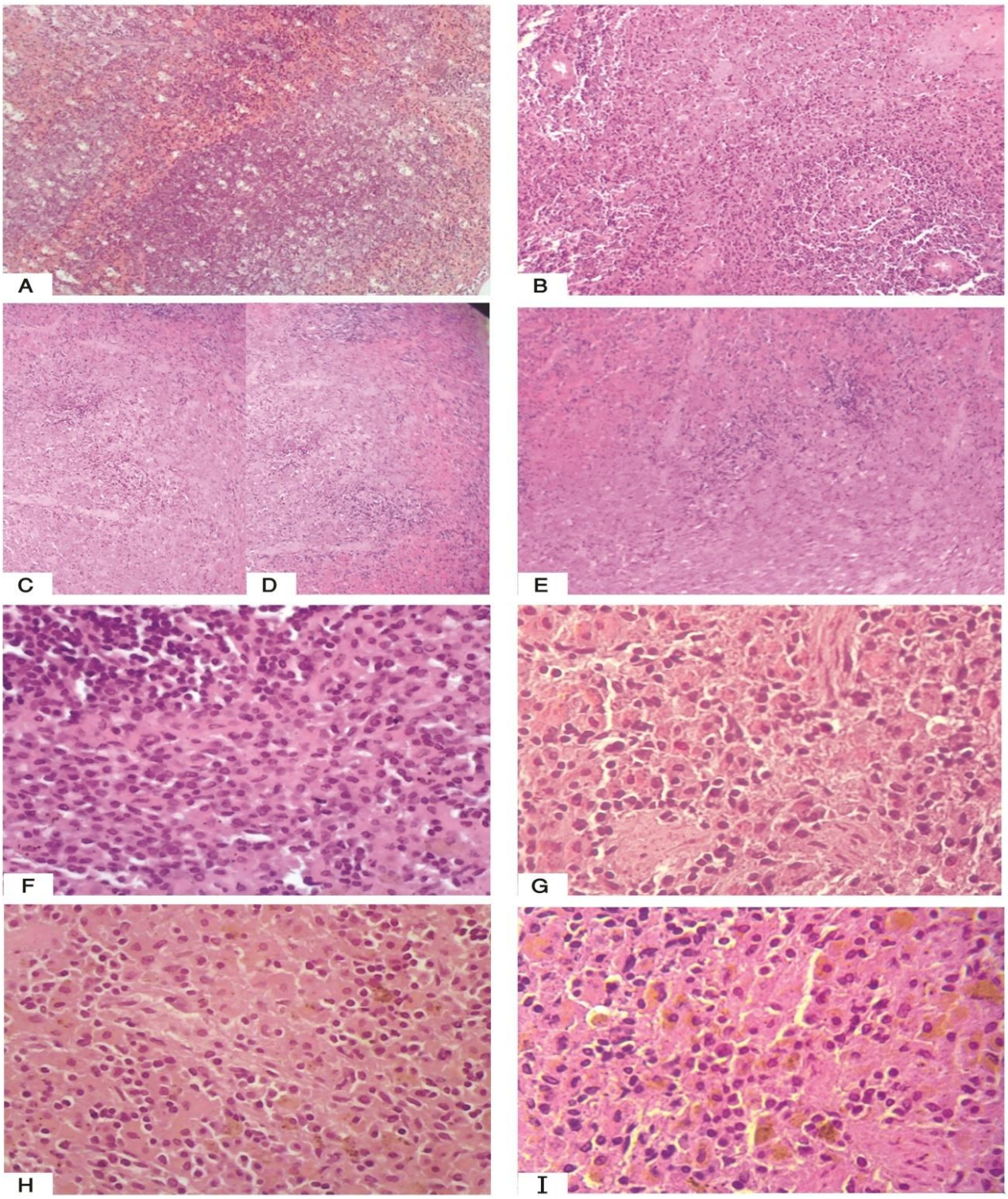


Figure 4: Histopathological findings of male Wistar rats spleen (transitional section) by H&E stain. Normal histological findings were seen in control group: [A (X100), F (X250)]. Male Wistar rats spleen histopathological appearance after intramuscular injection with 100 mg ZnO nanoparticles/kg daily [B

(X100), G (X250)], 200 mg ZnO nanoparticles/kg daily [C,D (X100), H (X250)] and 400 mg ZnO nanoparticles/kg daily [E (X100), I (X250)].

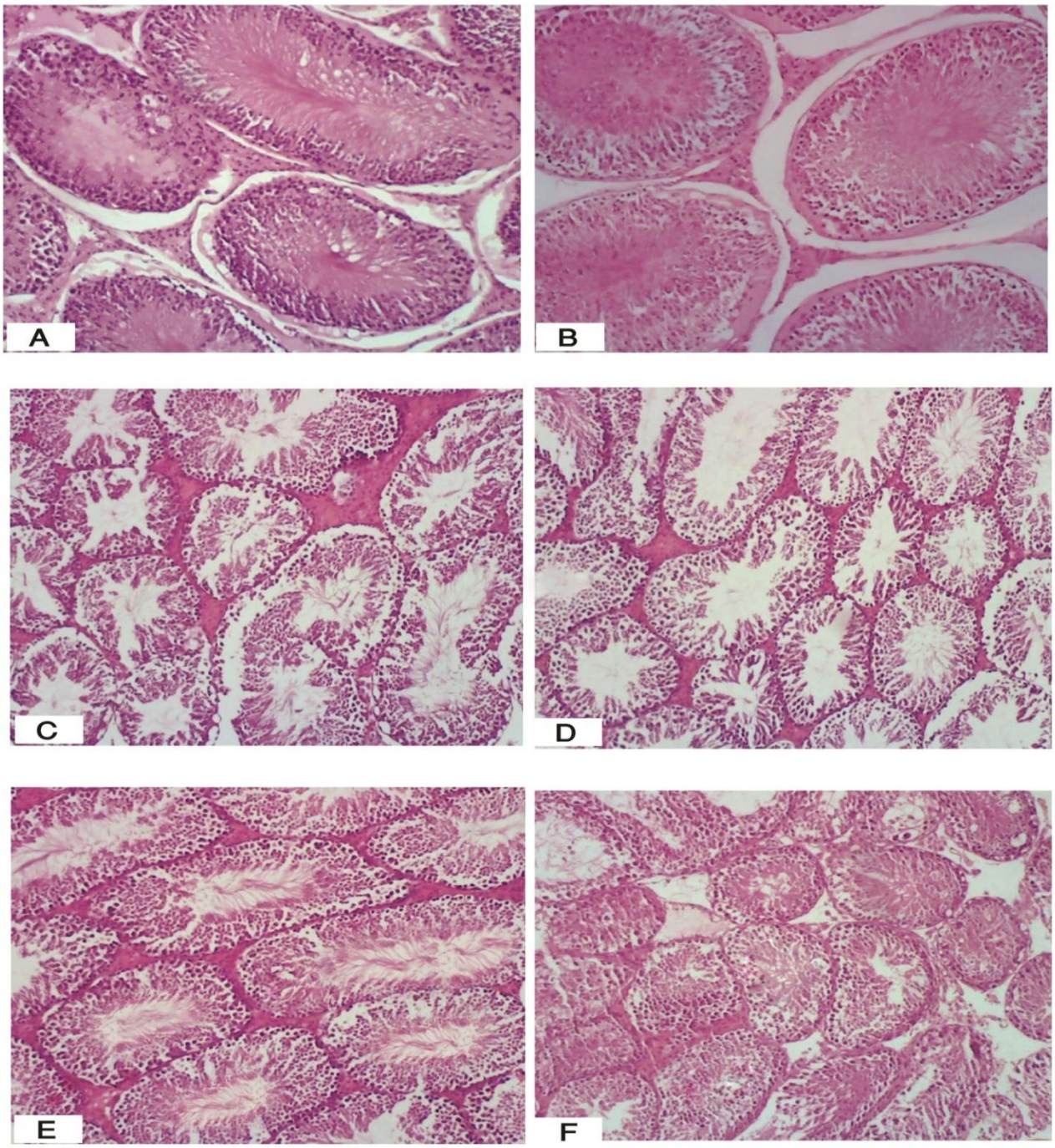


Figure 5: Histopathological findings of male Wistar rats testis (transitional section) by H&E stain. Normal histological findings were seen in control group: [A and B (X250)]. Male Wistar rats testis histopathological appearance after intramuscular injection with 100 mg ZnO nanoparticles/kg daily [C and D (X250)], 200 mg ZnO nanoparticles/kg daily [E (X250)] and 400 mg ZnO nanoparticles/kg daily [F (X250)].

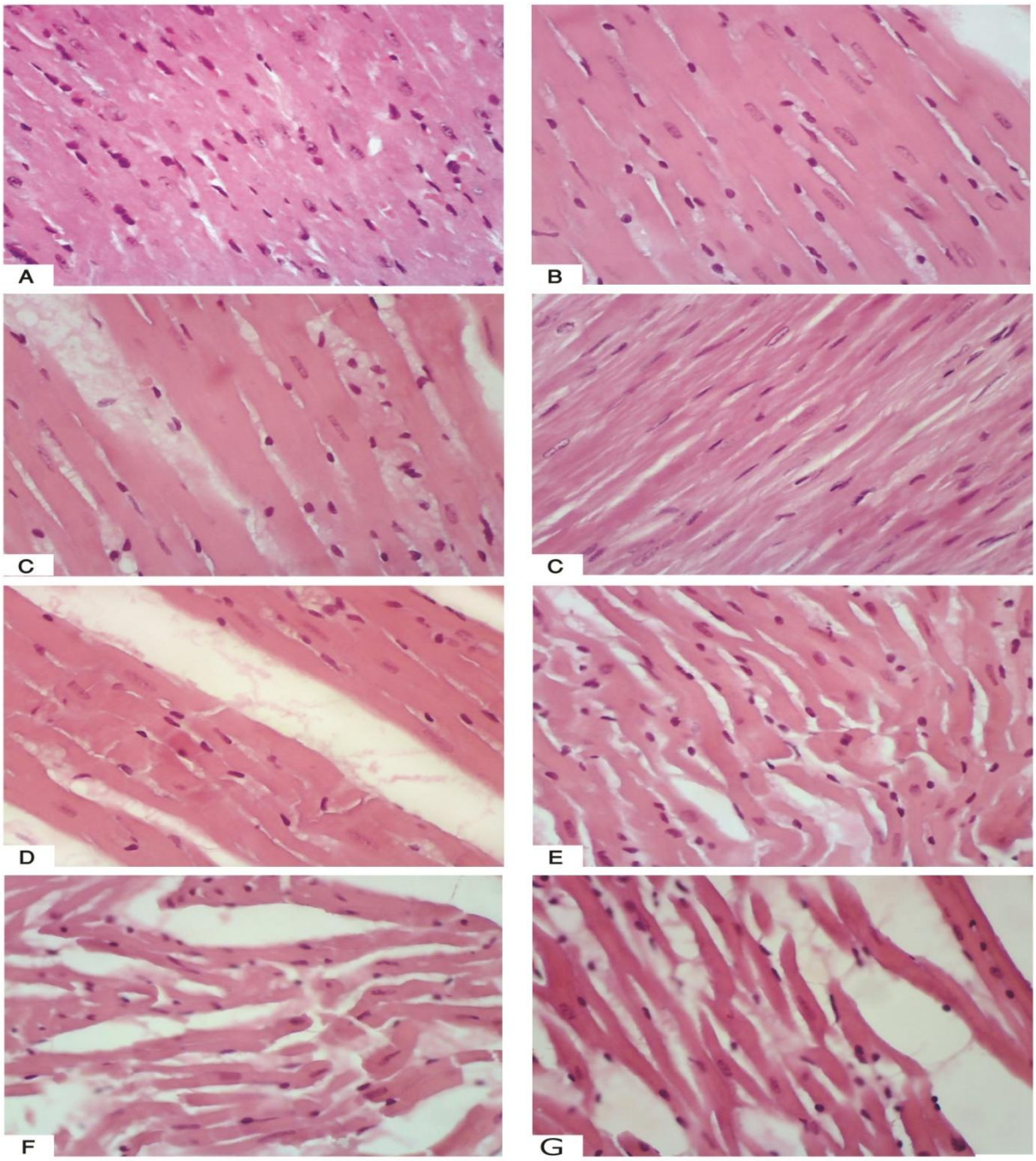


Figure 6: The histopathological findings of male Wistar rats heart muscle fibers (longitudinal section) by H&E stain. Normal histological findings were seen in control group: [A (X100)]. Male Wistar rats heart muscle fibers histopathological appearance after intramuscular injection with 100 mg ZnO nanoparticles/kg daily [B (X100)], 200 mg ZnO nanoparticles/kg daily [C (X100)] and 400 mg ZnO nanoparticles/kg daily [D (X100)].

DISCUSSION

ZnO nanoparticles are widely used in food industrial products as source of Zn and in cosmetics products as sunscreen as it was reported the effective dermal adsorption of ZnO nanoparticles [14]. Oral, inhalation, and intratracheal instillation routes have also been used to evaluate the acute toxicity of ZnO nanoparticles at high concentration [15]. The present results indicate that ZnO nanoparticles induce dose-dependent cytotoxicity on the tissues and cause histopathological damage. Compared with the control group, spleen, testis and heart tissues were normal, whereas those of treated groups showed histopathological alterations in their tissue composition.

Histopathological changes which induced by ZnO nanoparticles were dose dependent [16]. Decreasing particles size results in increasing nanoparticles specific surface area, which promotes not only the accumulation of nanoparticles in different tissues, but also increases the reactivity and enhance interactions between nanoparticle and tissue cells. In recent study, there was a direct proportional relationship between ZnO nanoparticles dose and cell uptake which leads to more accumulation and then causes cell death and irreversible histopathological damage. It has also been reported that oral administration of high doses of ZnO nanoparticles causes histopathological changes in liver and kidney tissues [17].

Exposure to ZnO nanoparticles in experimental albino rats after 7 days is accompanied with signs of toxicity [18]. Administration of 10 mmol/L of ZnO nanoparticles could cause necrosis and apoptosis in 75% of cells [19]. Therefore, ZnO nanoparticles pose an extraordinary hazard and the health precautions are necessary to those who are in continues and close contact with this material which containing ZnO nanoparticles specially in industrial cities and factories or those who always use cosmetic products.

CONCLUSION

We demonstrated ZnO nanoparticles ability to exert their cytotoxicity effects on spleen, testis and heart tissues after intramuscular injection in male Wistar rats with three different doses of ZnO nanoparticles. We concluded that ZnO nanoparticles administration in high dose could cause significant irreversible damages to body organs tissues and threat human health. This finding could be important as a health hazard to those who are in continuous exposure to ZnO nanoparticles. We recommended to have more attention for ZnO nanoparticles usage and its dose as further studies still need to predict ZnO nanoparticles toxicity specially on human health.

ACKNOWLEDGMENTS

We are grateful for faculty of medicine and applied medical science-Northern Borders University-KSA and King abdullah institute for nanotechnology-King Saud University-KSA for their support and their helpful efforts for doing this study.

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