INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 14, No. 1, p. 300-308, 2019

RESEARCH PAPER OPEN ACCESS

Zinc oxide nanoparticles induced histopathological alterations in the spleen of male sprague dawley rats

Nagina Rehman, Muhammad Ali, Farhat Jabeen*, Azhar Rasool, Muhammad Kashif Zahoor

Department of Zoology, Government College University Faisalabad, Pakistan

Key words: ZnO, Nano Particles, Histopathology, Spleen.

http://dx.doi.org/10.12692/ijb/14.1.300-308 Article published on January 26, 20199

Abstract

Considering the wide spread application of nanotechnology in a number of industries (cosmetics, dyes, rubber and food etc.), extensive role of Zinc oxide nanoparticles (ZnO-NPs) is reported in many fields of biology, but limited information is available regarding their toxic effects. The primary purpose of the current study was to assess the toxic effects of ZnO nano particles (ZnO-NPs) in the spleen of male Sprague Dawley rats via intraperitoneal injection @ of either 10 or 20 or 30 mg/kg bw on alternate day for 28 days. At the end of experiment, rats were sacrificed and spleen was collected for the investigation of metal accumulation and histopathological studies. Histological alterations included magakarycytes, macrophages along with the accumulation of zinc particles, congestion of white pulp and the cell necrosis in ZnO-NPs treated groups and their severity was dose dependent from low to high dose treated groups (10<20<30 mg/kg) in comparison with the control group. Likewise, accumulation of Zinc metal showed significant results within treated groups in an order of low< medium<high (10< 20 < 30 mg/kg). Results of the current study revealed the dose dependent toxicity and accumulation of zinc metal in the organs with their histological alterations in living organisms. So, further studies regarding ZnO toxicity is recommended.

* **Corresponding Author:** Farhat Jabeen ✉ farjabeen2004@yahoo.co.in

Introduction

Nanotechnology is a rapidly growing field with significant impacts on the environment, economy and society. Among various reported oxide nanoparticles, the most widely used nanoparticles are Zinc Oxide nanoparticles (ZnO-NPs), which are abundantly used in many products like textile, rubber industry, moisturizing creams, cosmetics and sunscreen lotions because of its effective UV absorption properties (Schilling *et al.,* 2010).

Rapid use of ZnO-NPs has increased their exposure with the result of getting more attention regarding their toxicity such as genotoxicity, cytotoxicity and proinflammatory effects (Hackenberg *et al.,* 2011; Teow *et al.*, 2011). These nanoparticles enter in human body through different ways like injection, inhalation and ingestion (Koeneman *et al.,* 2010). They cause adverse biological reactions in organs by entering to blood stream (Johnston *et al.,* 2010).

As a result of research conducted on nanoparticles, it is concluded that on the basis of reactivity nanoparticles are more reactive as compared to their bulk salt due to decreased surface to volume ratio (Nasu and Ostubo, 2006) which is the leading factor for their toxic reactivity. The nanoparticles of zinc are essential heavy metals in industries especially food industry but their over exposure cause accumulation in tissues which cause toxicity (Nations *et al.,* 2011).

On the basis of previous research it is considered that ZnO-NPs are materials with less toxicity because it is an essential trace element of human body and also present in food as a nutritional supplement. So, this is the reason why ZnO-NPs are getting more attention regarding its toxicity assessment (Wang *et al.,* 2008). Beside all these reasons, it is also known that Zinc is responsible for its toxic effects at a high concentration (Plum *et al.,* 2010). Moreover, it is recently reported that release of Zn^{2+} ions is the main reason of ZnO-NPs toxicity in microorganisms and rodents (Pujalte *et al.,* 2011).

Many studies conducted *in vivo* and *in vitro* investigated that most of the nanoparticles (NPs) are responsible for adverse toxic effects on male germ cells (Braydich *et al.,* 2005; Braydich-Stolle *et al.,* 2010). Previous studies investigated that exposure of NPs to mice resulted in their accumulation to various organs like testes and brain indicating the fact that these NPs can easily pass through the blood-testis barriers and blood-brain (Borm *et al.,* 2004). Therefore, NPs must be studied on the basis of their positive or negative effect on spermatogenesis because metal and their oxides nanoparticles are widely applicable in the fields of industries and medicines (Tyner *et al.,* 2004). In the current study, toxic effects of zinc oxide nanoparticles (ZnO-NPs) were studied in spleen by investigating the histological alterations and metal accumulation in adult male Sprague Dawley rats.

Materials and methods

Experimental animals

Twenty five male Sprague Dawley rats of 200-250g weight were obtained from the animal house of University of Agriculture Faisalabad, Pakistan. During experimental work, all the rats were acclimatized under 12/12h dark/light cycle, at the room temperature of 23± 4°C. All the animals were kept in stainless steel cages and fed with standard laboratory food and water. The experimental protocol was approved by the Ethical committee on animal experimentation of Government college University Faisalabad, Pakistan.

Grouping

Animals were divided into five groups (n=5). Control group was kept untreated. Placebo group was treated with saline (0.9g/100ml dist. Water). Groups namely G1, G2 and G3 were intraperitoneally treated with ZnO-NPs in normal saline at 10 or 20 or 30mg/kg body weight, respectively for 28 days on alternate day. On 29^{th} day, animals were weighed, sacrificed and target organ (Spleen) was collected for further laboratory analysis.

Nano particles

ZnO-NPs were purchased from Sigma Aldrich (CAS #

1314-13-2). Purity level was 99.9% and suspension was made in normal saline (0.9g/100ml distil water). Prepared suspension was kept on vortex and then sonicated for atleast one hour before injection to make sure the fine solution and complete dissolution.

Tissue collection, histology and atomic absorption spectroscopy

Spleen was excised, kept in ice-cold saline for the removal of attached adipose tissues and then washed thoroughly. Small pieces of 2-3 mm were fixed in 10% formalin immediately for histological study and remaining spleen tissue samples were further processed for digestion to assess accumulation of metals in splenic tissues by atomic absorption spectroscopy.

Tissue histology

Fixed tissues were treated under standard histological laboratory tools. Tissues were dehydrated with different grades of alcohol (70-100%) overnight. In paraffin, tissue blocks were made and 3-4 µm tissue sections were cut by using microtome (SLEE Rotary Microtome CUT5062 by Nikon Instruments Europe) and then stained with hematoxylin and eosin stain with standard staining technique (Hussein, 2015).

The slides were studied under light microscope (Nikon E200 POL) and photographed by a digital camera attached with the microscope.

Metal analysis

For the analysis of ZnO-NPs, 0.5g of spleen tissue was dried using Microwave Accelerated Reaction system (MARS, CEM) at 180°C for 15 minutes. The samples were digested in 5ml Nitric acid for 24hrs. After digestion in Nitric acid, samples were filtered by adding dH_2O and made final volume of each filtrate upto 10ml. Atomic absorption spectrophotometry was carried out on a Fast Sequential Atomic Absorption Spectrometer (Varian-AA-240) and the concentration of Zinc was determined. Standard curve against different absorbance was made. Results were interpreted in ppm and were converted to µg/g of tissue using following formula:

Statistical analysis

The results were analyzed by general linear model (GLM) using statistical program Minitab17 and Tukey's test was used for the comparison of means in different groups at the significance level of $P<0.05$.

Results

Table 1 shows the Zn accumulation in spleen tissues of control and treated groups. No significant difference in accumulation of Zn was observed at low dose (10mg/kg) compared with the control groups, while within the treated groups there was a significant difference in the Zn accumulation ($P<0.001$) as per dose exposure when compared with control in an order of 10< 20<30mg/kg, respectively.

Table 1. Mean concentrations (±SD) of Zn in spleen of male Sprague Dawley rats in control, placebo and treated groups.

Sr #	Groups	Mean concentration Zn (ppm) in Spleen
1	Control	0.101±0.012 [c]
2	Placebo	0.101±0.012 [c]
3	G1 (10mg/kg)	0.104±0.011 [c]
4	G2 (20mg/kg)	0.113±0.012 [b]
5	G3 (30mg/kg)	0.139±0.012 [a]

Table 2 shows the histological alterations in the spleen of male Sprague Dawely rats. In this study dose dependent histological alterations were observed and more severity was observed in high doses compared with control. In control group normal splenic architecture was observed with capsule. The internal components of the spleen composed of two pulp areas: white pulp area and red pulp area. Below the capsule, internal trabeculae are present in red pulp area (Fig. 1).

Low dose exposure of ZnO-NPs (10mg/kg) showed mild histological alterations when compared to control group revealing the accumulation of test particles along with the formation of macrophages (Mtp), congestion of white pulp (Cwp) with necrosis, megakaryocytes (Mk), hemorrhage (He) and formation of mast cells (Mc) (Fig 2).

Medium dose exposure of ZnO-NPs (20mg/kg) revealed histological abnormalities when compared to control group that were inflammation of blood vessels with necrotic detached cells (Ib) and expansion of blood vessels along with breakage (Fig. 3). High dose exposure of ZnO-NPs (30mg/kg) revealed histological abnormalities like formation of blood filled spaces enlined by endothelium in parenchymal portion of spleen named angectasis (Ang), mal-defined broken trabaculae (Ct) and high necrosis (Nc) (Fig. 4).

Dose dependent histological abnormalities in the nano treated groups and their severity in comparison with the control group is shown in Table 2.

Table 2. Histological alterations in spleen of male Sprague Dawley rats in control, placebo and treated groups.

Histological alterations	Groups				
	Control	Placebo	G1 (10mg/kg)	G2 (20mg/kg)	G3 (30mg/kg)
Congestion	-	-	+	++	+++
Megakaryocytes	-	-	+	++	+++
Macrophages with test particles	-	-	+	++	++
Degenerated blood vessels	-	-	+	++	++
Necrosis	-	-	+	++	+++
Internal dilation of white pulp	-	-	+	++	++
Mast cells	-	-	+	+	++
Degenerated blood vessels	-	-	+	++	+++
Hemorrhage	-	-	+	+	+++
Angectasis	-	-	-	+	+++

Note: (-) no histological alterations (Normal architecue of histology); (+) moderate histological alterations; (++) sever histological alterations; (+++) very sever histologial alteration in the spleen of Sprague Dawley rats.

Discussion

In the current study, accumulation of Zn concentration was observed in the experimental groups treated with ZnO-NPs. The order of accumulation within treated groups was low<medium<high (10mg/kg<20mg/kg<30mg/kg), respectively. Current study is in line with previous studies which revealed that nanoparticles can cross wall of intestines and then further can enter and accumulate in brain, blood, heart, lungs and spleen (Hilyer *et al.,* 2001).

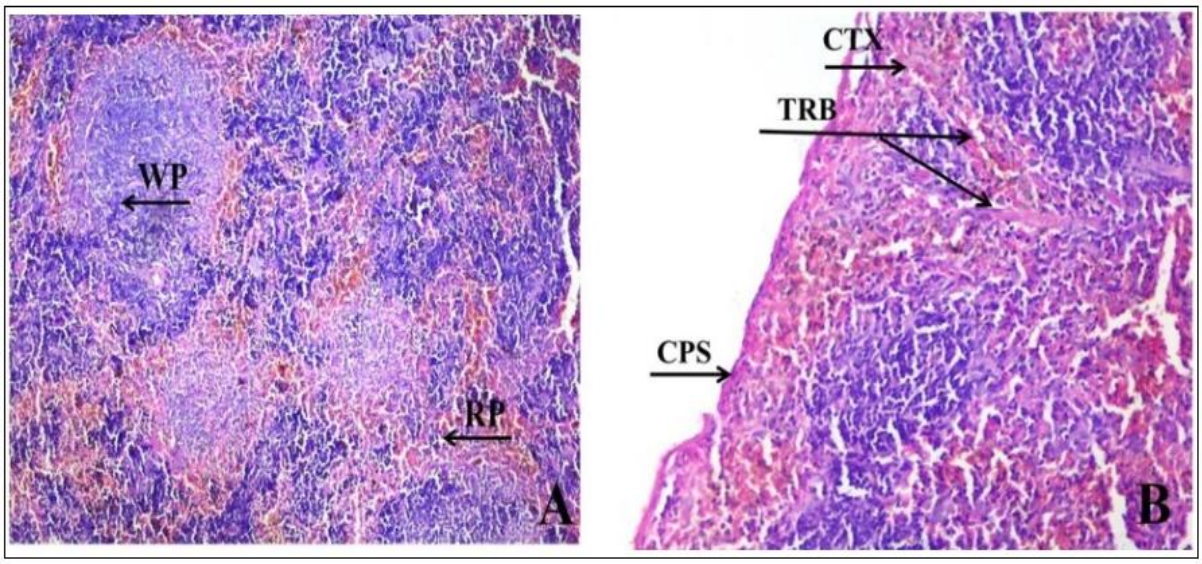

Fig. 1. Photomicrograph of spleen of control group (400X H & E stain) showing Normal histological architecture with white pulp area (WP), red pulp area (RP), outer covering of capsule (CPS), trabeculae (TRB) and cortex below capsule (CTX) (A-B).

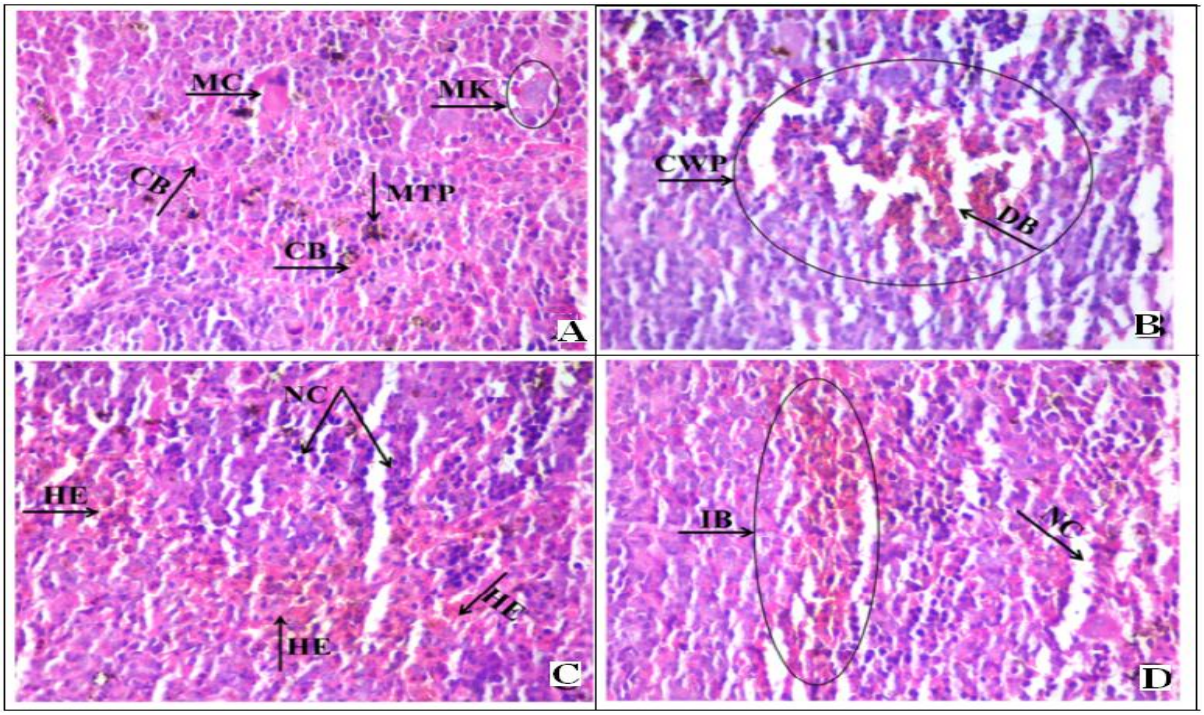

Fig. 2. Photomicrograph of spleen of male Sprague Dawley rats (400X H & E stain) treated with low dose (10mg/kg of ZnO-NPs) showing macrophages along with the accumulation test (zinc) particles (MTP), dilated blood vessels (DB), congestion of white pulp with inflammation of blood vessels (CWP), hemorrhage with the congestion of red pulp (HE), megakaryocytes (MK), congestion of blood vessels (CB), mast cells (MC) and necrosis (NC).

Current study is in good agreement with the study of Song *et al.* (2010), who investigated that Zn contents showed no significant accumulation at low dose while at high dose, they accumulate in liver, spleen, lungs, bone and brain at a significant level ($P<0.05$).

A study revealed that ZnO-NPs accumulates in spleen and lungs within one hour and the long term exposure resulted in peak accumulation in spleen after one day by dose dependent exposure via intravenous injection due to the formation of macrophages and megakaryocytes (Fujihara *et al.,* 2015). Tissue distribution of ZnO-NPs in rat spleen and brain is far less as compared to kidney and liver in highest dose group tissues when compared with the tissue distribution of titanium dioxide nanoparticles for 13 weeks exposure with a clear cut dose dependent response (Cho *et al.,* 2013).

Exposure of low (2mg/l) and high dose (12mg/l) for 48 hours in fish (large yellow croaker) resulted in organ specific effect in liver and spleen. Dose dependent accumulation observed with highest in liver and then in spleen and no significant accumulation at low dose (Zheng *et al.,* 2017). Above findings are in good agreement with the current study. Results of current study indicated cytotoxicity in spleen to produce histological damages due to ZnO-NPs. Histological alterations Induced by ZnO-NPs were dose dependant (De Berardis *et al.,* 2010). In the current ZnO-NPs treated groups showed the formation of megakaryocytes. Their abundance increased with the increase of dose in all treated groups. Production of megakaryocytes is linked with decrease in the production of blood cells and platelets (Josefsson *et al.,* 2011). Similar damage of spleen with increase production of megakaryocytes and distortion in spleen structure was observed when exposed to magnetic nanoparticles (MNPs) (Aziz, 2015). Other splenic abnormalities like red pulp disruption and necrosis with necrotic detached cells was observed in three treated groups and their severity increased with the increase of dose. Similar aberrations were found in spleen of mice exposed to Bisphenol A (Hussein,

2015). In the current study, mast cells formation was also observed and their severity was linked with the increase of dose (ZnO-NPs) in three nano treated groups which is in agreement with the previous study (Toyoshima *et al.,* 2017).

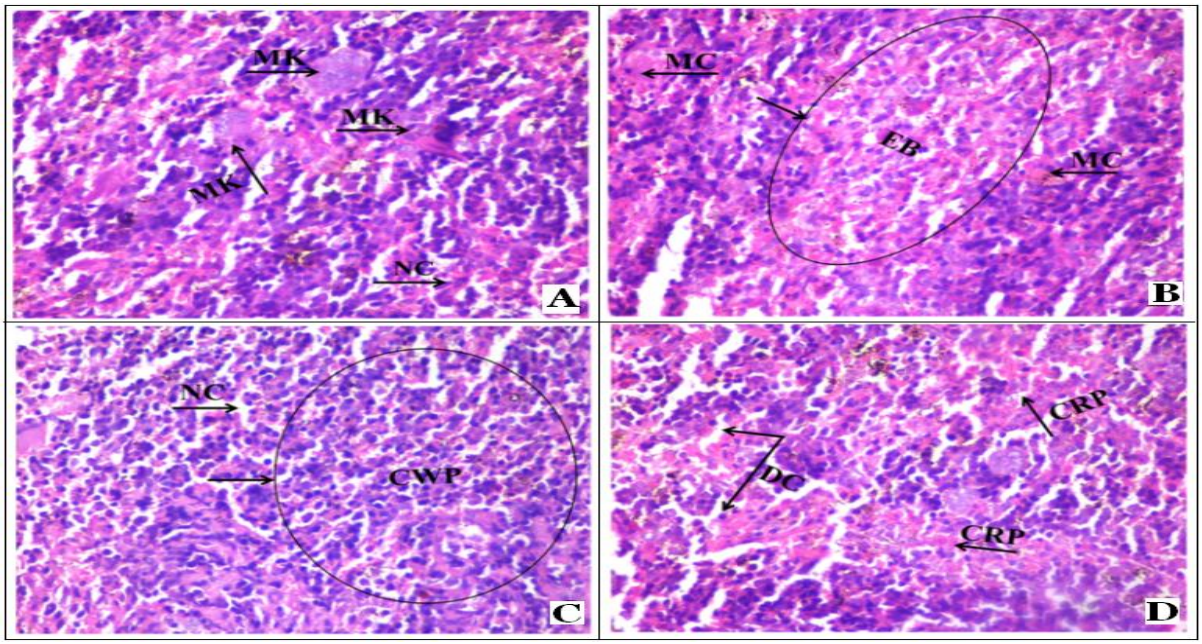

Fig. 3. Photomicrograph of spleen of male Sprague Dawley rats (400X H & E stain) treated with medium dose (20mg/kg of ZnO-NPs) showing dilated blood vessels (DB), megakaryocytes (MK), congestion of red pulp (CRP), degenerated mal-defined cells (DC), congestion of white pulp (CWP), inflammation of blood vessels with necrotic cell debris (IB), expansion of blood vessels (EB) and necrotic cells (NC).

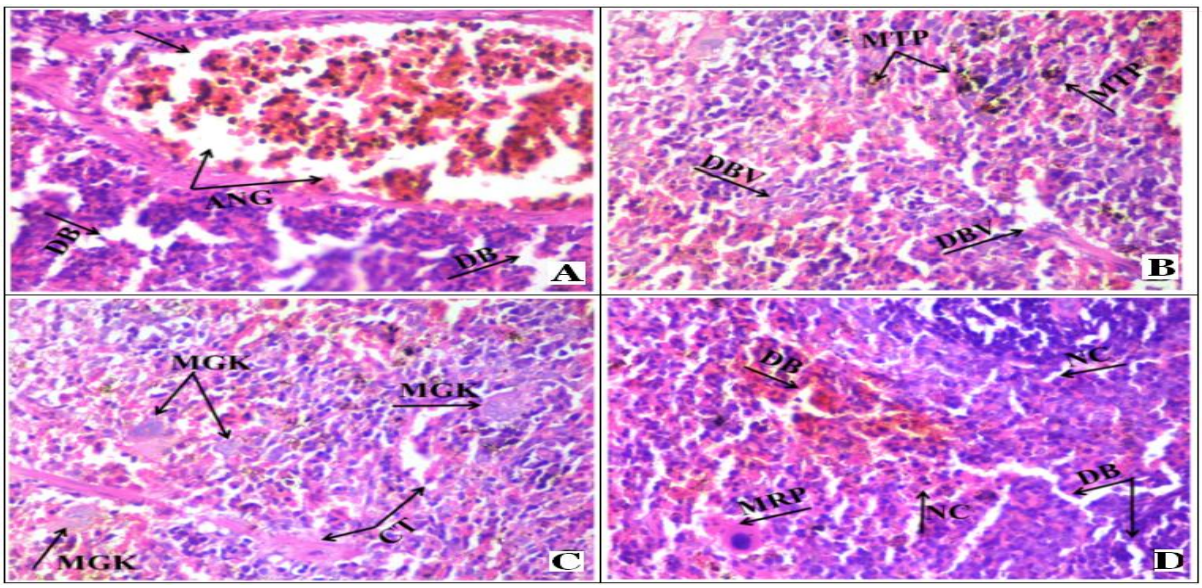

Fig. 4. Photomicrograph of spleen of male Sprague Dawley rats (400X H & E stain) treated with high dose (30mg/kg of ZnO-NPs) showing angectasis (blood filled spaces lined by endothelium in splenic parenchyma) (ANG), congestion of white pulp area of spleen (CWP), degenerated blood vessels (DGB), macrophages accumulated with test (Zinc) particles (MTP), inflammation of blood vessels (IB), dilated blood vessels with internal hemorrhage (DB), mast cells clustered with red pulp area (MRP), congestion of red pulp (CRP), necrotic cell debris accumulation (degeneration of cellular integrity) (NC), megakaryocytes (a sort of stem cells) (MGK) and congested trabecular artery (CT).

In treated groups of current study, accumulation of test nanoparticles was observed along with the formation of macrophages and the similar accumulation were studied in rats spleen exposed to silver nanoparticles (Mazen *et al.,* 2017). Moreover, in current nanotreated groups, congestion of white pulp remarkably increases with the increase of dose concentration of ZnO-NPs and similar disruption of white pulp and appearance of megakaryocytes were studied in rats exposed to gold nanoparticles (Ibrahim *et al.,* 2018).

The high nano zinc oxide treated group showed sever histological damage ratio in comparison with control group while the groups of medium and low nano treated (ZnO-NPs) showed comparatively moderate abnormalities as compared to the control group.

Conclusion

It is concluded that toxicity of ZnO-NPs increases with the increase of dose. At low dose, ZnO-NPs showed less accumulation of Zn concentration and less sever histological abnormalities as compared to the control and placebo groups. At high dose, Zn accumulation and the histological aberrations were high as compared to the control and placebo group.

References

Aziz A. 2015. Histopathological and immunological changes induced by magnetic nanoparticles in the spleen, liver and genital tract of mice following intravaginal installation. The Journal of Basic and Applied Zoology **71,** 32-47. https://doi.org/10.1016/j.jobaz.2015.03.003

Borm PJ, Kreyling W. 2004. Toxicological hazards of inhaled nanoparticles potential implications for drug delivery. Journal of Nanoscience and Nanotechnology **4(5),** 521–31.

Braydich-Stolle L, Hussain S, Schlager JJ, Hofmann MC. 2005. In Vitro cytotoxicity of nanoparticles in mammalian germline stem cells. Toxicological Sciences **88(2),** 412–9. https://doi.org/10.1093/toxsci/kfi256

Braydich-Stolle LK, Lucas B, Schrand A, Murdock RC, Lee T, Schlager JJ, Hussain SM, Hofmann MC. 2010. Silver nanoparticles disrupt GDNF/Fyn kinase signaling in spermatogonial stem cells. Toxicological Sciences **116(2),** 577-89. https://doi.org/10.1093/toxsci/kfq148

Cho WS, Kang BC, Lee Jk, Jeong J, Che JH, Seok SH. 2013. Comparative absorption, distribution and excretion of titanium dioxide and zinc oxide nanoparticles after repeated oral administration. Particle and Fiber Toxicology **10,** 9. https://doi.org/10.1186/1743-8977-10-9.

De Berardis B, Civitelli G, Condello M. 2010. Exposure to ZnO nanoparticles induces oxidative stress and cytotoxicity in human colon carcinoma cells. Toxicology and Applied Pharmacology **246,** 116–127. https://doi.org/10.1016/j.taap.2010.04.012.

Fujihara J, Miki T, Hashimoto H, Yamada T, Kimura-Kataoka K, Yasuda T, Fujita Y, Takeshita H. 2015. Distribution and toxicity evaluation of ZnO dispersion nanoparticles in single intravenously exposed mice. The Journal of medical investigation **62(1),** 45-50. https://doi.org/10.2152/jmi.62.45.

Hackenberg S, Zimmermann FZ, Scherzed A, Friehs G, Froelich K, Ginzkey C, Koehler C, Burghartz M, Hagen R, Kleinsasser N. 2011. Repetitve exposure to zinc oxide nanoparticles induces dna damage in human nasal mucosa mini organ cultures. Environmental and Molecular Mutagenesis **52(7),** 582-9. https://doi.org/10.1002/em.20661

Hillyer JF, Albrecht RM. 2001. Gastrointestinal persorption and tissue distribution of differently sized colloidal gold nanoparticles. Journal of Pharmceutical Sciences **90,** 1927-1936. https://doi.org/10.1002/jps.1143

Hussein AJ. 2015. Histopathological study of lung,

kidney, spleen and prostate in adult male rats treated with bisphenol A. Basrah journal of veterinary research **4(2),** 74-86.

Ibrahim KE, Al-Mutary MG, Bakhiet AO, Khan HA. 2018. Histopathology of the Liver, Kidney, and Spleen of Mice Exposed to Gold Nanoparticles. Molecules **23,** 1848. https://doi.org/10.3390/molecules23081848

Johnston HJ, Hutchison G, Christensen FM, Peters S, Hankin S, Stone V. 2010. A review of the in vivo and in vitro toxicity of silver and gold nanoparticles: particle attribute and biological mechanisms responsible for the observed toxicity. Critical Reviews in Toxicology **40(4),** 328-46. https://doi.org/10.3109/10408440903453074.

Josefsson EC, James C, Henley KJ. 2011. Megakaryocytes possess a functional intrinsic apoptosis pathway that must be restrained to survive and procedure platelets. The Journal of Experimental Medicine **208,** 2017-2031. https://doi.org/10.1084/jem.20110750

Koeneman BA, Zhang Y, Westerhoff P, Chen Y, Crittenden JC, Capco DG. 2010. Toxicity and celluler responses of intestinal cells exposed to titanium dioxide. Cell Biology and Toxicology **26(3),** 225-38. https://doi.org/10.1007/s10565-009-9132-z.

Mazen NF, Saleh EZ, Mahmoud AA, Shaalan AA. 2017. Histological and immunohistochemical study on the potential toxicity of silver nanoparticles on the structure of the spleen in adult male albino rats. The Egyptian Journal of Histology **40(3),** 374-387. https://doi.org/10.21608/EJH.2017.4662.

Nasu, A, Otsubo Y. 2006. Rheology and UV protection properties of suspensions of fine titanium dioxides in a silicone oil. Journal of Colloid Interface Science **296,** 558–564. https://doi.org/10.1016/j.jcis.2005.09.036

Nations S, Wages M, Canas JE, Maul J, Theodorakis C, Cobb GP. 2011. Acute effects of F_e2O_3, TiO_2, ZnO and CuO nanomaterilas on Xenopus laevis. Chemosphere **83(8),** 1053-61. https://doi.org/10.1016/j.chemosphere.2011.01.061.

Plum LM, Rink L, Haase H. 2010. The Essential Toxin: Impact of Zinc on Human Health. International Journal of Environmental Research and Public Health **7(4),** 1342-1365. https://doi.org/10.3390/ijerph7041342

Pujalte l, Passagne l, Brouillaud B, Treguer M, Durand E, Ohayon-Courtes C, L'Azou B. 2011. Cytotoxicity and oxidative stress induced by different metallic nanoparticles on human kidney cells. Particle and Fiber Toxicology **8,** 10. https://doi.org/10.1186/1743-8977-8-10

Schilling K, Bradford B, Castelli D, Dufour E, Nash JF, Pape W, Schulte S, Tooley I, Van den Bosch J, Schellauf F. 2010. Human safety review of "nano" titanium dioxide and zinc oxide. Photochemical and Photobiological Sciences **9(4),** 495-509. https://doi.org/10.1039/b9pp00180h.

Song W, Zhang J, Zhang J. 2010. Acute toxicity of different sized ZnO nanoparticles in mice after intratracheal administration. International Conference on Bioinformatics and Biomedical Engineering. https://doi.org/10.1109/ICBBE.2010.5515207

Teow Y, Asharani PV, Hande MP, Valiyaveettil S. 2011. Health impact and safety of engineered nanomaterials. Chemical Communication (Cambridge, England) **47(25),** 7025-38. https://doi.org/10.1039/c0cc05271j.

Toyoshima S, Wakamatsu E, Ishida Y, Obata Y, Kurashima Y, Kiyono H, Abe R. 2017. The spleen is the site where mast cells are induced in the development of food allergy. International Immunology **29(1),** 31-45.

https://doi.org/10.1093/intimm/dxx005.

Tyner KM, Schiffman SR, Giannelis EP. 2004. Nanobiohybrids as delivery vehicles for camptothecin. Journal of Controlled Release **95(3),** 501–14. https://doi.org/10.1016/j.jconrel.2003.12.027

Wang B, Feng W, Wang M, Wang T, Gu Y, Zhu M, Ouyang H, Shi J, Zhang F, Zhao Y, Chai Z, Wang H, Wang J. 2008. Acute toxicological impact of nano-and submicro-scaled zinc oxide powder on healthy adult mice. Journal of Nanoparticles Research **10(2),** 263-276. https://doi.org/10.1007/s11051-007-9245-3.

Zheng JL, Yan SS, Shen B, Wu CW. 2017. Organ-specific effects of low-dose zinc pre-exposure on high-dose zinc induced mitochondrial dysfunction in large yellow croaker *Pesudosciaena crocea*. Fish Psychology and Biochemistry **43(2),** 653-661. https://doi.org/10.1007/s10695-016-0319-y.