Current Topics in Toxicology
Vol. 13, 2017

Original Communication

Toxic effect of sub-chronic use of zinc oxide nanoparticles on the lymphatic system of adult albino rats

Said Said Elshama[1,5], Rasha R. Salem[2], Hosam-Eldin Hussein Osman[3,6] and Ayman El-Meghawry El-Kenawy[4,7,*]

[1]Department of Forensic Medicine and Clinical Toxicology, College of Medicine, Taif University, Taif, Saudi Arabia. [2]Department of Anatomy, Faculty of Medicine, Alexandria University, Egypt. [3]Department of Anatomy; [4]Department of Pathology, College of Medicine, Taif University, Taif, Saudi Arabia. [5]Department of Forensic Medicine and Clinical Toxicology, Faculty of Medicine, Suez Canal University, Ismailia City; [6]Department of Anatomy, Faculty of Medicine, Al-Azhar University, Cairo; [7]Department of Molecular Biology GEBRI, University of Sadat City, Sadat City, Egypt.

ABSTRACT

Zinc oxide nanoparticles have widespread applications in medicine and in several industries because of its specific physical and chemical properties. Nanoparticles have a greater toxicity in comparison to the large-sized materials because they are highly reactive causing oxidative stress in humans. The current study aims to evaluate the toxic effect of sub-chronic use of zinc oxide nanoparticles on lymphatic system of rats by the assessment of some hematological indices (total and differential white blood cell, red blood cell and platelet counts) and histopathological changes concerning the spleen, thymus gland, and lymph nodes. Sixty adult albino rats were divided into three groups, each group comprising twenty rats. The first control group received water *via* an oral gastric tube while the second and the third group received 200 and 400 mg/kg/day of zinc oxide nanoparticles, respectively *via* intraperitoneal injections for three months. Toxicity of zinc oxide nanoparticles caused a decrease in the rats' body, thymus and spleen weight, reduction in the total blood cell count, disturbance in the differential white blood cell count, histopathological and ultrastructural changes in thymus, spleen and lymph nodes. Thus, sub-chronic use of zinc oxide nanoparticles affects lymphatic system and some hematological indices depending on their dose.

KEYWORDS: zinc oxide, nanoparticles, sub-chronic, lymphatic system.

1. INTRODUCTION

In the last few years, there has been widespread application of nanoparticles in medicine and in several industries because of their unique physical and chemical properties, depending on their shape, size and the surface area to volume ratio [1].

Nanoparticles are toxic for humans because they can pass through the cell membrane and the blood-brain barrier easily, thereby affecting the different organs of the body based on the nature of the material and the doses used. Many studies have proved that the use of high dose of fine or big nanoparticles is considered dangerous for human health [2].

Many published articles have reported that the nano-sized material has more toxicity than the large-sized material because it is highly reactive leading to oxidative stress in humans and animals, and hence we need to clarify the possible side effects of nanoparticles on the body [3].

*Corresponding author: elkenawyay@yahoo.com

The environmental pollution by zinc oxide nanoparticles is very threatening because of its common use in different applications such as paints, dyes, rubber, electronics, sunscreens, cosmetics, personal care products and food additives [4]. Zinc oxide nanoparticles can pass into the human body *via* many routes such as oral, inhalation, intravenous injection and dermal penetration; it can interact with macromolecules in the cells leading to toxic effects on different organs. However, till now there are not enough available data on the toxicity of zinc oxide nanoparticles [5].

Therefore, the current study aims to evaluate the toxic effect of sub-chronic use of zinc oxide nanoparticles on lymphatic system of adult albino rats; it assesses histopathological changes in the spleen, thymus gland, and lymph nodes along with counting the total and differential white blood cells, red blood cells, and platelets.

2. MATERIALS AND METHODS

Sixty healthy male adult albino rats, aged about 16 weeks (weighing 200-250 g) were maintained on a 12-hr day and night cycles. They were fed with water and standard rat pellets during the experimental period. Rats were divided into three groups, each group comprising twenty rats. The first (control) group received 0.5 ml of distilled water *via* an oral gastric tube while the second and the third group received 200 and 400 mg/kg/day of zinc oxide nanoparticles respectively *via* intraperitoneal injections [6, 7]. The daily administration of distilled water and zinc oxide nanoparticles continued for three months.

The dispersion of zinc oxide nanoparticles was nearly 100 nm with an average particle size of 35 nm and a concentration of 50 wt.% in H_2O. The pH of the aqueous systems was 7 $\pm$ 0.1 (Lot/ No. MKBN3534V- Nano - Sunguard^{TM} in water). The zinc oxide nanoparticles were manufactured by Sigma-Aldrich Co., St. Louis, USA [8].

2.1. Histopathological studies

Twenty-four hours after the last administration of zinc oxide nanoparticles, the rats were sacrificed after being excessively anaesthetized. Neck, chest, and abdominal incisions were carried out; thymus gland, spleen, and lymph nodes were excised for weight measurement and histological studies. The tissue specimens were collected from the three groups and were fixed in 10% neutral buffered formalin. The fixed specimens were trimmed, washed and dehydrated in ascending grades of alcohol, cleared in xylene, embedded in paraffin, sectioned at 4-6 μm thickness and stained with haematoxylin and eosin for examination using the light microscope [9].

Ultrastructure studies were performed using the transmission electron microscope. The tissue specimens of the thymus gland, spleen, and lymph nodes were prepared by soaking and fixating the specimens in 2.7% glutaraldehyde solution in 0.1 M phosphate buffer for 1.5 hours at 4 ºC. They were washed in 0.15 M phosphate buffer (pH 7.2) and post-fixed in 2% osmic acid solution in 0.15 M phosphate buffer for one hour at 4 ºC. Dehydration was done by using acetone. They were then embedded in the soaked epoxy resin Epon 812. The tissue sections were cut with an ultramicrotome type LKB at 70 nm thickness. The sections were differentiated using the solutions of uranyl acetate and lead citrate for an electron microscopic analysis [10].

2.2. Statistical analysis

Statistical analysis was performed using SPSS version 17. The data were expressed as mean $\pm$ SD and the analysis was performed by using one-way analysis of variance (ANOVA) and post-hoc multiple comparisons test (TUKEY) to investigate the difference between the parameters among the different groups where the P value of 0.05 was considered statistically significant.

2.3. Ethical considerations

The most appropriate animal species was chosen for this research. Promotion of high standard care and animal well-being were exercised at all times. An appropriate sample size of animals for the experiment was calculated using the fewest number of animals to obtain the valid results statistically. Painful procedures were performed under anaesthesia to avoid any distress or pain that could be inflicted on the animals. The Institutional Animal Ethics Committee approved our standards of animal care and administration that are consistent with the requirements and standards of international laws and regulations.

3. RESULTS

3.1. The body, thymus and spleen weight changes in the different rat groups

Table 1 shows mean ± SD values of the rats' body, thymus and spleen weight. Mean ± SD values of the body weight in the control group that received distilled water, the second group which received 200 mg/kg/day of zinc oxide nanoparticles and the third group which received 400 mg/kg/day of zinc oxide nanoparticles are 195.45 ± 3.917; 182.2 ± 5.817 and 172.5 ± 3.435, respectively. The value of F indicates that the difference between the groups is 123.444 with the statistical significance at $P < 0.001$. Mean ± SD values of thymus weight in the control, second and the third groups are 681.8 ± 5.862; 667.8 ± 5.316 and 632.9 ± 9.34, respectively. The value of F indicates that the difference between the groups is 267.958 with the statistical significance at $P < 0.001$. Mean ± SD values of spleen weight in the control, second, and the third groups are 2.566 ± 0.374; 1.810 ± 0.239 and 1.656 ± 0.1607 respectively. The value of F indicates that the difference between the groups is 89.799 with the statistical significance at $P < 0.001$.

3.2. Hematological findings

3.2.1. Total blood cells count

Table 2 shows mean ± SD values of the total blood cell count in the rats. Mean ± SD values of total white blood cell count in the control, second and the third groups are 11.5 ± 0.334; 10.17 ± 0.608 and 7.63 ± 1.44, respectively. The value of F indicates that the difference between the groups is 138.476 with the statistical significance at $P < 0.001$. Mean ± SD values of red blood cell count in the control, second and the third groups are

Table 1. Mean + SD of the body, thymus and spleen weight in the different rat groups.

Group / Parameter	First M ± SD	Second M ± SD	Third M ± SD	F
Body (gm)	195.45 ± 3.917	182.2 ± 5.817 *	172.5 ± 3.435 **	123.444
Thymus (mg)	681.8 ± 5.862	667.8 ± 5.316 *	632.9 ± 9.34 **	267.958
Spleen (gm)	2.566 ± 0.374	1.810 ± 0.239*	1.656 ± 0.1607**	89.799

Number per group: 20; SD: standard deviation.
The first group (control) received distilled water.
The second group received 200 mg/kg/day of zinc oxide nanoparticles.
The third group received 400 mg/kg/day of zinc oxide nanoparticles.
*: $P < 0.001$ (significant difference in comparison with the first group).
**: $P < 0.001$ (significant difference in comparison with the second group).

Table 2. Mean + SD of the total blood cells count in the different rat groups.

Group / Parameter	First M ± SD	Second M ± SD	Third M ± SD	F
WBCs	11.5 ± 0.334	10.17 ± 0.608*	7.63 ± 1.44**	138.476
RBCs	7.54 ± 0.269	5.33 ± 0.311*	4.85 ± 0.4332**	261.225
PLAT	833.85 ± 31.003	631.1 ± 51.47*	519.2 ± 29.85**	431.462

Number per group: 20; SD: standard deviation.
WBCs: White blood cells; RBCs: Red blood cells; PLAT: Platelet.
The first group (control) received distilled water.
The second group received 200 mg/kg/day of zinc oxide nanoparticles.
The third group received 400 mg/kg/day of zinc oxide nanoparticles.
*: $P < 0.001$ (significant difference in comparison with the first group).
**: $P < 0.001$ (significant difference in comparison with the second group).

Table 3. Mean + SD of the differential white blood cells count in the different rat groups.

Group / Parameter	First M ± SD	Second M ± SD	Third M ± SD	F
Neutrophils	45.93 ± 1.64	41.35 ± 1.66*	27.64 ± 4.83**	648.279
Lymphocytes	46.33 ± 2.198	51.78 ± 1.29*	64.34 ± 5.708**	487.464
Monocytes	4.34 ± 0.268	4.595 ± 0.34*	4.76 ± 0.185**	5.569
Eosinophils	2.61 ± 0.366	2.32 ± 0.398*	1.395 ± 0.305**	56.074
Basophils	0.365 ± 0.421	0.52 ± 0.484*	0.575 ± 0.539**	15.552

Number per group: 20; SD: standard deviation.
The first group (control) received distilled water.
The second group received 200 mg/kg/day of zinc oxide nanoparticles.
The third group received 400 mg/kg/day of zinc oxide nanoparticles.
*: $P < 0.001$ (significant difference in comparison with the first group).
**: $P < 0.001$ (significant difference in comparison with the second group).

7.54 ± 0.269; 5.33 ± 0.311 and 4.85 ± 0.4332, respectively. The value of F indicates that the difference between the groups is 261.225 with the statistical significance at $P < 0.001$. Mean ± SD values of platelet count in the control, second and the third groups are 833.85 ± 31.003; 631.1 ± 51.47 and 519.2 ± 29.85, respectively. The value of F indicates that the difference between the groups is 431.462 with the statistical significance at $P < 0.001$.

3.2.2. The differential white blood cells count

Table 3 depicts mean ± SD values of the differential white blood cell count in the rats. Mean ± SD values of neutrophil count in control, second and the third groups are 45.93 ± 1.64; 41.35 ± 1.66 and 27.64 ± 4.83, respectively. The value of F indicates that the difference between the groups is 648.279 with the statistical significance at $P < 0.001$. Mean ± SD values of lymphocyte count in the control group, second and the third groups are 46.33 ± 2.198; 51.78 ± 1.29 and 64.34 ± 5.708, respectively. The value of F indicates that the difference between the groups is 487.464 with the statistical significance at $P < 0.001$. Mean ± SD values of monocyte count in the control, second and the third groups are 4.34 ± 0.268; 4.595 ± 0.34 and 4.76 ± 0.185, respectively. The value of F indicates that the difference between the groups is 5.569 with the statistical significance at $P < 0.001$. Mean ± SD values of eosinophil count in the control, second and the third groups are 2.61 ± 0.366; 2.32 ± 0.398 and 1.395 ± 0.305, respectively. The value of F indicates that the difference between the groups is 56.074 with the statistical significance at $P < 0.001$. Mean ± SD values of basophil count in the control, second and the third groups are 0.365 ± 0.421; 0.52 ± 0.484 and 0.575 ± 0.539, respectively. The value of F indicates that the difference between the groups is 15.552 with the statistical significance at $P < 0.001$.

3.3. Histopathological findings

3.3.1. Histopathological findings using a light microscope

3.3.1.1. Thymus gland

The examination of thymus gland tissues of the rats in the first (control) group shows normal histological structure of thymus gland (Fig. 1). But, the thymus gland tissues of the rats in the second group which received 200 mg/kg/day of zinc oxide nanoparticles shows slight degeneration in the peripheral cortex and medulla, hemorrhage, and congestion of blood vessels in the septa and medulla (Fig. 2). The transverse section of the thymus glands of the rats in the third group which received 400 mg/kg/day of zinc oxide nanoparticles shows severe congestion of blood vessels in the septa, outer cortex, and medulla, marked lymphocytic infiltration and vacuolated cytoplasm (Fig. 3).

3.3.1.2. Spleen

The examination of spleen tissues of the rats in the first control group shows normal histological structure (Fig. 4). The spleen tissues of the rats in the second group shows congestion and dilatation in the splenic blood sinuses of red pulp, hemorrhage, destruction, and atrophy of the red and white pulps, thickening and hyalinosis in the wall of

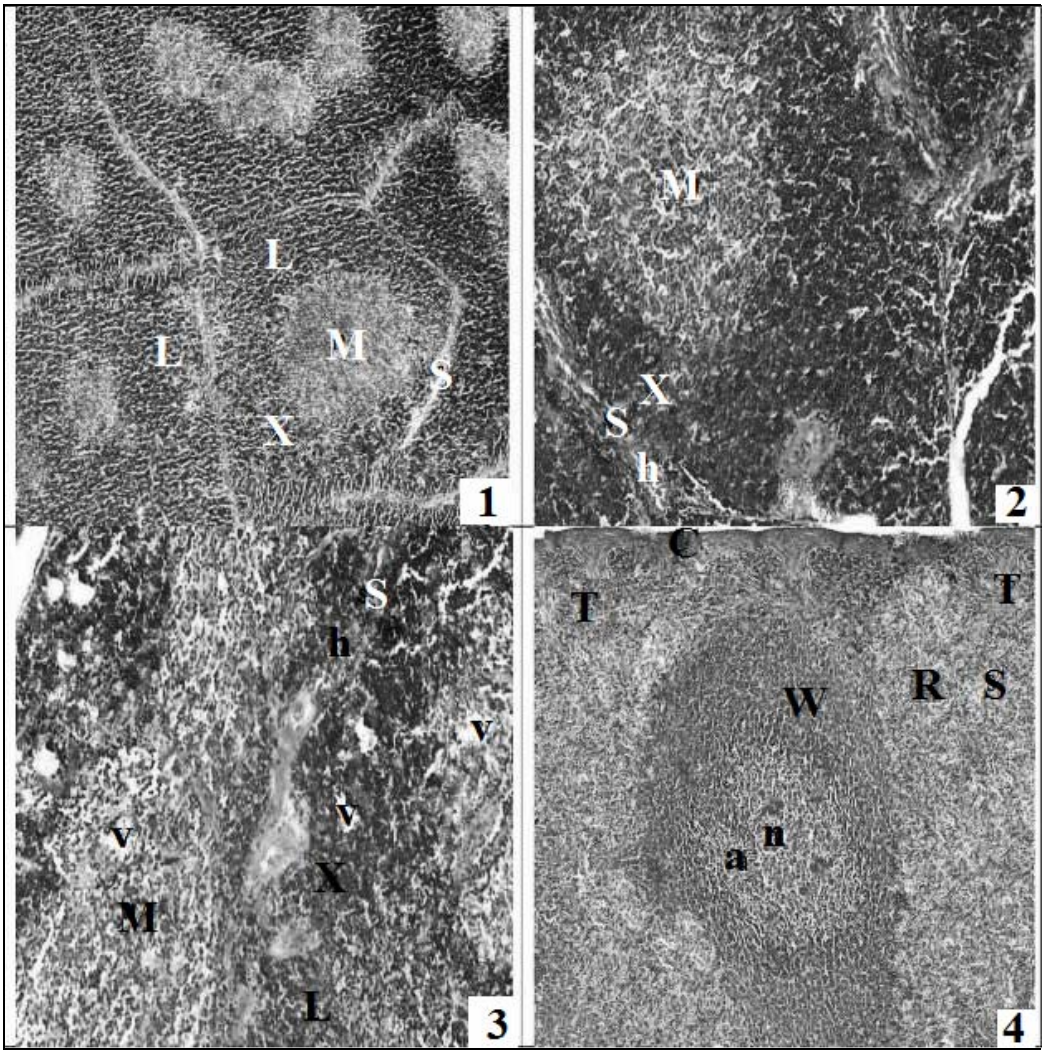

Fig. 1. Photomicrograph of a section in the control rat thymus gland showing the typical appearance of septa (S), incomplete lobules (L), peripheral cortex (X) and central medulla (M) (H&E X 80).
Fig. 2. Photomicrograph of a section in the second group rat thymus showing slight degeneration in the peripheral cortex (X), hemorrhage and congestion of blood vessels (h) in the septa (S) and medulla (M). (H&E X 80).
Fig. 3. Photomicrograph of a section in the third group rat thymus gland showing severe lymphocytes infiltration (L), vacuolated cytoplasm (v), severe congestion of blood vessels (h) in the septa (S), peripheral cortex (X) and medulla (M). (H&E X 80).
Fig. 4. Photomicrograph of a section in the control rat spleen showing the classic appearance of the capsule (C), trabeculae (T), red pulp (R) and white pulp (W). The splenic cord is separated by the splenic blood sinuses (S) in the red pulp while the lymphoid nodule (n) surrounds the central artery (a) of white pulp (H&E X 80).

central arterioles along with lymphocytic infiltration (Fig. 5). The spleen tissue of the rats in the third group shows severe hemorrhage, sub-capsular cloudy swelling, hyalinized capsule and trabeculae with lymphocytic infiltration, atrophy of white pulp, congestion, and dilatation in the splenic blood sinuses of red pulp, and hyalinosis in the wall of central arterioles (Fig. 6).

3.3.1.3. Lymph nodes

The examination of lymph node sections of the rats in the control group reveals entirely typical

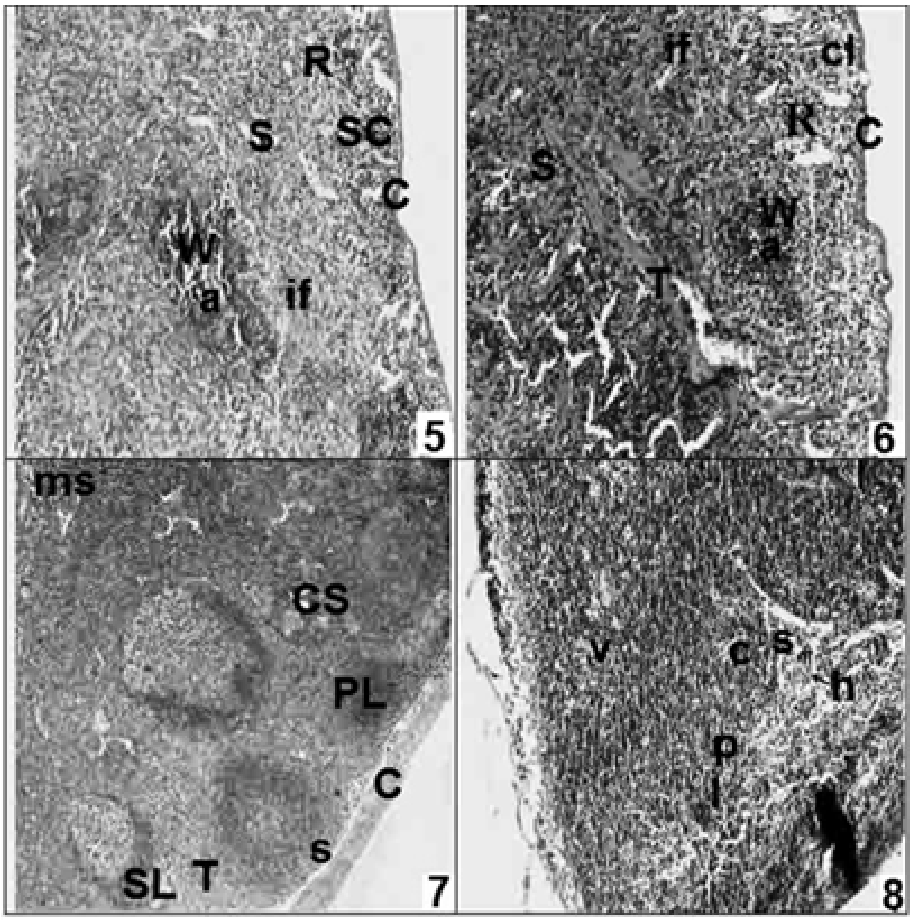

Fig. 5. Photomicrograph of a section in the second group rat spleen showing capsule (C), lymphocytic infiltration (if), sub-capsular hemorrhage (SC), congestion with the dilated splenic blood sinuses (S) of red pulp (R) and the destructed atrophied white pulp (W). The wall of central arterioles (a) is thickening and hyalinosis (H&E X 80).

Fig. 6. Photomicrograph of a section in the third group rat spleen showing hyalinized capsule (C), trabeculae (T) with lymphocytic infiltration (if) and sub-capsular cloudy swelling (cl). The splenic blood sinuses (S) of red pulp (R) are congested and dilated with the atrophied white pulp (W) and hyalinosis in the wall of central arterioles (a). (H&E X 80).

Fig. 7. Photomicrograph of a section in the control rat lymph nodes showing the classic capsule (C), trabeculae (T), subcapsular (s), cortical (cs) and medullary (ms) lymphoid sinuses, cortical primary (PL) and secondary lymphoid follicles (SL). (H&E X 80).

Fig. 8. Photomicrograph of a section in the second group rat lymph nodes showing reactive proliferation in the cortical (c) follicles with expansion, hyperplasia, and widening of the paracortical area (p) and intranodal sinuses (s). There are mild vascular (v) proliferation, activated lymphocytes (l) and histiocytes (h) (H&E X 80).

histological features, as illustrated in Fig. 7. The lymph node sections of the second group show mild vascular proliferation, hyperplasia and widening in the paracortical area and intranodal sinuses, activated lymphocytes and histiocytes, and reactive proliferation in the cortical follicles with expansion (Fig. 8). The lymph node sections of the third group show severe reactive proliferation in the cortical follicles, severe hyperplasia in the paracortical area and intranodal sinuses, severe vascular proliferation, lymphocytes and histiocytes infiltration (Fig. 9).

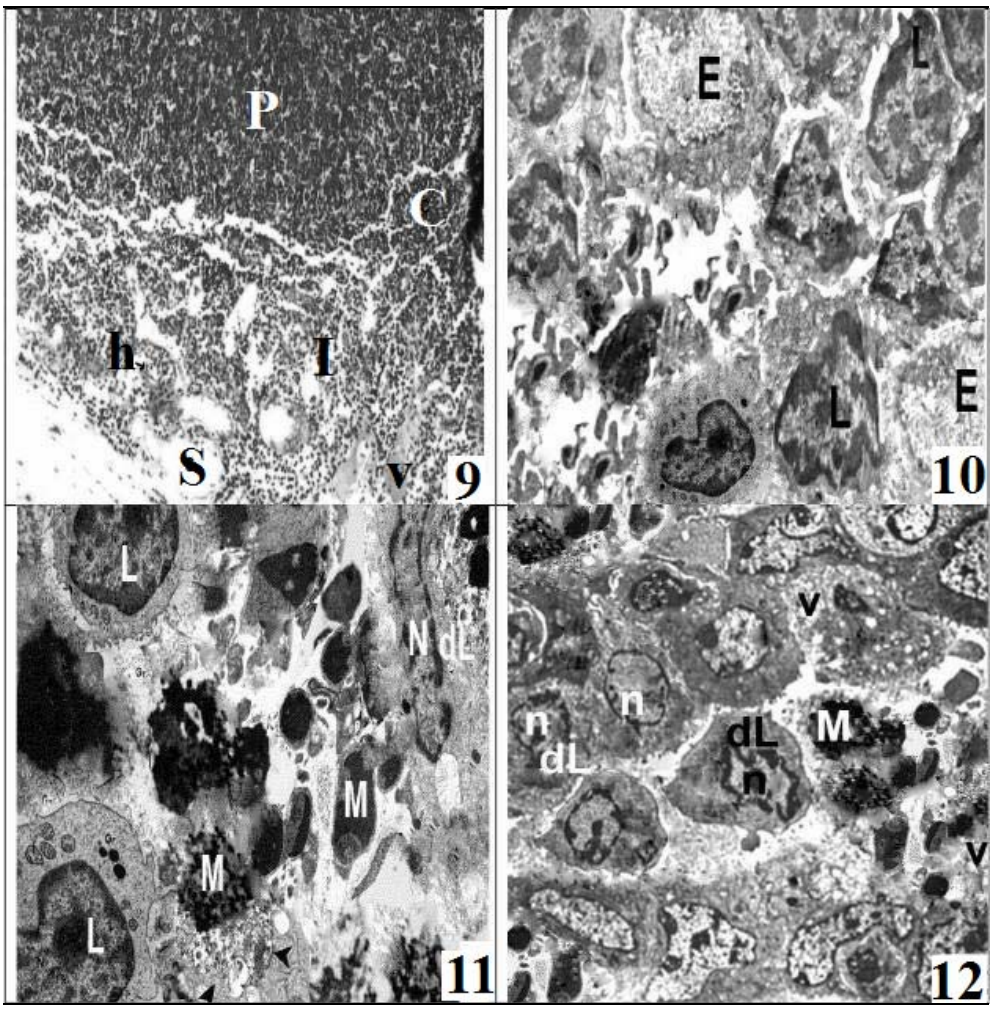

Fig. 9. Photomicrograph of a section in the third group rat lymph nodes showing prominent vascular (v) proliferation, severe reactive proliferation in the cortical (C) follicles with significant expansion, hyperplasia, and widening in the paracortical area (P) and intranodal sinuses (S). There are activated lymphocytes (l) and histiocytes (h). (H&E X 80).

Fig. 10. Electron microscopic picture of the control rat thymus gland showing normal cortex containing lymphocyte (L) and epithelial cell (E). (X8000).

Fig. 11. Electron microscopic picture of the second group rat thymus gland shows normal lymphocytic (L), degenerated lymphocytes (dL) with degenerated nucleus (N) and macrophage containing phagocytic materials (M) (X10000).

Fig. 12. Electron microscopic picture of the third group rat thymus gland showing marked degenerated lymphocytes (dL), pyknotic nuclei (n), vacuoles (v) and large sized macrophage containing phagocytic materials (M) (X10000).

3.3.2. Histopathological findings using an electron microscope

3.3.2.1. Thymus gland

The ultrastructure of the thymus glands of the control group rats shows conventional appearance (Fig. 10). But, the ultrastructure of the thymus glands of the second group rats shows a few numbers of degenerated lymphocytes and macrophage in the cytoplasm containing phagocytic materials (Fig. 11). The ultrastructure of the thymus glands of the third group rats shows marked degeneration in lymphocytes, pyknotic nuclei and non-identified micro-organelles in the cytoplasm of most cells, vacuoles and large sized macrophage containing phagocytic materials (Fig. 12).

3.3.2.2. Spleen

The spleen ultrastructure of the control group rats shows classic appearance (Fig. 13). But, the spleen

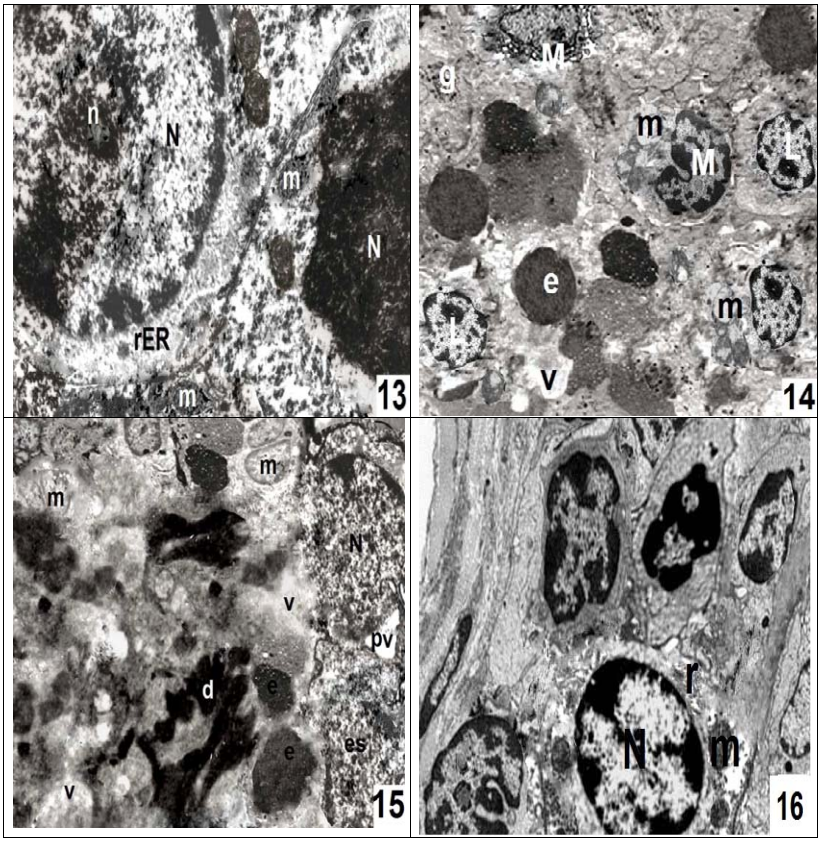

Fig. 13. Electron microscopic picture of the control group rat spleen showing the typical appearance of the nucleus (N), nucleolus (n), rough endoplasmic reticulum (rER) and mitochondria (m) (X12000).
Fig. 14. Electron microscopic picture of the second group rat spleen showing venous sinusoids filled with erythrocytes (e), macrophages (M), lymphoid cells (L) with eosinophilic ogranules (g) and vacuolation in mitochondria (m) and the cytoplasm of some cells containing vacuoles (v) (X10000).
Fig. 15. Electron microscopic picture of the third group rat spleen showing degeneration of nuclear envelop in most of the nuclei (N), perinuclear area (pv), swelling and vacuolation of mitochondria (m), vacuoles (v), erythrocytes (e) and eosinophils (es) in the lumen of blood sinusoids with electron-dense materials (d). (X10000).
Fig. 16. Electron microscopic picture of the control group rat lymph nodes showing heterochromatic nucleui (N) of lymphocytes, well-developed mitochondria (m) and cisternae of rough endoplasmic reticulum (r) (X12000).

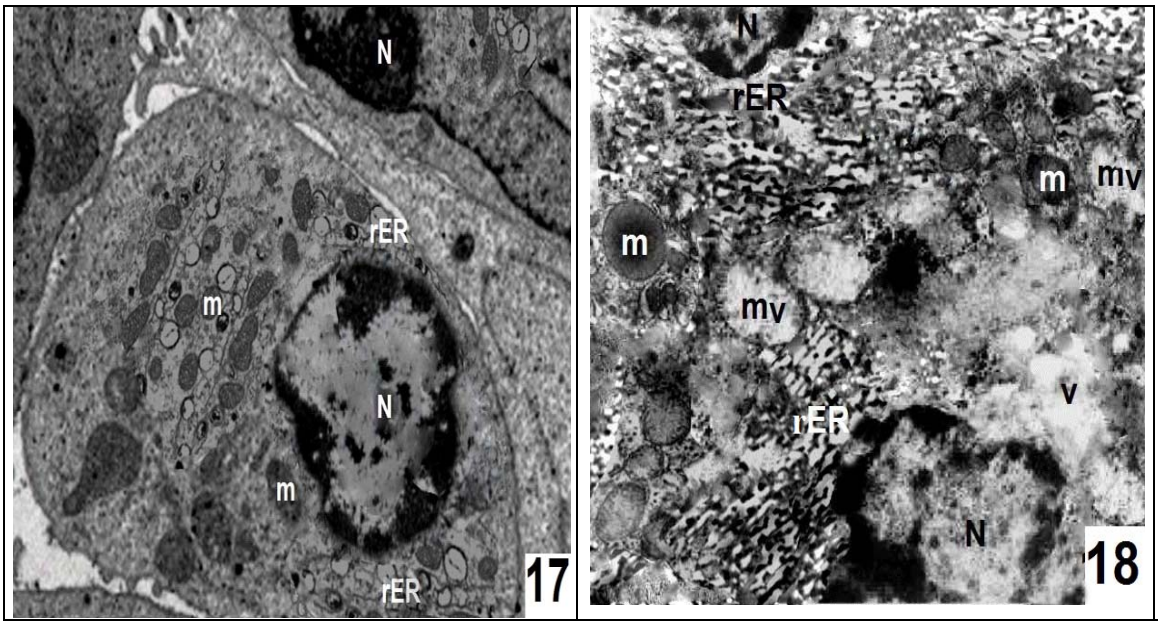

Fig. 17. Electron microscopic picture of the second group rat lymph nodes showing degenerated nucleus (N), disrupted endoplasmic reticulum (rER) and swelling in mitochondria (m). (X10000).
Fig. 18. Electron microscopic picture of the third group rat lymph nodes showing marked pyknotic nuclei (N), swollen rough endoplasmic reticulum (rER) and mitochondria (m), vacuolated cytoplasm (v) and mitochondria (mv) (X12000).

ultrastructure of the second group rats shows blood sinusoidal stasis in the red pulp, macrophage and lymphocyte infiltration, and vacuoles in the cytoplasm of some cells and erythrocytes (Fig. 14). The spleen ultrastructure of the third group rats shows marked degeneration in the cells of the spleen, loss of normal structure of nuclei and mitochondria, and vacuoles formation in the cytoplasm (Fig. 15).

3.3.2.3. Lymph nodes

The ultrastructure of lymph nodes of the control group rats shows normal appearance (Fig. 16). But, the ultrastructure of lymph nodes of the second group rats shows disorganization, degeneration in the nucleus and cytoplasmic organelles such as mitochondria and rough endoplasmic reticulum (Fig. 17). The ultrastructure of lymph nodes of the third group rats shows marked degeneration in the cells of lymph nodes, structural disturbance in the of cytoplasm and mitochondria with vacuole formation (Fig. 18).

4. DISCUSSION

The study of the possible toxicological effects of zinc oxide nanoparticles on different body systems becomes an urgent necessity because of the frequent use of zinc oxide nanoparticles in various applications in several areas. Thus, this study evaluates the toxic effect of zinc oxide nanoparticles on the lymphatic system of rats by the assessment of some hematological indices and histopathological changes in the spleen, thymus gland, and lymph nodes.

The current study showed that there is a significant decrease in the rats' body, thymus and spleen weight in the third group which received 400 mg/kg/day of zinc oxide nanoparticles in comparison to the second group which received 200 mg/kg/day of zinc oxide nanoparticles. There is also a significant decrease in the rats' body, thymus and spleen weight in the second group in comparison to the control group which received water only. In agreement with our results, Hong *et al*. [11] reported that there is a decrease in the weight of the body and some organs such as thymus gland based on the reduction in food consumption. In a different context, Ben-Slama *et al*. [12] showed that zinc oxide nanoparticles do not have any significant effect on the rats' body

weight and the relative organs weight, which is in contrast with our results.

The present study showed a significant statistical decrease in all blood cells' count (platelets, white and red cells) in the third group in comparison to the second group, and in the second group as compared to the control group. Regarding the differential white blood cells' count, there is a significant statistical increase in the counts of lymphocytes, monocytes and basophils in the third group in comparison to the second group. There is also a significant statistical increase in lymphocyte, monocyte and basophil counts in the second group as compared to the control group. Neutrophil and eosinophil counts decreased significantly in the third group in comparison to the second group that has also a statistically significant decrease in the neutrophil and eosinophil counts as compared to the control group.

Ben-Slama *et al*. [12] showed a decline in platelet and white cell counts, which is consistent with our results**.** But, they showed an increase in the red blood cell count, which is in contrast with our results and those of Wang *et al* [13], who showed that the increase in the red blood cell count may lead to the increase in blood viscosity. Furthermore, Somayeh and Mohammad [6] reported that there is an increase in white cell count due to the toxicity of zinc oxide nanoparticles, which is in contrast with our study, but they are in agreement with our results on the rise lymphocyte count. Similarly, Liu *et al*. [14] reported that zinc oxide nanoparticles may cause an increase in white and red blood cell counts, a decrease in lymphocyte count with an increase in neutrophil count, which is in contrast with our results. However they are in agreement with our results on the rise in monocyte count depending on the dose. In contrast with our results, Espanani *et al*. [15] showed a decrease in the counts of platelet and lymphocyte with an increase in white cell count without any effect on the red cell count by using the same doses and route of administration but with different periods of administration that are not more than 21 days. Moreover, Srivastav *et al*. [16] were in agreement to our results on the reduction in the red blood cell and platelet counts.

The current study showed many histopathological and ultrastructural changes in different lymphatic organs (thymus gland, spleen and lymph nodes) in the second and third groups; these changes are degeneration in the cells of various organs including the nucleus and micro-organelles of cytoplasm depending on the dose. Yang *et al*. [17] explained that the lymphatic tissues and blood capillaries have an important role in absorbability wherein they can carry and accumulate zinc oxide nanoparticles in the different organs leading to cell toxicity. According to Fan and Lu [18], the toxic effects of zinc oxide nanoparticles rely on their solubility leading to the increase in intracellular zinc causing cytotoxicity, oxidative stress, and mitochondrial dysfunction.

Furthermore, Xia *et al*. [19] showed that the mechanism of zinc oxide nanoparticle toxicity depends on the intracellular oxidative stress as a result of a disturbance in the oxidant and anti-oxidant balance. This imbalance leads to modification in proteins, lipids, and nucleic acids causing cell death. Moreover, zinc oxide nanoparticles cause DNA damage, an increase in the gene expression of the death receptor and ROS generation in lysosomes, which is in agreement with Saptarshi *et al.* [20]. On the other hand, Dhawan and Sharma [21] clarified that nanoparticle is more toxic than microparticle where the spleen is considered to be one of the target organs for engineered nanoparticles, which is consistent with the current study and Wang *et al.* [13] concerning the histopathological changes in spleen**.** On the contrary, Liu *et al*. [14] demonstrated that zinc oxide nanoparticles do not cause any alteration in the histological structure of spleen and thymus gland, which is in contrast with our results.

In agreement with Qian [22], the high activity of zinc oxide nanoparticles led to histological and ultrastructural changes in lymph nodes. The peak activity of zinc oxide nanoparticles affects their ability to penetrate the target tissue because of the thick coating formation in the nanoparticles that cannot be absorbed by phagocytes. And then, zinc oxide nanoparticles enter the lymphatic system and accumulate in the lymph nodes causing a toxic inflammatory reaction and degenerative changes, which is consistent with the present study. In addition to this, Sharma *et al*. [23] showed that the biological responses to toxic nanoparticles and distribution pathways are

different based on the doses, which is in accordance with this current study.

5. CONCLUSION

Sub-chronic use of zinc oxide nanoparticles led to toxicity manifestations depending on their dose. They caused a decrease in the rats' body, thymus and spleen weights, reduction in the total blood cell count, disturbance in the differential white blood cell count, along with histopathological and ultrastructural changes in thymus, spleen and lymph nodes.

6. RECOMMENDATION

Further researches on humans should be done in future to verify our results and confirm toxicity manifestations of sub-chronic use of zinc oxide nanoparticles on the lymphatic system and blood cell counts.

CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest.

REFERENCES

1. Heinlaan, M., Ivask, A., Blinova, I., Dubourguier, C. and Kahru, A. 2008, Chemosphere, 71, 1308-1316.
2. McAuliffe, M. E. and Perry, M. G. 2007, Nanotoxicol., 1, 204-210.
3. Fartkhooni, F. M., Noori, A., Momayez, M., Sadeghi, L., Shirani, K. and Babadi, V. Y. 2013, European Journal of Experimental Biology, 3, 145-149.
4. Kao, Y. Y., Chen, Y. C, Cheng, T. J., Chiung, Y. M. and Liu, P. S. 2012, Toxicol. Sci., 125, 462- 472.
5. Espanani, H. R., Fazilati, M., Sadeghi, L., Yousefi, B. V., Bakhshiani, S. and Amraie, E. 2013, Int. Res. J. Biological Sci., 2, 54-58.
6. Somayeh, B. and Mohammad, F. 2014, Int. Res. J. Biological Sci., 3, 65-70.
7. Saman, S., Moradhaseli, S., Shokouhian, A. and Ghorbani, M. 2013, Adv. Biores., 4, 83-88.
8. Kim, K. M., Kim, T. H. and Kim, H. M. 2012, Toxicol. Environ. Health Sci., 4, 121-131.
9. Bancroft, J. and Gamble, M. 2002, Theory and Practice Histological Techniques, 5th Ed., Churchill Livingstone, New York, USA. 173-175.
10. Graham, L. and Orenstein, J. M. 2007, Nat. Protoc., 2, 2439-50.
11. Hong, J. S., Park, M. K., Kim, M. S., Lim, J. H., Park, G. J., Maeng, E. H., Shin, J. H., Kim, M. K., Jeong, J., Park, J. A., Kim, J. C. and Shin, H. C. 2014, International Journal of Nanomedicine, 9, 159-171.
12. Ben-Slama, I., Mrad, I., Rihane, N., El Mir, L., Sakly, M. and Amara, S. 2015, J. Nanomed. Nanotechnol., 6, 1-6.
13. Wang, B., Feng, W. Y., Wang, M., Wang, T. C., Gu, Y. Q. and Zhu, M. T. 2008, J. Nanopart. Res., 10, 263-76.
14. Liu, H. L., Yang, H. L., Lin, B. C., Zhang, W., Tian, L., Zhang, H. S. and Xi, Z. G. 2015, Toxicol. Res., 4, 486-493.
15. Espanani, H. R., Faghfoori, Z., Izadpanah, M., Yousefi, B. V., Bakhshiani, S. and Amraie, E. 2015, Bratisl Lek Listy, 116, 616-20 .
16. Srivastav, A. K., Kumar, M., Ansari, N. G., Jain, A. K., Shankar, J., Arjaria, N., Jagdale, P. and Singh, D. 2016, Hum. Exp. Toxicol., 35, 1286-1304.
17. Yang, H., Liu, C., Yang, D. F., Zhang, H. S. and Xi, Z. 2009, J. Appl. Toxicol., 29, 69-78.
18. Fan, Z. and Lu, J. G. 2005, J. Nanosci. Nanotechnol., 5, 1561-1573.
19. Xia, T., Kovochich, M., Liong, M., Madler, L., Gilbert, B., Shi, H., Yeh, J. I., Zink, J. I. and Nel, A. E. 2008, ACS Nano, 2, 2121-2134.
20. Saptarshi, S. R., Duschl, A. and Lopata, A. L. 2015, Nanomedicine (Lond.), 10, 2075-92.
21. Dhawan, A. and Sharma, V. 2010, Anal Bioanal. Chem., 398, 589-605.
22. Qian, J. L. 2011, Appl. Surf Sci., 180, 308-314.
23. Sharma, V., Singh, P., Pandey, A. K. and Dhawan, A. 2012, Mutat. Res., 745, 84-91.