Original Article

Subacute Toxic Effects of Zinc Oxide Nanoparticles on Testis of Adult Male Albino Rats

Soheir A. Mohamed[1], Ahmed M. Said[1], Eman K. Ahmed[2], Hend G. Aref [1*], Esam M. A. Ali [1]

[1]Department of Forensic Medicine and Clinical Toxicology, Faculty of Medicine, Sohag University, Sohag , Egypt, [2]Department of Histology, Faculty of Medicine, Sohag University, Sohag , Egypt.

***Corresponding author**

Hend Gamal Aref

Email:

hend_gamal2000@yahoo.com

ABSTRACT

Introduction: Nowadays, zinc oxide nanoparticles are considered one of the commonly used nanoparticles. They are utilized in numerous fields as agriculture, industry and biomedicine. Zinc oxide nanoparticles are widely used as food additive and in food packaging because of their antibacterial properties. Many commercial products as sun protection creams and daily-care products contain zinc oxide nanoparticles. **Aim** : The aim of the study was to evaluate the subacute toxicity of different doses of orally administrated zinc oxide nanoparticles on testis. **Methods**: The study was conducted on forty adult male albino rats divided into four groups (10 rats per group); Group I: control group, Group II: received 10 mg/kg/day zinc oxide nanoparticles, Group III: received 100 mg/kg/day zinc oxide nanoparticles, Group IV: received 200 mg/kg/day zinc oxide nanoparticles orally for 28 days. Serum testosterone level and oxidative stress biomarkers in testicular tissue including malondialdehyde, glutathione peroxidase and superoxide dismutase were estimated. Histopathological examination of the testis by light microscope was also, performed. **Results**: Zinc oxide nanoparticles induced significant decrease in serum testosterone, elevation of malondialdehyde and decrease the activity of superoxide dismutase and glutathione peroxidase in testis in a dose dependent manner as toxicity was more obvious in high doses. Significant histopathological changes were detected. **Conclusion**: The study concluded that subacute oral exposure to zinc oxide nanoparticles might cause toxic effects on testis through oxidative stress and high doses have more toxic action. **Recommendations**: Great attention must be given about the field of nanotoxicology to evaluate the potential toxic effects of nanoparticles on human health.

Keywords : Zinc oxide nanoparticles, Oxidative stress, Toxicity, Testis.

I. Introduction :

Nanotechnology has recently grown in popularity and considered as one of the most promising technologies of the twenty-first century. It is concerned with the manufacture and applications of nanoparticles (NPs) (Bayda et al., 2020). Nanoparticles are tiny materials with a size of around (1 - 100 nm). Because of their high surface area and nanoscale size, they have unique chemical and physical properties (khan et al., 2019). Entrance of NPs into human body occurs through many routes as inhalation, ingestion and injection. They may then translocate to the blood stream, triggering harmful biological effects in several organs (De Matteis, 2017). The term nanotoxicology has gained interest from the last two decades onwards. It is a subfield of toxicology that is concerned with the adverse health effects of nanoscale structures or particles with a diameter of less than 100 nm (Krug, 2014 ; Singh et al., 2019). Previous researches studied the potential toxicity of many NPs (Amer et al., 2020; Elkhateeb et al., 2020 and Sakr et al., 2021).

Zinc oxide nanoparticles (ZnO NPs) are one of the widely utilized NPs in consumer items. The annual global manufacture of ZnO NPs is estimated to be between 0.1 to 1.2 million tons (Kumar et al., 2013). They are widely found in commercial items such as sun protection creams and personal care products. Furthermore, they are utilized in numerous fields as agriculture, industry and biomedicine. Zinc oxide nanoparticles have antimicrobial and fungicidal activity so, they are commonly used as food additive and in food packaging (Sruthi et al., 2018 ; Pinho et al., 2020).

Food and Drug Administration (FDA) graded ZnO as a generally recognized as safe (GRAS) substance. On the other hand, researchers detected that the toxicological properties of NPs are different from their corresponding bulk materials and have unique physicochemical characters. Recent research papers proposed that ZnO NPs may induce undesirable effects on human health (Morris and Salem, 2017 ; Mohd Yusof et al., 2019).

Zinc oxide nanoparticles possess the ability to cross the cell membrane and the blood- barrier of some vital organs. The solubility of these particles and ability to generate free radicals are the main factors that contributed to their toxicity. Some studies reported that oxidative stress, cytotoxicity and genotoxicity could be exhibited by ZnO NPs (Ng et al., 2017). Nemenqani et al. (2015) stated that small amounts of ZnO NPs can enter into the cells and lead to production of reactive oxygen species (ROS) in large amounts. Zinc oxide nanoparticles stimulate oxidative stress inside the cells by making imbalance between the oxidant and anti-oxidant mechanisms and this explain the low activity of antioxidant enzymes.

Nanoparticles can prompt toxicity on male reproductive system via crossing the blood–testis barrier and eventually producing damage in the spermatoza (Habas et al., 2021). Harmful effects as testicular lesions, sperm malformations, changes in serum levels of sex hormone and testis-specific gene expressions may occur due to accumulation of NPs (Baek et al., 2012 ; Gao et al., 2013 and Hong et al., 2015). Many studies were conducted to evaluate ZnO NPs toxicity on lungs, livers and kidney, while, in vitro and in vivo studies

about ZnO NPs toxicity on reproductive system started to be increased nowadays. Recent researches revealed that ZnO NPs were able to accumulate in the testis and epididymis (Tang et al., 2019 and Kong et al., 2020).

The present study aimed to evaluate the subacute toxic effects of different doses of orally administrated Zinc oxide nanoparticles on testis of adult male albino rats.

II. Material and methods

II.1. Chemicals:

ZnO NPs were purchased from Nano-Gate Company, Cairo, Egypt. ZnO NPs powders were white in color, spherical in shape with an average size of ≈ 20nm.

II.2. Animals:

The present study was conducted on 40 sexually mature male albino rats (7 weeks old) weighing (185–215gm) for 28 days. They were purchased from the animal Facility Centre of Faculty of Medicine Helwan University. Animals were fed with standard pellet food and water. They were acclimatized to the laboratory condition for one week before starting the treatment protocol. The protocol of ethics and husbandry conditions of animal research were considered according to the guide of laboratory animals care and use that approved by the ethical committee of Faculty of Medicine, Sohag University.

Rats were randomly divided into 4 groups, 10 rats per group:

Group I: control group, animals were not received any treatment.

Group II: received ZnO NPs in a dose of 10 mg/kg /day (about 1/500 of LD_{50}) (Wang et al., 2008).

Group III: treated with 100 mg/kg /day ZnO NPs (about 1/50 of LD_{50}).

Group IV: treated with 200 mg/kg /day ZnO NPs (about 1/25 of LD_{50}).

Zinc oxide nanoparticles powder was dissolved in distilled water and dispersed by sonicator for 10 minutes then administered orally to the rats by gavage tube once daily for 28 days.

II.3. Methods:

Collection of blood samples

After 28 days of treating animals with ZnO NPs , blood samples were taken before scarification of animals from retro-orbital plexus into clean dry tubes (Johnson, 2007). Later on , blood samples were centrifuged and serum was separated to be transferred into sterile screw capped vials for measurement of serum testosterone levels by using testosterone ELISA kits according to Tietz. (1995). .

Tissue samples:

Rats were sacrificed at the end of the study after being anaesthetized by ether inhalation then dissected to expose testes. According to the method of Kheradmand et al. (2009) , left testis samples were perfused via using a phosphate-buffered saline (PBS) solution pH 7.4 containing 0.16 mg/ml heparin for removing any red blood cells clots then stored at -80 oC for

measurement of oxidative stress biomarkers. Finally, 500 mg of testis tissue was homogenized in 3 ml of PBS and the homogenates were centrifuged at 300 rpm for 15 min then supernatants were used for estimation of malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GPx) enzymes activity. They were purchased from Bio-diagnostic company, Giza, Egypt.

II.4. Histopathological examination:

Right testis samples were cleaned with physiological saline and fixed in bouin's solution then absolute ethyl alcohol was used for dehydration of the samples. The specimens were embedded in blocks for sectioning at 5 micro thicknesses. Finally, sections were processed to be stained with hematoxylin and eosin (H&E) stain and examined by light microscope then photographed (Bancroft and Layton, 2013).

Statistical analysis:

The provided data were analyzed by using SPSS software version 24. One way ANOVA (analysis of variance) test and post-hoc Tukey HSD (honestly significant difference) test were used for comparing the differences between groups. Probability values of significance (P- values) < 0.05 were considered as significant groups. .

III. Results

III.1. Serum testosterone

This study detected a very highly significant decrease in serum testosterone hormone level between the studied groups as shown in table 1. Results revealed a non-significant difference in serum testosterone of group II (10 mg/kg /day ZnO NPs) compared to group I (control group). On the other hand, a highly significant decrease in serum testosterone level of group III (100 mg/kg /day ZnO NPs) in comparison to group I and a very highly significant decrease in group IV (200 mg/kg /day ZnO NPs) compared to group I were detected as illustrated in table 1. Statistical analysis between ZnO NPs treated groups showed a highly significant decrease in serum testosterone of group III in comparison to group II and a very highly significant decrease in group IV compared to group II. On the other hand, comparison between group IV and III revealed a highly significant decrease in serum testosterone level as shown in table 2.

III.2. Oxidative stress parameters

Estimation of oxidative stress biomarkers in testis showed a very highly significant increase in MDA level and a very highly significant decrease in the activity of SOD and GPx enzymes between the studied groups as illustrated in table 3.

Table 3 showed a non-significant difference in MDA level of group II compared to group I. On comparing group III to group I, results revealed a significant increase in MDA level. Furthermore, there was a very highly significant increase in MDA of group IV compared to group I .Comparison between ZnO NPs treated groups revealed a non-significant difference in

MDA of group III compared to group II, while, there was a very highly significant increase in group IV compared to group II. Comparison between group IV and III revealed a significant increase in the values of MDA as shown in table 4.

Regarding SOD enzyme activity, table 3 showed a non- significant difference in SOD activity in group II compared to group I. On the other hand, there was a highly significant decrease in the activity of SOD enzyme of group III in comparison to group I. Results also revealed a very highly significant decrease in the activity of SOD enzyme of group IV in comparison to group I. Statistical analysis between ZnO NPs treated groups showed a significant decrease in the SOD activity in group III compared to group II and a highly significant decrease in group IV compared to group II. On the other. hand, comparison between group IV and group III showed a non-significant difference as illustrated in table 4.

Regarding GPx enzyme activity, table 3 showed a non- significant difference in GPx enzyme activity in group II compared to group I, while a highly significant decrease in GPx enzyme activity of group III compared to group I was detected. Furthermore, comparison between group IV and group I revealed a very highly significant decrease. Results of comparison between ZnO NPs treated groups revealed a significant decrease in the activity of GPx in group III compared to group II and a very highly significant decrease in group IV compared to group II. Also, comparison between group IV and III revealed a significant decrease in GPx enzyme activity as shown in table 4.

Table (1): Statistical comparison between control and ZnO NPs treated groups regarding mean values of serum testosterone hormone level by using one way ANOVA test and post-hoc Tukey HSD test.

Groups / Testosterone	Mean± SD				ANOVA	p-value by Tukey-test		
	Group I (control)	Group II (10 mg/kg ZnO NPs)	Group III (100 mg/kg ZnO NPs)	Group IV (200 mg/kg ZnO NPs)		Group II versus Group I	Group III Versus Group I	Group IV versus Group I
Serum Testosterone (ng/ml)	2.82 ±0.19	2.49±0 .16	1.67 ±0.21	0.81 ± 0.19	< 0.001***	0.226♦	0.001**	<0.001 ***

p-values ♦ > 0.05 Non significant ** < 0.01 Highly significant *** < 0.001 Very highly significant SD: Standard deviation ANOVA: Analysis of variance ZnO NPs: Zinc oxide nanoparticles.

Table (2): Statistical comparison between ZnO NPs treated groups regarding mean values of serum testosterone hormone level by using post-hoc Tukey HSD test.

Groups / Testosterone	Mean± SD			p-value by Tukey-test		
	Group II (10 mg/kg ZnO NPs)	Group III (100 mg/kg ZnO NPs)	Group IV (200 mg/kg ZnO NPs)	Group III versus Group II	Group IV versus Group II	Group IV versus Group III
Serum Testosterone (ng/ml)	2.49 ± 0.16	1.67 ± 0.21	0.81 ± 0.19	0.003**	< 0.001***	0.002**

p-values: ** < 0.01 Highly significant *** < 0.001 Very highly significant SD: Standard deviation ANOVA: Analysis of variance ZnO NPs: Zinc oxide nanoparticles.

Table (3): Statistical comparison between control and ZnO NPs treated groups regarding mean values of testicular MDA, SOD and GPx by using one way ANOVA test and post-hoc Tukey HSD test.

Groups / Testis	Mean± SD				ANOVA	p-value by Tukey-test		
	Group I (control)	Group II (10 mg/kg ZnO NPs)	Group III (100 mg/kg ZnO NPs)	Group IV (200 mg/kg ZnO NPs)		Group II versus Group I	Group III Versus Group I	Group IV versus Group I
MDA Level (nmol/ gT)	33.36 ±3.07	38.50± 3.33	44.74 ± 2.96	55.15 ±2.19	<0.001***	0.215♦	0.006*	<0.001***
SOD Activity (U/gT)	359.92±17.2	354.25±11.8	297.95±13.6	284.7±14.4	<0.001***	0.961♦	0.003**	<0.001***
GPx Activity (U/gT)	59.06 ± 5.84	53.91± 3.71	42.04 ± 2.89	31.67 ±2.15	<0.001***	0.422♦	0.002**	<0.001***

p-values ♦ > 0.05 Non significant * < 0.05 significant ** < 0.01 Highly significant *** < 0.001 Very highly significant SD: Standard deviation ANOVA: Analysis of variance MDA: Malondialdehyde SOD: Superoxide dismutase GPx: Glutathione peroxidase ZnO NPs: Zinc oxide nanoparticles.

Table (4): Statistical comparison between ZnO NPs treated groups regarding mean values of testicular MDA, SOD and GPx by using post-hoc Tukey HSD test.

Groups / Testis	Mean± SD			p-value by Tukey-test		
	Group II (10 mg/kg ZnO NPs)	Group III (100 mg/kg ZnO NPs)	Group IV (200 mg/kg ZnO NPs)	Group III versus Group II	Group IV versus Group II	Group IV versus Group III
MDA Level (nmol/ gT)	38.50 ± 3.33	44.74 ± 2.96	55.15 ±2.197	0.114♦	<0.001***	0.010*
SOD Activity (U/gT)	354.25 ± 1.81	297.95 ±13.63	284.70±14.43	0.006*	0.002**	0.685♦
GPx Activity (U/gT)	53.91 ± 3.71	42.04 ± 2.89	31.67 ± 2.15	0.024*	<0.001***	0.044*

p-values ♦ > 0.05 Non significant * < 0.05 significant ** < 0.01 Highly significant *** < 0.001 Very highly significant SD: Standard deviation ANOVA: Analysis of variance MDA: Malondialdehyde SOD: Superoxide dismutase GPx: Glutathione peroxidase ZnO NPs: Zinc oxide nanoparticles.

III.3. Histopathological findings:

Light microscopic examination from testis sections of group I (control group) revealed normal appearance of seminiferous tubules that were separated by interstitial tissue. seminiferous tubules were lined with a sort of stratified epithelium which consist of spermatogenic or germ cells and sertoli cells in-between. The lining spermatogenic cells were spermatogonea, primary spermatocytes, spermatids and sperms as shown in (figure 1.A). Hematoxylin and eosin stained sections from testis of group II (10 mg/ kg ZnO NPs) revealed that seminiferous tubules have normal spermatogenic lining and mild irregular thickened basement membrane. The interstitial tissues have some homogenous exudate as shown in (figure 1.B). Hematoxylin and eosin stained sections from testis of group III (100 mg/ kg ZnO NPs) showed that seminiferous tubules are small and have few large vacuoles. Their spermatogenic cells are dislocated and disarranged. The basement membrane is irregular and thickened. Furthermore, Interstitial tissue shows acidophilic homogenous exudate, cellular hyperplasia and markedly dilated and congested blood vessels with thickened wall as in (figure 1.C). Hematoxylin and eosin stained sections of testis of group IV (200 mg/ kg ZnO NPs) revealed that seminiferous tubules are small with large vacuoles and few spermatogenic cells. Some tubules have multiple giant cells which have many nuclei and acidophilic cytoplasm. There is marked irregularity of the basement membrane surrounding the seminiferous tubules. Transudate edema, marked widening of interstitial tissue, cellular hyperplasia and congested, dilated blood vessels with thickened wall are obvious as in (figure 1.D)

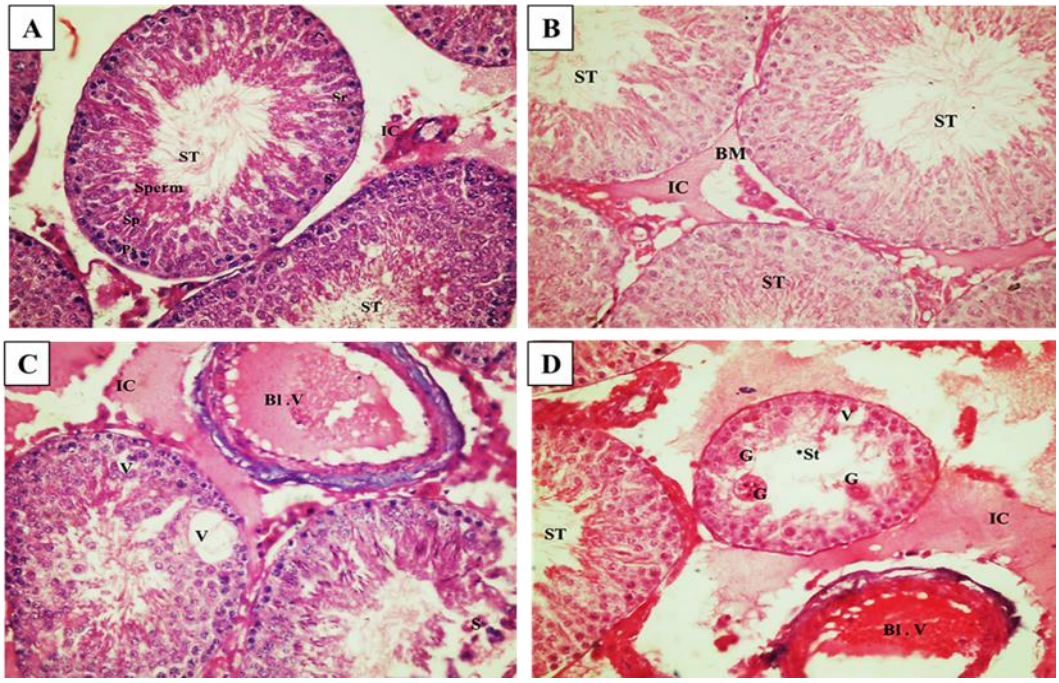

Figure (1): H&E-stained sections in the testes of the study groups. **A:** A photomicrograph of testis section of group I (control group) showing; parts of seminiferous tubules (ST). The lining spermatogenic cells are spermatogonea (s), primary spermatocytes (ps), spermatids (sp) and sperms. In between there are Sertoli cells (sr) Notes: interstitial tissue in between seminiferous tubules (IC) (H&E, x 400)**.** **B:** A photomicrograph of testis section of group II (10 mg/kg/day ZnO NPs) showing, seminiferous tubules (ST) with normal spermatogenic lining and mild irregular basement membrane (BM). Furthermore, interstitial tissues have some homogenous exudate (IC) (H&E, x 400). **C:** A photomicrograph of testis section of group III (100 mg/kg/day ZnO NPs) showing; parts of seminiferous tubules. Large vacuoles (v) and dislocated with disarrangment of spermatogenic cells (s) are observed Notes: transudate edema, widening of interstitial tissue (IC) and markedely dilated congested blood vessels (H&E, x 400). **D:** A photomicrograph of testis section of group IV(200 mg/kg/day ZnO NPs) showing; parts of seminiferous tubules. The tubules are small (ST) with large vacuoles (v) and few spermatogenic cells (*st) Notes: transudate edema , widening of interstitial tissue (IC) and dilated congested blood vessels. Multiple giant cells are seen in seminiferous tubule (G). Irregularity of the basement membrane surrounds the seminiferous tubules (H&E, x 400).

IV. Discussion

Male reproductive system has been known to be more susceptible to environmental stress including external toxicants and NPs (Tang et al., 2019). Zinc oxide nanoparticles could enter in the male reproductive system via crossing the blood–testis barrier, leading to disruption in the endocrine system and eventually causing reproductive toxicity (Lu et al ., 2013 ; Pinho et al., 2020). The present study evaluated effects of orally administrated ZnO NPs on serum testosterone level in adult male albino rats by using 3 different doses (10, 100 and 200 mg/kg/day) for 28 days.

Testosterone hormone plays a major role in stimulating spermatogenesis process . Suppression of testosterone hormone leads to apoptosis and changes in the structure of germinal epithelium (Blanco-Rodríguez and Martínez-García 1998).

Results of this study revealed that the higher the ZnO NPs dosage, the lower the serum testosterone level. There was a highly significant decrease in serum testosterone level in the group that received 100 mg/kg/day ZnO NPs and a very highly significant decrease in the group that received 200 mg/kg/day ZnO NPs compared to the control group. While, results of the group that received 10 mg /kg revealed a non-significant difference in comparison to the control group.

Our findings are in line with those recorded by Tang et al. (2019). They studied the change in serum testosterone level of male mice after administration of 50,150, and 450 mg/kg/day ZnO NPs orally for 14 days. The results showed that increasing the dosage of the ZnO NPs leads to more decrease in the serum testosterone level compared to the control group. Data revealed a significant decrease in testosterone level in the group that treated with 150 mg/kg/day and a highly significant decrease in those treated with 450 mg/kg/day.

Go in harmony with the present study, a significant decrease in serum testosterone level in male rats treated with 50 mg/kg/day ZnO NPs by oral route for 35 days was noticed by Rafiee et al. (2019). They stated that the significant decrease in the testosterone hormone indicates that ZnO NPs can affect the function of Leydig cells in androgen synthesis.

The results of the present study were in accordance with Moridian et al. (2015) who studied the effects ZnO NPs on the mouse testicular tissue at doses of 5, 50 and 300 mg/kg/day orally for 35 days. There was a significant decrease in serum testosterone level in the dose of 50 mg/kg and a highly significant decrease in the dose of 300 mg/kg comparing to the control group.

Male infertility is one of the first reproductive functions shown to be vulnerable to oxidative stress , as ROS and free radicals can affect the sperm function and DNA integrity (Aitken, 2020). Some studies have proposed that ZnO NPs can induce cytotoxicity in both , testis and male germ cells. Furthermore , this toxicity not only dependent on dose but also on the time of exposure (Pinho et al., 2020).

In the present study estimation of oxidative stress biomarkers in testicular tissues revealed a significant increase in MDA and a s decrease in SOD and GPx enzymes activity in the groups that received 100 and 200 mg/kg/day in a dose dependent manner as toxicity was more severe with increasing the dose .

In accordance with the present study Rafiee et al.(2019) stated that ZnO NPs induced oxidative stress in the testis of mice by decreasing the level of catalase (CAT) and SOD enzymes and increasing MDA level. Mice in this study treated with 50 mg/kg/day ZnO NPs orally for 35 days.

Go in harmony with the present study, the reproductive toxicity of ZnO NPs in male albino rats was studied by Hussein et al. (2016). Rats received 100 and 400 mg/kg/day ZnO NPs orally. Results revealed a significant increase in MDA as a lipid

peroxidation marker and decrease in the activity of GPx, SOD, and CAT in the testicular tissue of ZnO NPs treated groups.

Yousef et al. (2019) evaluated ZnO NPs reproductive toxicity in male rats after oral administration of 100mg/kg/day. Results showed a significant decrease in the activities of plasma and testes GPx, CAT, and SOD and a significant increase in MDA level comparing to control group.

Regarding histopathological changes , no abnormal finding was detected in the testis section of group I (control group). There was minimal histopathological changes in group II (10 mg/kg/day ZnO NPs). On the other hand marked changes in group III (100 mg/kg/day ZnO NPs) and group IV(200 mg/kg/day ZnO NPs) were observed . The severity of the changes observed was dose-dependent, as the changes were more severe at the highest dose. The histopathological changes in group II were mild irregularity in the basement membrane of seminiferous tubules and homogenous exudate in the interstitial tissues. While, group III showed large vacuoles in the seminiferous tubules and dislocated with disarrangment of spermatogenic cells. In addition, transudate edema , widening of interstitial tissue and markedly dilated and congested blood vessels were observed. Group IV showed that seminiferous tubules were small with large vacuoles and few spermatogenic cells. Transudate edema, widening of interstitial tissue , multiple giant cells and irregularity of the basement membrane were also observed .

Alazouny et al. (2014) stated that free radicals can affect the cell membrane by oxidative phosphorylation causing loss of its integrity. Furthermore, they enhance the lysosomal enzymes release in cytoplasm and oxidation of cellular protein causing their disruption. These effects lead to appearance of vacuoles through producing degeneration in germ cells and increasing the spaces in-between Sertoli cells.

Hussein et al. (2016) evaluated the histopathological changes in testis of male albino rats following oral administration of 100 and 400 mg/kg/day ZnO NPs. Pathological changes showed more damage with increasing the dose. The testes in the group that treated with 100 mg/kg ZnO NPs showed mild focal testicular degeneration of single or multiple layers of vacuolated spermatocytes and congested interstitial blood vessels. While, testes in the group that treated with 400 mg/kg ZnO NPs showed more lesions as seminiferous tubules were small, disorganized and appeared with irregularity in the basement membrane and incomplete spermatogenesis. In addition, some seminiferous tubules were empty from spermatids and spermatozoa .

Rafiee et al. (2019) studied the histopathological changes that occurred in testis of male mice after treatment with 50 mg/kg/day ZnO NPs orally for 35 days. There was degenerative changes in the form of loss of the elongated spermatid , germ cell layer disorganization, detachment and sloughing of germ cells and vacuolization and atrophy of germinal epithelium

V. Conclusion :

This study concluded that subacute oral exposure to ZnO NPs have toxic effects on testis functionally and pathologically and these effects were attributed to oxidative stress in testicular tissue. ZnO NPs have a dose

dependent toxicity and higher doses have more toxic action

VI. Recommendations :

- More studies to evaluate toxicity of ZnO NPs on different organs are needed.
- Great attention must be given about the field of nanotoxicology that was proposed as a new branch of toxicology , concerned with studying the adverse health effects caused by nanoparticles.
- The wide spread use of ZnO NPs in many applications as food industry, medicine and cosmetics ,increase the alarms about their potential adverse effects on human and animal health.

VII. References:

Aitken, R. J. (2020): Impact of oxidative stress on male and female germ cells: implications for fertility. Reproduction, 159(4): 189-201.

Alazouny, Z. M.; Mohamed, E. M. and Ahmed, G. A. (2014): Effect of cyclosporine A on the structure of adult albino rat testis and the role of lycopene supplementation: a histological and immunohistochemical study. Egyptian Journal of Histology, 37(2): 292-303.

Amer, M.; Farahat, F. and Hassan, H. (2020): Protective effects of cerium oxide nanoparticles on oxaliplatin induced neurotoxicity in adult male albino rats. Zagazig Journal of Forensic Medicine, 18(1): 52-67.

Baek, M.; Chung, H.E.; Yu, J.; Lee, J.A.; Kim, T.H.; Oh, J.M.; Lee, W.J.; Paek, S.M.; Lee, J.K.; Jeong, J. and Choy, J.H. (2012): Pharmacokinetics, tissue distribution, and excretion of zinc oxide nanoparticles. International Journal of Nanomedicine, 7: 3081-3097.

Bancroft, J.D. and Layton, C. (2013): The Hematoxylin and eosin. Suvarna, S.K.; Layton, C. and Bancroft, J.D. (editors). In: Theory Practice of histological techniques. 7th edition, El Sevier, Chapter 10, 173–186.

Bayda, S.; Adeel, M., Tuccinardi, T., Cordani, M., and Rizzolio, F. (2020): The history of nanoscience and nanotechnology: From chemical-physical applications to nanomedicine. Molecules, 25(1): 112-126.

Blanco-Rodríguez, J. and Martínez-García, C. (1998) : Apoptosis precedes detachment of germ cells from the seminiferous epithelium after hormone suppression by short-term oestradiol treatment of rats. International Journal of Andrology, 21(2):109-115.

De Matteis, V. (2017): Exposure to inorganic nanoparticles: routes of entry, immune response, biodistribution and in vitro/in vivo toxicity evaluation. Toxics, 5(4): 29-49

Elkhateeb, S. A.; Ibrahim, T. R.; El-Shal, A. S. and Abdel Hamid, O. I. (2020): Ameliorative role of curcumin on copper oxide nanoparticles-mediated renal toxicity in rats: An investigation of molecular mechanisms. Journal of Biochemical and Molecular Toxicology, 34(12): e22593.

Gao, G.; Ze, Y.; Zhao, X.; Sang, X.; Zheng, L.; Ze, X.; Gui, S.; Sheng, L.; Sun, Q.; Hong, J. and Yu, X. (2013):

Titanium dioxide nanoparticle-induced testicular damage, spermatogenesis suppression, and gene expression alterations in male mice. Journal of Hazardous Materials, 258: 133-143.

Habas, K.; Demir, E.; Guo, C.; Brinkworth, M. H. and Anderson, D. (2021): Toxicity mechanisms of nanoparticles in the male reproductive system. Drug Metabolism Reviews, 1-14.

Hong, F.; Zhao, X.; Si, W.; Ze, Y.; Wang, L.; Zhou, Y.; Hong, J.; Yu, X.; Sheng, L.; Liu, D. and Xu, B. (2015): Decreased spermatogenesis led to alterations of testis-specific gene expression in male mice following nano-TiO2 exposure. Journal of Hazardous Materials, 300,718-728.

Hussein, M. M.; Ali, H. A.; Saadeldin, I. M. and Ahmed, M. M. (2016): Querectin alleviates zinc oxide nanoreprotoxicity in male albino rats. Journal of Biochemical and Molecular Toxicology, 30(10), 489-496.

Johnson, M.D. (2007): The Rats. Gad, S.C. (editor). In: Animal models of toxicology. 2nd edition. CRC Press, Taylor & Francis Group, Chapter 3, 187–188.

Khan, I.; Saeed, K. and Khan, I. (2019): Nanoparticles: Properties, applications and toxicities. Arabian Journal of Chemistry, 12(7), 908-931.

Kheradmand, A.; Alirezaei, M.; Asadian, P.; RafieiAlavi, E. and Joorabi, S. (2009): Antioxidant enzyme activity and MDA level in the rat testis following chronic administration of ghrelin. Journal of Andrologia, 41(6): 335-340.

Kong, T.; Zhang, S. H.; Zhang, C.; Zhang, J. L. Yang, F.; Wang, G. Y. and Li, H. L. (2020): The effects of 50 nm unmodified nano-ZnO on lipid metabolism and semen quality in male mice. Biological Trace Element Research, 194(2), 432-442.

Krug, H. F. (2014): Nanosafety research - are we on the right track?. Angewandte Chemie International Edition, 53(46): 12304-12319.

Kumar, S.S.; Venkateswarlu, P.; Rao, V.R. and Rao, G.N. (2013). Synthesis, characterization and optical properties of zinc oxide nanoparticles. International Nano Letters, 3(1):1-6.

Lu, X.; Liu, Y.; Kong, X.; Lobie, P.E.; Chen, C. and Zhu, T.(2013): Nanotoxicity: a growing need for study in the endocrine system. Small,9(9-10):1654-1671.

Mohd Yusof, H.; Mohamad, R. and Zaidan, U. H. (2019): Microbial synthesis of zinc oxide nanoparticles and their potential application as an antimicrobial agent and a feed supplement in animal industry: a review. Journal of Animal Science and Biotechnology, 10(1): 1-22.

Moridian, M.; Khorsandi, L. and Talebi, A. R. (2015): Morphometric and stereological assessment of the effects of zinc oxide nanoparticles on the mouse testicular tissue. Bratislavske lekarske listy, 116(5), 321-325

Morris, A. S. and Salem, A. K. (2017): Nanotoxicity. Milane, L.S. and Amiji, M.M. (editors). In: Nanomedicine for Inflammatory Diseases, 1st edition, CRC press, chapter 3, 67-79.

Nemenqani, D.; El-Gharib, O.; Ahmed, A. M. and Baiuomy, A. R. (2015): The protective effects of antioxidant (vitamin C) against hepatic

oxidative damage induced by zinc oxide nanoparticals. International Research Journal of Applied and Basic Sciences, 2(9): 502-509.

Ng, C. T.; Yong, L. Q.; Hande, M. P.; Ong, C. N.; Yu, L. E.; Bay, B. H., and Baeg, G. H. (2017): Zinc oxide nanoparticles exhibit cytotoxicity and genotoxicity through oxidative stress responses in human lung fibroblasts and Drosophila melanogaster. International journal of nanomedicine, 12, 1621-1637

Pinho, A. R.; Rebelo, S. and Pereira, M. D. L. (2020): The impact of zinc oxide nanoparticles on male (in) fertility. Materials, 13(4): 849

Rafiee, Z.; Khorsandi, L. and Nejad-Dehbashi, F. (2019): Protective effect of Zingerone against mouse testicular damage induced by zinc oxide nanoparticles. Environmental Science and Pollution Research, 26(25), 25814-25824.

Sakr, S.; Abdelwahab, M. and Atef, M. (2021): The oxidative stress mediated toxicity and immunotoxic effect of Copper Oxide Nanoparticles on spleen of adult albino Rats. Zagazig Journal of Forensic Medicine, 19(1): 42-59.

Singh, A.V.; Laux, P.; Luch, A.; Sudrik, C.; Wiehr, S.; Wild, A.M.; Santomauro, G.; Bill, J. and Sitti, M. (2019): Review of emerging concepts in nanotoxicology: Opportunities and challenges for safer nanomaterial design. Toxicology Mechanisms and Methods, 29(5): 378- 387.

Sruthi, S.; Ashtami, J. and Mohanan, P. V. (2018): Biomedical application and hidden toxicity of Zinc oxide nanoparticles. Materials Today Chemistry, 10: 175-186.

Tietz, N. W. (1995): Clinical guide to laboratory tests. 3rd edition, Saunders, 268-273.

Wang, B.; Feng, W.; Wang, M.; Wang, T.; Gu, Y.; Zhu, M.; Ouyang, H.; Shi, J.; Zhang, F.; Zhao, Y.; Chai, Z.; Wang, H. and Wang, J. (2008): Acute toxicological impact of nano and submicro-scaled zinc oxide powder on healthy adult mice. Journal of Nanoparticle Research, 10: 263-276.

Yousef, M. I.; Al-hamadani, M. Y. I and Kamel, M. A. (2019). Reproductive toxicity of aluminum oxide nanoparticles and zinc oxide nanoparticles in male rats. Nanoparticle, 1(1): 3-12.

الملخص العربى

التأثيرات السّمية تحت الحاده لجزيئات أكسيد الزنك المتناهية الصغر على الخصية في ذكور الفئران البيضاء البالغة

سهير على محمد [1]، أحمد محمد سعيد [1]، إيمان خليفه أحمد [2]، هند جمال عارف[1]، عصام محمد عبدالله على[1]

[١] قسم الطب الشرعي والسموم الإكلينيكية - كلية الطب البشرى - جامعة سوهاج - مصر.
[٢] قسم الهستولوجى - كلية الطب البشرى - جامعة سوهاج - مصر.

المقدمة : تُعتبر جزيئات أكسيد الزنك المتناهية الصغر واحدة من الجزيئات النانوية شائعة الاستخدام في الوقت الحاضر . حيث يتم استخدامها في العديد من المجالات مثل الزراعة والصناعة والطب الحيوى. كما أنها تُستخدم على نطاق واسع كمضافات غذائية وفي تغليف المواد الغذائية نظرا لخصائصها المضادة للبكتيريا .كذلك تحتوي العديد من المنتجات التجارية مثل كريمات الحماية من الشمس ومنتجات العناية اليومية على جزيئات أكسيد الزنك المتناهية الصغر .

. **الهدف**: كان الهدف من الدراسة هو تقييم التأثيرات السُمية تحت الحادة للجرعات المختلفة من جزيئات أكسيد الزنك المتناهية الصغر على الخصية.

طريقة البحث : أجريت الدراسة على 40 فأرا من ذكور الفئران البيضاء البالغة مقسمة إلى 4 مجموعات و 10 فئران في كل مجموعة. المجموعة الأولى: المجموعة الضابطة ، المجموعة الثانية: تم إعطاء كل فأر 10 مجم / كجم / يوم من جزيئات أكسيد الزنك المتناهية . المجموعة الثالثة: تم إعطاء كل فأر 100 مجم / كجم / يوم من جزيئات أكسيد الزنك المتناهية ، المجموعة الرابعة: تم إعطاء كل فأر 200 مجم / كجم / يوم من جزيئات أكسيد الزنك المتناهية الصغر عن طريق الفم وذلك لمدة 28 يومًا. تم تقدير مستوى هرمون التستوستيرون في الدم و دلالات الإجهاد التأكسدي في أنسجة الخصية بما في ذلك المالوندالدهيد والسوبر أوكسيد ديسميوتيز والجلوتاثيون بيرأوكسيديز). كما تم إجراء فحص الأنسجة المرضية لأنسجة الخصية بالمجهر الضوئى

النتائج :أظهرت النتائج انخفاضا ذو دلالة إحصائية في هرمون التستوستيرون في الدم . كذلك لوحظ وجود ارتفاعًا ذو دلالة إحصائية في المالوندالدهيد وانخفاض في إنزيمات السوبر أوكسيد ديسميوتيز و الجلوتاثيون بيرأوكسيديز في الخصية بطريقة تعتمد على الجرعة حيث كانت السمية أكثر وضوحًا في الجرعات العالية. كما تم الكشف عن وجود تغيرات نسيجية مرضية في الخصية.

الخلاصة: خلُصت هذه الدراسة إلى أن التعرض الفموى تحت الحاد لجزيئات أكسيد الزنك المتناهية قد يسبب تأثيرات سامة علي الخصية عن طريق الاجهاد التأكسدى وأن الجرعات العالية لها تأثير أكثر سُمية.

التوصيات : توصى هذه الدراسة بضرورة إعطاء إهتمام كبير بمجال علم السموم النانوية لتقييم التأثيرات السُمية المُحتملة للجزيئات النانوية على صحة الإنسان