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Effect of Zinc oxide nanoparticles (ZnO-NPs) on weights of some reproductive organs and sperm abnormalities in the tail of epididymis of albino mice

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Abstract

Increasing use of zinc oxide nanoparticles (ZnO- NPs) in many products may lead to damage of different organ tissues. Therefore, this study was conducted to identify the effect of ZnO-NPs on testicular, epididymal and some accessory glands weights as well as spermatic abnormalities in the epidydimal tail of male albino mice. Animals were orally administrated by 0.1 ml of 100 and 200 mg/kg ZnO-NPs for 7 and 14 days respectively for each concentration. The control group was orally gavaged with 0.1 ml of distilled water. Statistical analysis of the results showed a significant decrease (P≤0.05) in the average body weights of animals after 7 and 14 days of exposure to each treatment compared to the control and the weight of the same animal before treatment. Losing of body weight was increased by elevation the concentration and the duration of exposure. Whereas, the weight of testes and epididymis (head and tail) showed a significant decrease (P≤0.05) with both concentration and duration of exposure compared to the control, with more decreasing in the weight with high concentration and long duration of exposure. The average weight of seminal vesicle and prostate was significantly increased (P≤0.05) with both concentration and duration of exposure compared to control group. The percentage of sperm abnormalities was significantly (P≤0.05) increased with 100 and 200 mg/kg ZnO-NPs for 7 and 14 days of exposure compared to the control. The high concentration of ZnO-NPs and long duration of exposure resulted in more severe changes in the percentage of sperm abnormalities in the epididymis tail. Thus, we can conclude that increasing concentrations of ZnO-NPs and durations of exposure leads to negative effects on the male reproductive efficiency of albino mice.

Key word: ZnO-NPs, male reproductive system, testes, epididymis, sperm quality, prostate, seminal vesicles.

INTRODUCTION

Nanoparticles (NPs) are very small, one-dimensional particles that vary in size from 1 to 100 nm[1]. Recently, they had great attention because of their unique characteristics that are useful in many medical and industrial applications [2]. Estimates show that 260,000-309,000 metric tons of nanoparticles were produced globally in 2010. [3] Other estimates predicts that, between 2014 – 2019, global consumption of NPs will increase from 225,060 to 58500 metric [4]. Zinc oxide nanoparticles (ZnO-NPs) represent the third most used nanoparticles, and their global annual production ranges from 550 and 33400 tons [5,6]. Zinc oxide nanoparticles are used in many products, including sun screens, biological sensors, food additives, dyes, rubber industry and electronic material [7]. It is expected that Zinc oxide nanoparticles will reach the environment and affect the nature of its living beings.[8]

Reproductive system is one of the most important systems in living beings, which aims into genital reproduction and conservation of species. This system is affected by different ZnO-NPs, as many recent studies showed that of ZnO-NPs accumulate in different tissues, including brain and testis, which means that these nanoparticles can easily diffuse through blood brain and blood testes barriers. [9,10] Most NPs have toxic effect on spermatogenesis, [11] due to their small size which allows them to occupy higher surface area, compared to larger particles, and hence affecting specific physical and chemical proprieties, example chemical reactions, durability and high conduction ability.[12] This small size allows NPs to penetrate cytoplasmic membrane and interfere with important cellular functions. Many reports have shown that NPs have more toxic effect than other larger part materials, due to their high reaction potential. Discussions previously concentrated on the positive aspects of ZnO-NPs, however, recently, more studies are dealing with their toxic effect. Yet, information about lines and toxicity of ZnO-NPs in environment is still limited. [13] That's why it is very important to spot the light on ZnO-NPs-related problems and identifying their toxic effects on male albino mouse' reproductive system secondary to oral ingestion of ZnO-NPs, and hence this study was conducted, in order to know the effect of ZnO-NPs on some reproductive organs (testes, epididymis, seminal vesicle and prostate) weight and sperm abnormalities in male of albino mice.

MATERIALS AND METHODS

The study was conducted on 30 male albino mice, with weights ranging from 30 – 35 g. They were divided on five groups, each one contains 6 animals. The first group was a control that ingested with distilled water. The second group was ingested with 100 mg/kg ZnO-NPs for 7 days, the third group was ingested with 100 mg/kg ZnO-NPs for 14 days, the fourth group was ingested with 200 mg/kg ZnO-NPs for 7 days, and the fifth group was ingested with 200 mg/kg ZnO-NPs for 14 days. Zinc oxide nanoparticles were prepared as the stored solution. All animals were exposed to normal laboratory conditions of temperature, light, air, and were provided with water and mice pelleted diet *ad libitum*. The concentration of 100 mg/kg ZnO-NPs was prepared by dissolving 325 mg of ZnO-NPs in 13 mL of distilled water and dissolving 650 mg ZnO NPs in 13 ml of distilled water to prepare a concentration of 200 mg/kg. The using Zinc oxide nanoparticles in this study were produced by Zhenghzou Dongyao nanomaterial company, and their characteristics were as follows: particle purity of 99.99%, mean particle size of 50 nm in diameter, have cube shaped and was a white powder form. The purity of the powder was tested by Energy Dispersive x-ray spectroscopy (SDX) device in AL-Nahrin University, faculty of science, physics department, electron microscopy lab. , which showed purity of 100% (Fig. 1). While the shape and size of the particles were determined by Transmission Electron Microscopy, in AL-Nahrin University, faculty of medicine. The results were almost identical to company descriptions (Fig. 2).

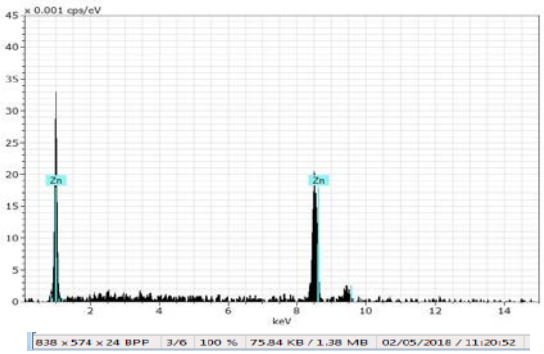

Figure (1) shows the purity of ZnO NPs powder by using SDX device .

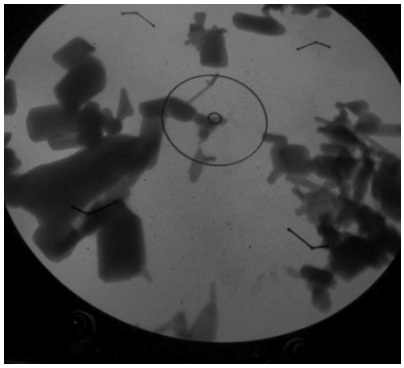

Figure (2) illustrates the shape of ZnO NPs by using TEM

Animals were obtained from animal's house in the commission of research and industrial development and then were transferred into animal's house under the life sciences department of Ibn AL-Haitham education for pure sciences. Animals were sacrificed after the end of the same duration and concentration of exposure by cervical dislocation, through fixing the head and pulling the tail, then inverted T-shaped abdominal incision was done to open abdominal cavity. Testes, epididymis, seminal vesicle and prostate were resected, and fatty tissue adhered to them were removed and dried by filtration paper. The weights of Testicles, epididymis (head and tail), seminal vesicles and prostates were taken by a sensitive 4 steps scale Analytic A 2005 Sartorius. Epididymis was separated from testicles after removal of surrounding fatty tissue, then, for purpose of studying their spermatic anomalies, tails of epididymis were resected, cut, crushed and mixed with 5 ml of saline solution. A drop of the solution was applied on a clean glass slide, then a drop of Eosin dye was added and mixed with a drop of the mash by Hancock method (1951) [14] through moving the glass rod continuously, then a swab epididymis of tail was taken. The slide was left to dry, then the swab was fixed by DPX to study abnormal changes in head, tail, and middle piece of sperm and the cytoplasmic droplet position. 100 sperm were extracted, and abnormal ones were examined using Krazanowska method[15] . All study slides were examined using the light microscope with the zoom power of 10 x 40.

Statistical analysis

Results were analyzed by SPSS program – version 20 , where t-test was used to find difference between totals of animals before and after the exposure, while f-test was used to calculate significant differences and approval of value of least significant difference to obtain differences between mean characteristics at the level of P ≤0.05 significance. [16]

RESULTS

Body weights

The results of statistical analysis showed a significant decrease (P≤0.05) in the average body weight of animals after 7 and 14 days of oral gavaged with 100 and 200 mg/kg ZnO-NPs compared to control group and to the animal body weight before each treatment (Table 1). Decreasing in the average body weight after the end of treatment was increasing with concentration (200 mg/kg ZnO-NPs) and duration of exposure (14 days) (Table 1).

Reproductive organs weight

Results of the statistical analysis showed a significant decrease (P≤0.05) in average testicular weights of animals exposed to ZnO-NPs for both concentrations (100 and 200 mg/kg), and duration (7 and 14 days), compared to control group (Table 2). The results showed also significant differences between concentrations and durations of exposure, with the lowest reduction in the testicular weight with 200 mg/kg ZnO-NPs and 14 days of exposure.

The average weight of the head and tail of epididymis showed significantly decrease (P≤0.05) in the animal treated with 100 and 200 mg/kg ZnO-NPs for 7 and 14 days of exposure compared to control group (Table 2). Decreasing the average epididymis weight was increased with elevation concentration and duration of exposure (Table 2).

The weight of seminal vesicle and prostate in animals treated with 100 and 200 mg/kg ZnO-NPs for 7 and 14 days showed significantly increased (P≤0.05) when compared to control group (Table 2). The elevation in the weight of seminal vesicle and prostate was increased with increasing the concentration and duration of exposure (Table 2).

Percentage of spermatic abnormalities in the epididymis tail

The results showed a significant increase (P≤0.05) in the percentage of sperm abnormalities in mice tails with both concentrations (100 and 200 mg/kg) of ZnO-NPs and duration of exposure (7 and 14 days) when compared with control group (Table 3 and Figure 3). The rate of abnormalities was increased with increasing the concentration and duration of exposure.

Table 1. shows animals body weights before and after exposure to different Zinc oxide nanoparticles (ZnO-NPs) concentrations for different durations.

Treatments	Number of animals	duration of exposure (days)	Animal bodies weights(gm)	
			Before exposure	After exposure
Control	6	-	34.36 ± 0.31 a	35.42± 0.37 a
100 mg/kg ZnO-NPs	6	7	33.05 ± 0.21 a	28.97 ± 0.38 b
	6	14	33.50 ± 0.30 a	24.33 ± 0.40 b
200 mg/kg ZnO-NPs	6	7	33.97 ± 0.35 a	23.35 ± 0.31 b
	6	14	34.30 ± 0.32 a	20.25 ± 0.17 b

Values represent mean ± standard error
Similar horizontal letters indicate no significant change (P ≤0.05)
Different horizontal and vertical letters indicate significant change (P ≤0.05)

Table 2. Changes in the testes, Epididymis (head and tail), and accessory glands (seminal vesicles and prostate) after exposure to 100 and 200 mg/kg of ZnO NPs for 7 and 14 days of exposure.

Treatments	Number of animals	Duration of exposure/day	Weights (mg)				
			Testes	Epididymis		Seminal vesicles	Prostate
				Head	Tail		
Control	6	-	94.29 ± 0.54 a	36.58 ± 0.29 a	21.06 ± 0.42 a	0.179 ± 0.002 a	0.025 ± 0.001 a
100 mg/kg ZnO-NPs	6	7	86.94 ± 0.41 b	32.54 ± 0.49 b	13.19 ± 0.49 b	0.205 ± 0.006 b	0.044 ± 0.001 b
	6	14	81.42 ± 0.56 c	28.23 ± 0.36 c	17.39 ± 0.21 c	0.232 ± 0.002 c	0.061 ± 0.001 c
200 mg/kg ZnO-NPs	6	7	77.51 ± 0.52 d	24.67 ± 0.22 d	14.97 ± 0.21 d	0.267 ± 0.003 d	0.071 ± 0.001 d
	6	14	71.62 ± 0.52 e	21.79 ± 0.41e	19.38 ± 0.19 e	0.352 ± 0.005 e	0.082 ± 0.002 e

Values represent mean ± standard error
Different horizontal letters indicate significant change (P ≤0.05

Table 3. Shows changes in the percentage of sperm abnormalities in epididymis tail of albino mice treated with 100 and 200 mg/kg ZnO NPs for 7 and 14 days.

Treatments	Number of animals	duration of exposure (days)	(%) of spermatic abnormalities in epididymis tail
Control	6	-	7.17 ± 0.31 a
100 mg/kg ZnO-NPs	6	7	75.33 ± 0.76 b
	6	14	79.33 ± 0.88 c
200 mg/kg ZnO-NPs	6	7	86.17 ± 1.25 d
	6	14	92.50 ± 0.62 e

Values represent mean ± standard error
Different horizontal letters indicate significant change (P ≤0.05)

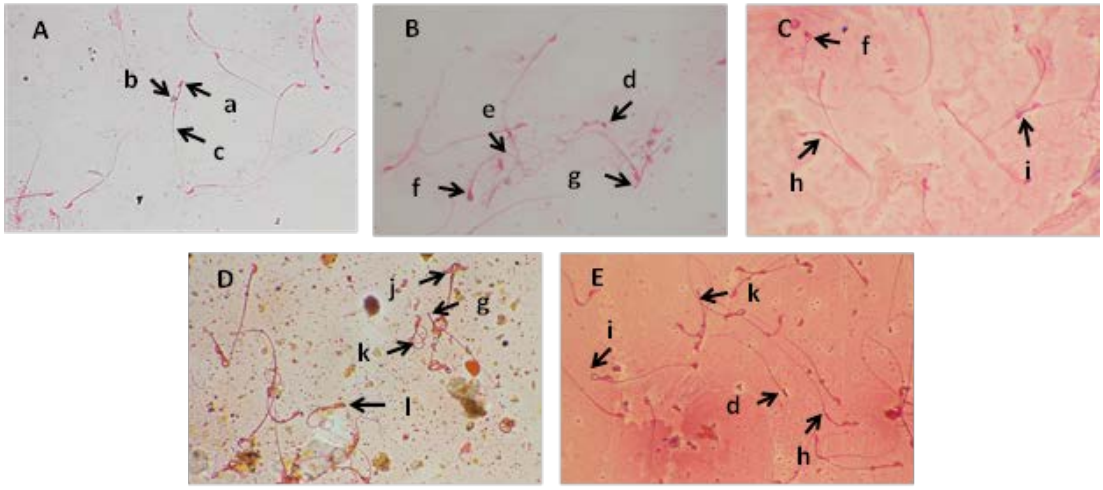

Figure 3 (A) shows the normal sperm in albino mice of the control group. (B) abnormal sperm in mice treated with 100 mg / kg of ZnO-NPs for seven days. (C) abnormal sperm in mice treated with 100 mg / kg of ZnO-NPs for 14 days. (D) abnormal sperm in mice treated with 200 mg / kg of ZnO-NPs for seven days. (E) abnormal sperm in mice treated with 200 mg / kg of ZnO-NPs for 14 days. The letters refers to: (a) sperm head containing the spine. (b) median piece containing the cytoplasmic droplets. (c) sperm tail. (d) head atrophy. (e) tail curvature. (f) Head twisting. (g) Lack of sperm head. (h) the appearance of the cytoplasmic droplets in the median piece. (i) median piece warp .(j) Splitting of the head and median piece. (k) tail wrap (l) deformation of the head. Eosin Dye.(400x)

DISCUSSION

The harmful effect of different Zinc oxide nanoparticles (ZnO-NPs) concentrations was obvious on animals' weights before and after exposure, evidenced by the absence of significant differences in weights of animals in control group, before and after exposure. However, animals' body weights kept decreasing with increasing concentration of Zinc oxide nanoparticles, to reach its lowest weight at 200 mg/kg concentration (the highest concentration) of Zinc oxide nanoparticles. Also, such correlation occurred with increasing duration of exposure from 7 to 14 days (Table 1), which indicate the negative effect of Zinc oxide nanoparticles on animal's structure, which represent the vital efficacy of its internal organs. This suggests that exposure to metallic nanoparticles, including silver nanoparticles, could cause GIT disturbances in animals, loss of appetite and decrease fatty tissue in organs, and eventually decrease animal's body weight.[17]

Furthermore, The effect of different ZnO-NPs concentrations was obvious on lowering animals' testicular weights, especially with increasing the ZnO-NPs concentration into 200 mg/kg, and duration of exposure from 7 to 14 days (Table 2).

These effects result from the accumulation of nanoparticles in different tissues, including brain and testicular tissues, which lead to damage of testicular tissue and increasing apoptotic cells, known as programmed cell death, and hence decrease testicular weights, compared with control group[17]

Badkoobeh et al. [18] showed that giving 5 mg/kg Zinc oxide nanoparticles to male rats, resulted in decrease of their testicular weights. They attributed such effect to the histopathological changes such as necrosis and degenerations. These results are consistent with what Shirvani et al.[19] concluded, when they showed that giving 25, 50 and 100 mg/kg Zinc oxide nanoparticles to male rats, resulted in bilateral decrease in their testicular weight, after 15 days of exposure, compared with control group.

The hypertrophy of seminal vesicles and prostates in animals exposed to Zinc oxide nanoparticles, especially at 200 mg/kg concentrations and with increasing duration of exposure from 7 to 14 days (Table 2), may be due to seminal vesicles inflammation and prostate hypertrophy. This result correlates with what Mohammed et al.[20] concluded, as they noticed seminal vesicles dilatation and epithelial sloughing, plus prostatic hypertrophy, in animals given silver nanoparticles.

The high increase in spermatic abnormalities was associated with increasing the concentration of Zinc oxide nanoparticles and duration of exposure, as spermatic abnormalities reached 75% at 100 mg/kg ingestion of Zinc oxide nanoparticles, and 95% at 200 mg/kg, which shows the strong effect of Zinc oxide nanoparticles on sperm structure (Table 3) and (Fig. 3). These abnormalities occurred by creating gaps in the cytoplasm of sertoli cells, which play important role in the formation and preservation of sperms [21]. Furthermore, the structure of Zinc oxide nanoparticles enables them to diffuse and enter the blood and different body tissues, including testis. Figures 3 show the presence of deformities in head and tail of sperms, damage of acrosomes, and the presence of cytoplasmic droplets at a middle piece. These changes can be due to the formation of reactive oxygen species (ROS) by Zinc oxide nanoparticles [22].

CONCLUSION

We conclude from the results of this study that Zinc oxide nanoparticles have a harmful effect by increasing the concentration and duration of exposure on the animal bodies

weights and the weight of testis, epididymis (head and tail), and accessory reproductive glands (seminal vesicles and prostate), as well as on the sperm quality, thus, impact on the functional activity of male reproductive system.

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