



The impact of nano zinc oxide particles on the histology of the male reproductive system of adult male rabbits

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Abstract

The goal of our research is to evaluate the histological impact of nano ZnO particles (NZnO) on the testis and epididymis of adult male rabbits treated with I.P of 600mg/kg body weight of nano ZnO particles twice a week for three weeks and to the protective effect of vitamin E versus the effect of nano zinc oxide particles. Twenty-four adult male rabbits were used in this research and divided into four groups. The first group was the control group injected with intraperitoneal distal water, and the second group was injected with 600mg/kg body weight of nano ZnO particles I.P. twice a week for three weeks, the third group was injected with 600 mg/kg body weight of nano ZnO particles I.P. and co-administrated with 100 mg/kg body weight of vitamin e orally, while the fourth group received 100mg/kg body weight of vitamin e orally. The histological results showed that the nano ZnO particles treatment causes noticeable changes in the testis and epididymis. These changes are characterized by thickening of tunica albuginea of testis, degenerative and necrotic changes of germ cells lining the seminiferous tubules, arrest of spermatogenesis, giant cell formation, degeneration, and necrosis of epithelial cells lining epididymis canals. The canals are free from sperms is observed. As for the group of animals treated with nano ZnO particles co-administrated with vitamin e showed improvement in the histological changes compared with the control and group treated with vitamin e only showed normal architecture of testis and epididymis. Moreover, there is a decrease in the level concentration of Testosterone of the animals treated with nano ZnO particles compared with other groups.

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Introduction

The Nano ZnO particles are substances of a size of 1-100 nm (1,2). The small size of these particles will cause an increase in the surface area. This will induce a single and specific physiochemical characterization like reaction and resistance with high conduction compared to the bulk material (3-6). Because of the tiny size of Nano ZnO particles, they can penetrate the cell membrane. This will have a profound effect on severe cell function. Several studies have proved that nanoparticles have a more toxic effect than larger particles according to their small size. For

this reason, nanoparticles are more highly reactive (7,8). Many studies revealed that nanoparticles have a toxic effect on the vascular endothelium. Also, nanoparticles can come across the blood-brain barrier and blood testes barrier (9-11). Nano, zinc oxide particles have comprehensive spread manufacturers such as food additives, electronic materials, glasses cleaning, and biosensors (12,13). Vitamins such as vitamin e is considered a primary antioxidant additive commonly used in the food industry and has shown the ability to lower oxidative stress in animals (14). In recent years, researchers have been focusing their efforts on determining how to use antioxidant substance mechanisms

and enhancing the antioxidant system efficiency (15). Vitamins, notably vitamin e is extensively used in many organisms as ameliorative aims (16-18). There are numerous applications for nano ZnO particles in biomedicine due to the numerous benefits afforded by the physicochemical features of these nanoparticles. On the other hand, they have a strong cytotoxic effect on spermatogenesis. These cytotoxic effects are dosage and duration-dependent, implying that higher concentrations and longer exposure times result in increased toxicity, increased ROS production and inhibition of the cell's antioxidant response usually cause toxicity, which causes DNA damage, cell cycle arrest, and apoptosis in male germ cells (19-22).

Additionally, because of the broad uses of nano ZnO particles in many industries, it is necessary to evaluate the toxic impacts of nano ZnO particles on the vital organs, especially their impacts on the reproductive system of the male rabbits. For this reason, our research aims to evaluate the histological impact of intraperitoneal injection of nano ZnO particles at the dose of 600 mg/kg body weight on the testis and epididymis of the male rabbits.

Materials and methods

Ethical approval

The scientific committee has approved this study of the department of pathology and poultry diseases of collage of veterinary medicine- University of Mosul at the first congress dated 13/9/2021, that the concurrent conducting experiment did not violent the laws of animal rights and the euthanasia is applied in accordance of this guidelines.

Animals

This study was set on 24 adult male rabbits (obtained from a rabbit breeding farm in Gogjali quarter in Mosul city), weighing 1.5 to 2 kg. They were kept in clean, well-ventilated cages throughout the experiment, with unrestricted access to food and water.

Chemicals

Zinc oxide nanoparticle (>100nm) the powder was obtained from Sigma-Aldrich chemicals (color: white, form: rod-shape, x-ray diffraction: conforms, particle size: \leq 50nm. Vitamin E from Poland- Pharmacy laboratories.

Experimental design

the rabbits were randomly divided into four groups, each containing 6 rabbits. The first group; was given distal water intraperitoneal as a control group. The second group was treated with nano ZnO particles I.P. 600 mg/kg body weight twice weekly for 3 weeks. The third group; was injected with nano ZnO particles I.P. 600 mg/kg body weight twice weekly in addition to vitamin e orally 100 mg/kg body weight twice weekly for 3 weeks (23). Fourth group; treated with vitamin e orally 100 mg/kg body weight twice weekly for 3 weeks.

Blood sampling

On the 21st day, the rabbits' blood was obtained aseptically using disposable syringes. Afterward, the blood was transferred to a clean tube containing gelatin to be separated at room temperature by centrifugation at 3600 RPM for 15 minutes. A Pasteur pipette aspirated the serum to Eppendorf tubes, then stored in a freezer at -20°C for estimation of testosterone level (24).

Tissue sampling

Rabbits were euthanized after 21 days from the beginning of the experiment, and testes were obtained from each of them. Then swilled with saline and fixed in 10% neutral buffered formalin for 72 h. then the testis was processed by dehydrating in ethyl alcohol, clearing in xylol, then infiltrating and embedding in paraffin wax. The wax blocks have been trimmed at 25 microns and then sectioned at 5 μ thick with an automatic microtome. Then the slides were stained with hematoxylin and eosin for routine staining (25,26).

Laboratory analysis

The concentration of Testosterone in serum has been calculated using an ELISA kit (Monobind Inc., Lake Forest, CA 92360, USA, product code: 3725-300). Elisa reader has read the microplate at 450nm wavelength. These were performed at 25°C.

Statistical analysis

Analysis of variance has been done using the SPSS program Ver.26 (SPSS Inc., Chicago, IL, USA) and using ANOVA (one-way analysis of variance), and the Duncan test has been used in data analysis. The P-value has been estimated at $P \leq 0.05$ whether there was a significant difference between groups (27).

Results

Testosterone concentration by using ELISA

The results of the analysis revealed a significant difference in the concentration of Testosterone, where it was the lowest concentration in the second group treated with nano ZnO particles I.P. 600 mg/kg body weight two times weekly and then the third group treated with nano ZnO particles I.P. 600 mg/kg body weight two times weekly in addition to vitamin e orally 100 mg/kg body weight two times weekly compared with the first and fourth control groups (Table 1).

Testis

The microscopic examination of histological sectioning of a control group was treated with distilled water I.P. and with vitamin e only revealed a normal histological appearance of the testis, composed of tunica albuginea surrounding several seminiferous tubules separated by

interstitial tissue containing connective tissue and Leydig cells. The seminiferous tubules are lined by germinal epithelial cells, composed of layers of spermatogenesis cells (Figures 1 and 2). The histological examination of the testicular section of treated animals with 600mg/kg B.W. of nano ZnO particles twice weekly for three weeks showed severe histological changes, including distortion in the normal appearance of seminiferous tubules (Figure 3). The lumens of seminiferous tubules are free from spermatozoa, and the sperms are not seen (Figure 4). Degeneration and necrosis of spermatogenic cells, with the formation of giant cells (Figures 5 and 6). Congestion and thickening in the wall of blood vessels in the interstitial tissue with deposition of eosinophilic in between the seminiferous tubules (Figure 7). degeneration and necrosis of Leydig cells depletion in the spermatogenic cells and the Leydig cells (Figure 8), thickening of tunica albuginea (Figure 9). While the group of animals was treated with Nano ZnO particles and vit. E showed improvement in the histological appearance of the testicular structure, which revealed normal seminiferous tubules lined by normal spermatogenic cells (Figure 10).

Table 1: Effect of nano ZnO particles and vitamin E on the concentration of Testosterone in blood serum

Groups	Testosterone concentration (ng/ml)
First group	4.02±0.07a
Second group	0.53±0.19c
Third group	1.32±0.02b
Fourth group	4.59±0.02a

(a-c) the letters are different vertically, indicating a significant difference at level $P \leq 0.05$. c means lower value followed by b then a.

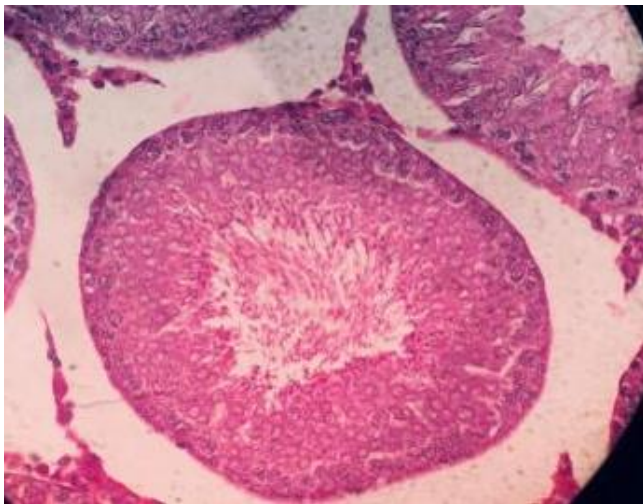


Figure 1: Micrograph of rabbit testis of control group showed normal testis architecture. H&E, 10X.

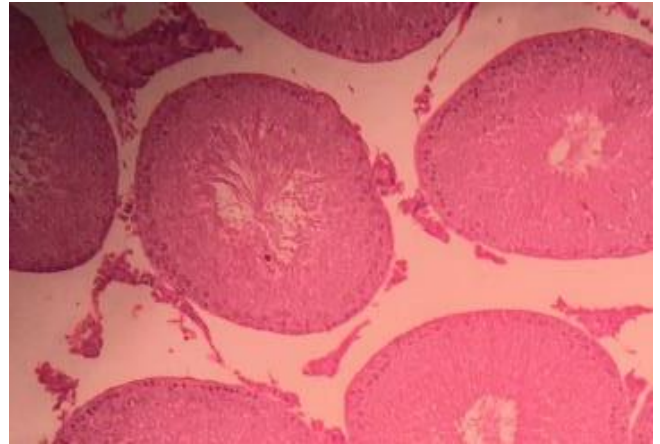


Figure 2: Micrograph of rabbit testis of vitamin E group showed normal architecture of testis. H&E, 10X.

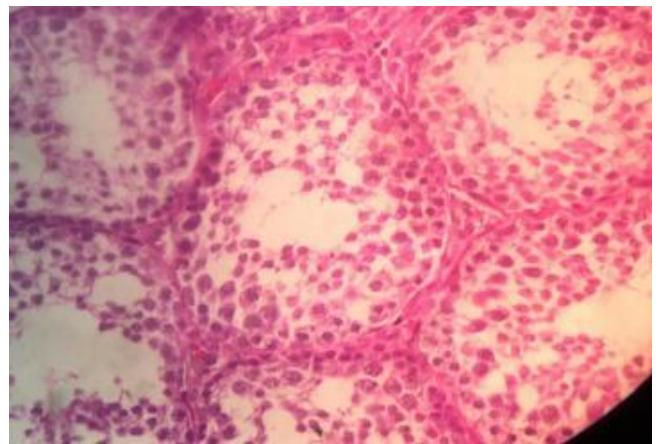


Figure 3: Micrograph of rabbit testis of treated group with Nano ZnO particles showed distortion in the normal appearance of seminiferous tubules. H&E, 40X.

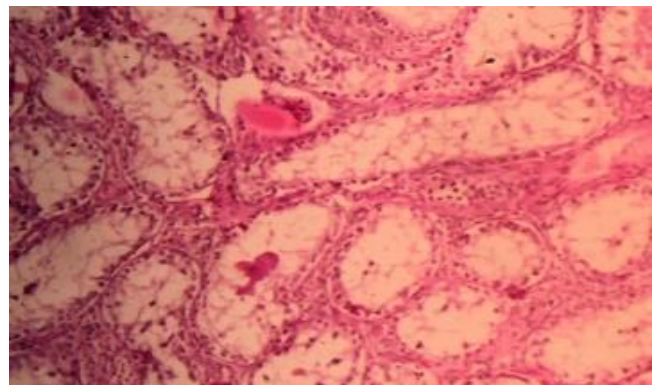


Figure 4: Micrograph of rabbit testis of treated group with Nano ZnO particles showed the lumens of seminiferous tubules are free from spermatozoa, and the sperms are not seen. H&E, 10X.

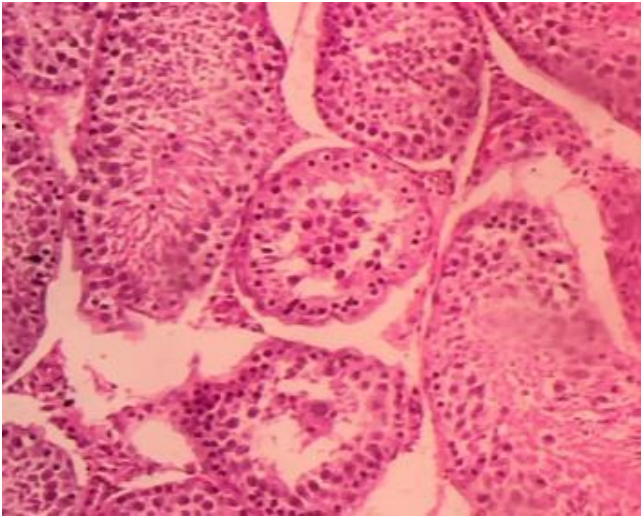


Figure 5: Micrograph of rabbit testis of treated group with Nano ZnO particles showed degeneration and necrosis of spermatogenic cells, with the formation of giant cells. H&E, 10X.

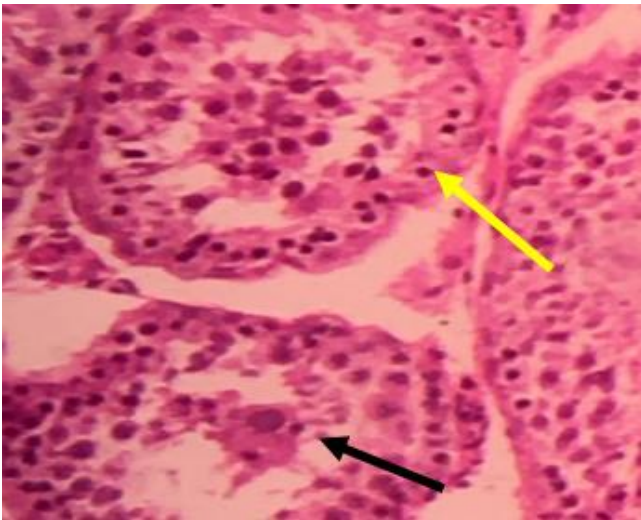


Figure 6: Micrograph of rabbit testis of treated group with Nano ZnO particles showed giant cells (black arrow), necrosis of Sertoli cells (yellow arrow). H&E, 40X.

Epididymis

The histological examination of the epididymis of the control group and group was treated with vitamin e only showed a normal histological appearance characterized by ciliated pseudostratified columnar epithelium with the presence of mature sperms in the epididymal lumen (Figure 11). The histological examination of the H&E epididymal section of animals treated with nano ZnO particles showed hyperplasia of epithelial cells lining the epididymal canal, which led to stenosis in the lumen of the canal. Few or no

spermatozoa were seen in some of the epididymal lumens compared with control animals (Figure 12). Other sections showed changes in the epididymis's epithelium lining, including vacuolation and necrosis of epithelial cells, and dilatation of epididymal tubules, without stereotyped mitotic activity (Figures 13 and 14). As for the group of animals treated with Nano ZnO particles and vitamin e showed improvement in the histological appearance of the epididymal structure, which revealed the normal appearance of epididymis similar to the histological images of the control group (Figure 15).

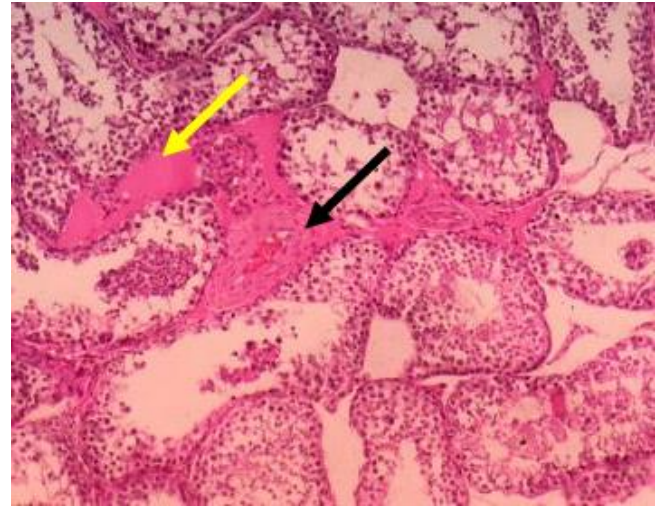


Figure 7: Micrograph of rabbit testis of treated group with Nano ZnO particles showed congestion and thickening in the wall of blood vessels (black arrow), deposition of homogenous eosinophilic material between the somniferous tubules (yellow arrow). H&E, 10X.



Figure 8: Micrograph of rabbit testis of treated group with Nano ZnO particles showed degeneration and necrosis of Leydig cells (yellow arrow). H&E, 40X.

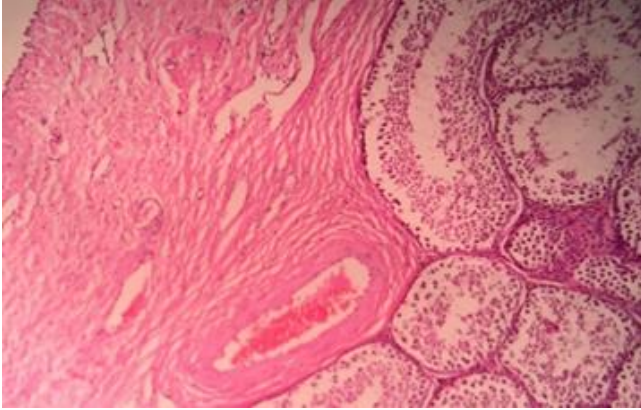


Figure 9: Micrograph of rabbit testis of treated group with Nano ZnO particles showed thickening of tunica albuginea. H&E, 10X.

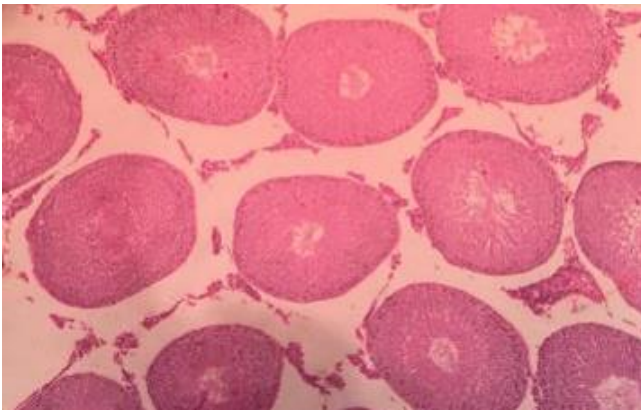


Figure 10: Micrograph of rabbit testis of treated group with Nano ZnO particles and vitamin E showed improvement in the histological appearance of testicular structure. H&E, 10X.

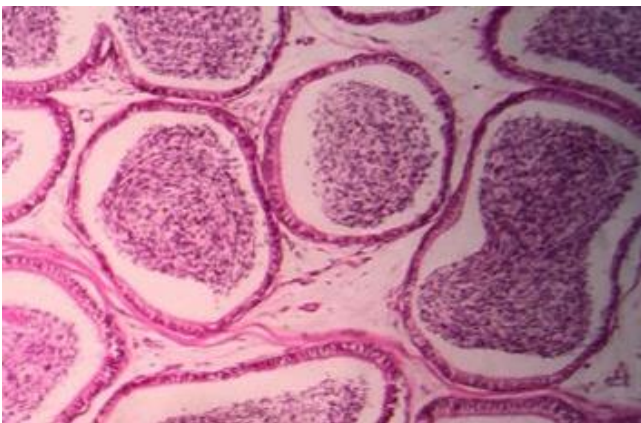


Figure 11: Micrograph of rabbit epididymis of control group showed the normal histological appearance of the epididymis. H&E, 10X.

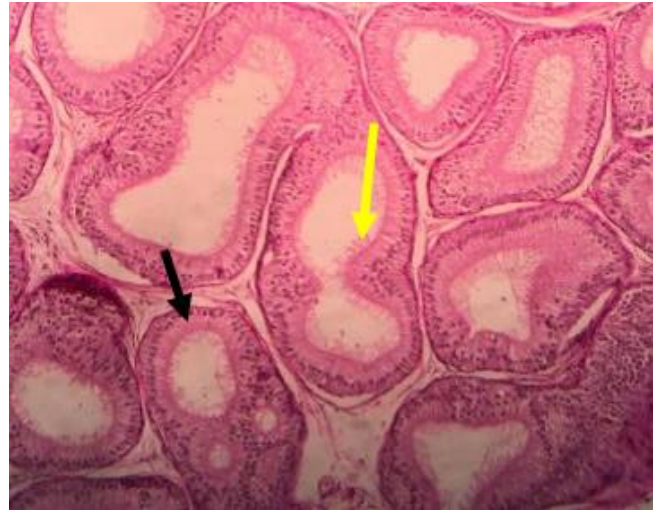


Figure 12: Micrograph of rabbit epididymis treated with Nano ZnO particles showed hyperplasia of epithelial cells (yellow arrow) stenosis in the lumen of (black arrow). H&E, 10X.



Figure 13: Micrograph of rabbit epididymis treated with Nano ZnO particles showed dilatation of epididymal tubules (black arrow) without stereotyped mitotic activity. H&E, 10X.



Figure 14: Micrograph of rabbit epididymis treated with Nano ZnO particles showed vacuolation (yellow arrow) and necrosis of epithelial cells (black arrow). H&E, 40X.

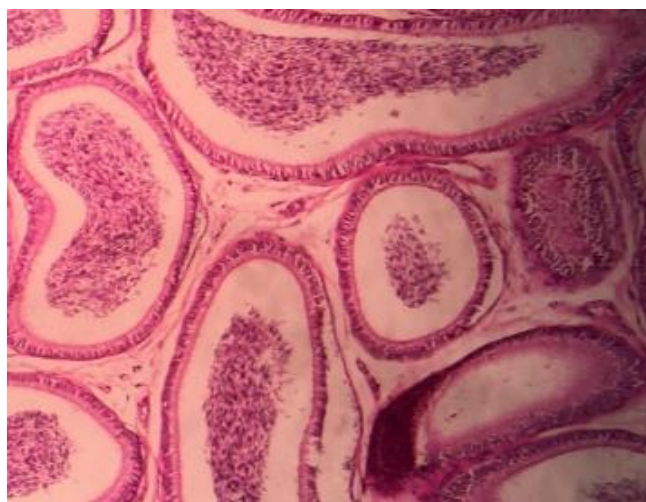


Figure 15: Micrograph of rabbit epididymis treated with Nano ZnO particles and vitamin E showed improvement in the histological appearance of epididymal structure. H&E, 10X.

Discussion

Nano ZnO particles have exclusive physical and chemical properties that enable them to have a severe impact on the vital organs, like the brain, liver, heart, kidney, and testes, due to their ability to pass via the BBB and BTB (11,28). The histological alteration in testis and epididymis might be revealed by the oxidative stress, variation between the production of ROS, and the antioxidant mechanisms' ability to disrupt them (29). Many reactive oxygen mediators led to cell organelles injury, which led to the production of other toxic substances (30).

The free radicals cause the triggering of the nuclear kappa B-factor (NF-KB), a redox-sensitive transcription factor that excites the inflammation gene and produces the chemical mediators of inflammation (31,32). This research showed the light microscopic examination of testis of animals treated with 600mg/kg body weight showed thickening of tunica albuginea. This occurs due to the increase in the number of collagen fibers. This occurs due to the increase in the production of collagen fibers by fibroblast or depletion in the rate of phagocytosis of collagen fibers (33). Also, results seen in this work showed deposition of eosinophilic materials in the interstitial tissue; this was reported by Ibrahim (34). Degenerative and necrotic changes observed in the germ cells lining the seminiferous tubules occur due to oxidative stress induced by nano ZnO particles. Furthermore, there was the arrest of spermatogenesis with giant cells in some lumen of seminiferous tubules, which occurs due to a failure of spermatids to separate. These are agreed with the results of Foster (35), Mozaffari (36), Talebi (37). Also, there are disturbances in spermatogenesis, which discredit the development of stages of spermatogenesis. This occurs due to oxidative stress or damage to Sertoli cells (38).

Oxidative stress induced by nano ZnO particles will affect the epididymal tissue and the secretory action of the epithelium lining the epididymal canal. This led to disturbances in sperm maturation (39). Degeneration of epididymal epithelium may result from toxicant-producing phospholipidosis-mediated degeneration (40). As for the level of Testosterone, this work showed that the treatment with 600mg/kg of nano ZnO particles causes a decrease in the level of Testosterone; this occurs as a result of the formation of superoxide molecules and oxidation of protein, this will cause depletion in the production of testosterone (41).

Nano ZnO particles may affect Leydig cells and suppress testosterone synthesis. Our work showed that co-administration of it. E cause ameliorative effect against nano ZnO particles induced histological changes because vitamin e is the most robust antioxidant. The overproduction of reactive oxygen species by nano ZnO particles will impact fertility and increase the damage to the male reproductive system (42). So, vitamin e can prevent histological alteration through its antioxidant ability (43).

Although nano ZnO particles have been widely used, there has been an increase in the reports on the toxicity of nano ZnO particles. However, the study on the mechanism is in vivo conditions insufficient. Several in vitro studies have demonstrated the toxic effect of nano ZnO particles, such as induction of oxidative stress, autophagic cell death, inflammatory responses, cellular damage, and genotoxicity. Zn⁺² induced damages, the excessive levels of ROS are known to affect membrane potential change, induce lipid peroxidation, affect lipoprotein structure, and cause plasma membrane injuries, leading to cell death (44).

ROS is one of the direct causes of nano ZnO particles mediated cell death in vivo and in vitro models. Largely dissolution and generation of ROS appear to be the most common model of nano ZnO particles which cause cytotoxic effects. Moreover, the shape of nano ZnO particles may have a role in the nano-bio interaction and bioactivity (45).

ROS generation generally plays a role in cellular damage from exposure to nano ZnO particles (46-48). Similar toxic effects were revealed in the testis may result from ROS production (49-51). Vitamin E has a powerful impact on reproductive health, vitamin e plays an essential role in maintaining both male and female fertility. Many studies suggested that vitamin e has a vital role in the testicular activity and production of Testosterone, so the deficiency of vitamin e can cause suppression of testicular activity and production of testosterone (52-54). Vitamin E plays a role in the production of steroid hormones, including Testosterone, so the more consumption of vitamin e gives a more chance to boost testosterone levels.

Conclusions

Based on the results of our work, the treatment with 600 mg/kg B.W. intraperitoneally causes apparent histological alteration in testis and epididymis and impacts the level of Testosterone. This suggests that the widespread application of nano ZnO particles in biomedicine should be assessed with great attention to prevent male infertility in humans and animals because the nano ZnO particles have a cytotoxic impact on the reproductive system of male's rabbits, and the consumption of vitamin e is recommended.

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Conflict of interest

No conflict of interest.

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التأثيرات المحدثة بواسطة جزيئات أكسيد الخارصين على نسيج الجهاز التناسلي لذكور الأرانب

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الخلاصة

الهدف من بحثنا هو تقييم التأثير النسيجي لجزيئات أكسيد الخارصين النانوية على الخصبة والبربخ عند ذكور الأرانب البالغين بعد المعالجة بالحقن داخل غشاء الخلب بجرعة 600 ملغم / كغم من وزن الجسم من جزيئات أكسيد الخارصين النانوية مرتين في الأسبوع لمدة ثلاثة أسابيع وكذلك تقييم التأثيرات المحسنة لفيتامين هـ للحد من تأثير جزيئات أكسيد الخارصين النانوية. تم استخدام أربعة وعشرين ذكر أرنب بالغ في هذا البحث وتم تقسيمهم إلى أربع مجموعات لكل مجموعة ست أرانب. المجموعة الأولى هي مجموعة السيطرة تم حقنها بالماء المقطر

الجرثومية المبطنة للأنابيب المنوية وتوقف تكوين الحيوانات المنوية وتكوين الخلايا العملاقة والتنكس ونخر الخلايا الظهارية المبطنة لقنوات البربخ. لوحظ كذلك خلو القنوات المنوية من الحيوانات المنوية. أما بالنسبة لمجموعة الحيوانات التي تم إعطائها جزيئات أكسيد الخارصين النانوية مع فيتامين هـ فقد أظهرت تحسناً في التغيرات النسيجية مقارنة بالمجموعة التي عولجت بفيتامين هـ فقط حيث أظهرت البنية الطبيعية للخصية والبربخ. علاوة على ذلك، هناك انخفاض في مستوى تركيز هرمون التستوستيرون للحيوانات التي تم إعطائها جزيئات أكسيد الخارصين النانوية مقارنة بالمجموعات الأخرى.

داخل غشاء الخلب، المجموعة الثانية تم حقنها بـ ٦٠٠ ملغم / كغم من وزن الجسم من جزيئات أكسيد الخارصين النانوية داخل غشاء الخلب مرتين في الأسبوع لمدة ثلاثة أسابيع. المجموعة الثالثة تم حقنها بـ ٦٠٠ ملغم / كغم من وزن الجسم من جزيئات أكسيد الخارصين النانوية داخل غشاء الخلب بالإضافة إلى إعطائها جرعة ١٠٠ ملغم / كغم من وزن الجسم من فيتامين هـ عن طريق الفم. بينما تم إعطاء المجموعة الرابعة ١٠٠ ملغم / كغم من وزن الجسم من فيتامين هـ عن طريق الفم. أظهرت النتائج النسيجية أن إعطاء جزيئات أكسيد الخارصين النانوية تسبب تغيرات ملحوظة في الخصية والبربخ. تتميز هذه التغيرات بسماكة الغلالة البيضاء في الخصية والتغيرات التنكسية والنخرية للخلايا