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RESEARCH ARTICLE

Effects of Silica Nanoparticles on Some Indicators of Fertility and Histological Changes in Male Rats

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Abstract

The current study has been designed to evaluate the effect of silica Oxide nanoparticles (SiO2NPs) exposure on the reproductive performance of male rats . Forty adult *norvegicus rattus* (aged 60-70 days with body weight130-140 g), were randomly divided into four equal groups , 10 rats per group : control group were received physiological saline, and three treated groups were administered by gavage at dose (1 mg , 10 mg and 100 mg/kg Body weight) of SiO2NPs suspension daily for 22 days, Body weight , some characters of sperms (sperm count, percentage of motility , abnormality , sperms viability) and histological changes in testis and epididymis were investigated. The results showed insignificant decrease ($p \geq 0.05$) in body weight of three treated groups in comparison with control group. Experimental results obtained from SiO2NPs treated male rats showed significant decrease ($p<0.05$) in sperm count , percentage of motility and viability sperms , while a significant increase ($p<0.05$) in percentage of dead and abnormality sperms compared to control group, histopathological results revealed changes in tissues of testes such as atrophy in some seminiferous tubules with expanded lining . Also noted tubules in both testis and epididymis are empty from sperms with hyperplasia and damage in stereocilia of tubules lining cells of epididymis.

Keywords: *SiO2NPs, Rats, Reproductive, Sperm, Testis, Epididymis.*

Introduction

Nanotechnology is one of the most important of the most effectiveness research, it's applications have been extensively developed in the past two decades [1].Nanoparticles are used in many fields such as medicine, electronics [2] and batteries, agriculture, food industries, cosmetics, in addition to water treatment. [3], so they are wide spread in the environment and the human body and cause toxicity through the skin, nose, mouth and lung or through other ways. As well as nanotechnology is based on the fact that nanoparticles have distinct and unique properties [4].Also the effect of nanomaterials depends on many factor, including level of dose , duration and rout of exposure [5].

Also, nanomaterials possess large surface area due to its small diameter compared with micron-particles therefore are more toxic and more effective than large-size materials [6]. Silica has attracted great importance due to its basic properties, which include it's size ranging from 5-1000 nm, unique optical characteristics, high surface area ,low density ,absorption ability and compatibility with life as well as low toxicity [7], there for, it have wide applications biotechnological applications such as DNA transfection, and biomedical applications as drug delivery and cancer therapy [8] Nanoparticles are able to pass the cell membrane and penetrate the blood barrier in the brain and the blood barrier in the testis [9] , therefore its effect on all organs of the body [10].Other studies have been showed that nanoparticles have the ability to penetrate the testicular -blood barrier and accumulate in testicular tissue after exposure to nanoparticles by intravenously [11], Silica nanoparticles decreased the number of sperm and percentage of sperm motility in male rats [12],also the results of other studies showed to damage of Sertoli cells and spermatogenic cells in male rats testis treated with nanosilica [13]. In addition to causing dysfunctional and oxidative stress, this is led to reproductive toxicity [14].

Materials and Methods

Preparation of SiO2NPs

In this study used SiO2NPs (White nanopowder) were purchased from (Sky Spring Nanomaterials, INC), with diameters of 10-30 nm and purity 99%, Treated with Silane coupling Agent. The different concentrations of silica suspended were prepared by dissolving each of (0.1,1,10 gm) of SiO2NPs with 1 litter of distal water to prepare each of the level doses (1,10,100 mg/kg) respectively .When The silica nanopowder suspended in distal water , sonicated for 5 min , and then mixed by vortex for 1min before using it. Optical properties of silica nanoparticle was measured by spectrophotometer in (Environment Research Unit affiliated to College of Science, University of Al-Qadisiyah)

Experiment Animals

The current study has been conducted at the animal house of science college, University of Al-Qadisiyah. *norvegicus Rattus* were used in this study as atypical sample for mammals .The period of the administration is 22 days and they were bred under suitable laboratory conditions, at temperature between (20 - 25° C) and with average14 hours of light to 10 hours of darkness during the study period. The animals were fed on a standard laboratory food (19% protein and 3000 calories) and water *ad libitum*.

Design Experimental

In this experiment, 40 adult males aged 60 - 70 were used, and their weights ranged from 130 to 140 g. Rats were randomly divided into four groups. Each group consists of 10 rats. The rats of first group control (C) daily received 1ml of normal saline orally for 22 days and rats of three treated groups were administered with silica nanoparticles by orally gavage at levels of doses (1,10,100 mg / kg body weight), respectively, daily for 22 days. A body weight of male rats for each group has been monitored at beginning and the end of the experiment. At the end of the experiment, all rats of each groups were sacrificed by anesthetized by injecting *ip* a mix of 0.3 ml ketamine and 0.1 ml Xylazine per kg of body weight , testes, right epididymis were dissected and kept directly in formalin (10%) for histological studies ,while left epididymis for all male rats were removed to be used for sperm analysis including (sperm concentration ,viability , sperm motility, percentage live and dead sperm).

Sperm Analysis

After the removal of the left epididymis, it's placed in a warm concave watch glass containing 5 ml of normal saline. It was cut by scalpel blade into very small pieces to release the sperm in it to determine the concentration, vitality and motility of sperms.

Estimated the Percentage of Motility, Viability and Dead Sperms

The percentage of motility sperm is calculated using method of [15], the percentage of viability and dead sperms are estimated by using method of [16].

Calculate Sperm Concentration

The haemocytometer for sperm count was used based on the method described in[17].The slide chamber was installed on the optical microscope and covered with cover slip , 10 microliters of diluted sample were taken by a pipette and slowly inserted under the cover slip on both sides of the slide chamber , and then left about 5-10 minutes to settle the sperm on the slide and was counted five squares by used 40x microscope objective, The concentration of sperm was calculated by multiplying the mean of sperm count calculated in the multiplier factor (10^6)

Abnormality of Sperm

The viability, dead sperms and percentage of sperms morphology were determined by using Nigrosin - Eosin stain at 40x microscope objective .the stained sperms were considered as dead ,while those not stained were considered as alive [17]. The same prepared slides were used to count the viability and dead sperms used for calculate the percentage of abnormality sperms and they were examined at 40x microscope objective. The sperm, which were differed from the normal appearance of the sperms, were considered as abnormal sperm [18]. The percentage abnormalities are calculated according to the following equation:

$$\text{Percentage of abnormality sperms} = \frac{\text{Number of abnormality sperms}}{* \text{ Total number of sperms (abnormality and non} - \text{abnormality)}} \times 100$$

Histological Study

Procedure of [19] has been used to Preparation of histological sections of the reproductive organs, which are including (testes and epididymis), the histological sections were staining with hematoxylin - eosin according to method that described by [20].

Statistical Analysis

The results of experiment were analyzed by using completely randomized design depending on Statistical Analysis System [21]. Dun cane multiple range tests are used to estimate the significant differences among means of treatments [22].

Results

Optical Properties of Silica Nanoparticles

UV spectrum of silica nanoparticles observed an absorption maximum at wave length 400 nm (Figure1).

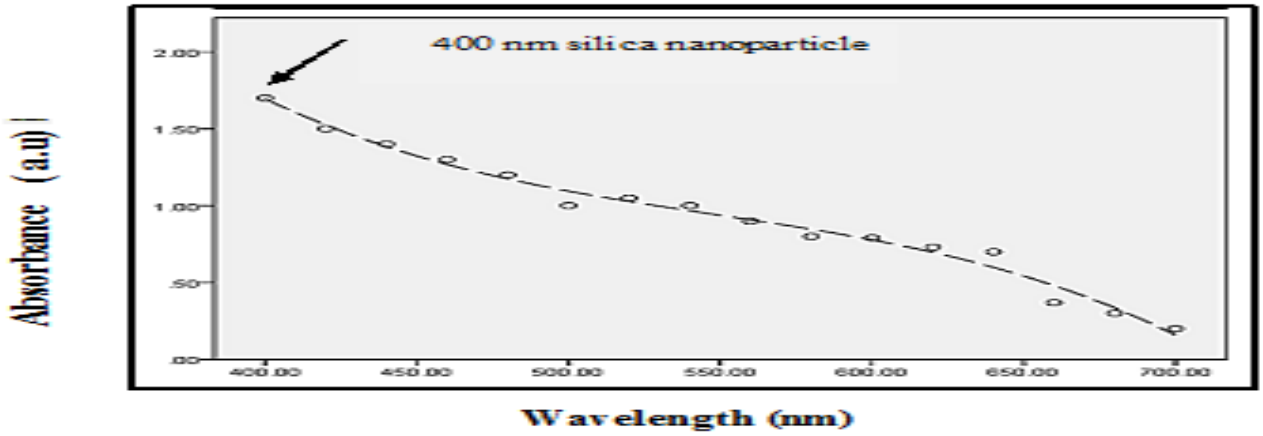

Figure 1: UV spectra of SiO2 NPs showed an absorbance maximum at 400 nm

Body Weight

The results showed there were no significant decreases ($p \geq 0.05$) in gain body weight of the all treated groups with SiO2NPs compared to the control group (Table 1)

Table 1: Effect of SiO2 NPs on body weight (gm) for male rats

groups	Mean of Initial body weight (gm)	Mean of Final body weight (gm)	Mean of Weight gain (gm)
C	135.300 ± 0.989 a	162.200 ± 1.236 a	26.900 ± 0.781 a
T1	134.400 ± 0.806 a	159.400 ± 0.884 a	25.00 ± 0.730 a
T2	133.800 ± 0.940 a	158.600 ± 1.204 a	24.800 ± 0.727 a
T3	133.000 ± 0.615 a	157.200 ± 1.48 a	24.200 ± 0.975 a

***Values represent Mean ±SE**
***similar small letters indicate to there is no significant p≥0.05 among groups**

Characteristics of the Epididymis Sperms

Table (2) that male rats treated with SiO2 NPs suspension revealed significantly decrement ($P<0.05$) in sperm concentration, percentage of motility and viability sperms compared to control group, further significant decrease ($p<0.05$) have been recorded in sperm concentration, percentage of motility and viability sperms of treated group with SiO2NPs at dose (100mg/kg) compared to treated groups with SiO2NPs at dose (1 and10mg/kg) respectively. On the other hand ,the result of percentage of dead and abnormality sperm proved increase significantly ($P<0.05$)in all treated group compare to control group, and this higher was more in treated group with SiO2NPs at dose (100mg/kg) in comparison with treated groups with SiO2NPs at dose (1 and10mg/kg respectively (Table2). The recorded sperm's abnormalities in silica expose group involved loss of sperm tail, loss of sperm head, double head and coiled tail Figure (2).

Table 2: Effect of SiO2NPs on Characteristics of the epididymis sperms of male rats

Groups	Sperms concentrations x10⁶/mm³	Percentage of motility %	Percentage of abnormalities sperms %	Percentage of sperm viability %	Percentage of dead sperm %
C	2.210 ± 0.110 a	73.000 ± 0.471 a	15.000 ± 0.298 a	83.200 ± 0.711 a	16.800±0.711 a
T1	1.920 ± 0.033 b	65.000 ± 0.667 b	24.200 ± 0.389 b	75.000 ± 0.365 b	25.000± 0.365 b b
T2	1.670 ± 0.059 c	59.000 ± 0.471 c	32.000 ± 0.333 c	63.000 ± 0.649 c	37.000± 0.649 c
T3	0.910 ± 0.053 d	55.200 ± 0.467 d	41.400 ± 0.371 d	55.100 ± 0.547 d	44.900± 0.547 d

* Values represent Mean ± SE.
*Similar small letters indicate to there is no significant among groups.
* Different small letters indicate to there is significant P<0.05 among groups.

Histological study

The results of the histological examination showed the presence of histological changes in all studied organs (testis and epididymis) of the treated groups with SiO2NPs compared to the control group. Control male testis and epididymis section shown in Figures (3 ,7) respectively, revealed normal structure, while sections of testis that obtained from treated group with SiO2NPs Figures (4,5,6) demonstrated presence of seminiferous tubules were empty from sperms with expanded lining , degeneration in spermatogenesis and there were a few number of leydig cells in interstitial space of testicular tissue especially in treated group with SiO2NPs at dose (100mg/kg) Figure (6) compare to treated group with SiO2NPs at dose (1and10mg/kg) respectively Representative images of the epididymis tissue slices in Figure (8,9,10) showed to simple degeneration in the lining of epididymal tubule and completely empty from sperms in lumen Figures(9,10) compared to control and first treated group Figures (7,8).Also observed damage in stereocilia and degeneration with Sloughing in lining cells of epididymal tubules, Figure (10).

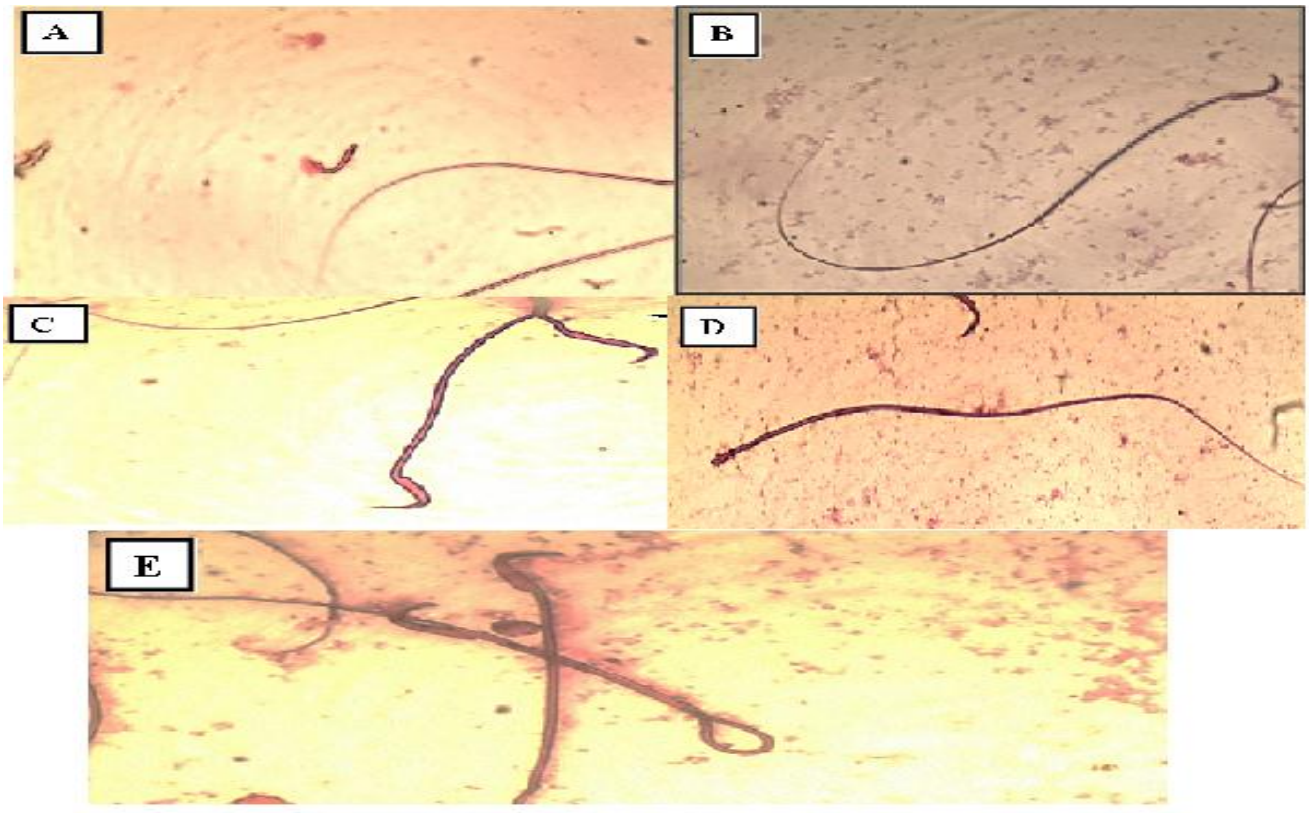

Figure 2: Sperms of silica nanoparticles- exposed rats showing increased incidences of head and tail abnormalities : (B) normal sperm shape consist of head with spine, middle piece, and tail.; (A); Loss of sperm tail (D) Loss of sperm head ; (C) Double head; (E) coiled tail.(100X, Nigrosin- Eosin)

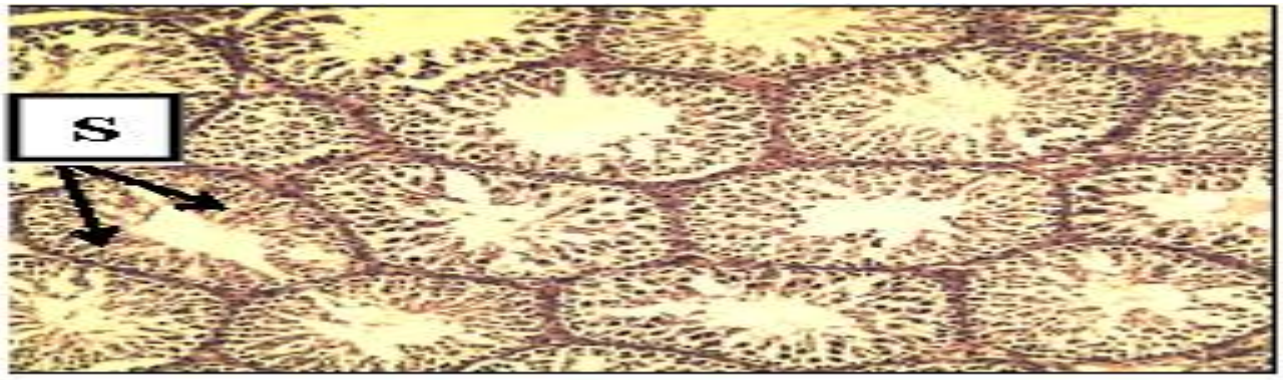

Figure 3: Section of rat testis of control group (C) shows normal tissue and complete spermatogenesis(S). (H&E stain X100)

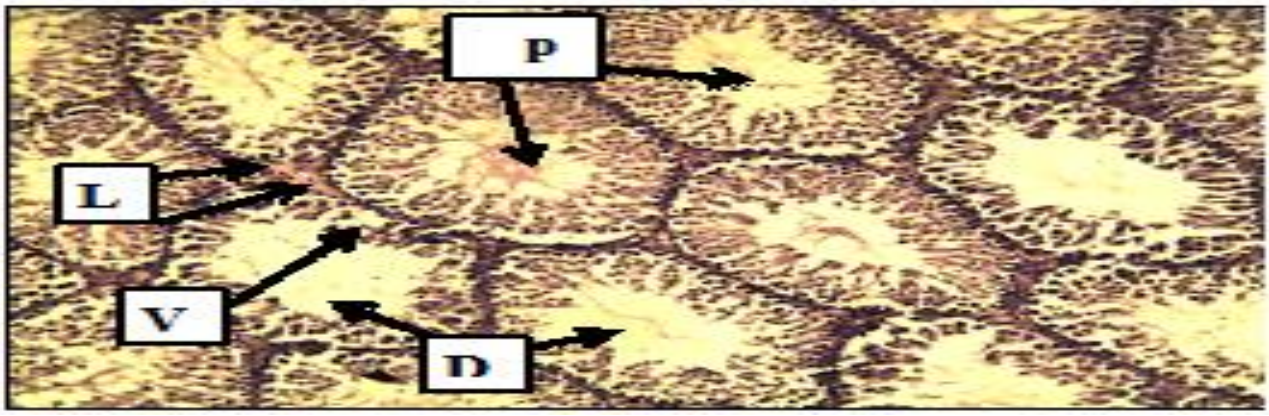

Figure4:Section of rat testis of (T1).shows some tubules contain sperms (P),also there are a simple condensing vacuoles in spermatogonia (V),also there are a few tubules appear with a wide lining and empty from sperms (D), and It's noticed a few number of leydig cells in the interstitial tissue.(L). (H&E stain X100).

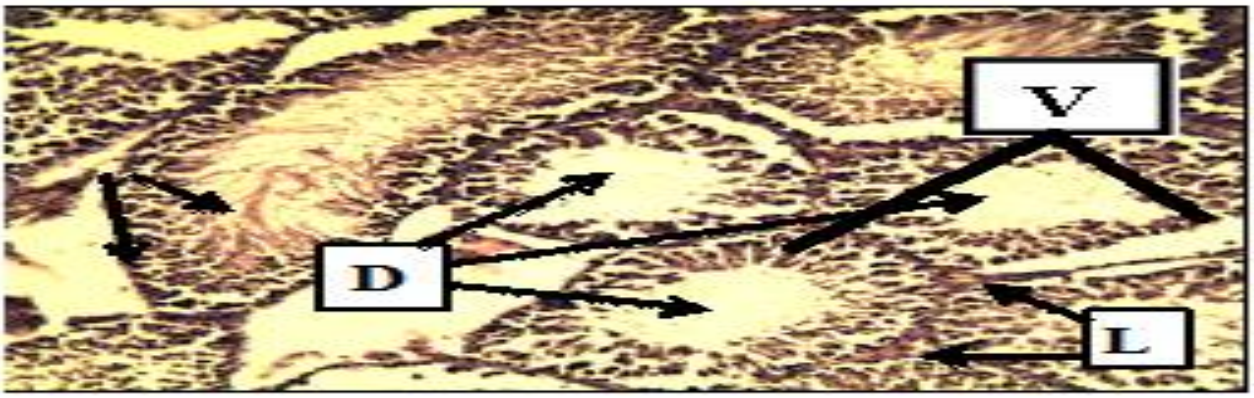

Figure 5: Section of rat testis of (T2).shows a few tubules are empty from sperms and appear with a wide lining (D). also there are a simple condensing vacuoles in spermatogonia (V),also show a few number primary and secondary spermatocytes .and it's noticed a few number of leydig cells in the interstitial tissue (L).(H&E stain X 100)

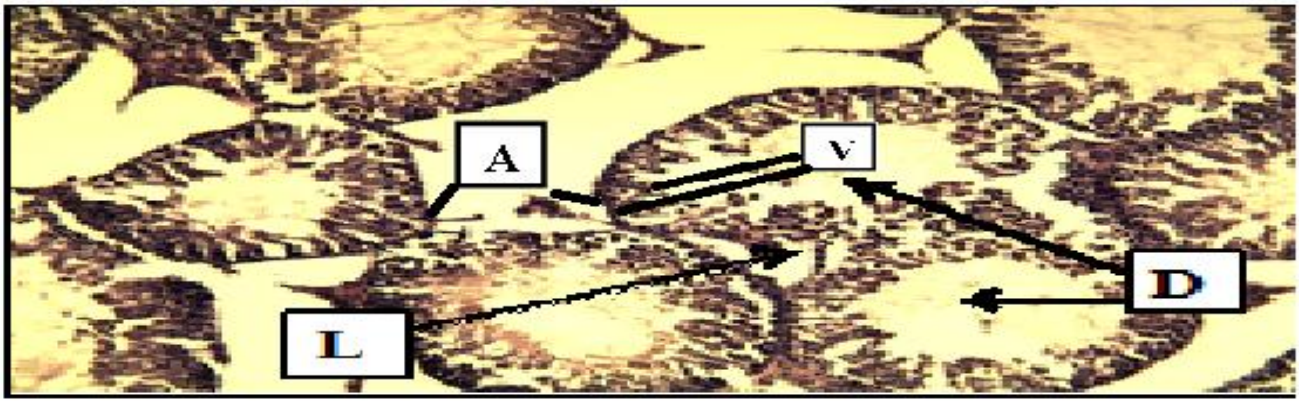

Figure 6: Section of rat testis of (T3).shows an atrophy in some seminiferous tubules (A),It's also noted that these seminiferous tubules are empty from sperms with expanded lining (D),also a clear condensing vacuoles in spermatogonia (V) with a few number of primary and secondary spermatocytes also it's noticed that there are a few number of leydig cells in interstitial space of the testicular tissue (L) .(H&E stain X100).

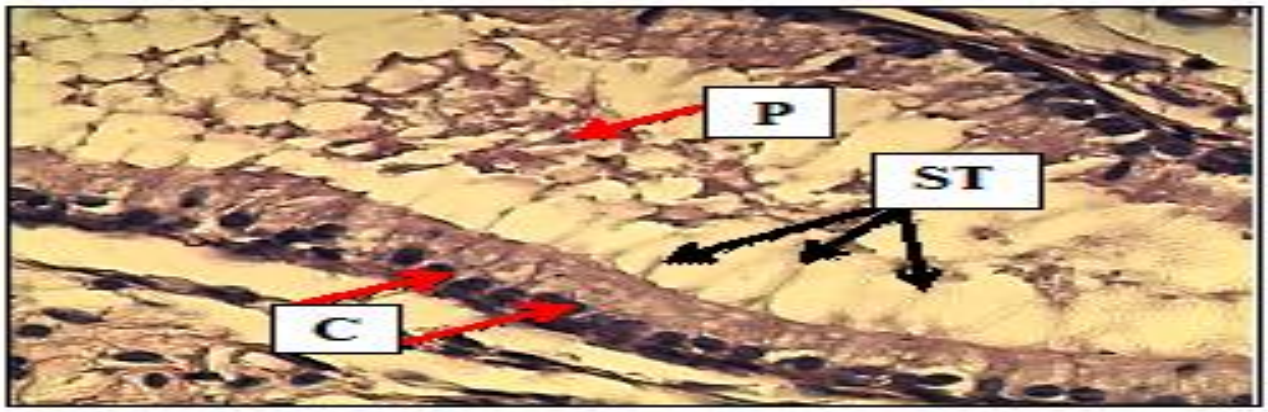

Figure 7: Section of rat epididymis of group control (C) shows expanded epididymal tubules where they appear full of sperms (P) lined with one row of columnar cells (C),with long stereocilia (ST) .(H&E stain X400)

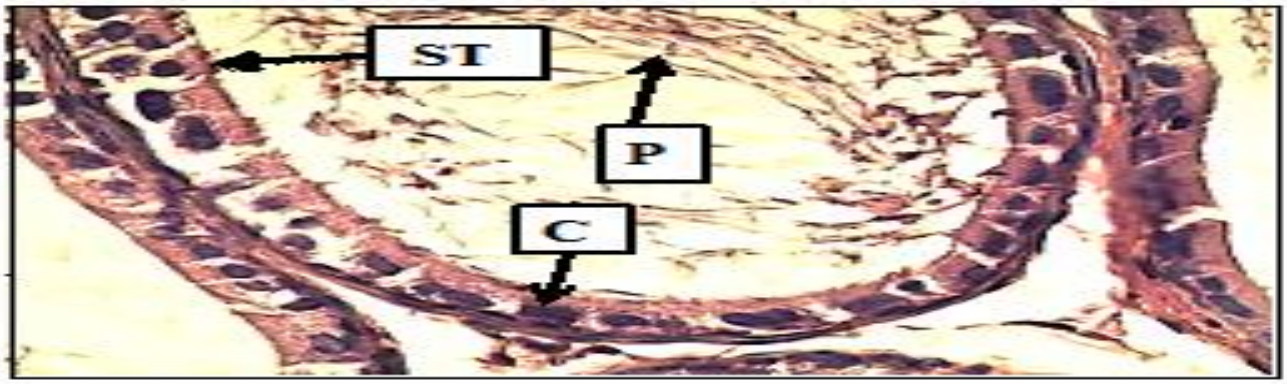

Figure 8: Section of rat epididymis of first treated group (T1) shows epididymal tubules appear full of sperms (P) and lined with one row of columnar epithelium cells (C), with long stereocilia (ST). (H&E stain X400)

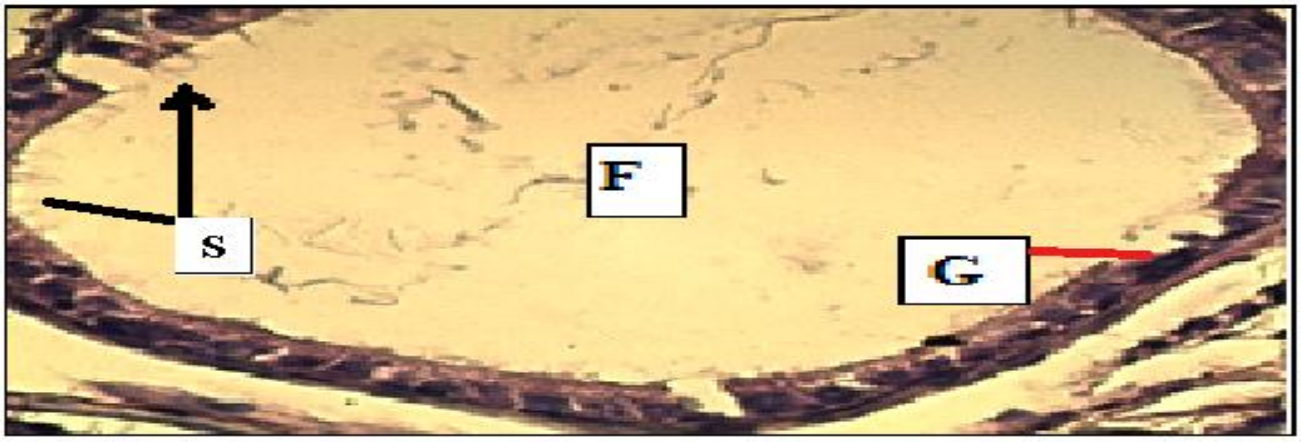

Figure 9:Section of rat epididymis for second treated group (T2).Shows epididymal tubule is a complete empty from sperms(F) also the lining of epididymal tubule where they appear consisting of one layer cells with a slight degeneration (G) ,also there are a few of stereocilia with in epididymis tissue (S). (H&E X400)

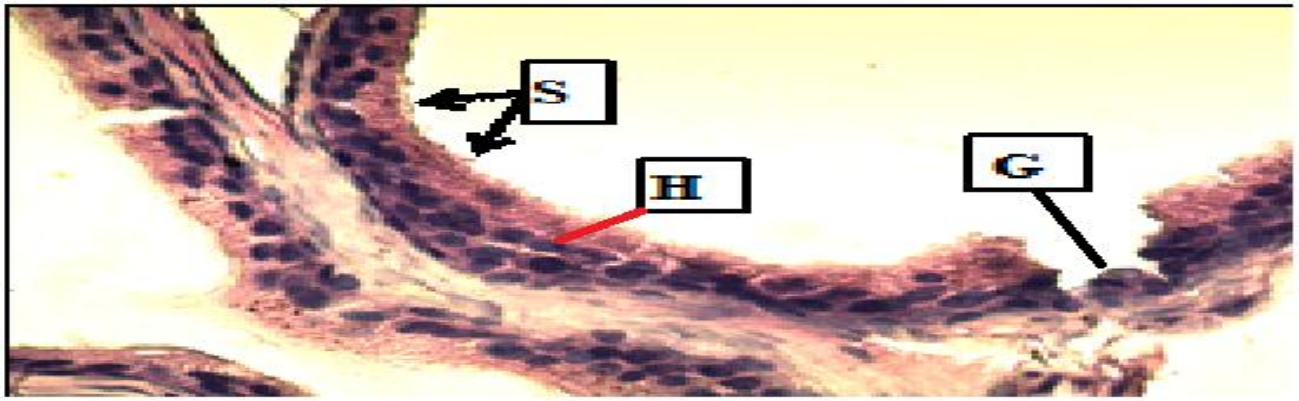

Figure10:Section of rat epididymis of third treated group (T3).Shows epididymal tubules appear empty from sperms also shows a clear hyperplasia of tubules lining cells (H)with damage in stereocilia (S), also a clear degeneration (G) and Sloughing in lining cells of epididymal tubules. (H&E stain X400).

Discussion

Effect of SiO2NPs on Body Weight Gain

Result of male rats body weight gain revealed insignificant differences between treated groups with silica nanoparticles and control group, which may be attributed to the low toxicity of silica indicating that the rats continued to mature. This result was in agreement with other studies [23, 24] who found that exposure to silica and silver nanoparticles do not cause a change in body weight compared to the control group, while the results of the current study do not agreement with [25], who they have been shown significant decrease in body weight when exposure to nanoparticles**.**

Effect of Silica Nanoparticles on Some Semen Parameters

The results of the current study has been revealed that a significant decrease in the sperm concentration, the percentage of viability and motility of sperms. While recorded significant increase in the percentage of abnormality and dead sperms of the treated groups with compared to the control group this may be due to effect of silica nanoparticles on germ cells, which lead to influence in spermatogenesis and imbalance of sperms, these results were similar to that reported by [26], who they found a decrease in sperm concentration and the percentage of viability sperm, as well as an increase in the percentage of abnormality sperms in the testis and epididymis after administrated of male mice with silver nanoparticle.

The effect of silica nanoparticles may be due to cause oxidative stress, resulting in an imbalance between reactive Oxygen Species (ROS) and antioxidants. ROS, also induce the oxidation of vital compounds such as lipid, where membranes of sperms are rich with unsaturated fatty acids which are making them susceptible to oxidative damage, then apoptosis of sperm, and decrease its motility [27]. Excessive increase in oxidative stress and (ROS) are an important mechanism for the toxicity of silica nanoparticles, resulting in damage of sperms membranes [28].This is agreement with that reported by [29] who they observed decrement in quantity and quality of sperms in epididymis of male rats treating with nano-silica.

Effect of Silica Nanoparticles on Histological Changes

Light microscopy investigation of sections of testis and epididymis from treated groups with SiO2NPs demonstrated histopathological change including degeneration in spermatogenesis in seminiferous tubules with expanded lining of testis. Also, degeneration in the lining of epididymis tubule and completely empty from sperms in lumen In contrast, testis and epididymis sections of control group revealed that normal architecture. The results of current study are consistent with [30], who reported that histological changes, including epithelial cell dysfunction, necrosis and atrophy of a number of spermatozoa, Spermatocytes and Spermatids of testicular testis in male rat due to treated it's with nanoparticles . Disorganization in seminiferous tubules may be due to the overlap of nanoparticles directly with spermatogenesis because it has the ability to penetrate the blood –testicular barrier and accumulation in Sertoli cell. Also these results are in agreement with [31], who showed to the change in seminiferous tubules by titanium nanoparticles. Spermatogenesis degeneration in seminiferous tubules of testis of treated groups with SiO2NPs may be due to the low levels of testosterone which is caused by lower number of leydig cells in interstitial space of testicular tissue and then its activity, this is confirmed by the results of the present study (Unpublished), where the levels of genes expression of steroidogenic enzymes including (*Cyp19a1* and *Cyp17a1*) were decrement.

Testosterone hormone, which is a hormone that directly affects on spermatogenesis and any imbalance in the level of this hormone negatively reflected on spermatogenesis [32] This is confirmed by [33] who have been shown to effect of exposed to nickel nanoparticles in male rats lead to decrease in the level of testicular lipids, then testosterone level and this reflects on damage of spermatogenesis and the testis dysfunction. Low number of leydig cells may be due to Oxidative stress triggered by SiNPs and release of (ROS), which causes the death of leydig cells and therefore decline in their number. Furthermore, SiNPs could increase levels of genes expression of caspase and RIPK3/MLKL-dependent proteins which are main proteins Responsible for apoptosis and necroptosis, respectively [34]. Our results are consistent with [35] who have found the decrement number of leydig cells when the

rats were administered with titanium oxide nanoparticle.

Conclusion

In this study, We demonstrated that SiO2NPs causes decrement of quantity and quality of sperms in male rat, in addition to disorganization of testis and epididymis with impairment of spermatogenesis, this suggest that reproductive system consider as target organs to SiO2NPs and dysfunction of reproductive efficiency.

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