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Research Article

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Effects of Silica Nanoparticle Administration on Testis Structure

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Abstract

Objective: This study aimed to investigate the effects of oral and local application of silica-based nanoparticles on spermatogenesis. The study also investigated the potential toxic effects of silica-based local and systemic applied nanoparticles on spermatogenesis, which will be used as a different delivery system in our future studies.

Materials and Methods: Sprague-Dawley male rats were randomly divided into 3 groups: control group, local silica nanoparticle-administered group (L group), and oral silica-administered group (G group). Gavage group rats were administrated with oral silica nanoparticles, and the local group rats were topically administrated with local silica nanoparticles of 2 mg/cm². The testicular samples were prepared for light and electron microscopic analysis at the end of the experiment. The total antioxidant status (TAS) and total oxidant status (TOS) were measured in the blood serum.

Results: Normal testis morphology was observed in the control group. Seminiferous tubules were more disrupted in the G group compared with the L group. Serum TAS levels were increased in the L group compared with the G group. Degenerated testis morphology, increased TOS levels, and decreased occludin immunoreactivity were observed in the G group. These morphological and biochemical parameters were reduced in the L group.

Conclusion: This study showed that oral administration of silica-based nanoparticles increased tissue damage and antioxidant levels. Local administration protected the testis tissue damage compared with oral administration by modulating the oxidant/antioxidant balance.

Keywords Testis · Drug delivery system · Silica nanoprticle · Microscopy · Toxicity

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INTRODUCTION

Nanopharmacology is based on the principle of administering small doses of an agent to reduce systemic exposure and optimize delivery directly to the target site, thereby minimizing adverse effects and maximizing therapeutic effects. Nanoparticles are colloidal structures with different morphologies, such as prism, rod, cube, sphere and needle.[1,2] Nanoparticles have a broad range of applications in physics, chemistry, biology, and medicine. Although nanomaterials have been widely applied, information regarding their toxic effects on human health, especially in reproductive organs, is lacking. Nanotechnology has found a frim place in therapeutic medicine, and ongoing research is evaluating its utility in several diseases.[1,3-7] There are a few small animal studies on the potential use of nanoparticles in urology. It has been reported that nitric oxide (NO)-releasing nanoparticles are beneficial in wound healing, ulcer treatment and angiogenesis.[3,8,9] In a study, Blecher et al. demonstrated the benefit of topical NO-releasing platforms to improve wound healing in a mouse model.[7] The key mediator in penile erections is NO, and NO accumulation is also the main mechanism of action of phosphodiesterase type-5 inhibitors, which leads to smooth muscle relaxation in cavernous penile tissue. In a recent *in vivo* murine study, NO-releasing nanoparticles were found to be effective when used as a penile rehabilitation strategy in a cavernous nerve injury model.[10] In a recent study, Draganski et al. showed that topically applied curcumin-loaded nanoparticles effectively treated erectile dysfunction in a rat model of Type-2 Diabetes.[8] Another study reported that transdermal sildenafil, tadalafil, and nitric oxide-loaded nanoparticles increased the erectile function.[8] These studies clearly highlight the potential use of nanoparticles in urology.

NO-releasing nanoparticles are mediators in penile erections.[10] NO in the physiologic level improves sperm motility, but excessive NO degenerates sperm activity and its could be decrease fertility and also excessive NO exacerbates the oxidative stress.[11] In drug delivery, silica nanoparticles are used as carriers because they show good stability and biocompatibility and require simple modifications. However, their potential toxic effects remain unclear. Therefore, further studies are needed to investigate the toxicity and reliability of nanoparticles, given that their clinical use is likely to increase in the future development and testing of new release systems. In this study, the oxidant/antioxidant balance using total antioxidant status (TAS) and total oxidant status (TOS) parameters were also investigated. TAS and TOS levels play a role in testis tissue degeneration.

Silica nanoparticles are used as a drug delivery system. Silica nanoparticles negatively affect sperm motility, degenerate sperm morphology, and cause apoptosis in the testis tissue of the rats[12], although low concentrations of nanoparticle-rich diesel exhaust increase plasma testosterone levels.[13] Oxidative stress negatively affects male fertility. Therefore, it is related to the testis tissue degeneration mechanism. Several studies showed that the relation between the silica nanoparticles and the oxidative stress.[14,15] Spermatogenesis process is approximately takes 35 days in rats. Silica nanoparticles could affect this process. The potential toxic effects of nanoparticles by intravenous administration on spermatogenesis was examined.[16,17] Oral administration (1 mg/kg) of nanoparticles with different sizes was also investigated, and the results showed that significant body weight change was not seen but nanoparticle accumulation was detected in the testis.[12] So, these results showed that nanoparticles could cross the blood-testis barrier (BTB). BTB integrity deformation causes decreased fertility.[15] In the regulation of BTB, occludin, a protein located in the tight junction barrier, plays an important role.

This study aimed to investigate the putative adverse effects of oral and local administration of silica-based nanoparticles on testis morphology in an *in vivo* experimental rat model. These effects were analysed using histopathologic, immunohistochemical, electron microscopy, and biochemical methods.

MATERIALS AND METHODS

Animals

A total of 24 male Sprague-Dawley rats (6–8 weeks old and weighing 250–300 g) were obtained from the Acibadem University Laboratory of Animal Application and Research Centre. Male Sprague-Dawley rats (n=8 in each group) were randomly divided into three groups: control group (C), local silica nanoparticle administration group (L group), and oral silica nanoparticle administration group (G group). The dosage was chosen according to the previous experimental studies.[15,18-21] Silica nanoparticles were administered every 3 days for 35 days at doses of 20 mg/kg in the oral administration group,[15] and topically to the penis at 2 mg/cm^2 in the local administration group.[22] Dermal application of nanoparticles in the previous experimental study showed that titanium dioxide nanoparticles (2 mg/cm^2) were effective,[23] and the toxicity was low in dermal exposure. The experimental protocol of this study was approved by the Animal Ethics Committee of Acıbadem University (Approval number: 2015/11).

Nanoparticles Preparation

Silica nanoparticles, 80 nm in diameter (NanoXactTM Silica, NanoComposix, Inc.) were prepared as a 10 mg/dL solution in distilled water. To prevent the aggregation of the nanoparticles, the solution was first sonicated for 10 min. For oral administration, the required volume of nanoparticle solution at dose of 20 mg/kg was prepared using the stock nanoparticle solution (10 mg/mL) and sonicated again for 10 min. On the other hand, for the local administration group, a nanoparticle solution of 4 mg/mL (4:6, distilled water: coconut oil, v:v) was prepared and then sonicated for 10 min.[22] In both the G and L groups, the nanoparticle solution was administered every 3 days for 35 days as 20 mg/kg dose in the oral administration group via the gavage method and 2 mg/cm² topically to the penis in the local administration group.

Experimental Design

At the end of 35 days, the rats were sacrificed after anaesthesia (100 mg/kg ketamine and 5 mg/kg xylazine were given intraperitoneally). Blood samples were obtained by heart puncture, and the serum was used for biochemical analyses. The testis tissue was removed through a bilateral orchidectomy, and the abdominal fascia was closed using 3/0 PDS with a skin stapler. Tissue samples were prepared for histopathological and ultrastructural evaluations.

Histological Analysis of Preparatins and Scoring

Testis tissue samples were fixed in 10% buffered neutral formalin. The samples were then dehydrated with alcohols, and the following processes, including clearing and paraffin incubation, were performed using an automated tissue processor (Thermo Citadel 2000). Paraffin sections (approx. 5 µm thick) were stained with haematoxylin and eosin (H&E) and Periodic acid Schiff (PAS) for histopathological evaluation. H&E staining was applied to the sections for histological evaluation. PAS was used to show alterations in the basement membrane of the seminiferous tubules. Occludin immunohistochemistry was used to evaluate BTB integrity. The H&E-stained sections of all groups were analysed by a blinded observer, and images taken from five similar areas were scored. The histopathological scoring was evaluated by modifying Hess's data and categorised as normal, regressive, degenerative, or atrophic seminiferous tubules.[24] All tissue sections were examined and photographed using a Zeiss AxioCam MRc5 camera with a photomicroscope.

Occludin Immunohistochemistry

Occludin immunohistochemistry was performed on tissue sections to determine occludin localisation in testicular tissue. Tissue sections (5 µm-thick) were fixed with 4% paraformaldehyde and deparaffinized in xylene for 40 min. The samples were then rehydrated through a graded alcohol series (100%, 96%, and 70%). Subsequently, the sections were heated in a microwave oven at 100 W for 20 min in citrate buffer solution (pH 6, 1X). After allowing the sections to cool for 20 min at room temperature, they were washed thrice for 5 min each in phosphate-buffered saline (PBS) solution. Samples were incubated in 5% goat serum for 1 h for blocking. Rabbit anti-occludin antibody (Abcam, USA) was diluted 1:200 in blocking solution. All sections were incubated overnight at +4°C, followed by three washes in PBS for 5 min each. Subsequently, a 1:500 diluted fluorescently conjugated goat anti-rabbit secondary antibody (DyLight™ 488, Invitrogen, USA) was applied to the sections and incubated for 1 h at room temperature. After washing all sections three times in PBS for 5 min each, the coverslips were placed in mounting medium containing 4'6-Diamidino-2-phenylindole (DAPI) (Abcam, Cambridge,UK). Sections were examined and photographed using a fluorescence microscope (Zeiss Axio Scope, Germany).

Transmission Electron Microscopy (TEM)

The testicular tissue samples were fixed with 2.5% glutaraldehyde in PBS (0.1 M, pH 7.2), processed for routine electron microscopic analysis, and embedded in Epon 812 (Fluka, Sigma–Aldrich Chemical, Steinheim, Switzerland). Ultrathin sections (approximately 600 Å) were contrasted with 2%-uranyl acetate and lead citrate. The ultrathin sections were analysed using TEM (JEM-1011, Jeol, Tokyo, Japan) and photographed using the Olympus Soft Imaging camera system (Olympus, Tokyo, Japan).

Measurement of Antioxidant Status

Serum TAS and TOS levels were evaluated using a kit developed by Erel (RelAssay Diagnostics kit). TAS levels were measured using a method based on the disappearance and discolouration of the characteristic colour of the dark blue-green 2,2'-azino-bis (3-ethylbenz-thiazoline 6-sulfonic acid) cation. The overall antioxidant level of the sample was correlated with the change in absorbance at 660 nm. The assay was calibrated using a stable antioxidant standard solution, commonly referred to as Trolox equivalent, which is an analog of vitamin E. The TAS results were expressed in mM/L.

Measurement of Total Oxidant Status

Total oxidant status (TOS) of serum samples was determined using an automated colorimetric method developed by Erel (Rel Assay Diagnostics, Türkiye). This method is based on the oxidation of a ferric ion-chelator complex to ferric ion by oxidant species present in the sample. The oxidation process is enhanced by specific molecules abundantly present in the reaction medium. In an acidic medium, the resulting ferric ions form a colored complex with a chromogenic substrate. The intensity of the color, which is directly related to the total oxidant concentration, was measured spectrophotometrically at 530 nm. The test was calibrated using hydrogen peroxide, and results were expressed as micromolar hydrogen peroxide (H_2O_2) equivalents per liter (μmol H_2O_2 equivalent/L).

Statistical Analysis

All data were analysed using one-way or two-way analysis of variance. Post-hoc tests were performed using Tukey's test for multiple comparisons, and the significance of differences was assumed at the level of $p<0.05$. The Instant Statistical Analysis Package (Prism 6.0 GraphPad Software, San Diego, CA, USA) was used for all calculations.

RESULTS

Histopathological Changes

Histochemical examination of the C group revealed normal testicular tissue morphology. The lumen of the seminiferous tubules contained abundant spermatozoa (Figure 1). Seminiferous tubules were extensively damaged with a prominent cellular loss in H&E-stained sections of the G group. Cell-to-cell junctions were dilated and luminal cell deposits were observed in the seminiferous tubules. The basal membranes of seminiferous tubules showed decreased PAS positivity with nodulated contours (Figure 1). The number of regressive, degenerative, and atrophic tubules was increased in the G group, whereas the number of normal tubules was significantly decreased (Figure 2) compared with the C group ($p<0.05$). Microscopic examinations of the L group revealed less tissue damage in the testis structure compared with the G group. Cell-to-cell junctions on the seminiferous tubular wall demonstrated a limited number of vacuoles. Abundant spermatozoa in the luminal compartments indicated normal spermatogenesis cycles. Basal membranes of seminiferous tubules with regular contours showed high PAS positivity (Figure 1). Histopathological staining of the L group revealed more normal tubules and decreased number of regressive tubules compared with the G group.

Occludin Immunohistochemistry Findings

High occludin immunoreactivity (occludin-IR) was observed at the basolateral site of the Sertoli cells in the C group (Figure 3). Occludin immunostaining intensity at basolateral sites of Sertoli cells was decreased in the G group compared with the C group (Figure 3 and Table 1). Occludin immunoreactivity was slightly decreased in the L group compared with the C group (Figure 3 and Table 1).

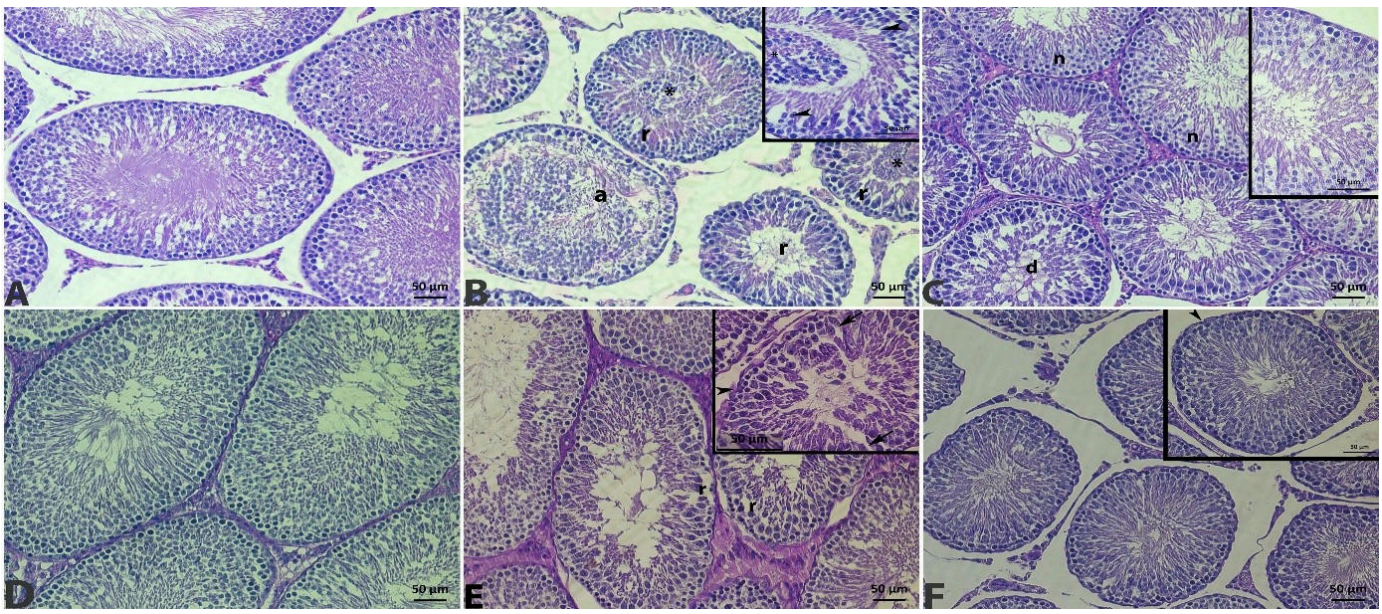


Figure 1. Photomicrographs of H&E-stained (upper) and PAS-stained (lower) testis tissue in the experimental groups. Normal testis morphology in the control group (**A** and **D**); some regressive (r) and atrophic (a) seminiferous tubules with irregular cell borders (arrowhead) and *: cellular debris in tubule lumens in the gavage group (**B** and **E**); normal seminiferous tubules (n) and some degenerated (d) tubules in the local group. (**C** and **F**). Magnifications, A-F : 20x, insets:40x.

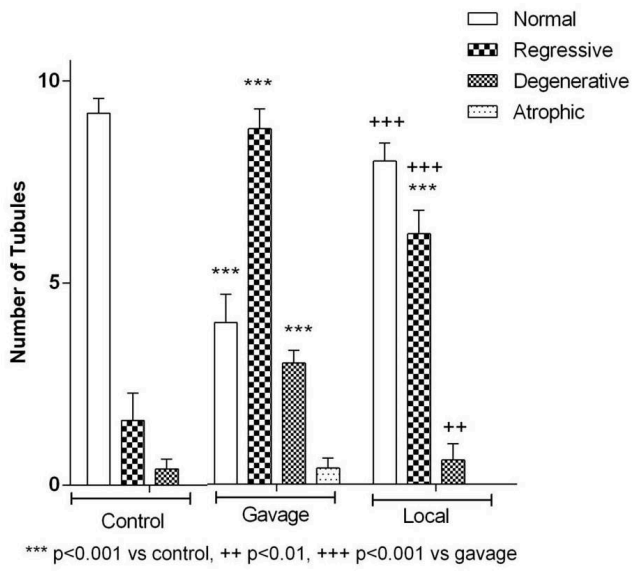


Figure 2. The number of normal, regressive, degenerated, and atrophic seminiferous tubules. ***p<0.001 vs control, **p<0.01 and ***p<0.001 vs gavage group.

Table 1. Intensity of occludin immunoreactivity in experimental groups' seminiferous tubules

	Control	Gavage	Local
Occludin-IR	+++	+	++

+: very low, ++: moderate, +++: intense

Electron Microscopy Findings

The C group presented normal testis tissue ultrastructure with a regular germinal epithelium and basal lamina (Figure 4A). In G group, a moderate number of seminiferous tubules displayed vacuolizations and separations of intercellular areas between adjacent Sertoli cells. Additionally, degenerated spermatogenic cells and Sertoli cells were observed on the seminiferous tubule wall of G group rats. Additionally, the basal lamina surrounding the seminiferous tubules exhibited a mostly thickened lamellar appearance (Figure 4B). Sections from seminiferous tubules of L group rats revealed scanty dilated vacuolar areas in the intercellular areas between germinal epithelial cells and Sertoli cells. With limited degenerative findings, spermatogenic and Sertoli cells mostly reflected a normal ultrastructure in L group. A number of nanoparticles were partially detected in the cytoplasm of Sertoli cells. Local degeneration in the intracellular region of epithelial cells was observed in the L group (Figure 4C). TEM analyses also showed that BTB integrity was disrupted more in the G (Figure 5A) group than in the L (Figure 5B) group. Nanoparticles, approximately 80 nm in size as determined by TEM (Figure 5B, inset), were observed in both G and L groups (Figure 5).

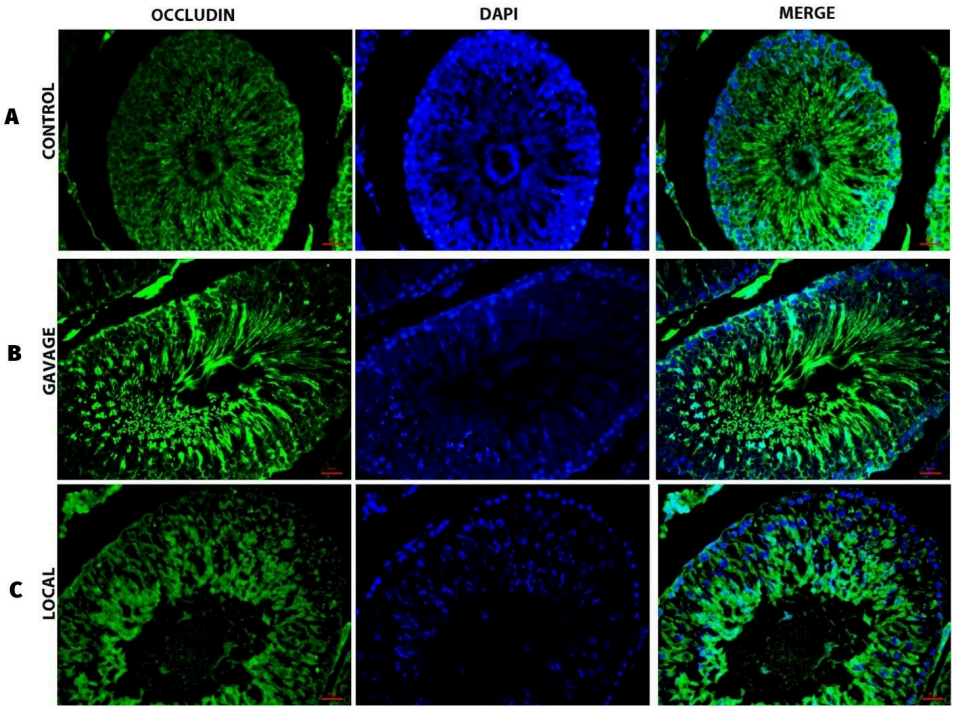


Figure 3. Photomicrographs of testis tissue immunostained with occludin in the experimental groups. Occludin immunoreactivity (green) in the control group **(A)**. Occludin IR was lower in the gavage group **(B)** than in the local group **(C)**. Green: Occludin positivity, Blue: Nuclear staining with DAPI. Scale bar: 50 µm.

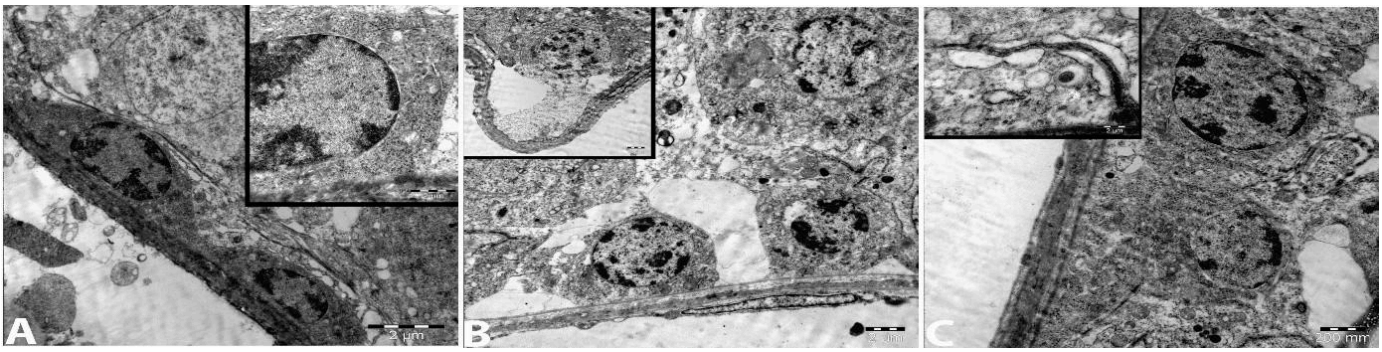


Figure 4. Electron micrographs of the testis tissue in the experimental groups. Regular ultrastructure of spermatogenic and Sertoli cells in the control group **(A)**. Vacuoles in the cytoplasm and detached regions between the germinal epithelium and Sertoli cells were observed in the gavage group **(B)**. The normal morphology of the basal lamina and Sertoli- Sertoli cell junctions were observed in the local group **(C).** Scale bars: A, B, and their insets =2 µm; C=200 µm; inset in C=2 µm, electron micrographs contrasted with uranyl-acetate-lead citrate.

Anti-Oxidant Levels

Serum TAS levels in G (1.01±0.28 mmol/L) and L groups (1.44±0.31 mmol/L) were significantly decreased (Figure 6A) compared with the C group (2.49±0.44 mmol/L) ($p<0.001$). Parallel to these results, the serum TOS levels in both G (7.13±1.56 µmol/L) and L groups (5.45±0.82 µmol/L) were significantly increased (Figure 6B) compared with the C group (4.60±0.43 µmol/L) ($p<0.0001$ and $p<0.05$, respectively). On the other hand, this increase was higher in the G group, and there was a statistically significant difference between them compared with the L group ($p<0.05$).

DISCUSSION

Silica nanoparticles are known to provide potential advantages in drug delivery systems, gene therapy and molecular imaging.[1,4-6] While they offer advantages in the modification of surface reactivities, their potential toxic effects on human health are a matter of concern. The use of silica nanoparticles in drug delivery systems is preferred due to their ease of modification, good stability, and biological compatibility.[8] The present study aimed to identify the optimal method of administering silica nanoparticles, either oral or local application, to minimise any effects on testis tissue ultrastructure and spermatogenesis.

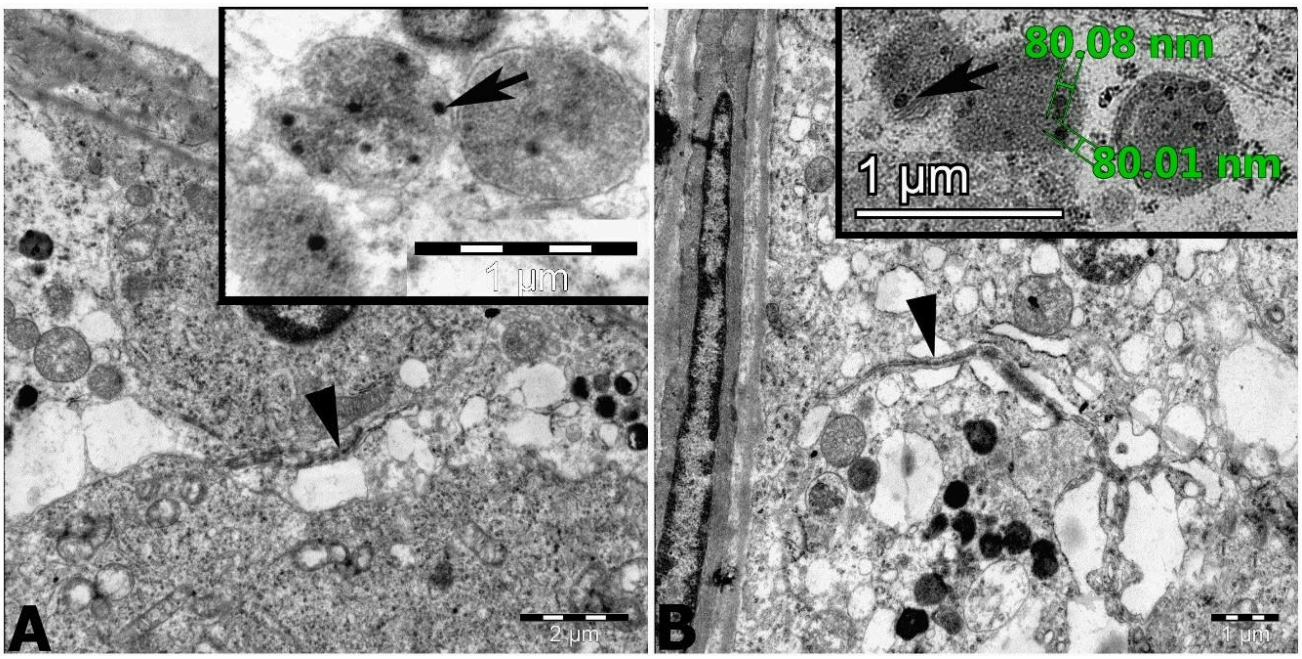


Figure 5. Electron micrographs of testis tissue in the gavage **(A)** and local **(B)** groups. Accumulation of silica nanoparticles (arrow) and blood-testis barrier (arrowhead) in testis tissue. Nanoparticle size (80 nm) can be recognise in lysosomes of spermatogenic cells. Scale bars: A=2 µm; inset A=1 µm, B=1 µm , inset B=1 µm, electron micrographs contrasted with uranyl-acetate-lead citrate.

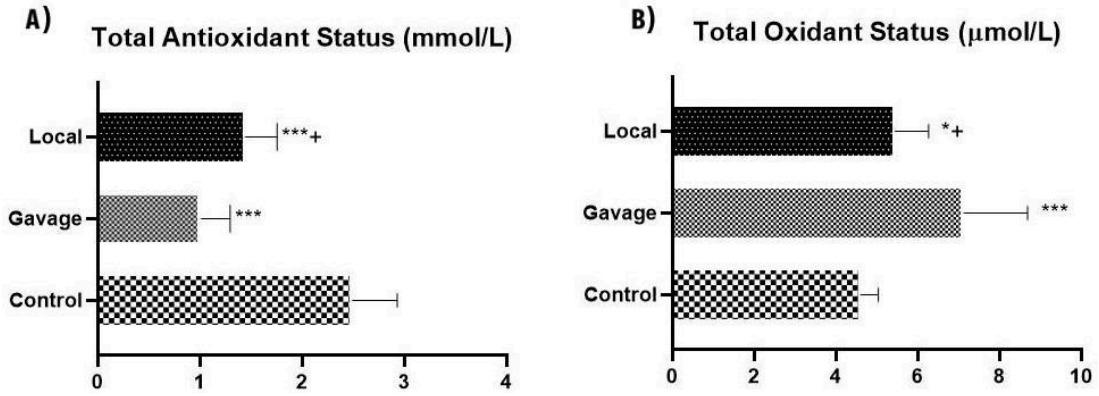


Figure 6. Total antioxidant (TAS) (**A**) and total oxidant (TOS) (**B**) levels in the experimental groups. *p<0.05 and ***p<0.001 compared to the control group, p⁺<0.05 compared to gavage group.

The toxic effect of silica nanoparticles on the reproductive system has been shown to result in DNA damage within fibroblasts and cause differentiation in embryogenic stem cells.[8,9] In another study, nanoparticles were found to affect viability, proliferation, and gene expression upon being taken up by Leydig cells.[24] It is known that the toxic effects tend to increase with decreasing diameter of the silica nanoparticles. Damage has been identified with those measuring 10–30 nm in size, whereas no damage was noted with those measuring 80–400 nm.[2] Based on the prior data, we used the nanoparticles of 80 nm diameter. Thakur et al. emphasised the importance of the size (surface/mass ratio) as the most important parameter in the toxity of nanoparticles.[12] Previous studies have shown that nanoparticles could impair the male reproductive system and that silica nanoparticles could damage the germinal epithelium of the seminiferous tubules. Short-term exposure to nanoparticles may not affects reproduction.[25] So, when using nanoparticles as a drug delivery agent, the dosage, concentration, and duration of the experiment are important. Nanoparticles could pass through BTB and accumulate in the testis after exposure.[26] They indicated in that study, 20 mg/kg nanoparticles had deleterious effects on the testicular structure of rats, which increases progressively with time.[10] In the present study, we confirmed that the oral administration of nanoparticles had a negative impact on spermatogenesis. We showed that local administration of nanoparticles attenuated this adverse effect. The 2-week period required for the rat spermatogenetic cycle suggests that spermatogenesis is appropriate for an investigation of toxic effects.[10] It was shown that silica nanoparticles decreased the number and motility of sperm, caused sperm malformation, and increased the apoptotic index in rat spermatogenetic cells. Li et al. detected a decrease in the weight of rat testis following exposure to nanoparticles, with the additional finding of reduced serum levels of testosterone due to nanoparticles,[27] while Ramdhan et al. found that a low dose of nanoparticles increased serum levels of testosterone.[13] These conflicting findings in the literature may be due to the differences in nanoparticle size and modifications.

Xu et al. demonstrated that silica nanoparticles first affect the maturation phase of sperm in the epididymis and cause oxidative stress and mitochondrial damage, leading to energy metabolism dysfunction.[15] The hypothesis was that damage to sperm maturation would primarily occur in the epididymis and be reversible, based on the observation that testicular damage peaks between 15 and 35 days after administration, with recovery beginning around day 60. The authors did not specify whether damage increased with repeated doses.[13] Oxidative stress plays role in the deterioration of the antioxidant defence system.[28] The total oxidant and antioxidant measurements are useful methods to evaluate the complicated oxidative mechanisms.[28] In this study, we determined the total antioxidant and oxidant effects of silica nanoparticles on the testis tissue. We revealed that the local administration of silica nanoparticles has a minor degenerative effect compared with oral administration. Oxidative stress is considered a triggering event for reproductive toxicity. The development of oxidative stress implies an imbalance between oxidative and antioxidative systems.[26] Oxidative stress plays an important role in male infertility.[29] Oxidative stress in the male reproductive system negatively affects sperm concentration, motility, and morphology, damaging sperm and affecting semen parameters, causing infertility.[30] Consistent with the literature, we observed cellular membrane damage and degenerative organelles in germinal epithelial cells in both the L and G groups, with the most prominent changes seen in the G group.

Local drug delivery maximises efficacy of drug at the application site and limits systemic side effects.[3,17] In Tar et al., NO-releasing nanoparticles were administered

locally onto the penis of Sprague-Dawley rats.[22] Although this application method has contributed to erections, the potential toxic effects of the administered nanoparticles were not tested.[22] Occludin, located in the tight junction barrier, plays an important role in the regulation of BTB integrity. Impaired BTB integrity could be caused by nanoparticle penetration into the seminiferous tubules.[31] Occludin shows high immunoreactivity in normal seminiferous epithelium.[32] When occludin immunoreactivity decreases, BTB integrity would probably degenerate. As a parallel finding, a decreased intensity of occludin immunoreactivity at basolateral sites of Sertoli cells was revealed in both the gavage and local groups compared with the C group. Most prominently in the gavage group, the disrupted ultrastructure of BTB was correlated with the decreased intensity of occludin immunoreactivity. Exposure to silica nanoparticles in male mice led to significant structural damage in the testis and epididymis, reduced sperm quality, and increased rates of sperm abnormalities, which were linked to oxidative stress, inflammation, and apoptosis within testicular tissue.[33] Silica nanoparticles also impaired the BTB by downregulating integral membrane proteins, increasing barrier permeability, and promoting inflammatory responses, partly through the activation of the p38 MAPK pathway.[34] Although these studies have limited direct evidence for specific changes in occludin, ZO-1, and claudin at the BTB, the observed barrier dysfunction and increased testicular permeability to toxins and inflammatory mediators strongly suggest that silica nanoparticles compromise tight junction integrity. In the present study, it was revealed that oral administration of silica nanoparticles caused significant ultrastructural damage to testicular tissue, including spermatogonia and spermatocyte degeneration, nuclear pyknosis, and disorganisation or reduction of germ cell layers within the seminiferous epithelium. Sertoli cells exhibited swollen mitochondria, vacuolisation, and loss of tight junctions with neighbouring cells after exposure to silica nanoparticles. Additionally, basement membrane thickening or irregularity in seminiferous tubules might be due to silica nanoparticles. These structural changes are closely linked to oxidative stress, as silica nanoparticles increase the generation of reactive oxygen species (ROS), leading to DNA damage, lipid peroxidation, and apoptotic pathway activation in testicular cells.[35] The resulting oxidative imbalance and inflammation further intensify cell death and tissue disruption, ultimately impairing spermatogenesis and reducing sperm quality and quantity.[36] Mechanistically, silica nanoparticles can induce hypermethylation of key regulatory genes, disrupt cell cycle progression, and activate pro-apoptotic signalling pathways, all of which contribute to testicular dysfunction.[36] Some studies have also reported that these effects may be partially reversible after cessation of exposure, but the potential for long-term reproductive harm remains a concern, especially with chronic or high-dose exposure to silica nanoparticles.[33,35,36] The present study revealed that local application instead of systemic administration can overcome the negative effects of silica nanoparticles. Light microscopy examinations of the L group revealed less tissue damage compared to the G group. Numerous spermatozoa, indicating normal spermatogenesis cycles, were observed in the seminiferous tubular luminal compartments (Figure 1). High PAS positivity of the basal membranes of seminiferous tubules with regular contours was observed in the L group (Figure 1F). Histopathological scoring of the L group revealed more normal tubules and a decreased number of regressive tubules when compared with the G group (Figure 2). These microscopical data were quite parallel to the biochemical data, including the oxidant and antioxidant levels in both groups. This study suggest that local administration instead of systemic administration can overcome the negative effects of nanoparticles. Based on both light and TEM examinations, the present study revealed that the local administration of silica nanoparticles affected the seminiferous tubular epithelium and Leydig cells less than the gavage administration.

CONCLUSION

Nanopharmacology relies on the strategy of giving small quantities of a drug to decrease whole-body exposure and optimize its delivery to target sites, thereby lowering harmful effects and increasing treatment efficiency. Any unwanted systemic side effects are discarded by topical application of the drug. The ability of nanoparticles to pass through the skin barrier offers potential advantages in terms of drug delivery. The use of nanotechnologies for local treatment is widely accepted as promising, although the adoption of such advantages in clinical practice has been slow, despite much ongoing research. Technological developments will advance future studies and help clarify the unresolved questions regarding nanoparticles. As the type, size, chemical content, surface characteristics, concentration, exposure time and dose of the nanoparticles, as well as the study design, used in nanoparticle researches are different, the results might be different.

The main goal of this study was to evaluate the effect of nanoparticle administration method, oral or local application, on the testis tissue. The elimination of silica nanoparticles from the liver and kidneys is reduced when administered orally, depending on the dose and particle size, aside from through spermatogenesis in the testicles. One of the

limitations of the present study is its failure to include the effects on other organs, which has been mentioned in several previous studies.

The toxicity of applied silica nanoparticles on the male reproductive system is low using both types of application, with lesser structural and spermatogenesis effects using local application. Although the toxicity of silica nanoparticles on testis tissue is known, experiments generally focus on the oral administration or vein injection of nanoparticles. Because of the suspicions of dermal penetration, local administration studies are few. In this study, we observed the accumulation of silica nanoparticles in the testis indicating their penetration across the skin into testis tissue via local application. Additionally, silica nanoparticles were passed into the BTB. The testis tissue degeneration in the L group was lower than that in the G group. TAS and TOS levels were also used as oxidative stress parameters. The TAS level in the L group was higher than that in the G group. We concluded that local application of silica nanoparticles could be a promising approach for drug delivery system to the testis tissue.

Ethics Committee Approval	This study was approved by the ethics committee of the Animal Ethics Committee of Acıbadem University (Approval number: 2015/11).
Peer Review	Externally peer-reviewed.
Author Contributions	Conception/Design of Study- S.D., D.Y. M.A.E.; Data Acquisition- S.D., D.Y. M.A.E., M.S.K., S.D., O.B.; Data Analysis/Interpretation- S.D., D.Y. M.A.E., M.S.K., S.D., O.B.; Drafting Manuscript- S.D., D.Y. M.A.E., S.A. ; Critical Revision of Manuscript- S.D., D.Y. M.A.E., S.A.; Final Approval and Accountability- S.D., D.Y. M.A.E., S.A., M.S.K., S.D., O.B.
Conflict of Interest	Authors declared no conflict of interest.
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