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Effect of Moringa Oleifera Lam Extract (MOLE) on Testicular Ultra Structure in Cryptorchidism Model

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Abstract

Moringa Oleifera Lam Extract (MOLE) is an antioxidant plant that belongs to the Moringaceae family. It also has antimicrobial, antidiabetic, anticarcinogenic, cardio-protective and sexual potential-enhancing effects. Cryptorchidism causes infertility with sperm DNA disorder and germ cell apoptosis with the production of ROS and increased oxidative stress. In the present study, the histopathological and ultrastructural changes in experimental Cryptorchidism-induced rat testes, and the effects of Moringa Oleifera Lam Extract (MOLE) on these changes were investigated at two different doses. A total of 26 male 15-17-day-old Sprague Dawley rats were used in the present study. The rats were divided into 4 groups as Control, Cryptorchidic, Cryptorchidic + 400mg/kg MOLE, and Cryptorchidic + 800mg/kg MOLE. The rats were sacrificed on the 15th day of the trial. Light and electron microscopic examinations were evaluated in the testicular tissues. It was determined as a result of the light microscopic examination after Cryptorchidism that there were ruptures in the interstitial area, separation of germ cells from basal membrane, cell loss into the lumen, and cytoplasmic vacuolization. In electron microscopy, it was determined that the basal membrane was intact, the tight cytoplasmic connections and the bridges between Sertoli Cells were broken, and there were mitochondria with abnormal crystals. It was determined that both doses of the MOLE plant, which were administered for treatment purposes, had a repairing effect on these pathological damages, and this effect was more pronounced in Dose 2.

INTRODUCTION

Cryptorchidism is defined as the defect in unilateral or bilateral descent of testes towards the scrotum and in the associated structures. Testicular descent is a series of complex events that require nervous system, hormones, and the basic mechanisms of this descent to work collectively [1]. Its congenital form is detected in 2-4% of those born in due period [2], the incidence in childhood is 1-3% [3]; and 85% of Cryptorchidism is unilateral. It was shown in previous studies that pathologies like Cryptorchidism result in germ cell apoptosis in the testes. Cryptorchidism affects spermatogenesis and reduces sperm density, which eventually leads to infertility [4].

Moringa Oleifera is usually known as "Tree of Life" or "Miracle Tree". It is a plant variety which is used in each part, and has a value in almost every part [5,6]. M. Oleifera has been used in nutrition and in industrial and medical fields for a long period. It was reported in previous studies that it has antitumor, antiepileptic, antidiuretic, and anti-inflammatory properties. In addition to these, it was also shown that its extracts inhibit the 6-β-hydroxylation of the testosterone [7].

The purpose of the present study was to investigate the light and electron microscopic structure of the testes in Cryptorchidism, and to examine the role of M. Oleifera Lam Extract (MOLE) on these effects. It was also aimed to investigate any possible degenerative effects of unilateral Cryptorchidism on the testes, and to evaluate the testicular findings on both sides with intragroup comparisons with the Control Group.

MATERIALS AND METHODS

Animals

According to the Power Analysis, a total of 26 rats were used in the present study. The Sprague-Dawley rats were 15-16-day-old, weighed 27-31 g, and were obtained from Gaziantep University, Experimental Animals Research Center. The testicles of the rats did not descend onto the scrotum in this period. All animals were weighed, and the MOLE doses that would be administered to the rats every day were calculated. All the experiments were done following a protocol approved by the Animal Care and Ethics Committee of Gaziantep University.

Extract preparation (aqueous extract of Moringa Oleifera Lam)

The powder of the M. Oleifera Lam was obtained from Eczacibasi Pharmaceutical Company, Turkey. The first dose was arranged as 400 mg/kg MOLE, and the second dose was arranged as the maximum dose (800 mg/kg MOLE) so that 16 days old rats could receive orally. For simplicity, the maximum dose will be referred to as 800 mg/kg MOLE (i.e. the second dose) in

subsequent part of the manuscript. The powder of the M. Oleifera Lam was dissolved in distilled water.

Experimental protocol

Twenty-six rats were divided into four randomized groups as 5 in the Control Group, and 7 in the other groups. The rats were given distilled water, a MOLE solvent for placebo purposes, for 14 days/po only. The rats in the Cryptorchidic group were given MOLE solvent by applying left-unilateral Cryptorchidism as 14 days/po. Dose 1 Group rats received 400mg MOLE/kg/po after left-unilateral Cryptorchidism; and Dose 2 group rats received 800mg MOLE/kg/po for 14 days after left-unilateral Cryptorchidism. The rats were sacrificed under anesthesia at the end of the experiment.

Surgical Method

The rats to which Unilateral Cryptorchidism was induced were anesthetized intraperitoneally with ketamine/xylazine hydrochloride mixture. The inguino-scrotal areas of the rats were cleaned with povidone iodine, and the skin was opened. The entry was achieved with blunt dissection towards the left gubernaculum. The left gubernaculum was isolated from tissues and pushed back into the abdominal cavity from the external inguinal ring. The left gubernaculum was fixed with external inguinal ring (Dündar et al., 2001).

Histological examination

In the 14th day of the trial, the rats were weighed and testicular tissues were removed. The testes were fixed with lanthanum nitrate-containing fixation to test the integrity of the Blood-Testis Barrier (BTB) at electron microscope level in permeable electron microscopic studies (TEM) [8]. Then, 1/3 of these was taken to the 5% glutaraldehyde fix for electron microscopic examinations, and was fixed for 1 night at +4 degrees. Then, the remaining part was placed into 10% formaldehyde with a buffer with pH=7.0. After routine follow-up and staining, the sections were examined with JEOL-JEM 1400 TEM (Japan); and the micrographs of the testicular tissues were obtained. Routine histological tissue tracking procedure was performed and Hematoxylin-Eosin (H-E) staining was applied for light microscopic examination.

STATISTICAL ANALYSIS

The statistical analysis of the numerical data obtained in the study was made with the IBM SPSS 22.0 Windows (Statistical Package for Social Sciences) Program. Shaphiro Wilk test was used to test the compliance of the numerical variables to normal distribution. Mann Whitney U-test was used to compare the variables that did not comply with normal distribution in two groups, Kruskal Wallis and Pairwise Tests were used in comparisons of the four groups. $P<0.05$ was considered to be significant.

RESULTS AND DISCUSSION

Light Microscopic findings

Control Group: In the left testis examinations, it was observed that the testes were in normal and regular structure. The seminiferous tubular integrity was preserved, and the germ cells formed regular series (Figure IA).

In the left Cryptorchidic testes of the Cryptorchidic Group: It was seen that normal testicular morphology was impaired. Early and prolonged spermatids were not detected. It was also seen that current spermatocytes were impaired in the tubule. It was detected that germinative epithelial was separated from basal membrane, the germ cells were split into the seminiferous tubular lumen, the seminiferous tubules were separated from each other in interstitial area, and edema was formed (Figure IB-D).

In the right scrotal testes of the Cryptorchidic Group; similar damage findings were observed compared to the descending testis images of the same group, although it was less (Figure IE).

In left-cryptorchidic testes in Dose 1 Treatment Group: Compared to the Control Group, germ cell spill continued into the seminiferous tubular lumen, the separation of the tubules from each other in the interstitial area decreased (Figure IF, IG).

In Dose 1 Group right scrotal testes: Compared to the Control Group and the right cryptorchidic testes of the same animals, there was a decrease in the edema in the interstitial area (Figure IH).

In left-cryptorchidic testes in Dose 2 Treatment Group

Compared to the Control Group, basal membrane was intact, and significant improvements were detected in the interstitial area of the testicle, such as in the separation of seminiferous tubules, and in the pathologies, the spill of the germs cells to the lumen was observed to continue very little (Figure IJ,IK). In the right scrotal testes of Dose 2 Group: Similar to the Control Group testes, normal histological structures were observed (Figure IL).

Electron microscopic findings

In the left testis of the Control Group: It was observed that the Membrane Propria, which consisted of basal lamina, myoid cells, and a layer of epithelioid cells, had regular structures. The nucleus was flat and contained peripheral chromatin masses in myoid cells that had normal electron density, clinging together firmly, and showing single-layer pattern. There were several mitochondria and peripheral micropinocytosis vesicles in their cytoplasm. The nuclei of the Sertoli cells that had normal electron density on basal lamina contained 1-2 nucleolus with deep invagination. There were common SERs, tubular and cup-shaped mitochondria, RERs, several lipid droplets, electron-dense structures, and glycogen granules in their cytoplasm. The zonula occlude connection complexes between the Sertoli cells were normal. Spermatogonium that had global and centrally-located nuclei sat on basal lamina. Their cytoplasms were not rich in organelles, but contained several mitochondria and ER cisterna. The spermatocytes with normal electron density attached to each other with cytoplasmic bridges among germinative epithelial cells of seminiferous tubules were distinguished by cytoplasms containing spherical-shaped nuclei and grouped mitochondria, microtubules and ER cisterns (Figure IIA, IIB).

In left-cryptorchidic testes in cryptorchidic group

The Membrane Propria that consisted of basal lamina and myoid cells was observed in the normal slim structure. However, serious degenerative changes were observed in the epithelia of

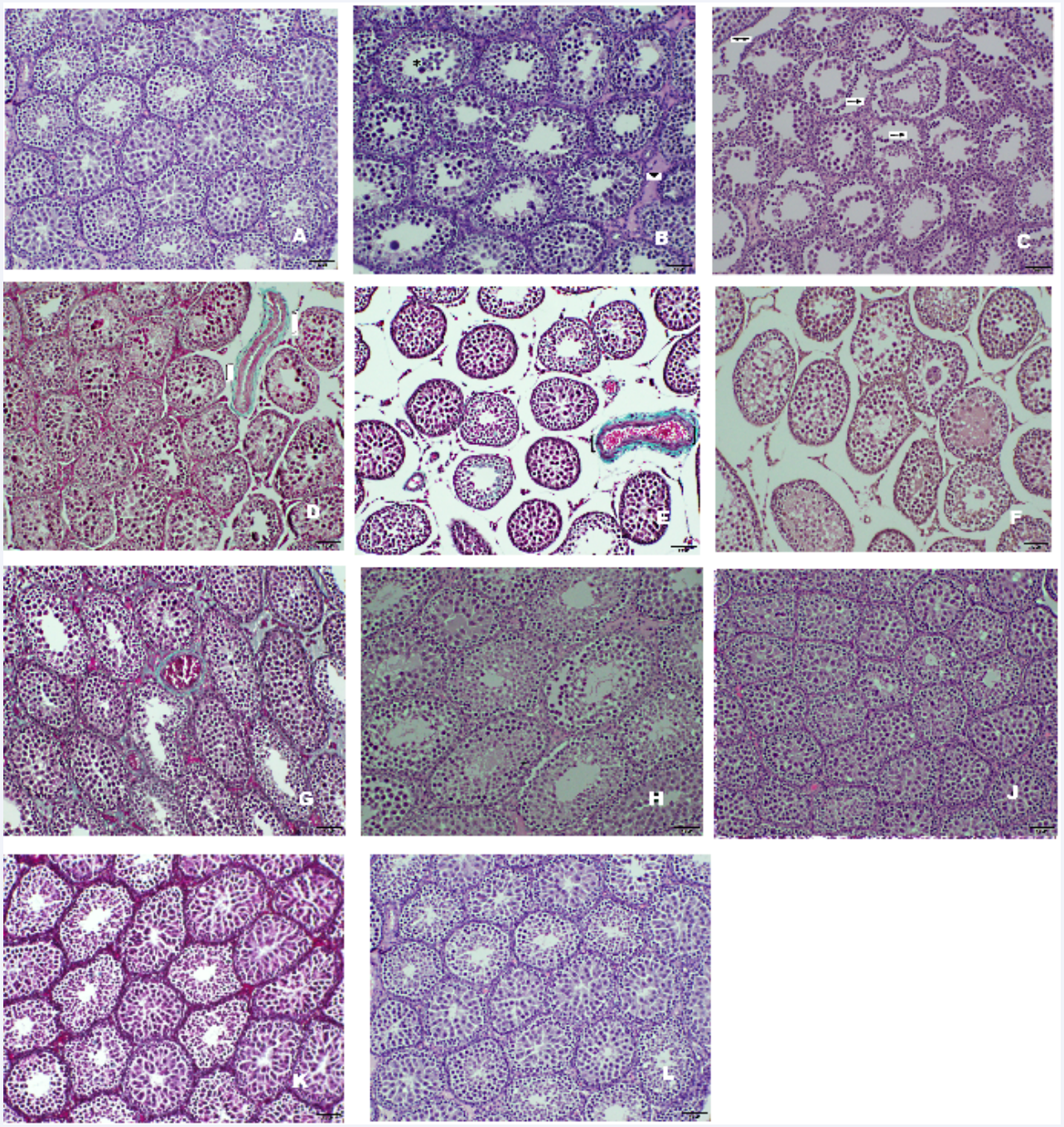

Figure 1

A) Image of the control testis (H-E, 200x).

B) Cryptorchitis group left cryptorchid testis (H-E 200x). (*germ cell spill into lumen, Edema).

C) Cryptorchid group left cryptorchid testis(H-E 200x) (↔Separation in the interstitial space, → separation from the basement membrane).

D) Cryptorchid group left cryptorchid testis (TRI 200x).

E) Cryptorchid group right scrotal testis (TRI 200x)

F) MOLE (400 mg/kg) Doz1 grubu sol kriptorşid testis (H-E 200x)

G) MOLE (400 mg/kg) Doz1 grubu sol kriptorşid testis (TRI 200x)

H) MOLE (400 mg/kg) Doz1 grubu sağ skrotal testis (H-E 200x)

J) MOLE (800 mg/kg) Doz2 grubu sol kriptorşid testis (H-E 200x)

K) MOLE (800 mg/kg) Doz2 grubu sol kriptorşid testis (TRI 200x)

L) MOLE (800 mg/kg) Doz2 grubu sağ skrotal testis (H-E 200x)

seminiferous tubule. Giant gaps left by germ cells that spilled were seen between the Sertoli cells that made up the epithelium and the spermatid cells. It was seen that the cytoplasmic bridges between the Sertoli cells, occludes, and spermatogenic serial cells with each other lost their integrity. It was also observed that the electron density of Sertoli cells sitting on basal lamina increased. There were vacuolated SERs in their cytoplasms, abnormal Krista structures, and giant lipid droplets in their mitochondria. Spermatogonia were shrunken, there were autolytic changes in cytoplasms, and irregular gaps around them. It was observed that there were decomposition and dissolution in the nucleus membrane of the spermatocytes characterized by the presence of synaptonemal complex, and there were mitochondria, which were degenerated and vacuolated in their cytoplasms (Figure IIC, IID). In the right scrotal testes of the cryptorchidic group: Mildly-degenerated changes were seen in normal appearance in the seminiferous tubule epithelium wrapped with Membrana Propria, the Sertoli cells, and spermatogenic cells. The giant gaps between tubular epithelial cells were fewer compared to the left cryptorchidic testes. The cytoplasmic bridges between the spermatogenic serial cells, Sertoli cells, and zonula occlude preserved their integrity in some areas. SERs were vacuolated, and mitochondria had abnormal Krista appearance in the cytoplasm of the normal electron-dense Sertoli cells with indentation nuclei. Similar organelle disruption was seen in the cytoplasms of the spermatocytes (Figure IIE).

In left-cryptorchidic testis of Cryptorchidic+Dose 1 (400 mg/kg) MOLE Treatment Group: It was seen that the Membrana Propria had normal appearance, similar to the Control Group. Basal lamina was regular, and myoid cells were in normal electron density. The connections between the seminiferous tubular epithelial cells were impaired, and the large gaps were still there. It was noteworthy that many tubules had reduced electron density in Sertoli cells; however, SER vacuolization continued in their cytoplasms. It was also detected that Spermatogonia, which are characterized with organelle-poor cytoplasms and spherical nuclei, maintained their cytoplasmic integrity relatively in many tubules. The spermatocytes, which reflect spermatogenesis process, were in normal electron density, were dissolved at places, and there were autolytic changes in the nucleus membrane (Figure IIF, IIG). In right-scrotal testis of Cryptorchidic + Dose 1 (400 mg/kg) MOLE Treatment Group: It was detected that the Sertoli cells and spermatogenic cells surrounded by Membrane Propria were in normal structure. It was also noted that the tight connections between Sertoli cells and the cytoplasmic bridges between spermatogenic cells remained their integrity. It was determined that the Sertoli cells, Spermatogonia, and the nucleus and nucleus membrane structures of spermatocytes were normal, and they had mitochondria and ER cisterns of a similar nature to the control group in their cytoplasms (Figure IIH).

In left-cryptorchidic testis of cryptorchidic +Dose 2 (800 mg/kg) MOLE Treatment Group: It was determined that Membrane Propria resembled that of the Control Group. The tight connections between the Sertoli cells and spermatogenic cells, which formed the tubule epithelium, and the cytoplasmic bridges between spermatogenic cells maintained their integrity. The Sertoli cells characterized by nuclei containing deep invaginations with pronounced nucleolus that sat on basal lamina had normal electron density, and had tubular mitochondria and ER cisterns in normal structure. Spermatogonia had similar characteristics with the Control Group with oval-shaped nuclei that had chromatin patches and organelle-poor cytoplasms. The cytoplasms of the spermatocytes that had nucleus and nucleus membrane in normal appearance characterized by the presence of synaptonemal complexes had organelle contents of a similar nature to the Control Group (Figure IIK, IIL). In right-scrotal testis of Cryptorchidic + Dose 2 (800 mg/kg) MOLE Treatment Group: It was determined that Sertoli and spermatogenic cells surrounded by Membrane Propria in normal appearance were similar to those of the Control Group. It was observed that the tight connection between the Sertoli cells that had indentation nuclei and the cytoplasmic bridges between spermatogenic cells maintained their integrity. The nuclei and nuclei membrane structures of the Sertoli cells, Spermatogonia and spermatocytes were normal, and there were mitochondria and ER cisterns of similar nature to the Control Group in their cytoplasms (Figure IIM).

Cryptorchidism is the most common developmental disorder in boys. Cryptorchidism is seen at a rate of %85 unilaterally, and ends up with infertility in adult years due to the disruption in the maturation of the male gametes. In addition, it is also already known that spermatozoon counts are reduced in individuals with Cryptorchidism. It was reported in previous studies that the increase of reactive oxygen types with the effect of cryptorchidism, decreased sperm mobility, DNA damage in sperms, decreased cell proliferation, and apoptosis in germinative cells might be the underlying effective mechanisms in reduced sperm density [4,9].

Since the result was precise among the different models made to create Cryptorchidic animal model, the surgical method was preferred, and the Inguinoscrotal Method was used [10,11]. The biggest advantage of this method is that the abdominal cavity is not opened as in other surgical methods. After the animals are anaesthetized, the surgery takes 15 minutes for each animal, and there is no loss of animal in the surgery. The results obtained in the present study were found to be clear and successful to create a model.

When the literature was reviewed, it was observed that there were various studies conducted on the antioxidant effects of MOLE plant used for treatment purposes. However, there are few studies on the presence of the therapeutic properties of the plant in testicular damage.

In previous studies, it was reported that both spontaneous and empirical Cryptorchidism caused germ cell loss [12] and this loss in germ cells occurred with apoptosis [12,13].

Tao et al. examined the effects of Cryptorchidism on testes in a day-dependent manner in Cynomolgus Macaque monkeys between the ages of 5-8 as 3 animals in each group by sacrificing the animals on the 1st, 3rd, 5th, 7th, 10th and 15th days. They showed that the seminiferous tubular epithelial structure began to deteriorate after the 5th day, multi-nuclei giant cells began to increase on the 10th day, and after day 15, seminiferous tubular germ cell loss increased after the induction of Cryptorchidism [14].

General electron microscopic images of the left and right testes of the Control and Experimental Group were evaluated

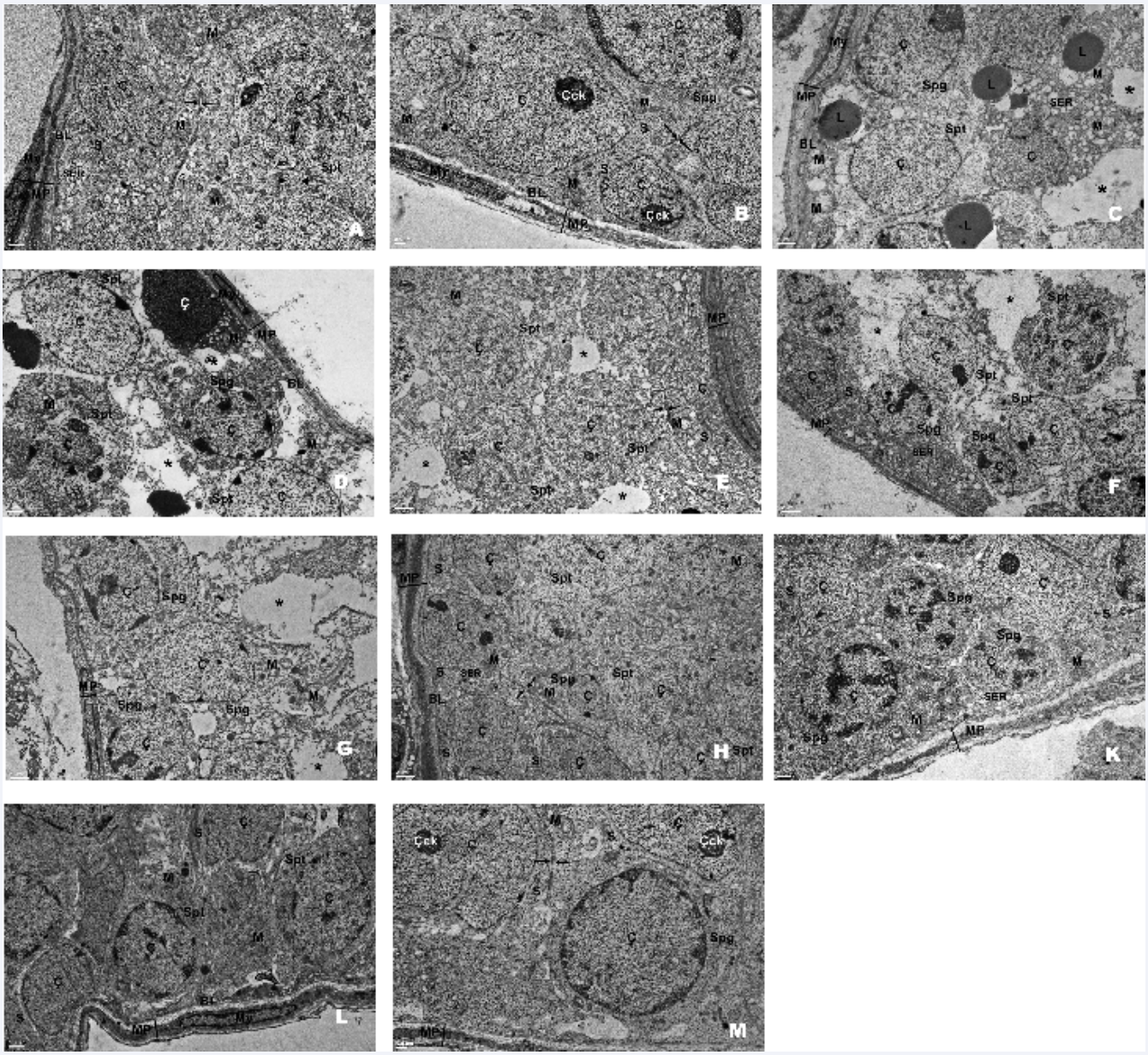

Figure 2

A: Electron microscopic view of control group left testicle tissue samples. Propria (MP), Sertoli cell (S) and spermatocyte (Spt) are seen on the normal structure membrane. Tight connections (arrow) between the Sertoli cells and cytoplasmic bridges (arrowhead) between the spermatogenic cells are observed in the normal structure. Nucleus (Ç), Basal lamina (BL), Myoid cell (My), Mitochondria (M), Agranular Endoplasmic reticulum (SER). Bar: 1 µm.

B: Electron microscopic view of the control group right testicle tissue samples. Membrane ppropria (MP), Sertoli cell (S) and spermatogonium (Spg) have normal appearance. The tight connections (arrow) between the Sertoli cells are normal. Nucleus (Ç), Nucleolus (Çck), Basal lamina (BL), Myoid cell (My), Mitochondria (M). Bar: 0.5 µm.

C: Electron microscopic view of cryptorchid group left cryptorchid testis tissue samples. In the relatively normal view, giant spaces (*) left by the germ cells pouring in the tubular epithelium surrounded by the membrane propria (MP) are observed. Sertoli cell cytoplasm (S) shows excessive agranular endoplasmic reticulum (SER) vacuolization, mitochondria (M) with abnormal crystals and giant lipid droplets (L). Nucleus (Ç), Spermatogonium (Spg), Spermatocyte (Spt), Basal lamina (BL), Myoid cell (My). Bar: 1 µm.

D: Electron microscopic view of cryptorchid group left cryptorchid testis tissue samples. Propria (MP) has a normal appearance on the membrane consisting of myoid cells (My) and basal lamina (BL). Giant gaps (*) are seen between tubular epithelial cells. In the cytoplasm (S) of electron dens Sertoli cells, agranular endoplasmic reticulum (SER) vacuolization and degenerate mitochondria (M) are observed. Autolytic changes in spermatogonium (Spg) and spermatocytes (Spt) and dissolution in the core (Ç) membranes are seen. Nucleus (Ç), Nucleolus (Çck), Basal lamina (BL), Myoid cell (My), Mitochondria (M). Bar: 1 µm.

E: Electron microscopic view of cryptorchid group right scrotal testis tissue samples. Membrane propria (MP), Sertoli cells (S) and spermatocytes (Spt) are close to normal. Huge gaps (*) are seen in places between tubular epithelial cells. Degenerated mitochondria (M) are observed in the cytoplasm (S) of Sertoli cells. The core (O). Bar: 2 µm.

F: Electron microscopic view of MOLE (400 mg/kg) dose 1 group of left cryptorchid testis tissue samples. In the relatively normal view, giant cavities (*) are seen in the tubular epithelium surrounded by membrane propria (MP). Agranular endoplasmic reticulum (SER) vacuolization is observed in the Sertoli cell cytoplasm (S). Nucleus (Ç), Spermatogonium (Spg), Spermatocyte (Spt). Bar: 2 µm.

G: MOLE (400 mg/kg) dose1 group left cryptorchid testis electron microscopic view. Giant spaces (*) are observed between the tubular epithelial cells surrounded by membrane propria (MP). Degenerated mitochondria (M) are seen in the cytoplasm (S) of Sertoli cells. The core (O). Bar: 1 µm.

H: Electron microscopic view of MOLE (400 mg/kg) dose 1 group of right scrotal testicular tissue samples. Membrane propria (MP), Sertoli cells (S), spermatogoniums (Spg) and spermatocytes (Spt) are in normal appearance. In the cytoplasm (S) of Sertoli cells, mitochondria (M), agranular endoplasmic reticulum (SER) are normal. Tight connections (arrow) between the Sertoli cells are intact. Core (Ç), Basal lamina (BL). Bar: 2 µm.

K: Electron microscopic view of MOLE (800 mg/kg) dose2 group left cryptorchid testis tissue samples. Sertoli cells (S) and spermatogoniums (Spg) are normal in the tubular epithelium, which is surrounded by propria (MP) to the membrane in a relatively normal view. Agranular endoplasmic reticulum (SER), Core (Ç). Bar: 1 µm.

L: Electron microscopic view of MOLE (800 mg/kg) dose2 group left cryptorchid testis tissue samples. In normal view, the membrane is propria (MP); It consists of basal lamina (BL) and myoid cells (My). Sertoli cells (S) and spermatocytes (Spt) are seen in the tubular epithelium. Nucleus (Ç), Mitochondria (M). Bar: 1 µm.

M: Electron microscopic view of MOLE (800 mg/kg) dose 2 group right scrotal testicle tissue samples. Membrane propria (MP), Sertoli cells (S), spermatogoniums (Spg) and spermatocytes (Spt) are in normal appearance. The tight connections (arrow) between the Sertoli cells (S) are intact. Nucleus (Ç), Mitochondria (M). Bar: 1 µm.

in the present study. It was determined that the Control Group testes were normal and regular, and the germ cells formed regular series from basal to apical of the seminiferous tubules.

It was determined that the normal testicle morphology of the Cryptorchidic group animals was impaired in the left Cryptorchidic testes. It was observed that the order of the existing spermatocytes in the seminiferous tubule was largely impaired, and the germ cells spilt into the tubular lumen in some areas. In addition, it was also observed that there were histopathological findings like the separation of germinative epithelia from basal membrane, and increasing edema. Similar signs of damage were observed in the right scrotal testes of the Cryptorchidic group animals compared to the left Cryptorchidic testes images. Although the healing effect of the 400 mg/kg MOLE dose used for treatment on Cryptorchidic-induced damage, it was not in the effect size that could repair all damage. It was observed that nearly all damage caused by Cryptorchidism was repaired by doubling the MOLE dose used (800 mg/kg).

Again in this study, it was also observed that there were degeneration areas in the cytoplasms of the Sertoli cells of the left Cryptorchidic testicles of the Cryptorchidic Group, and the slim structures were impaired. Similar signs of damage were observed in the right scrotal testes of the same group, although less than the Cryptorchidic testicles. It was considered that the degeneration in the slim structure of the Sertoli cells might be an indication that the functions of the Sertoli cells, which play roles in the spermatogenesis process, were also impaired. Similar opinions were reported in previous studies [15].

In another study, in a surgery-induced Cryptorchidism model, large vacuoles were detected in the tubule basal with the separation of connection complexes between the Sertoli cells, and there were large spherical lipid deposits and small dilated ER vesicles in the Sertoli cells [16].

In our study, there were decreases in the electron density of the Sertoli cells in many tubules in the left Cryptorchidic testes of the Dose 1 Group; but SER vacuolization continued in their cytoplasms. It was determined that Spermatogonia maintained their relatively cytoplasmic integrity in many tubules. The slim structures of the right scrotal testicle in this dose group were determined to be similar to those of the control testicular structures. Both the left Cryptorchidic testicles and the right scrotal testicular images of Dose 2 Group were similar to those of the Control Group. Based on these results, it was determined that the MOLE used in the 1st Dose partially repaired the damage on the left testicle caused by Cryptorchidism, and nearly all of the damage in the right scrotal testicle was healed. It was observed that the 2nd Dose of the MOLE repaired the entire damage in both left cryptorchidic testicle and right scrotal testicle caused by Cryptorchidism.

In the present study, perfusion fixation was carried out with the fixative solution that contained Lanthanum Nitrate, which is considered to be an effective method for testing the Blood-Testis Barrier (BTB) [8,17]. It was observed that the right Cryptorchidic testicles leaked Lanthanum in the electron microscopic examinations of the animals of the Cryptorchidic group. In addition to the leaking, there were also openings between the cells in the BTB area, and dilatations in the mutual membranes. It was also observed that the germ cells separated from the Sertoli cells. Similar damage was detected in the right scrotal testes of the same group, although it was at lower levels. In his study, Bergh showed that the intercellular area was dilated in the electron microscope. He also reported that the dilatation between the Sertoli cells and germ cells was an indication of deterioration in the connection complexes [18].

In their isolated germ cell culture study, Fujioka et al. found that the apoptosis in germ cells that were cultured with Sertoli cells was lower at significant levels than the germ cells that were cultured alone. They used two types of cultural media as

the mutual culture. In the first one, they used a system in which germ cells were separated by a membrane from the Sertoli cells in the same medium; and in the other type, they placed the germ cells directly on the Sertoli cells. They showed that the decrease in apoptosis in germ cells, which were in direct contact with the Sertoli cells, was more than that of the other group. This study also shows that germ cells whose contact with the Sertoli cells is cut off have apoptosis [19].

In their study, Wiebe et al., injected glycerol to the Study Group, and showed that the localization of occlude in the BTB deteriorated with the deterioration in the microfilament and microtubule structures, which altogether made up the cell skeleton. They reported that this was caused by the dissolution of the Sertoli cell skeletal structure, and the permeability of BTB. They also reported that one of the reasons for the degeneration in the spermatogenesis observed in their study might be the deterioration in the BTB as a result of the disintegration in the skeletal structure [20].

In Cryptorchidic cases, the direction of the changes in the right scrotal testicle are still a matter of dispute. The presence of degenerative changes was detected in the light and electron microscopic evaluation of the right scrotal testicles of the Cryptorchidic Group; and there were degenerative changes, although it was less than the kryptonite testes compared to the control testes.

When all these findings are evaluated together, it is considered that spermatogenesis may have been affected not only in the left Cryptorchidic testicle, but also in the right scrotal testicle. In unilateral Cryptorchidic cases, most of Cryptorchidic adults were subfertile/infertile [21]. Although normal sperm production continued by the descended scrotal testicle, it was considered that the formation of autoimmune response with the circulating of germ cell antigens because of the impaired BTB had roles in the creation of subfertility/infertility of these cases. As a matter of fact, anti-sperm antigens were examined in the unilateral Cryptorchidism model by Srinivas et al., and the anti-sperm antigens were found to be negative in the Control Group; however, it was also reported that this antigen was positive in the Experimental Group [22].

When planning this study, the purpose was to investigate how the damage anticipated in experimental Cryptorchidism affected the slim structure of the tissue, and to examine the presence of the effect of Moringa Oleifera plant on these damages. In addition, two (400-800mg/kg) doses were used with the knowledge that the effect of an antioxidant substance can turn into an oxidant effect with the increase in its dose. However, it was determined that the antioxidant effect of MOLE continued in both doses, and even at the 2nd dose, it was observed that the Cryptorchidic testes samples had an even more healing effect at the fine structure level.

CONCLUSION

Briefly, our findings suggest that the juicy extract of M. Oleifera can repair the damage in histopathological and ultrastructural structure in rats with unilateral Cryptorchidism, and can be used as a new therapeutic agent for Cryptorchidism.

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Approval for the study was obtained from the local ethics committee of Gaziantep University with the decision number 2016/13.

REFERENCES

1. Abe R, Ohtani K. An ethnobotanical study of medicinal plants and traditional therapies on Batan Island, the Philippines. J Ethnopharmacol. 2013; 145: 554-565.
2. Bakre AG, Aderibigbe AO, Ademowo OG. Studies on neuropharmacological profile of ethanol extract of Moringa oleifera leaves in mice. J Ethnopharmacol. 2013; 149: 783-789.
3. Connell CJ. A freeze-fracture and lanthanum tracer study of the complex junction between Sertoli cells of the canine testis. J Cell Biol . 1978; 76: 57-75.
4. Dündar M, Koçak I, Çulhaci N. A new experimental model for cryptorchidism: inguinoscrotal approach. Urological research. 2001; 29: 178-181.
5. Fawzy F, Hussein A, Eid MM, Kashash AME, Salem HK. Cryptorchidism and fertility. Clinical Medicine Insights: Reproductive Health. 2015; 9: CMRH. S25056.
6. Foresta C, Zuccarello D, Garolla A, Ferlin A. Role of hormones, genes, and environment in human cryptorchidism. Endocr Rev. 2008; 29: 560-580.
7. Fujioka H, Fujisawa M, Tatsumi N, Kanzaki M, Okuda Y, Okada H, et al. Sertoli cells inhibited apoptosis of pachytene spermatocytes and round spermatids. Endocr Res. 2001; 27: 75-90.
8. Kerr JB, Rich KA, de Kretser DM. Effects of experimental cryptorchidism on the ultrastructure and function of the Sertoli cell and peritubular tissue of the rat testis. Biol Reprod. 1979; 21: 823-838.
9. Klonisch T, Fowler PA, Hombach-Klonisch S. Molecular and genetic regulation of testis descent and external genitalia development. Dev Biol. 2004; 270: 1-18.
10. Koçak I, Dundar M, Hekimgil M, Okyay P. Assessment of germ cell apoptosis in cryptorchid rats. Asian J Androl. 2002; 4: 183-186.
11. Kvist K, Thorup J, Byskov A, Høyer P, Møllgård K, Yding Andersen C. Cryopreservation of intact testicular tissue from boys with cryptorchidism. Hum Reprod. 2006; 21: 484-491.
12. Levy S, Serre V, Hermo L, Robaire B. The effects of aging on the seminiferous epithelium and the blood—testis barrier of the Brown Norway rat. J Androl. 1999; 20: 356-365.
13. Monera TG, Wolfe AR, Maponga CC, Benet LZ, Guglielmo J. Moringa oleifera leaf extracts inhibit 6β-hydroxylation of testosterone by CYP3A4. J Infect Dev Ctries. 2008; 2: 379.
14. Ofordeme KG, Aslan AR, Nazir TM, Hayner-Buchan A, Kogan BA. Apoptosis and proliferation in human undescended testes. BJU Int. 2005; 96: 634-648.
15. Shikone T, Billig H, Hsueh AJ. Experimentally induced cryptorchidism increases apoptosis in rat testis. Biol Reprod. 1994; 51: 865-872.
16. Shono T, Zakaria O, Imajima T, Suita S. Extraabdominal fixation of the gubernaculum inhibits testicular descent in newborn rats. J Pediatr Surgy. 1996; 31: 503-506.
17. Srinivas M, Agarwala S, Gupta SD, Das S, Shaha C, Mitra D. Fertility and unilateral undescended testis in the rat model II. Pediat Surg Int. 1998; 13: 392-395.
18. Tao S, Guo J, Zhang X, Li Y, Hu Z, Han C, et al. Germ cell apoptosis

induced by experimental cryptorchidism is mediated by multiple molecular pathways in Cynomolgus Macaque. Front Biosci. 2006; 11: 1077-1089.

19. Van Brakel J, Kranse R, de Muinck Keizer-Schrama S, Hendriks A, De Jong F, Bangma C, et al. Fertility potential in men with a history of congenital undescended testes: a long-term follow-up study. Andrology. 2013; 1: 100-108.

20. Van Bergh Weerman MA, Dingemans KP. New techniques rapid deparaffinization for electron microscopy. Ultrastruct Pathol. 1984; 7: 55-57.

21. Watts LM, Hasthorpe S, Farmer PJ, Hutson JM. Apoptotic cell death and fertility in three unilateral cryptorchid rat models. Urolog Res. 2000; 28: 332-327.

22. Wiebe JP, Kowalik A, Gallardi Rl, Egeler O, Clubb BH. Glycerol Disrupts Tight Junction—Associated Actin Microfilaments, Occludin, and Microtubules in Sertoli Cells. J Androl. 2000; 21: 625-635.

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