Original Article

Histological Study of Alteration in Testes and Epididymis of Domestic Rabbits Caused by Tribulus Terrestris and Vitamin E

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ABSTRACT

Tribulus Terrestris (TT) is a common herbal plant with different categories that grows in many countries of the world. Traditional Chinese and Indian therapies have used TT for infertility treatment and also as a powerful antioxidant agent. Therefore, this study aimed to use this plant supplemented with vitamin E to study their combined effects on the histological condition of the testicle and epididymis of rabbits. This study was performed on 28 healthy male rabbits (445-950 g, 2.0-3.0 months old) that were randomly divided into four groups (n=7). All animals were subjected to clinical examination to ensure that they were free of external and internal parasites with the use of some preventive treatments. The animals were housed individually (cage size: 50 cm×50 cm×40 cm) over the 60-day study period starting from January 2022, with an adaptation period of two weeks. *Tribulus Terrestris* and vitamin E treatments were as follows: the first group (G1) was daily fed on a standard diet and kept as the control group, the second group (G2) was daily fed on the same ration plus 1 g of TT (animal/daily), the third group (G3) was daily fed on the same ration plus 1 g of TT supplemented with 60 mg of vitamin E (orally) (animal /daily), and the fourth group (G4) was daily fed on the same ration, with the addition of 60 mg vitamin E per animal (orally). The morphometric investigation, macroscopic variables (including body weight, testicular weight, and volume), and the microscopic parameters of the testicular seminal tubule were measured. The histological section showed the absence of negative effects after the oral administration of TT at a dose of 1 g per day and 60 IU vitamin E for each animal. However, there was a positive effect on spermatogonia and spermatocytes in all animals, while the spermatogonia in the experimental groups were more dense, especially in the second and third groups, compared to the control group. The seminiferous tubules were significantly lined with spermatogonia, spermatocytes, and round spermatids (P<0.5) in the experimental groups, compared to the control group. Nevertheless, the epididymis tissue did not show traces of histological changes, such as epididymal hyperplasia. Sperms were more frequent in the lumens of the epididymis as well as the lumens larger than those of the control. Based on the results of this study, it can be concluded that the pole plant and vitamin E have a positive effect on the epithelial lining of the seminiferous tubules and the epididymis with an increase in sperm formation and differentiation towards maturity.

Keywords: Epididymis, Histology, Rabbits, Spermatogenesis, Testis, Tartus tratus, Vitamin E

1. Introduction

Tribulus Terrestris (TT) is a common herbal plant with different categories that grows in many countries of the world. It is a drought-tolerant, summer-growing annual herb with a horizontal hairy structure that belongs to the Zygophyllaceae family. Some types of TT are classified as a noxious weed. Traditional Chinese and Indian therapies have used TT for infertility treatment and as a powerful antioxidant agent (1). This plant is found worldwide in the tropic and subtropic regions (2). The TT is well-known for its ability to treat sexual disorders, impotence, and hormonal imbalances in animals. It should be mentioned that it can also be used as a sexual stimulant. Interest in the use of TT is growing as a result of its active compounds, which can be used to treat infertility and other diseases, and it is now one of the most studied medicinal herbs (1, 3). *Tribulus Terrestris* has been used in folk medicine for thousands of years; however, more research is needed to understand its targets, effects, and mechanisms of action as it impacts various targets, physiological processes, and diseases (4).

Vitamin E is a non-toxic fat-soluble vitamin with antioxidant and non-toxic characteristics that impacts animal performance and outputs. Although it is only required in small amounts in the diet, its responsibilities in livestock production are critical since it is necessary to improve animal health and productivity, nutritional quality, and yield (5). Vitamin E supplementation, either dietary or oral, is critical for lowering cholesterol levels, boosting antioxidant status in animals, and minimizing lipid oxidation in muscles, eggs, and dairy products.

The bioavailability of vitamin E-enriched animal products has been proven to offer a valuable nutritional benefit to consumers, especially in resource-constrained areas where vitamin E deficiency poses a risk to some cellular functions. As a result, and due to the importance of this vitamin, many researchers have focused on the effects of vitamin E as an antioxidant that plays a vital role in the performance of livestock and their products (5). Addition of vitamin E to the diet feed of rabbit bucks at a different dose can improve their growth, hematology, and serum biochemical profile (6).

However, there is a lack of studies related to TT in its use in animal nutrition. Therefore, this study aimed to use this plant supplemented with vitamin E to study their combined effects on histological conditions of the testicle and epididymis of rabbits.

2. Materials and Methods

2.1. Experimental Design

In total, 28 healthy male rabbits (445-950 g, 2.0-3.0 months old) were used in this study. The animals were randomly divided into four groups (n=7). All animals were subjected to clinical examination to ensure that they were free of external and internal parasites with the use of some preventive treatments. They were housed individually (cage size: 50 cm×50 cm×40 cm) over the 60-day study period starting from January 2022, with an adaptation period of two weeks.

Tribulus Terrestris and vitamin E treatments were as follows: the first group (G1) was daily fed on a standard diet and kept as the control group, the second group (G2) was daily fed on the same ration plus 1 g of TT per animal, the third group (G3) was daily fed on the same ration plus 1 g of TT supplemented with 60 mg of vitamin E per animal (orally), and the fourth group (G4) was daily fed on the same ration with the addition of 60 mg of vitamin E per animal (orally).

2.2. Drug and Treatments

At the beginning of the experiment, clinical tests were carried out on all animals, including mucous membranes, skin, scalp, and feed intake evaluations. Moreover, the animals should have been free from any abnormalities and infectious. The rabbits were treated with anti-ecto- and anti-endo- parasites by subcutaneous administration of 0.1 ml/animal of ivermectin, oxytetracycline 20%, Clostridium Chauvoei-Septicum-Haemolyticum-Novyi-Sordellii, and perfringens types C and D.

2.3. Histological Examination

This study was carried out on the testis and epididymis of rabbits which were obtained immediately after slaughtering two animals from each group. The left testes and proximal epididymis were dissected and fixed in 10% formalin (10 ml of 37% formaldehyde+90 ml of distilled water+4 g of sodium phosphate monobasic+6.5 g of sodium phosphate dibasic) in labeled containers. After washing out the residual blood and debris with normal saline, the tissue was trimmed into pieces of 5 mm, and the specimens were washed with tap water for 4-6 h to remove the formalin solution and the histological evaluation was performed according to the previously published method by Pousty and Adibmoradi (7).

3. Results

The recorded data showed that the animals in the control group had normal testis tissue (architecture of seminiferous tubules) (Figure 1), with no pathological lesion on each part of the testis and central tubules. Testicular structure of rabbits revealed a thick spermatogenic epithelium in the interstitial tissue in seminiferous tubes.

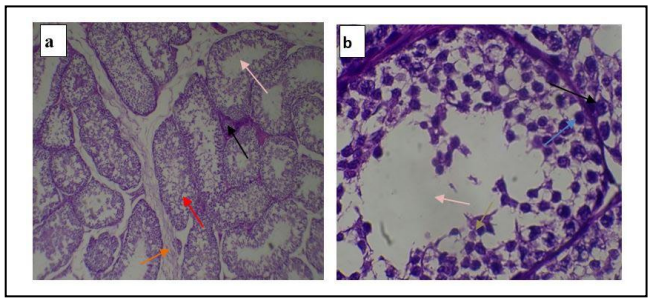

Figure 1. Histological section of testis in the control group (a and b testis structure) showing normal architecture of seminiferous tubules (red arrow), Lumen (pink arrow), interstitial tissue (orange arrow), Spermatogonia (blue arrow), Sertoli cells (green arrow) and Leydig cells (black arrow) with no abnormal lesion (H & E a×40, b×400)

The epididymal duct is lined by a pseudostratified columnar epithelium, with tall columnar cells and shorter basal cells giving the appearance of two rows of nuclei. This pseudostratified epithelium lines not only the epididymis but also the vas deferens. The columnar cells are characterized by apical stereocilia (giant microvilli, not true cilia; "stereo" means "solid"). The basal cells are believed to be precursors of the columnar cells (Figure 2).

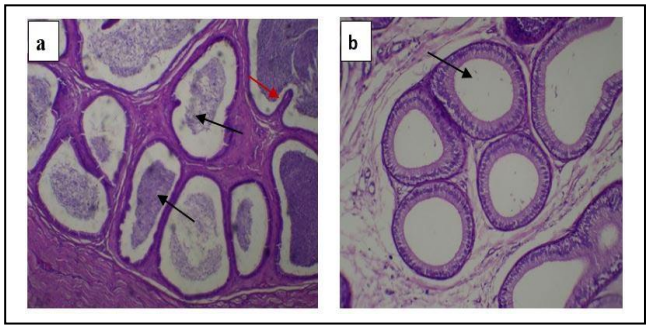

Figure 2. Histological section of epididymis (a-caput; b-cauda) in control group with showing normal architecture Epididymal tubule lined (black arrow) by ciliated pseudostratified columnar epithelium with numerous sperms (red arrow) in the lumen. With no abnormal lesion (H & E a×100, b×100)

3.1. *Tribulus terrestris* Group (G2)

3.1.1. Testis Tissue

The results showed that the animals in the *TT* group did not have any pathological lesions on each part of the testis and central tubules (Figure 3). Testicular structure of rabbits revealed a thick spermatogenic epithelium. Moreover, interstitial tissue in seminiferous tubes was also observed. In addition, there was a thick epithelium containing whole cell mosaic spermatogenesis from the spermatogonia stage to matured spermatozoa production. In rabbits that received 1 g/head/day of TT for 8 weeks, the seminiferous tubules were fused to gather and be filled with spermatogonial cells.

The epididymis duct of the *TT* group was lined by a pseudostratified columnar epithelium, with tall columnar cells and shorter basal cells giving the appearance of two rows of nuclei. This pseudostratified epithelium lines not only the epididymis but also the vas deferens. The columnar cells are characterized by apical stereocilia (giant microvilli, not true cilia; "stereo" means "solid"). Some of the epididymal

tubules of TT group rabbits were highly dilated with atypia or mitotic activity. These alterations were observed in the epididymis of rabbits exposed to TT as feed additives (Figure 4).

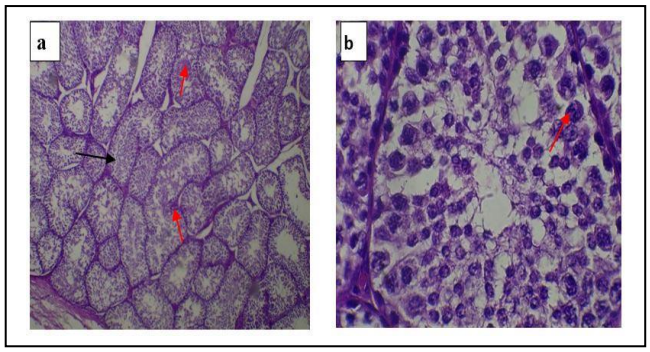

Figure 3. Histological section of testis in group treated with *Tribulus terrestris* showing active spermatogenesis (red arrow) in most seminiferous tubules (black arrow) of testis for all layers with no abnormal lesion (H & E a×40, b×400)

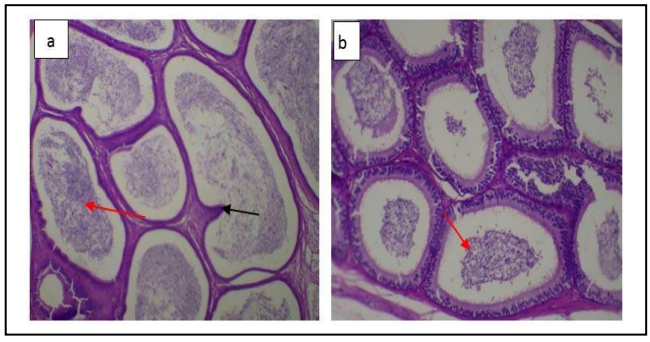

Figure 4. Histological section of epididymis in group treated with *Tribulus terrestris* showing normal architecture folded of epithelia lining (black arrow) epididymis with fulling with spermatozoa (red arrow) in the lumen (H & E a×100, b×100)

The results showed that the animals in the TT group had a tissue of testis architecture of seminiferous tubules, with no pathological lesion on each part of the testis and central tubules (Figure 5). Testicular structure of rabbits revealed a thick spermatogenic epithelium. Moreover, interstitial tissue was observed in seminiferous tubes. In addition, there was a thick epithelium containing whole cell mosaic spermatogenesis from stage spermatogonia to stage sperm. In rabbits that received 1 g/head/day of TT and 60 mg/head/day of vitamin E for 8 weeks, the seminiferous tubules were fused to gather with an increase in their diameters.

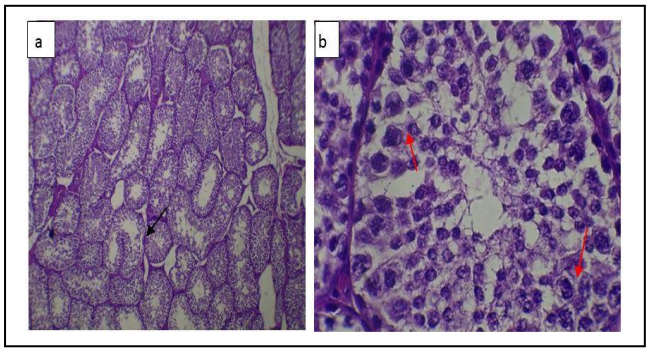

Figure 5. Histological section of testis in group treated with *Tribulus terrestris* and vit E showing few number of active spermatogenesis (black arrow) in most seminiferous tubules of testis for all layers with the degenerative change of some seminiferous tubules and less number of Sertoli with a very large number of abnormal space between neighboring Sertoli cells (red arrow) (H & E a×40, b×400)

The epididymis duct of TT with vitamin E group was lined by a pseudostratified columnar epithelium, with tall columnar cells and shorter basal cells giving the appearance of two rows of nuclei. This pseudostratified epithelium lined not only the epididymis but also the vas deferens. The columnar cells are characterized by apical stereocilia (giant microvilli, not true cilia; "stereo" means "solid"). Some of the epididymal tubules of rabbits in the TT with vitamin E group were epididymal epithelium alterations and highly dilated with atypia or mitotic activity. This alteration was observed in the epididymis of rabbits exposed to TT with vitamin E as feed additives (Figure 6).

The results showed that the animals in the vitamin E group revealed tissue of the testis (architecture of seminiferous tubules) (Figure 7), with no pathological lesion on each part of the testis and central tubules. Moreover, the testicular structure of rabbits revealed a thick spermatogenic epithelium and an interstitial tissue in seminiferous tubes was also observed. Besides, there was a thick epithelium

containing whole cell spermatogenesis from stage spermatogonia to stage sperm. In rabbits that received 60 mg/head/day of vitamin E for 8 weeks, seminiferous tubules fused to gather with an increase in diameter.

The epididymis duct of the vitamin E group was lined by a pseudostratified columnar epithelium, with tall columnar cells and shorter basal cells giving the appearance of two rows of nuclei. Some of the epididymal tubules of vitamin E group rabbits were epididymal epithelium alterations and dilated with atypia or mitotic activity. This alteration was seen in the epididymis of rabbits exposed to vitamin E as feed additives (Figure 8).

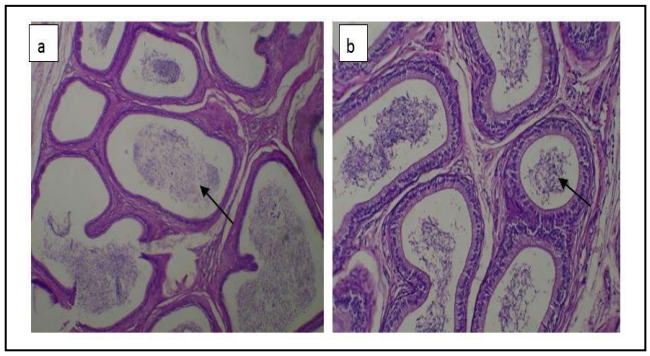

Figure 6. Histological section of epididymis in group treated with *Tribulus terrestris* and vit. E A showing a slight number of spermatozoa in the lumen (black arrow) (H & E a×100, b× 100)

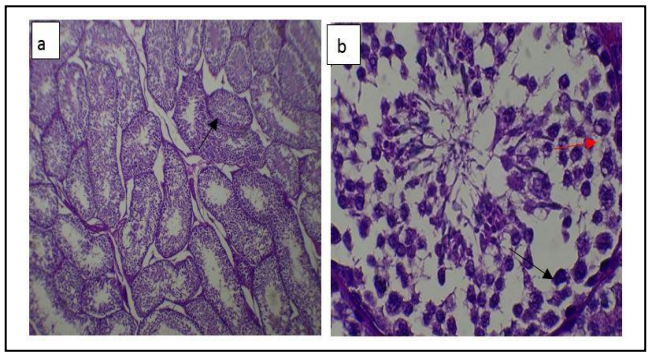

Figure 7. Histological section of testis in group treated with it. E showing few number of active spermatogenesis (black arrow) in most seminiferous tubules of testis for all layers with the degenerative change of some seminiferous tubules and less number of Sertoli with abnormal space between neighboring Sertoli cells (red arrow) (H & E a×40, b×400)

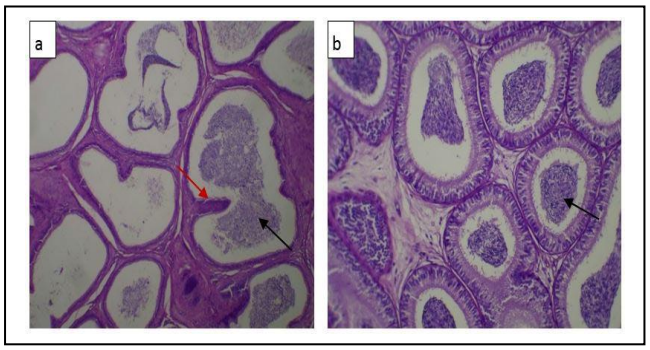

Figure 8. Histological section of epididymis in group treated with vit E A showing normal architecture (red arrow). With slight number of spermatozoa in the lumen (black arrow) (H & E a×100, b×100)

4. Discussion

Based on the results of the histological examination of the testes and epididymis of the control group, the testis section showed normal morphology and absent lesion (Figures 1 and 2). The histological section of all treated groups with moderate alteration reflected the effect of the nutritional addition of TT and vitamin E on the function and activity of the testes. This is due to the role of the herb TT and vitamin E in the improvement of fertility, as indicated by previous researchers (8, 9).

The second group was characterized by extensive epithelium containing whole cell mosaic spermatogenesis from stage spermatogonia, seminiferous tubules fused to gather and be filled with spermatogonial cells (Figures 3 and 4). The reason for this may be the effects of the active substances of the pole plant, including substances that stimulate hormonal activity as well as antioxidants, as these substances improve the activity and effectiveness of the secretory testicular tissue. This result is consistent with those of previous research performed on the use of the TT plant, which indicated that TT extract exhibited enhancement of dihydrotestosterone levels, sperm concentration, and motility in infertile men in a clinical trial (10).

Similarly, in patients with partial androgen deficiency, usage of TT resulted in a significant

increase in serum total, free testosterone, and erectile function (11). Gauthaman and Ganesan (12) mentioned that a TT extract of 5 mg/kg taken orally for eight weeks enhances intracavernous pressure and sexual behaviors in castrated rats and has an aphrodisiac effect due to increased androgen levels. In this study, there were no significant differences among the groups, in terms of the free testosterone levels. However, a higher value or concentration of testosterone was recorded in the experimental groups, compared to the control group, based on the results of histological cutting.

The histopathological sections indicated in the 2nd and 3rd groups were due to the effects of TT and vitamin E which positively influenced the development of gonadal tissue (Figures 5 and 6). Administration of TT at the dose of 2.50 mg/kg of body weight positively influenced the development of gonadal tissue. Moreover, in the aforementioned group, the population of cells in the lumen of the seminiferous tubules was higher in comparison with the control group (13). Moreover, sperm cells were noticed in the lumen of the seminiferous tubules of the rabbits receiving TT in the dose of 5.00 mg/kg of body weight according to Abadjieva, Grigorova (13).

The TT herb is also effective in the maintenance of testicular tissue against a fatty diet, according to Akdoğan, Nasir (14) due to the remedial effects of TT extract against the testicular damage induced by a high cholesterol diet in rats. At an optical microscopy level, no atrophic changes were noticed in testicular tissues. The overdose of steroid saponins from the additive probably inhibits the proliferation of Leydig cells and the production of testosterone, which negatively affects the differentiation of spermatocytesTremblay (15).

The present investigation revealed that the higher doses of TT as extraction (7.50 mg/kg and 10 mg/kg of body weight) provoked hypertrophia in the epididymis (Figure 6). According to McGavin and Zachary (16), probably the higher doses of herb affect the reproductive tissues that were sensitive to the extract of TT and led to cell proliferation. Therefore, it is important to determine the correct dosage of VemoHerb-T as a feed additive in the diet of field animals in order to obtain the positive effects of the herb.

Abadjieva, Grigorova (13) indicated that TT can affect the body weight of rabbits and testicle weight. It should be noted that this effect is dose-dependent; therefore, the low doses (2.50 mg/kg of body weight) used in the present investigation can be relied upon as the most effective and harmless doses, as they ensure maximum body weight gain as well as the normal development of histological structures in the testes and epididymis. High doses of the tested product may cause abnormal morphological changes in the testes and epididymis that can affect spermatogenesis and lead to decreased sperm quality in mature rabbits.

Regarding the improvement in animals that took vitamin E in terms of this testicular tissue (Figures 7 and 8), it depends on the importance of the vitamin and its potential protective effects against the harmful effects of free radicals in the systems and tissues of various organs. This effect of vitamin E and other antioxidant vitamins has become the subject of increased interest recently. Vitamin E is a powerful antioxidant and fat-soluble vitamin that is present in cell membranes to maintain their biological integrity (17). Vitamin E inhibits the production of reactive oxygen species (ROS); accordingly, it effectively prevents germ-cell damage and destruction resulting from this oxidation process (18, 19). Indeed, Hsu, Liu (19) reported that vitamin E prevents the mobility loss of spermatozoa by causing a reduction in ROS production. However, the present study was limited to mild histological and ultrastructural changes in the testis. In addition to these effects, a number of biochemical parameters can be monitored after treatment with another antioxidant agent, ascorbic acid, which should also provide more information regarding the system examined in this study.

Natural antioxidants, such as vitamin E, are widely used as dietary supplements due to their capacity to protect tissues from oxidative stress caused by ROS (20, 21). In addition to the importance of vitamin E at

the level of testicular cells, vitamin E supplementation can help repair Leydig cells and reduce damage caused by a high-fat diet (HFD). Vitamin E supplementation also significantly reduced fat weight, adiposity index, and lipid profile in obese rats and healthy mice. Although further studies are needed to elucidate the mechanism of testicular damage caused by elevated HFD and the mechanism of vitamin E repair, these results suggest that vitamin E supplementation for individuals consuming HFD could be beneficial (22).

The other part of the testes is the epididymis in all groups of rabbits, which is essentially a long, highly coiled tube. The epididymis is the initial part of the duct which eventually continues as the vas deferens. This tube is surrounded by smooth muscles and embedded within the stroma of the soft blood vessels, the epididymis is somewhat similar to the testicle. However, in the epididymis, the tubes are larger, fewer in number, and less crowded; moreover, the tubule epithelium lacks germ cells. The stroma lacks both dense fibrous connective tissue septa and smooth muscle cells (23).

Results of the current study showed that exposure to vitamin E and TT causes the dilation of the epididymis, which is an increase in the number of cells. This may be the result of an increase in the rate of cell division; therefore, it may improve the function of the epididymis in the storage and maturation of sperm (16, 24). This event may indicate that the ability to generate the epithelium is fine and may represent a type of epididymis response to feed additive caused by this plant as well as vitamin E. As shown in the results of this study, the presence of sperm cells in masses in the lumen of the epididymis may be more effective in the treatment of aggregation in the epididymis and may be caused by adhesion between these cells and/or natural intracytoplasmic bridges of ligands that give more maturity (25).

The location of the sperm cells in the cytoskeleton is dependent on testosterone as the junction between the sperm-producing cells dispersion in the absence of or lack of testosterone, as indicated by some studies (26, 27). It should be noted that the level of testosterone is low in the presence of a disorder of Leydig cells (27). On the basis of the results of the current study, it can be concluded that daily use of TT at a dose of 1 g and vitamin E through food for 60 days has a positive effect on testicular nevus and epididymis, which may affect raising fertility rates in males.

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Authors' Contribution

Study concept and design: K. R. H.
Acquisition of data: K. R. H.
Analysis and interpretation of data: J. E. Q. A.
Drafting of the manuscript: J. E. Q. A.
Critical revision of the manuscript for important intellectual content: J. E. Q. A.
Statistical analysis: K. R. H.
Administrative, technical, and material support: K. R. H.

Ethics

The animal studies were approved by the ethics committee of the University of Baghdad, Baghdad, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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