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The Protective Effect of Vasopressin on Sperm Parameters and Histopathology of Testicular and Epididymal Tissue in a Rat Model of Testicular Torsion-Detorsion

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TESTICULAR torsion-detorsion (TD) can lead to infertility. In the present study, an attempt was made to investigate the protective role of vasopressin (AVP) on histopathological changes in a rat model of testicular TD.We divided 50 rats into the following five groups (n=10 per group): healthy control, TD control, sham, TD + 0.1 µg/kg AVP, and TD + 0.2 µg/kg AVP. After 24 hours, the rats were anesthetized and their testis were removed. The effects of AVP on Morphologic manifestations were evaluated using H&E staining. Furthermore, sperm quality (sperm cell count and vitality) and spermatogenesis (tubule differentiation index (TDI), spermiation index (SPI), and repopulation index (RI)) were assessed. There were significant improvements in histopathological changes (epididymis and seminiferous tubules injury, the interstitial tissue edema). Furthermore, AVP could significantly enhance the parameters of sperm quality such as sperm cell count and vitality. Moreover, a significant increase was observed in TDI, SPI and RI. Our results showed that AVP could attenuate factors involved in the pathogenesis of testicular TD, including histopathological changes, sperm quality and spermatogenesis. Hence, the use of AVP is advisable for the amelioration of damage caused by the testicular TD.

Keywords: Vasopressin, Sperm cell, Testicular Torsion-Detorsion, Testis, Rat.

Introduction

The rotation of the testicle around the spermatic cord (its vascular axis) is called testicular torsion [1]. This condition is one of the medical emergencies and needs immediate action for diagnosis and treatment [2]. Delay in treating the testicular torsion, particularly after 4 hours of spermatic cord closure can cause irreversible damage to the testicles. The testicular torsion occurs at different ages, but it is most common in young men and between the ages of 12 and 20. The evidence have revealed that one in every 4000 men has experienced the testicular torsion in their life [3]. Researchers have exhibited that various diseases such as tumors of testicle, cryptorchidism and testicles with horizontal lie play role in the development of testicular torsion

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[4]. The testicular torsion could lead to loss of the living tissue through reduction or even complete stoppage of blood flow to the testicles and other contents of the scrotum. Spermatic cord twisting causes progressive obstruction of the testicular veins, interstitial edema, arterial occlusion, and subsequently ischemia of the testicles [3]. There are a number of reasons why blood does not reach the testicular tissue, which in turn causes both the lack of oxygen to the tissue, which causes hypoxia and the accumulation of waste products [5]. Reduction or cessation of blood flow to the testicles increases the production of reactive oxygen species (ROS) in the testicles [6]. ROS have a destructive effect on DNA, as well as disrupting the function of proteins and the lipid peroxidation of membrane [7-9]. Mammalian sperm are rich in unsaturated fatty acids, so they are very sensitive to ROS invasion, which results in reduced fertility in males [10]. Re-establishment of blood flow following testicular detorsion increases blood flow to ischemic tissue and intensifies the production of ROS, activation of inflammatory cells and mediators and finally cell death and apoptosis [11]. Although standard treatment for testicular TD injury is surgery, some interventions have recently been suggested to reduce injury; including ROS scavenging agents, anti-inflammatory agents, natural products, and synthetic drugs [4]. Antioxidants are able to protect cells and tissues against various diseases particularly disorders caused by ROS [12-16]. Therefore, prescribing antioxidants to reduce oxidative stress seems important and necessary [17-22]. The researchers have been described the antioxidant role of vasopressin previously [23]. Arginine Vasopressin, or antidiuretic hormone (ADH), is a non-A peptide hormone which is released from the posterior pituitary of mammals, including humans. AVP is structurally similar to the oxytocin, and this molecular similarity can explain their relative functional similarity [4]. Previous studies have revealed the protective effect of AVP against ischemia-reperfusion (I/R) injury in various tissues [4, 23]. Therefore, the present study was conducted to evaluate the protective effect of AVP on semen criteria, testicular and epididymal histopathological changes in experimental testicular TD male rat.

Materials and Methods

Ethical Approval

The Animal Ethics Committee of Lorestan University of Medical Sciences approved all the experimental protocols (IR.LUMS.REC.1398.067).

Chemicals

The Human Tubal Fluid Medium (HTF) and Bovine Serum Albumin (BSA) were obtained from Sigma-Aldrich Company (USA).

Study Design

The experimental procedures were conducted at Razi Herbal Medicines Research Center, affiliated to Lorestan University of Medical Sciences, Khorramabad, Iran. A total of fifty adult male Sprague-Dawley rats (aged 8-12 weeks; weight: 220±10 g) were purchased and kept in standard cages. The rats were housed under appropriate environmental conditions that included a temperature of 22°C, 12 h light-dark cycles, and with free access to standard laboratory food and tap water. Razi Institutional Animal Care Committee at Lorestan University of Medical Sciences confirmed all of the study protocols and experimental procedures. The rats were randomly divided into the following five groups (10 rats per group): control (healthy) rats without testicular TD surgery (group 1); sham (healthy) rats got testicular surgery without TD (group 2); control (TD) rats got testicular TD surgery without receiving any treatment (group 3); TD + AVP rats receiving 0.1 µg/kg AVP (group 4), and TD + AVP rats receiving 0.2 µg/kg (group 5).

Testicular TD Surgery

First, the rats were anesthetized via intraperitoneal injection of ketamine (50 µg/kg) and xylazine (5 µg/kg). Then, the prepared doses of vasopressin (0.1 and 0.2 µg/kg) were injected through the tail vein. After 15 minutes, the induction of ischemia model was performed. Testicular TD surgery (I/R) was carried out using 720-degree twisting of testicles for 30 minutes in accordance to previous studies [24].

Sample Preparation

After 35 days the treatment period, all animals were anesthetized after ketamine injection and subsequently killed by Co_2 gas inhalation. After weighing the rats, the testicles were removed by dissection and the tail of the epididymis was separated from the testis and after making several incisions placed into a petri dish at the same temperature as the incubator containing 1 ml of HTF medium and 4 mg mL-1 bovine serum albumin (BSA; Sigma, St. Louis, USA) [25]. In order to remove sperm from the tail of the epididymis, the Petri dish was placed in an

incubator (37 °C, 5% Co_2). After 30 minutes, the sperm cells were removed and dispersed in the HTF medium. Finally, the testicular and epididymis tissue samples were transferred to 10% formalin fixation solution. After fixation, dehydration of the testicular and epididymis tissue samples was conducted using ascending concentrations of ethanol. Then, the samples were embedded in paraffin, cut into 4 µm thick, and stained with hematoxylin–eosin (H&E), respectively. Finally, the sections were examined under an optical microscope (Olympus Company, Japan) at a magnification of 40× [4].

Histomorphometric Evaluations of the Testicles

To count spermatogenesis cells (spermatogonia type A, spermatogonia type B, primary spermatocytes, spermatids and spermatozoa), three slides were prepared from each testicle in all groups. Three fields of view from each slide were randomly selected and examined. The number of active Sertoli cells (in which each of the evolving sperm accumulations in the seminal vesicles was considered as an active Sertoli cell) was also counted in three seminiferous tubules in each tissue sample [24]. Moreover, the average number of Leydig cells was counted per 1mm^2 of testicular tissue. Furthermore, the sections were examined for any edema, hyperemia, and irregularity of cell arrangements in the seminiferous tubules, and for fibrosis, loose connective tissue, and the presence of the sperm in the epididymis. Score of edema was shown as a qualitative method used previously [24].

Evaluation of Sperm Characteristics

Sperm Count

A 1:20 dilution of sperm sample was prepared. This was accomplished by adding of 10 µl of sperm sample to 190 µl of the HTF medium containing BSA. Then, 10 µl of the solution was placed on a Neubauer chamber to count the sperms [25].

Evaluation of Sperm Vitality

To evaluate the vitality of the sperms, the Eosin-Nigrosin staining was used. For this purpose, 20 µl of sperm sample was mixed with an equal volume of eosin solution (20 µl) on a slide. After 20-30 seconds, 20 µl of Nigrosin solution was added. Then, a smear was made and let air dry. Finally, the slides were examined for determination of the percentage of live sperm (colorless) and dead sperm (stained) were studied an optical microscope at a magnification of (10-40×).

Evaluation of Spermatogenesis in Testicular Tissue

To evaluate spermatogenesis, an assessment of the TDI, SPI, and RI was conducted in one hundred seminiferous tubules of each testicle. To determine TDI, the percentage of the seminiferous tubules that had three or more spermatogenesis cells differentiated from spermatogonia A cells was calculated. The dark and light cytoplasm cells are categorized as B and A types spermatogonium cells, respectively.

The SPI indicates the percentage of seminiferous tubules with normal spermiogenesis (containing sperm). To determine RI, the mean percentage of active cells to inactive spermatogonia was examined [25].

Statistical Analysis

Statistical analysis was performed using SPSS (Version 26; SPSS Inc., Chicago, Illinois, USA). The results were expressed as Mean ± SD. The data were analyzed by one-way analysis of variance for comparing the pair groups independently and followed by Tukey's multiple comparison test. $P<0.05$ indicated statistical significance.

Results

Testicular Histological and Morphological Parameters

Histopathology of Epididymis Tissue

Histological changes in the epididymis were studied by hematoxylin-eosin staining. In the control group (Group I), the tissue was normal and the lumen of the epididymis was filled with sperm cells (Fig.1). No histopathological changes were observed in the epididymal epithelium and connective tissue. The thickness of the epithelial was normal and no edema and hyperemia were seen. The epididymal study in the sham group did not show any significant difference compared to the control group.

The histological analysis of epididymis showed damage, thickness, swelling, lack of spermatogenesis in the TD group. Generally, no sperm and connective tissue were replaced (Fig. 2).

There was also a low irregularity in the epididymis epithelial cell arrangements and hyperemia in the TD+0.1 µg/kg AVP group. Likewise, low sperm was observed inside the tubule (Fig. 3).

The irregularity in the cell arrangements, cell destruction and loss was observed in the TD+0.2 µg/kg AVP group, but was very low compared to the TD+0.1 µg/kg AVP group (Fig. 4).

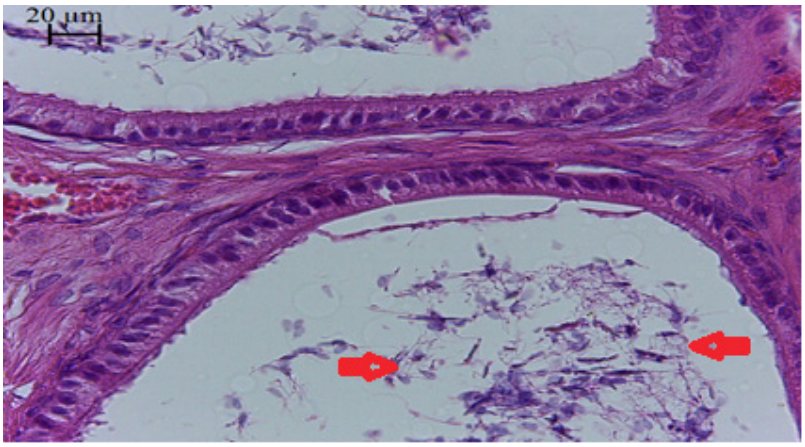

Fig. 1. Photomicrograph of the body of epididymis in the control and sham groups, showing a normal histological structure. The red arrows show sperm (H&E) staining.

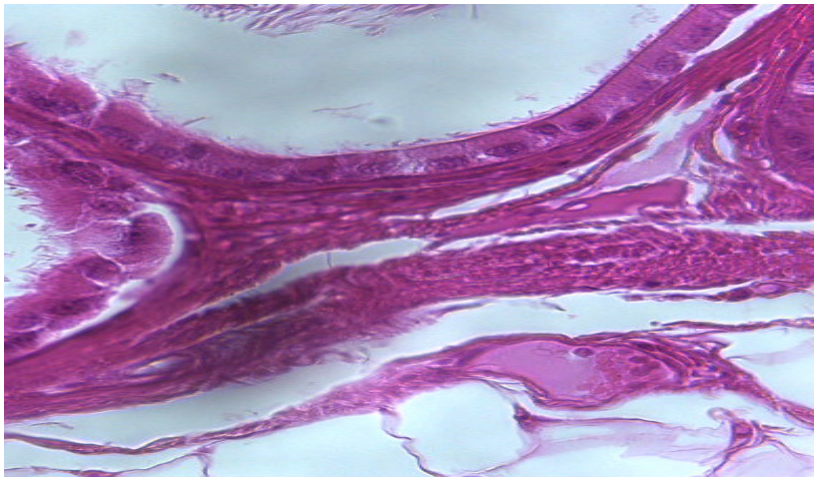

Fig. 2. Photomicrograph of the body of epididymis in in the TD group, Epithelium and connective tissue were abnormal and no sperm cells were observed (H &E) staining.

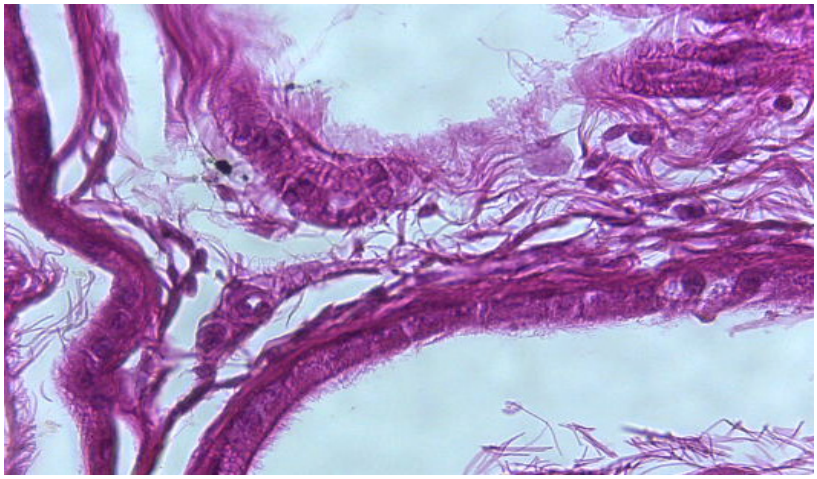

Fig. 3. Photomicrograph of the body of epididymis TD+0.1 µg/kg AVP group, Epithelium and connective tissue were abnormal and low sperm cell population was observed (H &E) staining.

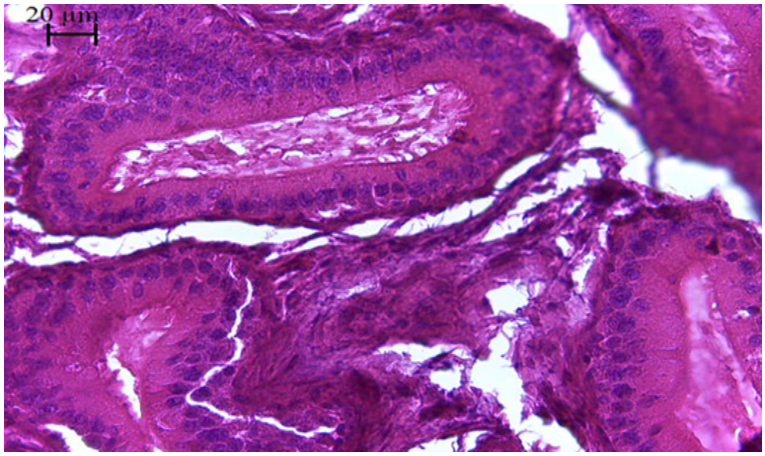

Fig. 4. Histopathological changes of the body epididymis in the TD+0.2 µg/kg AVP group (H & E) staining.

Histopathology of Testicular Tissue

The histological results of the testicular tissue exhibited normal histology, routine staining, and normal capsule thickness in the control group. Moreover, the testicular tissue was free of any edema and hyperemia. The seminiferous tubules were seen with normal cellular arrangement and mostly TDI positive. It was observed that testicular tissue was almost normal and close to the control group, and the TDI of most tubules was positive and contains sperm. Hyperemia and swelling were observed in some areas of testicular tissue in the sham group. The irregularity in the cell arrangements of the seminiferous tubules, swelling and hyperemia were seen in the TD group. The TDI of the seminiferous tubules was mostly negative. The tubules were destroying and cell loss occurred. There were no active tubules containing sperm and seminiferous tubules in some animals and replaced with connective tissue. The irregularity in the cell arrangements of the seminiferous tubules, and hyperemia were seen obviously in the TD+0.1 µg/kg AVP group. Negative TDI was seen in most tubules. Moreover, the testicular tissue had completely lost its histology and the connective tissue had been replaced in some animals. Low testicular tissue damage, hyperemia and swelling were observed in the TD+0.2 µg/kg AVP group. However, TDI of the most tubules were positive and active spermatogenesis tubules were visible.

This figure indicates the presence of edema in the interstitial tissue of the TD group. Furthermore, the germinal epithelium had a large rupture in most of the seminiferous tubules. The spermatogonia cells attachment to the basal layer remains in some tubules and other cells were isolated and stretched toward the middle cavity of the tubules. A considerable distance was observed between most of the spermatogenesis cells in a number of seminiferous tubules. This rupture was common among spermatogenesis cells in all samples of this group (Fig. 5).

The results of the histological analysis revealed a relative improvement in the seminiferous tubules of the TD+0.1 µg/kg AVP group. However, a rupture was observed between spermatogenesis cells in some seminiferous tubules. The edematous response also decreased in interstitial tissue compared to the control group (Fig. 6).

It was observed that increasing the dose of treatment in the TD+0.2 µg/kg AVP group significantly reduced the interstitial tissue edema. Furthermore, fewer tubules showed intercellular rupture in the spermatogenesis cell of seminiferous tubules (Fig. 7).

It was shown that there was no significant edema in the interstitial tissue of the control and sham groups. The seminiferous tubules had a continuous germinal epithelium in most of the samples of this group, but in some parts of the testicular tissue, discontinuity was observed in the germinal epithelium (Fig. 8).

Leydig and Sertoli Cell Count

The results showed that the mean number of the Leydig cells per 1 mm^2 of testicular tissue decreased significantly following TD compared to other groups ($P < 0.05$). However, there was a non-significant increase in the number of the Leydig cells in TD+VAP treated group (Table 1).

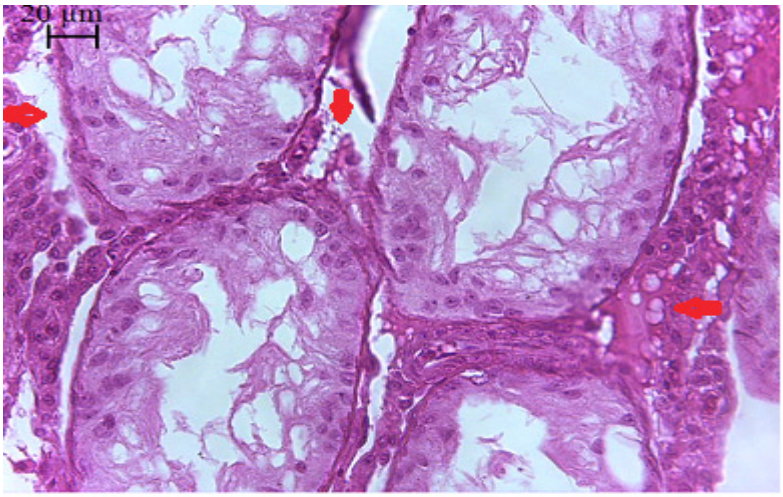

Fig. 5. Histopathological changes of the seminiferous tubules in the TD group. The red arrows show edema (H&E) staining.

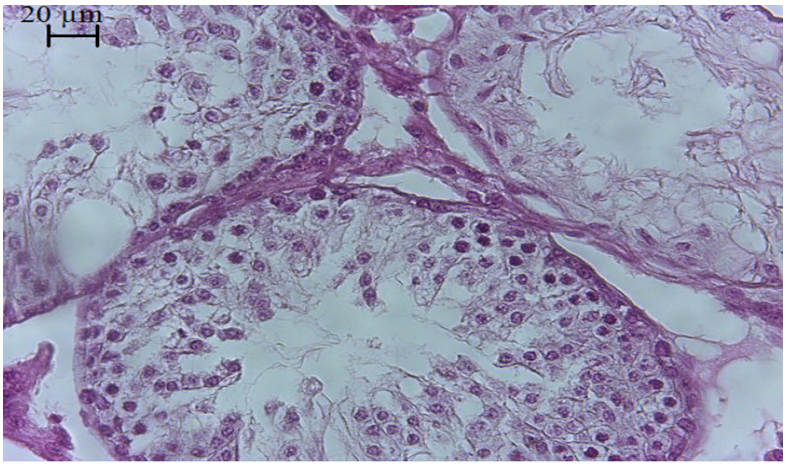

Fig. 6. Histopathological changes of the seminiferous tubules in the TD+0.1 µg/kg AVP group (H&E) staining.

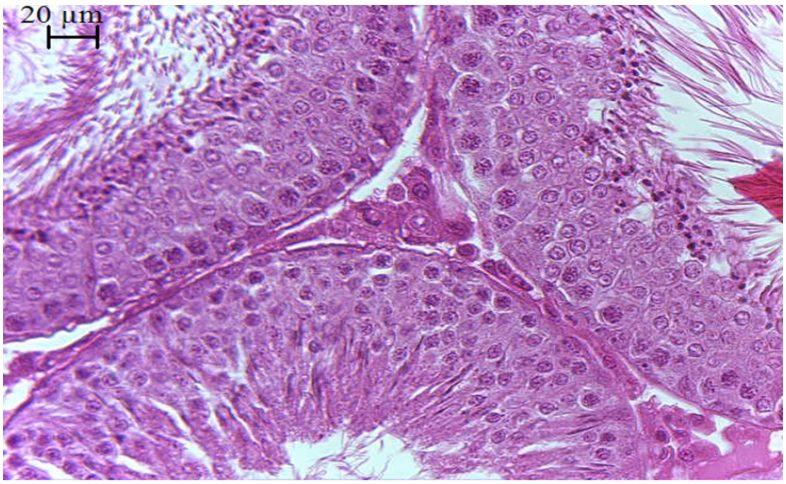

Fig. 7. Histopathological changes of the seminiferous tubules in the TD+0.2 µg/kg AVP group (H&E) staining.

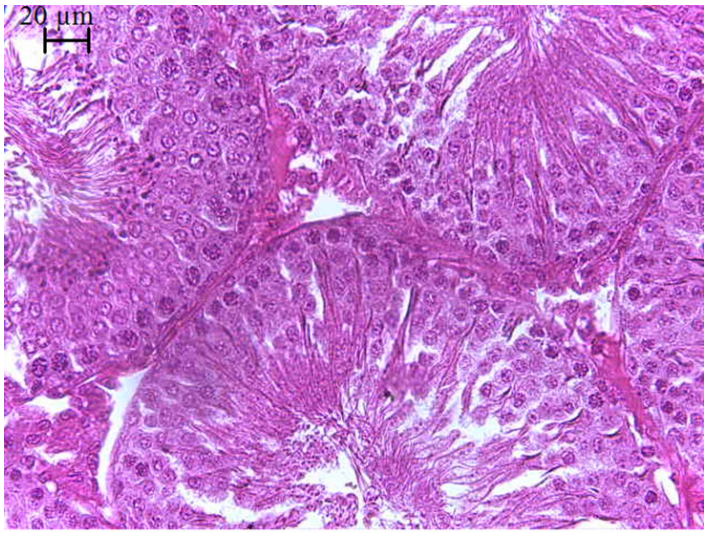

Fig. 8. Histopathological changes of the seminiferous tubules in the control group (H&E) staining.

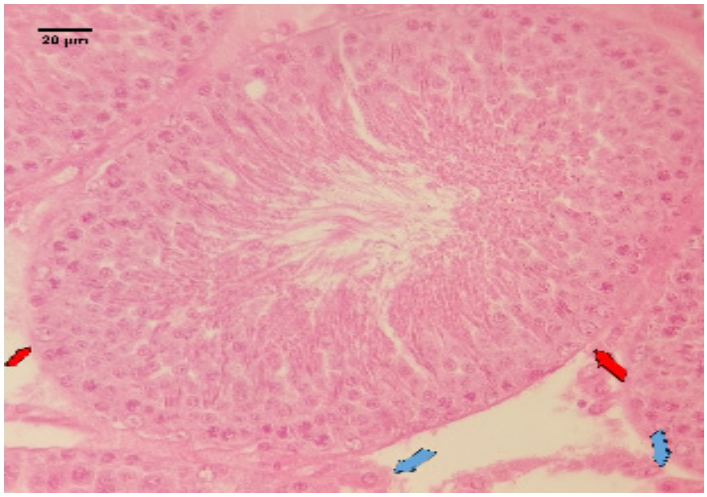

Fig. 9. Photomicrograph of the Leydig and Sertoli Cells. The red and blue arrows show Sertoli, and Leydig cells, respectively.

The findings exhibited that the mean number of the Sertoli cells decreased significantly in the wall of the seminiferous tubules of TD group compared to the control group ($P < 0.05$). Furthermore, the number of the Sertoli cells could increase significantly following treatment with 0.2 µg/kg AVP compared to the TD group (Table 1) (Fig. 9).

Spermatocyte Cell Count

The mean number of the spermatocytes decreased significantly in the TD group compared to the control group ($P < 0.05$). Furthermore, the number of the spermatocytes raised significantly following treatment with AVP in the TD+0.2 µg/kg AVP group compared to the TD group ($P < 0.05$) (Table 1).

Spermatid Cell Count

The mean number of the spermatids diminished significantly in the TD group compared to the control group ($P < 0.05$). Furthermore, the number of the spermatids elevated significantly in the TD+0.2 µg/kg AVP group compared to the TD group ($P < 0.05$) (Table 1).

Spermatozoa Cell Count

The mean number of the spermatozoa reduced significantly in the TD group compared to the control group ($P < 0.05$). Furthermore, the number of the spermatozoa increased significantly following treatment with AVP in the TD+0.2 µg/kg AVP group compared to the TD group ($P < 0.05$) (Table 1).

TABLE 1. The mean number of the different types of the testicular cells per 1 mm².

Group	Spermatogony A	Spermatogony B	Spermatocyte	Spermatid	Spermatozoa	Leydig cells	Sertoli Cells
Control	39.88±5.710 [a]	51.66±8.514[a]	94.33± 21.777[a]	183.44±26.149[a]	173.44±28.636[a]	27±3.807[a]	42.88±3.551[a]
TD+ AVP 0/2	25.33±5.545 [b]	35.66±5.408[b]	91.77±22.900[a]	157.55±30.590[a]	136±30.385[a]	23.77±4.146[ab]	39±3.708[a]
TD+ AVP 0/1	16.33±4.555 [b]	15.44±8.973[c]	40.66±19.956[b]	65.22±21.516[b]	41±24.377[b]	18.55±3.468[b]	21±9.096[b]
TD	21.44±8.001 [b]	16.66±9.733[c]	28.44±14.423[b]	46.88±9.662[b]	17.77±10.802[b]	12.77±4.024[cb]	14±7.193[b]
Sham	36.55±4.746 [a]	46.116.622±[ab]	105.66±17.860[a]	166.88±27.080[a]	197.55±31.024[a]	26.44±3.940[a]	43.88±6.990[a]

[a-c] Similar letters show non-significant difference.

Spermatogenesis in Testicular Tissue

TDI

As shown in Table 2, the results of the evaluation of TDI% revealed that this parameter of spermatogenesis decreased significantly in the TD group compared to the control group (TDI%=23% vs. TDI%=98%) (P<0.05). Moreover, the TDI% elevated significantly in the TD+0.2 µg/kg AVP group compared to the TD group (TDI%=82% vs. TDI%=23%) (P <0.05).

RI

As shown in Table 3, the results of the evaluation of RI indicated that this parameter of spermatogenesis decreased significantly in the TD group compared to the control group (0.798±0.504 vs. 1.305±0.199) (P <0.05). Moreover, the RI elevated significantly in the TD+0.2 µg/kg AVP group compared to the TD group (1.272±0.198 vs. 0.798±0.504) (P <0.05).

SPI

According to Table 3, the results of the evaluation of SPI indicated that this parameter of spermatogenesis decreased significantly in the TD group compared to the control group (20 vs. 98) (P <0.05). Moreover, the RI elevated significantly in the TD+0.2 µg/kg AVP group compared to the TD group (95 vs. 20) (P <0.05).

Sperm Characteristics

Sperm Count

As shown in Table 4, a significant decrease was observed in the mean number of sperms in the TD group compared to the control group (27.42±1.71 vs. 52.71±1.25) (P <0.05). Furthermore, there was a significant increase in the mean number of sperms in the TD+0.1 and 0.2 µg/kg AVP groups compared to the TD group (61.85± 1.57 and 63.57±1.90 vs. 27.42±1.71, respectively) more than the mean number of sperms in the control group (52.71±1.25) (P <0.05) (Fig. 9).

Sperm Vitality

According to Table 4, the results showed a significant elevation in the percentage of dead sperm of TD group compared to the control group (95± 0.004 vs. 2 ± 0.01) (P <0.05). Furthermore, a significant decrease was seen in the TD+0.1 and 0.2 µg/kg AVP groups compared to the TD group (83 ± 0.16 and 78 ± 0.29 vs. 95± 0.004, respectively) (P <0.05).

Discussion

Infertility can be a consequence of the testicular TD occurrence. The testicular TD disrupts blood flow to the testicular tissue and consequently develops the ischemia [4]. I/R is defined as a complex condition and as its name implies includes a stage of reduction or cessation of blood flow to the tissue and after a while reestablishment of the blood flow and may affect various organs of the body [26, 27]. Restoration of blood flow following the initial phase of ischemia causes a new wave of organ damage [28, 29]. Researchers have reported the loss of germ cells and the destruction of the epithelium of the seminiferous tubules after testicular I/R injury [4]. Surgery is currently the only treatment available to cure the testicular TD [3]. Several studies have been suggested application of various compounds with protective and ameliorative effects against the testicular TD in animal models [3, 4]. Vasopressin is a one of these compounds with proven effects against I/R injuries in various types of the organs of the body [23]. Therefore, the present study was conducted to investigate the protective effect of vasopressin on sperm parameters and histology of epididymis in rat model of the testicular TD.

Our results demonstrated that the AVP treatment has protective effects on TD damages in testicular tissue. The histological analysis of epididymis in testicular TD group showed tissue

TABLE 2. Comparison of the testicular TDI% and scores of edema in different groups.

Group	TDI %	Edema
Control	98 [a]	-
TD+ AVP 0/2	82 [a]	+
TD+ AVP 0/1	29 [b]	++
TD	23 [b]	+++
Sham	95 [a]	+

[ab] Different letters indicate significant difference between groups ($p < 0.05$).

TABLE 3. Comparison of the testicular RI in different groups.

Group	SPI	RI
Control	98	1.305±0.199[a]
Sham	86	1.434±0.203[a]
TD+ AVP 0.1	25	0.932±0.449[b]
TD	20	0.798±0.504[b]
TD+ AVP 0.2	95	1.272±0.198[a]

[ab] Different letters indicate significant difference between groups ($p < 0.05$).

TABLE 4. Comparison of the sperm characteristics in different groups.

Group Parameter	Control	TD	TD+ AVP 0.1	TD+ AVP 0.2	Sham
Sperm count (10^6/ml)	52.71±1.25[a]	27.42±1.71[b]	61.85± 1.57[c]	63.57±1.90[c]	39.28 ± 1.79[d]
Dead sperm %	2 ± 0.01[a]	95± 0.004[b]	83 ± 0.16[c]	78 ± 0.29[d]	7 ± 3.67[e]

All data were expressed as Mean ± SD.
[a-e] Similar letters show non-significant difference.

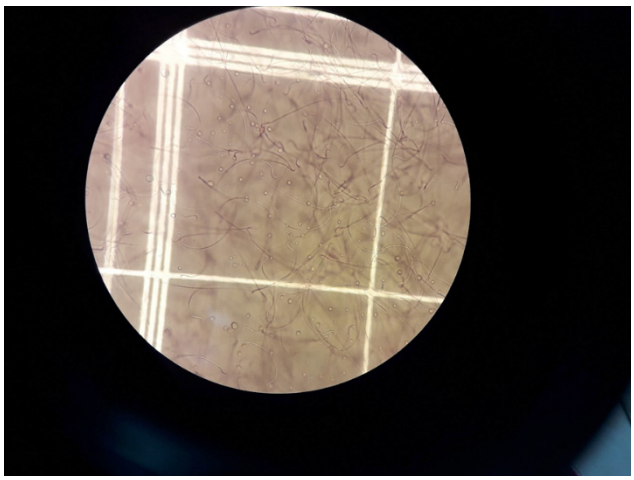

Fig. 9. Sperm count in the TD+0.2 µg/kg AVP group on a Neubauer chamber.

damages, and lumen of epidermal tubules were observed with low or no sperm count (Fig. 2). Furthermore, the result of our study showed that, the testicular TD destroyed the structure of seminiferous tubules and in some places, replacement of testicular tissue with connective tissue occurred (Fig. 5).

Likewise, the histopathological analysis showed the edema in the interstitial tissue and large rupture in the germinal epithelium. The testicular TD induced tissue damage has similarly been described in previous reports [3, 4]. Increased ROS generation mediated by the testicular TD can be propose as a possible mechanism for the testicular tissue damage [4]. The AVP treatment especially with a dosage of 0.2 µg/kg could improve above histopathological changes mediated by testicular TD compared to the TD group. The improvement in histological parameters could be due to the AVP antioxidant property via ROS scavenging. The results of our study complied with the study of Hasanvand A and the colleagues representing the protective effects of vasopressin on testicular TD in experimental animal model. The results of their histological analysis demonstrated the improvement in tissue changes of seminiferous tubules following administration of vasopressin [4]. Some pieces of evidence have approved the protective role of vasopressin against various types of I/R injuries. For instance, the attenuating effect of AVP against I/R injury in rat heart has been documented by Nazari and the colleagues. They concluded that the ameliorative effect of AVP against I/R injury returns to its capability to scavenge ROS. Furthermore, they represented this hypothesis that AVP exerts its cardio-protective effect via NO production [23]. According to that, it has been shown that several compound including natural antioxidants, drugs and hormones contribute to prevention of damage caused by testicular TD [4]. There are several supports from the evidence that induction of the testicular TD contributes to decrease in the mean number of the testicular tissue cells such as Leydig, Sertoli, spermatocyte, spermatid, and spermatozoa. Furthermore, decrease in the mean number of the sperms and increase in the percentage of dead sperm could occur following induction of the testicular TD [30]. The results of our study supported similar results for significant decrease in the above testicular tissue cells. Several recent studies have proposed the crucial role of ROS in decreasing the number testicular tissue cells and their vitality [31, 32]. In this study, AVP could significantly reverse changes in the number of sperms and increase their vitality. This protective effect could be due to the antioxidant property of AVP which protects the sperms against damage caused by ROS. The results of our study complied with the investigation of Bakhtiary et al. demonstrating the ameliorative effects of crocin on sperm quality in cyclophosphamide treated mice. They found that administration of crocin improved the parameters of sperm quality including sperm count and vitality via suppressing free radicals [33]. Zarei et al. reported the protective effects of vitamin E and Cornus mas fruit extract on cytotoxicity in sperms mediated by methotrexate in mice. Similar to the results of our study, they understood that antioxidant compounds exert protective effect on sperms via scavenging property [25]. Furthermore, it has been shown that intravenous injection of vasopressin plays role in significant elevation of both volume and sperm content in the ejaculates of rabbits in accordance to our results [34]. Our findings revealed that the parameter of spermatogenesis including TDI, RI, and SPI decreased significantly after testicular TD. While, administration of AVP contributed to significant elevation in the spermatogenesis. The findings of the study of Ghanbari and the colleagues approved the protective role of antioxidant compounds on TDI and SPI parameters. They concluded that royal jelly could significantly increase TDI and SPI parameters through its antioxidant property similar to protective effect of AVP [35]. The incidence of various diseases from the onset of genital diseases is spreading [39-51]. The use of natural and herbal products to protect the body from diseases is recommended due to its cheapness and morbidity [48-51].

Conclusion

Our results showed that AVP could attenuate factors involved in the pathogenesis of testicular TD, including histological changes, sperm quality and spermatogenesis. AVP could improve the histological changes caused by the testicular TD. Furthermore, AVP enhanced the parameters of sperm quality such as sperm count and vitality. Moreover, AVP played a significant role in the elevation of spermatogenesis parameters. Hence, the use of AVP is advisable for the amelioration

of damage caused by the testicular TD. Increasing the number of doses of AVP and evaluating the effect of AVP on hormones including FSH and LH are advisable for further studies.

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Authors' Contribution

AK and LZ designed the project. MAF collected the data and participated in primary drafting. LZ wrote the manuscript and performed critical revision. All the authors signed the manuscript.

Conflicts of Interest

The authors declare that they have no conflict of interest.

Ethical Considerations

The Animal Ethics Committee of Lorestan University of Medical Sciences approved all the experimental protocols (IR.LUMS. REC.1398.067).

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