EXPERIMENTAL STUDY

Oxytocin for preventing injury due to testicular torsion/detorsion in rats

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ABSTRACT

BACKGROUND: We aimed to demonstrate the effectiveness of oxytocin on the testes for treating ischemia-reperfusion injury.

METHODS: A total of 24 male Wistar albino rats weighing 250–320 g were used. The rats were randomized into three groups of eight rats. Group 1 was assessed as the control group. In Group 2 rats, testicular torsion was first performed, followed by testicular detorsion to induce reperfusion injury. In Group 3, following testicular torsion and detorsion, oxytocin was administered before inducing reperfusion. Testicular tissues were histologically evaluated, spermatogenic parameters were assessed using the Johnsen scoring system, and the mean Johnsen score was calculated.

RESULTS: Histological tests revealed significantly different results between the testicular torsion group and the oxytocin-treated torsion and control groups as well as between the oxytocin-treated torsion group and the control and testicular torsion groups (p=0.010 and 0.012, respectively). Biochemical test results revealed that superoxide dismutase and glutathione peroxidase levels were significantly lower in Group 2 than in Group 1 (p=0.007 and 0.007, respectively). Malondialdehyde and nitric oxide levels were significantly lower in Group 3 than in Group 2 (p=0.017 and 0.014, respectively).

CONCLUSION: These results indicate that oxytocin can be considered as an alternative agent for treating testicular torsion in clinical practice to minimize tissue damage.

Keywords: I/R injury; oxytocin; torsion.

INTRODUCTION

Testicular torsion is known as the rotation of the spermatic cord around its axis; its incidence is especially high during childhood and adolescence, with approximately 1 in 4000 males affected by 25 years of age.[1,2] In testicular torsion, spermatogenesis is affected secondary to the impairment of testicular blood flow, which may subsequently cause infertility. The fundamental approach for treating testicular torsion, which is accepted as an emergency pathology, is manual or surgical detorsion of the testes.[3] However, depending on the duration of the torsion, permanent testicular damage can occur. Reactive oxygen species (ROS) can be released depending on this pathology, which comprises processes of ischemia caused by torsion and further reperfusion due to detorsion.[4]

Under normal conditions, ROS, which assume certain physiological tasks, are generated in tissues and are eliminated by antioxidants. However, in cases of smoking, diabetes, cancer, trauma, intracranial pathologies, varicocele, infection, and testicular torsion, they can be produced in excessive amounts.[4–6] Accordingly, the high amounts of ROS released in stress-

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ful conditions cannot be eliminated by antioxidants in the human body. Excessive amounts of ROS and those formed in cases wherein antioxidants remain incapable adversely affect many anatomical entities, including lipid membranes and protein-containing structures, consequently leading to potential disruption of cellular integrity and functional impairments. Sperms contain polyunsaturated fat and are therefore very much vulnerable to oxidative injury.[7] Antioxidants are used as a classical approach for eliminating oxidative damage. Although surgical management is the fundamental treatment approach in testicular torsion, many treatment modalities have been attempted to minimize destructive changes, which may occur secondary to oxidative injury, following the surgical management of testicular torsion. To date, many pharmaceutical agents have been used in cases of testicular torsion or oxidative injury; however, limited number of studies have been conducted on the testicular effects of oxytocin, the receptors of which have been identified in the urinary system.[8,9] Oxytocin is generally known as a female hormone involved in lactation and childbirth. However, in recent years, its presence in the male reproductive system has been revealed, and in various studies, its antioxidative, anti-inflammatory, and anti-apoptotic effects have been demonstrated.[10]

This study aimed to evaluate the effects of oxytocin on testicular tissue and spermatogenesis in rats with induced testicular torsion.

MATERIALS AND METHODS

A total of 24 male Wistar albino rats weighing 250–320 g were used. All procedures were performed in compliance with the provisions of 1986 Strasbourg Universal Declaration on Animal Welfare and were approved by the Ethics Committee (2015 HADYEK 25). Rats were housed in standard rat cages, with maximum three rats in each cage. Rats were provided with standard pellets prepared for rodents and tap water ad libitum.

Experimental Method

The rats were randomized into three groups, each containing eight rats. Group 1 was assessed as the control group. In Group 2 rats, testicular torsion was first performed, followed by testicular detorsion to induce reperfusion injury. In Group 3, following testicular torsion and detorsion, oxytocin was administered before inducing reperfusion.

The rats were anesthetized using intraperitoneal injections of ketamine hydrochloride (50 mg/kg) and xylazine (0 mg/kg). Subsequently, in Group 1, orchiectomy was performed; in Group 2, testes were removed under sterile conditions through the left inguinoscrotal incision, rotated 720° counterclockwise, and left testicle fixated to the scrotum using 5.0 prolene sutures for 3 h. At the end of 3 h, the testes were detorsioned, and after 3 h of reperfusion, left testes were extracted for histopathological analysis and blood samples were drawn for biochemical analysis. Group 3 rats underwent torsion and detorsion as described for Group 2 rats but were intraperitoneally injected with oxytocin (80 IU/kg) injected 30 min before detorsion. After the procedure, the rats were sacrificed with cervical dislocation under ketamine and xylazine anesthesia. Testicular tissue samples were obtained and placed in 4% buffered neutral formaldehyde solution for histopathological analysis. Serum levels of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), malondialdehyde (MDA), and nitric oxide (NO) were measured.

Biochemical Analysis

Measurement of Plasma Thiobarbiturate Reactive Substances (TBARS) Levels

Plasma TBARS levels were determined by a method[11] based on the reaction with thiobarbituric acid (TBA) at 90–100°C. In the TBA test reaction, MDA or MDA-like substances and TBA react with each other and produce a pink pigment with maximum absorbance at 532 nm. This reaction was performed at pH 2-3 and 90°C for 15 min. The sample was mixed with two volumes of cold 10% (w/v) trichloroacetic acid to precipitate protein. The precipitate was pelleted by centrifugation, and an aliquot of the supernatant was reacted with an equal volume of 0.67% (w/v) TBA in a boiling water bath for 10 min. After cooling, the absorbance was read at 532 nm. The results were expressed as micromole per liter plasma (mmol/l) according to a standard graph, which was prepared based on serial dilutions of standard 1,1,3,3-tetramethoxypropane.

Measurement of Plasma NO Levels

NO has a half-life of only a few seconds because it is readily oxidized to nitrite (NO_2) and subsequently to nitrate (NO_3), which serve as index parameters of NO production. The method used for measuring plasma nitrite and nitrate levels was based on the Griess reaction.[12] Samples were initially deproteinized with Somogyi reagent. Total nitrite (nitrite + nitrate) was measured by spectrophotometry at 545 nm after converting nitrate to nitrite using copperized cadmium granules. A standard curve was obtained using a set of serial dilutions (108–103 mol/l) of sodium nitrite. Linear regression analysis was performed using the peak area obtained using nitrite standards. The resulting equation was used to calculate the unknown sample concentrations. Results were expressed as micromole per liter plasma (mmol/l).

Determination of Plasma SOD Activity

Total (Cu–Zn + Mn) SOD (EC 1.15.1.1) activity was determined according to the method of Sun et al.[13] The principle of the method is based on the inhibition of NBT reduction by the xanthine–xanthine oxidase system as a superoxide generator. The activity was assessed in the ethanol phase of the plasma sample after 1.0-ml ethanol/chloroform mixture (5/3, v/v) was added to the same volume of plasma and centrifuged. One unit of SOD was defined as the amount of enzyme caus-

ing 50% inhibition in the NBT reduction rate. SOD activity was also expressed as units per liter plasma (U/l).

Determination of Plasma GSH-Px Activity

Plasma GSH-Px (EC 1.6.4.2) activity was measured using the method of Paglia et al.[14] The enzymatic reaction in the tube, which contained NADPH, reduced glutathione, sodium azide, and glutathione reductase, was initiated by the addition of H_2O_2, and the change in absorbance at 340 nm was monitored using a spectrophotometer. The activity was expressed in units per milliliter plasma (U/ml).

Histopathological Analysis

Following the experiment, the testes were removed from the deeply anesthetized rats and were kept in suitable amounts of buffered 4% neutral formaldehyde solution for 36 h for fixation. Subsequently, following successive steps of routine tissue processing and follow-up protocol, which comprised rinsing, dehydration, permeabilization, and impregnation, the samples were embedded in paraffin blocks. Tissue samples embedded in paraffin blocks were cut into 4–5-µm-thick sections using a rotary microtome (LEICA RM2125RT, China), which were then placed on glass slides. The cut sections were kept overnight in an incubator at 58°C–60°C for deparaffinization. Subsequently, the sections were stained with hematoxylin–eosin and covered with a coverslip. These testicular preparations were histopathologically analyzed under a light microscope (Nikon Eclipse, Japan). Histopathological analyses were realized as a double-blind study, and the slides were randomly numbered. From each group containing eight rats, eight sections from each rat and an average of 25 seminiferous tubuli from each section were evaluated. Testicular tissues were evaluated with respect to histological and spermatogenic parameters using the Johnsen scoring system, and the mean Johnsen score was calculated. The Johnsen scoring system evaluates a total of 10 histological criteria as follows:[15]

Johnsen scoring criteria:

Score 10: Multilayered germinal epithelium, multiple spermatozoa

Score 09: Disorganized germinal epithelium piling up toward the lumen, spermatozoa are present

Score 08: Multilayered germinal epithelium, less than 10 spermatozoa in the lumen

Score 07: Spermatozoa are absent, multiple spermatids

Score 06: Spermatozoa are absent, less than 10 spermatids

Score 05: Spermatozoa and spermatids are absent, spermatocytes are seen

Score 04: Spermatozoa and spermatids are absent, less than five spermatocytes

Score 03: Only spermatogonia are present as germ cells

Score 02: Germ cells are absent, only Sertoli cells are seen

Score 01: Seminiferous tubuli do not contain any cell

Statistical Analyses

The statistical comparison of the mean Johnsen scores of the groups was performed using IBM SPSS 20 Windows Statistical Package for Social Sciences. The intergroup comparisons of mean Johnsen scores were performed using one-way ANOVA, followed by Tukey's HSD multiple comparison test.

RESULTS

Biochemical Test Results

SOD and GSH-Px levels were significantly lower in the testicular torsion group than in the control group (p=0.007 and 0.007, respectively). MDA and NO levels were increased in testicular torsion group compared to control group, but this increases were not statistically significant (p>0.05). In the testicular torsion group treated with oxytocin, MDA and NO levels decreased statistically significantly compared with those in the testicular torsion group (p=0.017 and 0.014, respectively), whereas increases in SOD and GSH-Px levels were not significantly different between these groups (p>0.05) (Table 1).

Histological Findings

Light microscopy analyses revealed that the histological

Table 1. Biocehemical results of the groups

	Groups			p
	Control	Testicular torsion	Oxytocin-treated torsion	
	Mean±SD	Mean±SD	Mean±SD	
Superoxide dismutase (U/mL)	5.82±0.73	4.07±0.94[a]	4.71±1.5	0.009
Malondialdehyde (µmol/L)	0.58±0.19	0.72±0.13	0.5±0.14[b]	0.021
Nitric oxide (mmol/L)	66.09±8.86	72±3.56	63.58±3.26[c]	0.016
Glutathione peroxidase (U/L)	460.58±89.73	310.39±100.42[d]	372.21±93.43	0.009

[a]A significant difference was found when compared with the control group (p=0.007). [b]A significant difference was found when compared with the testicular torsion group (p=0.017). [c]A significant difference was found when compared with the testicular torsion group (p=0.014). [d]A significant difference was found when compared with the control group (p=0.007). SD: Standard deviation.

appearance of the testicular tissues of control group rats generally resembled that of the testicular tissues of normal rats. Testicular microscopy of the control group rats completely demonstrated normal seminiferous tubuli (Fig. 1), germ cells, Sertoli cells, and Leydig cells without any signs of infiltration or bleeding. In the testicular torsion group, disorganization of the germinal epithelial cells of seminiferous tubuli, empty tubuli, dilatations in the interstitial tissue, patchy areas of severe edema, diffuse blood cells at the basement membrane, vascular dilatations, and vascular congestion were found. Coagulation necrosis in spermatogenic cells lining the tubular lumen and empty tubuli or tubuli containing very scarce number of germinal cells were observed (Fig. 2). Patchy areas of dehiscence were observed between germinal epithelial cells of seminiferous tubuli and basal lamina surrounding the tubuli. Histopathological findings observed in the oxytocin-treated torsion group were found in a mild form in certain areas of the testicular tissue in the testicular torsion group. In addition, slightly deformed seminiferous tubules containing all stages of the spermatogenic series were remarkable. However, patchy areas of detachment were observed between the basal lamina surrounding tubuli and the germinal epithelium of some seminiferous tubuli. Slightly edematous areas of intertubular interstitial tissue and some mildly congested vessels were observed. In general, intertubular loose connective tissues had preserved their histological architecture, and Leydig cells had retained their normal appearance (Fig. 3). Consistent with these histopathological findings, Johnsen scores, which indicate epithelial deformity of seminiferous tubuli, and spermatogenesis were found to be significantly lower in the testicular torsion group than in the other groups ($p<0.05$) (Table 2). Johnsen scores in the oxytocin-treated torsion group were in between those of the control and testicular torsion groups and significantly different from both ($p<0.05$). In other words, the three groups were significantly different from each other, and milder destructive changes were detected in the oxytocin-treated torsion group than in the testicular torsion group.

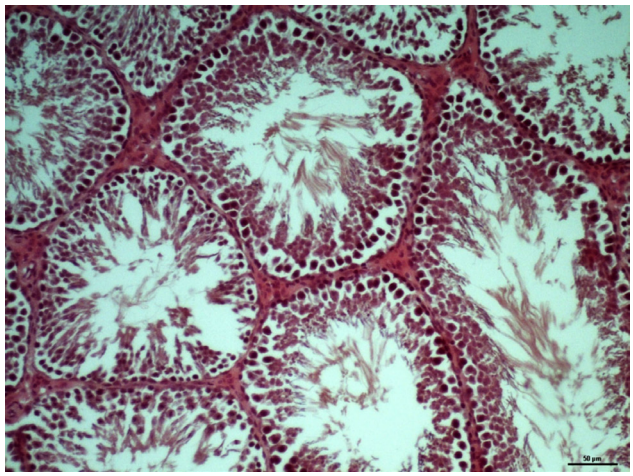


Figure 1. In the control group, seminiferous tubuli and intertubular connective tissue preserved their normal integrity. Sertoli cells and cells of all spermatogenic stages are present in a specific array in the seminiferous epithelium. Numerous spermatozoa are observed in the tubular lumen, and the testicular tissue retained its normal histological structure (H&E staining, Bar: 50 µm).

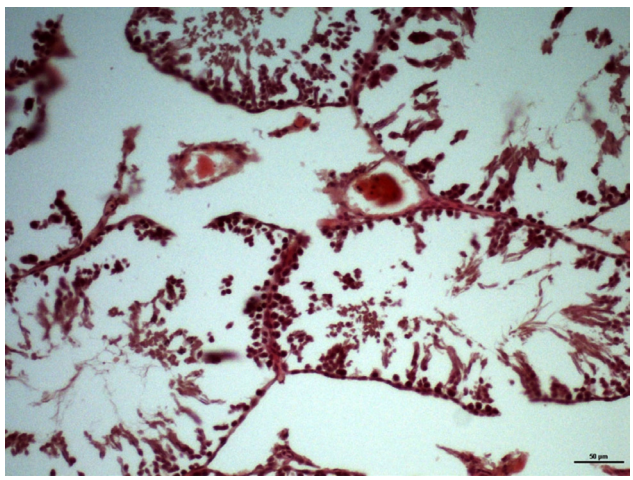


Figure 2. Integrity of seminiferous tubuli and intertubular connective tissue was disrupted. Vascular dilatation in the interstitial connective tissue and severe tissue loss; deformities and detachment of seminiferous tubuli; and disorganization and desquamations in the seminiferous tubuli epithelium are observed in Group 2. In general, very small amount (even absence) of spermatozoa, consistent with impaired spermatogenic cells, in the tubular lumens, which somewhat preserved their integrity, is observed. Overall, severe tissue damage is present in testicular tissues (H&E staining, Bar: 50 µm).

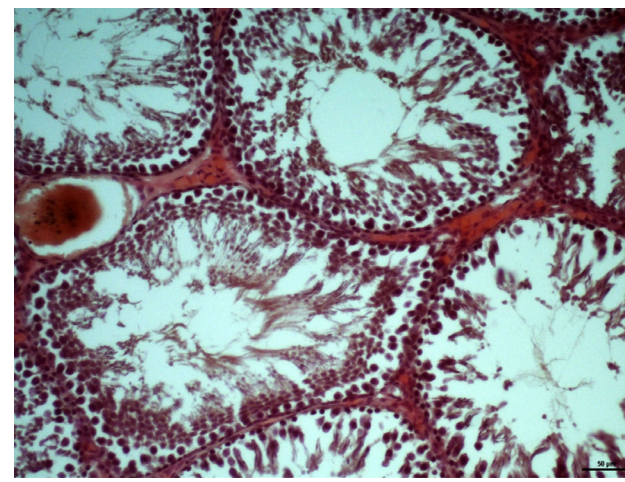
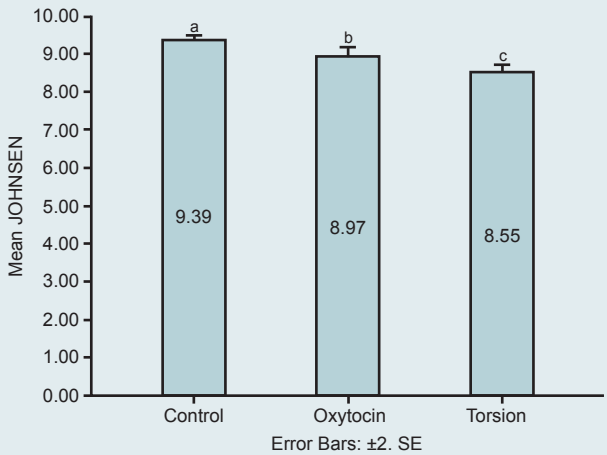


Figure 3. Histologically, Oxytocin-treated torsion group resembles the testicular structure in the control group rather than in the testicular torsion group wherein a slight but incomplete improvement is observed. The integrity of seminiferous tubuli and interstitial tissue is generally preserved. However, vascular dilatation, moderately severe congestion, and bleeding foci are observed in the interstitial tissue. Although epithelial deformities of some seminiferous tubuli are observed, spermatogenic cells and Sertoli cells demonstrate a tendency to preserve their unique epithelioid sequence. Less number of spermatozoa are observed in the tubular lumen (H&E staining, Bar: 50 µm).

Table 2. Mean Johnsen scores of the groups

	Control	Testicular torsion	Oxytocin-treated torsion
	Mean±SD	Mean±SD	Mean±SD
Johnsen Scores	9.39±0.13	8.55±0.28	8.96±0.44

SD: Standard deviation.



Graphic: Comparison of mean Johnsen scores of the groups. (Each letter on the bar signifies statistically significant difference; when compared with c b:p=0.012, a:p=0.000)

DISCUSSION

Oxytocin was first introduced as a peptide hormone in 1953.[16] It is produced in magnocellular neurons and is stored in posterior hypophysis.[17] The expression of oxytocin receptors has been shown not only in the uterine myometrium and mammary glands but also in the endometrium, decidua, ovary, thymus, pancreas, adipocytes, heart kidney, and brain.[18,19] Oxytocin plays a role in successful milk ejection, cardiovascular regulation, analgesic effects,[20] motor activity,[21] thermoregulation,[22] gastric motility,[23] natriuresis, osmoregulation,[24] and sexual behavior. It is well documented that levels of circulating oxytocin increase during sexual stimulation and arousal and peak during orgasm in both men and women.[25] In rats, oxytocin exerts potent anti-stress effects, such as decrease in blood pressure and corticosterone/cortisone levels and increase in insulin and colecystokinin levels. In addition, stress-induced central release of oxytocin can ameliorate stress-associated symptoms, such as anxiety.[26] Synthesis of oxytocin in the male reproductive system was first revealed by Nicholson et al in 1984.[27] Oxytocin is present at higher concentrations in the prostate than in the plasma and can increase the resting tone of prostatic tissue. Oxytocin is involved in prostate contraction and in the resulting expulsion of prostatic secretions at ejaculation.[28] Oxytocin is a potent stimulator of spontaneous erections in rats.[19] Recently, its presence as an endocrine and a paracrine hormone in the male urinary system, i.e., in the testis and epididymis, has been reported. In addition to ejaculation and penile erection, two primary functions have been ascribed to testicular oxytocin, namely the regulation of seminiferous tubule contractility and the modulation of steroidogenesis. In the testis, seminiferous tubules are surrounded by smooth muscle-like myoid cells. Their function is necessary for sperm transport and maturation.[29] Overall, the regulation by gonadal and adrenal steroids is one of the most remarkable features of the oxytocinergic system.[30] In this context, the effects of oxytocin on the penis, testes, and kidney have been evaluated in several studies.

Testicular torsion, which is a typical ischemia-reperfusion (I/R) injury, may cause male infertility. Therefore, early diagnosis and treatment of testicular torsion are quite important for preserving testes and fertility capacity. Surgical detorsion is the gold standard treatment approach for testicular torsion and provides reperfusion of testicular tissues. As reported above, the main pathophysiology of testicular torsion–detorsion is I/R injury of the testes, which causes overproduction of ROS.[31] It has been clearly revealed that reperfusion of the ischemic tissue is associated with oxidative stress.[32] Therefore, treatment by detorsion may further damage the testes. ROS are short-lived reactive molecules having one or more unpaired electrons, rendering them highly unstable and highly reactive.[33] In normal conditions, ROS expression plays physiological roles in cellular differentiation, sperm capacitation, acrosome reaction, and maintenance of fertilizing ability, whereas oxygen free radicals at concentrations beyond physiological limits result in oxidative stress.[34,35] ROS overexpression secondary to oxidative stress can negatively affect proteins, lipids, nucleic acids, carbohydrates, and other molecules and leads to cell membrane lipid peroxidation, protein denaturation, DNA damage, inflammation, cell proliferation, cell dysfunction, and apoptosis.[36] Thus, ROS may play a role in the pathogenesis of several diseases, such as atherosclerosis,[37] cancer,[38] diabetes mellitus,[39] infection,[40] central nervous system disorders,[41] and testicular torsion due to its involvement in lipid peroxidation.[38] In addition, negative effects of oxidative stress on the testes after the initiation of reperfusion have been reported in the testicular torsion induced rat model.[39] Indeed, it has been reported that activities of antioxidant enzymes are diminished and ROS production is increased in the testicular torsion induced rat model. The present study also showed that testicular I/R caused testicular damage, as evidenced by biochemical and histological changes. The levels (expression) of MDA as a marker of lipid peroxidation and NO were found to be high in Group 2. Levels of antioxidant enzymes, such as SOD and GSH-Px, decreased, whereas those of MDA and NO, which are indicators of oxidative stress, increased in the testicular torsion group (Group 2) compared with those in the control group. This indicated complete realization of the I/R injury.

Antioxidants break the oxidative chain reaction, thereby reducing the effects of oxidative stress.[42] Positive effects of

antioxidants on oxidative stress parameters and different tissues in oxidative stress conditions, such as atherosclerosis, hypertension,[43] type 2 diabetes mellitus,[44] ulcerative colitis,[45] and testicular torsion, have been shown. In this context, for preventing harmful effects of oxidative stress, many antioxidant agents, such as vitamin E, melatonin, retinol, β-carotene, omega-3, resveratrol, allopurinol, melatonin, N-acetylcysteine, zinc aspartate, and caffeic acid phenethyl ester, vitamin C, coenzyme Q10, and acetylcysteine have been used with different success rates.[46–50] Although numerous experimental animal studies have confirmed the efficacy of antioxidants in reducing short-term damaging effects of testicular torsion, different antioxidant agents have been investigated to reduce the short- and long-term reperfusion damage on the testes. Anti-apoptotic, anti-inflammatory, and antioxidant properties of oxytocin have been reported. The effects of oxytocin on I/R have been reported in limited number of studies.[51,52] Oxytocin reduces I/R injury in rat kidney, with improved renal function, as indicated by decreased serum creatinine and BUN levels in addition to improved antioxidant status and reduced ROS.[53] Moreover, the protective effect of oxytocin on I/R injury in the liver, kidney, stomach, and urinary bladder have been reported.[54–58] In addition, the anti-apoptotic effect of oxytocin in the heart and ovaries has been revealed.[51,59] However, to our knowledge, the effect of oxytocin on testicular tissues in I/R injured rat model has been reported in only one study. In an experimental study, Ghasemnezhad et al. demonstrated that Johnsen scores were higher in the oxytocin-treated torsion group than in the testicular torsion group.[10] Similarly, in our study, Johnsen scores were significantly different between the oxytocin-treated torsion group and the testicular torsion group.

In the oxytocin-treated torsion group (Group 3), levels of antioxidant enzymes, such as SOD and GSH-Px, increased and approached the levels in the control group. Similarly, levels of MDA and NO in this group decreased below those in the control group. These outcomes revealed that oxytocin increases antioxidant capacity and relieves the effects of oxidative stress.

In conclusion, although this is an experimental study, the results indicate that oxytocin can be considered as an alternative agent for treating testicular torsion in clinical practice to minimize tissue damage. However, additional experimental and clinical studies are necessary to confirm our findings.

Ethics Committee Approval

Ethics committee approval was received for this study from the ethics committee of Gaziosmanpaşa University School of Medicine.

Peer-review

Externally peer-reviewed.

Financial Disclosure

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Conflict of interest: None declared.

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DENEYSEL ÇALIŞMA - *ÖZET*

Sıçanlarda testiküler torsiyon/detorsiyon nedeniyle oluşan hasarın önlenmesinde oksitosin

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AMAÇ: Testislerdeki iskemi reperfüzyon hasarı üzerine oksitosinin etkilerini göstermeyi amaçladık.

GEREÇ VE YÖNTEM: Çalışmamızda ağırlıkları 250–320 gr arasında değişen toplam 24 adet Wistar-Albino cinsi sıçan kullanıldı. Sıçanlar, sekiz sıçandan oluşan randomize üç gruba ayrıldı. Grup 1 kontrol grubu olarak değerlendirildi. Grup 2'de önce testis torsiyonu gerçekleştirildi. Sonrasında detorsiyone edilerek reperfüzyon hasarı oluşturuldu. Grup 3'de ise torsiyon ve detorsiyon işlemlerini takiben reperfüzyondan önce oksitosin uygulandı. Testiküler dokular, Johnsen skorlama sistemi kullanılarak histolojik ve spermatogenik parametrelere göre değerlendirildi ve ortalama Johnsen skoru hesaplandı.

BULGULAR: Histolojik test sonuçları torsiyon grubu tedavi ve kontrol gruplarından istatistiksel olarak anlamlı derecede farklıydı. Oksitosin ile tedavi edilen grup hem kontrol hem de torsiyon gruplarından farklıydı (p=0.010 ve p=0.012). Biyokimyasal test sonuçları testis torsiyonu oluşturulan grupta süperoksit dismutaz ve glutatyon peroksidaz düzeyleri kontrol grubuna göre istatistiksel olarak anlamlı derecede düşük bulundu (p=0.007 ve p=0.007). Daha sonra oksitosin ile tedavi edilen testiküler torsiyon grubunda malondialdehit ve nitrik oksit düzeyleri yalnızca testis torsiyonu yapılan grupla karşılaştırıldığında istatistiksel olarak anlamlı şekilde azaldı (p=0.017 ve p=0.014).

TARTIŞMA: Bu sonuçlara göre, oksitosin klinik uygulamada testiküler torsiyon tedavisinde doku hasarını en aza indirgemek için alternatif bir ajan olarak düşünülebilir.

Anahtar sözcükler: İskemi reperfüzyon hasarı; oksitosin; torsiyon.

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