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Protective Effects of Capsaicin on Experimental Testicular Torsion and Detorsion Injury

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What's known on the subject? and What does the study add?

Recent investigations have demonstrated that there are many studies on the antioxidant mechanism of capsaicin (CAP), there is no study showing how CAP has an antioxidant effect in experimentally induced testicular torsion/detortion. In this study, we found that CAP will be an alternative treatment method for eliminating the pathological conditions resulting from ischemia.

Abstract

Objective: Testicular torsion is one of the most common genital diseases in males in adolescence and it should be treated as soon as possible without ipsilateral testicular dysfunction. We investigated the protective effect of Capsaicin, which is an active ingredient of red-hot pepper, an antioxidant substance, on tissue damage caused by ischemia/reperfusion (I/R).

Materials and Methods: Forty male, 250-300 g adult male rats were divided into 4 groups, 10 in each group. The torsion was created by rotating the spermatic cord of both testicles counterclockwise by 720°. In the experimental group where we applied capsaicin, detorsion was performed after 2 h of torsion. Malondialdehyde (MDA), superoxide dismutase (SOD), and catalase (CAT) were evaluated. Testicular tissue was stained with hematoxylin/eosin for histopathological evaluation.

Results: In the Capsaicin group showed reduced tubular damage and seminiferous tubules in a structure similar to ischemia and spermatogenic cell series in the tubular wall and decreased edema in the interstitial area. SOD, MDA and CAT levels evaluated for the determination of lipid peroxidation were observed to be close to the control group values in the Capsaicin administered group.

Conclusion: Capsaicin had a protective effect on I/R injury in the testicle.

Keywords: Capsaicin, ischemia/reperfusion, testis, torsion/detorsion

Introduction

Testicular torsion is a urological emergency characterized by the rotation of the spermatic cord and its anatomical structures. It is one of the most common genital diseases in men in adolescence and it should be treated as soon as possible without ipsilateral testicular dysfunction (1). Torsion occurs when the blood flow to the region decreases or is stopped completely. Increasing edema leads to arterial obstruction and subsequently results in ischemia and gonadal necrosis (2). Because of ischemia in the

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tissue, it causes cell death, energy deficiency, deterioration in signal pathways and accumulation of reactive oxygen species (ROS) (3). While testicular torsion causes ischemic damage in the tissue, detorsion causes reperfusion damage, which is the main cause of tissue damage. Simultaneously, the duration of ischemia is critical for the damage to occur. Because of the long ischemia process, irreversible damage and necrosis occur in the cells (4,5). Studies have shown that the critical ischemia time should not be more than 4 h to avoid irreversible damage to the germinal and tubular epithelium in rat testicles (6,7). Studies have shown that ROS can have adverse effects by disrupting the structural elements of the tissue and it has been reported that these effects can be reduced with antioxidant therapy (8). Many antioxidant substances have been used to prevent tissue damage in the experimentally created testicular torsion and detorsion (T/D) models.

Capsaicin (CAP) (trans -8- methyl-N-vanillyl-6-nonenamide) belongs to the Capsicum plant family and is the active ingredient of red-hot pepper (Figure 1) (9). CAP has many pharmacological properties. In this study, we use its antioxidative properties but simultaneously, it is a very effective anticarcinogenic and antimutagenic agent (10). Protect cells against free radical-mediated damage caused by exogenous chemicals (10), inhibition of the generation of ROS (11) and induction of apoptosis (12,13). CAP protects against testicular damage through mTOR-dependent mechanism (14). The mammalian target of rapamycin (mTOR) is a kinase that humans are encoded by the mTOR gene (15). mTOR is a member of the phosphatidylinositol 3-kinase-related kinase family of protein kinases (16). The mTOR pathway plays an important role in cell growth, cell proliferation, protein synthesis. Phosphorylation of mTOR is increased in pathological conditions such as testicular torsion. Studies have shown that CAP reduces mTOR phosphorylation, thus proving that it is effective in the survival of cells in testicular tissue (14).

Evidence shows that CAP decreases the activities of superoxide dismutase (SOD), which is an important antioxidant enzyme involved in the scavenging of free radicals (10). Although there are many studies on the antioxidant mechanism of CAP, there is no study showing how CAP has an antioxidant effect in experimentally induced testicular T/D.

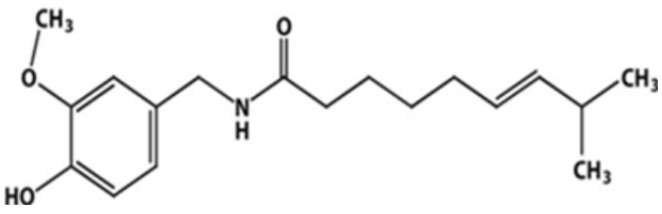

Figure 1. Chemical structure of capsaicin

In this study, we examined the effect of CAP on testicular T/D injury, by determining biochemical parameters and evaluating histopathological examinations.

Materials and Methods

Animals

The rats were obtained from the Medical and Experimental Research Center of Eskişehir Osmangazi University, Eskişehir, Turkey. For this study, 40 male Wistar-albino rats weighing 250-300 g were used and were housed in polycarbonate cages. The animal cages were maintained at 22±2 °C with a 12-hour light/12-hour dark cycle and the rats were fed with laboratory pellet shows and water was provided *ad libitum.* The treatment of the animals and experimental procedures were approved by the Experimental Animals Ethics Committee of Eskişehir Osmangazi University the decision no: 687/2018. After the surgical intervention, they were placed in individual polycarbonate cages to prevent intragroup injuries.

Groups

The rats were randomly separated into four equal groups and there were 10 rats in each group. Group I was the sham operated group. Only a scrotal skin incision was made in the rats in this group to evaluate the biochemical and histopathological basal values. Group II was the ischemia group in which we created testicular torsion. In this group, torsion was performed for 2 hours by 720-degree extravaginally testis. Group III was ischemia and reperfusion group. This group was designed to study the effect of detorsion after 2 h of torsion. Group IV was designed to determine the effect of CAP after ischemia. After surgical procedures as in group III, CAP (0.5 mg/kg, sc) (SİGMA Aldrich, Germany, M2028-250 MG) was administered before 30 min of detorsion. The CAP dose was injected subcutaneously as 0.5 mg/kg, which Zık et al. (17) determined as a safe and effective dose in rats.

Surgical Procedures

During all surgical procedures and euthanasia at the end of the experiment, the animals were placed under general anesthesia with xylazine/ketamine injection. Ketamine (Ketalar®) was injected intramuscularly (i.m.) at a dose of 50 mg/kg, and Xylazine (Rhompun®) was injected as 10 mg/kg i.m. After disinfecting the scrotal region, a midline vertical incision was made on the skin of the scrotal region and the testicular tissue was separated from the surrounding tissues (Figure 2A). To create testicular torsion, the double-sided testis was rotated 720 degrees counterclockwise along the longitudinal axis of the spermatic cord, and the testis was secured to the scrotum with a 4/0 non-traumatic suture passing through the tunica

albuginea and dartos (Figure 2B). After 2 h of ischemia (Figure 2C), the suture was removed and the testis was replaced in the scrotum for 2 h of reperfusion (Figure 2D). The rats were sacrificed by cervical dislocation under general anesthesia at the end of the experiment. Consequently, bilateral orchiectomies were performed for histopathological examination.

Histopathological Analyses

After the testicular tissues were removed, they were immersed in Bouin's fixative (7.5 mL of saturated picric acid, 2.65 mL of glacial acetic acid and 2.5 mL of 7% formaldehyde). The samples were processed through routine and standard paraffin embedding. After that they were sectioned into 5µ thickness and stained with hematoxylin and eosin (H&E) for histological analyses. Standard light microscopy (NIKON, Japan) was used for microscopic examination of rat testis tissue.

Biochemical Analysis

After the surgical intervention, blood was collected from the rats by the intracardiac route under general anesthesia. Biochemical analysis in blood; to determine lipid peroxidation (MDA), antioxidant enzyme levels such as SOD and CAT were evaluated. Erythrocyte hemolysate was prepared for the biochemical analysis to be made (18).

A. Measurement of Catalase Activity

Order to determine the catalase (CAT) activity, the procedures specified in the Cayman enzyme kit were applied for the preliminary preparation of the samples (CAYMAN, United States, Cat. no: 707002). At the last step of the procedure, a standard graph was drawn with the formaldehyde concentrations corresponding to the absorbance values read from the standard wells. The obtained values were calculated using the following formula. CAT activity (nmol/min/mL)=µM/20* sample dilution.

B. Measurement of Superoxide Dismutase Activity

SOD activity was studied with the water-soluble tetrazolium salt reaction-based Sigma SOD detection kit (SİGMA Aldrich, Germany, Cat. No:19160). The obtained values were calculated using the specified formula. (% inhibition rate) = {[(A blank 1-A blank3)- (A sample- A blank 2)]/(A blank 1- A blank 3)}.

C. Measurement of Malondialdehyde Levels

The measurement of the amount of malondialdehyde (MDA), which is effective in the determination of lipid peroxidation, is made using thiobarbituric acid (TBA). 0.1 mL homogenate, 3 mL of 1% phosphoric acid, 0.5 mL of distilled water and 1.0 mL of 0.6% 2-TBA were added. The mixture was boiled in the water bath for 45 min, followed by cooling in an ice. After the addition of 4 mL of n-butanol/pyridine, homogenate, and hemolysate MDA levels were measured spectrophotometrically at 532 nm and expressed as mmol MDA/mL (19).

Statistical Analysis

Statistical analyses were performed using the version of IBM SPSS 21 (Statistical Package for Social Sciences). Shapiro-Wilk Normality test and Kolmogorov-Smirnov test were used to determine whether the groups possessed normal distribution. The groups, which showed a normal distribution, were analyzed using One-Way analysis of variance (ANOVA) test. Variables that did not show normal distribution were analyzed with the Kruskal-Wallis test. All the data were expressed as mean ± standard deviation. The results were considered within 95% confidence bounds and a $p<0.05$ was considered to statistically significant.

Results

Because of the biochemical evaluations, SOD, MDA and CAT values, which are important parameters for the determination

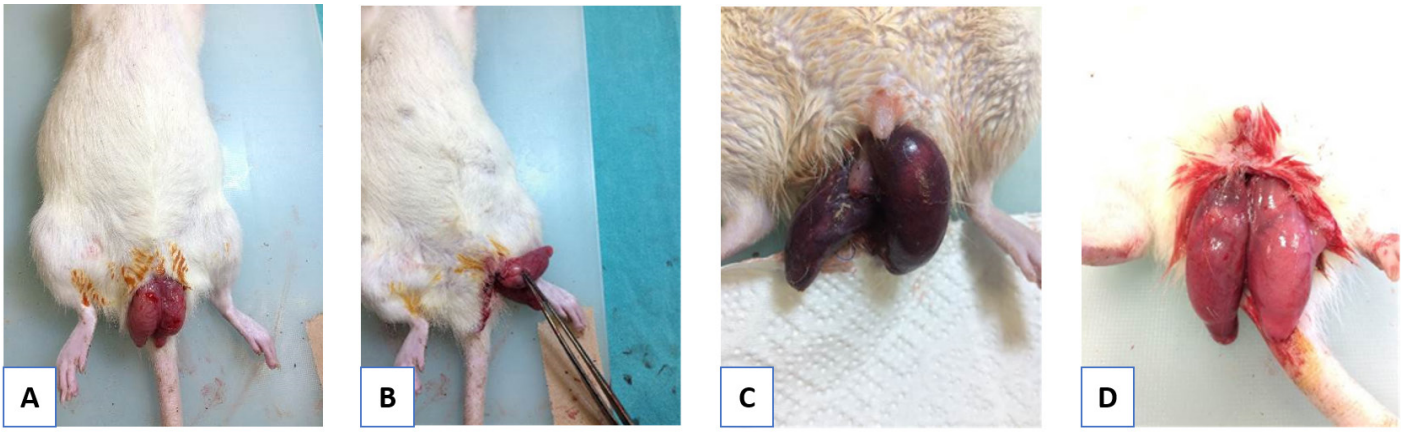

Figure 2. Rat testis image with experimental testicular torsion. (A) A midline vertical incision was made on the skin of the scrotal region and the testicular tissue was separated from the surrounding tissues. (B) The double-sided testis was rotated 720 degrees counterclockwise along the longitudinal axis of the spermatic cord for create testicular torsion. (C) Image of testis 2 hours after ischemia. (D) Image of testis 2 hours after reperfusion

of lipid peroxidation, are shown in Table 1 and Figure 3. MDA levels were significantly increased compared with groups II and group III in the group I and group IV. Additionally, MDA levels were significantly decreased in the CAP-treated group compared with the groups II and group III ($p<0.05$). But, there was no significant difference between the group II and group III ($p>0.05$). The SOD and CAT levels were significantly decreased in groups II and III when compared with groups I and IV, but after CAP administration, SOD and CAT levels increased and there weren't any difference between groups I and IV ($p>0.05$).

The histopathological examinations for each group are shown in Figure 4. The sham group showed a normal testicular structure. regular seminiferous tubular morphology and spermatogenic cells in the tubule wall, interstitial area and Leydig cells were seen in the normal structure in this group (Figure 4.A1-A3). In the ischemia group, it showed intense tubular damage, epithelial shedding, atrophic tubule structures, edema in the interstitial area and vascular congestion (Figure 4.B1-B3). In the ischemia/reperfusion (I/R) group, intense tubular damage, epithelial spills, edema in the interstitial area and vascular congestion were observed (Figure 4.C1-C3). CAP-treated group

Table 1. Antioxidant enzyme levels in testes tissue

Group	Group number	MDA (mmol/mL)	SOD (Inh%)	CAT (kU/L)
Group I (Sham)	GI	4.244±0.06	74.825±4.43	1.848±0.05
Group II (Ischemia)	GII	5.645±0.09	64.644±2.62	1.699±0.08
Group III (Ischemia+Reperfusion)	GIII	6.252±0.09	58.157±2.68	1.376±0.09
Group IV (Ischemia+Reperfusion+Capsaicin)	GIV	4.338±0.12	71.670±2.67	1.954±0.02
p values and multiple comparison of the groups	GI-GII	<0.001	<0.001	<0.001
	GI-GIII	<0.001	<0.001	<0.001
	GI-GIV	0.141	0.139	0.010
	GII-GIII	<0.001	<0.001	<0.001
	GII-GIV	<0.001	<0.001	<0.001
	GIII-GIV	<0.001	<0.001	<0.001

All of the data were expressed as means ± SD. Differences between groups were evaluated by One-Way analysis of variance (ANOVA) followed by Post-hoc comparison test. The significance was tested as n.s p>0.05, p<0.05, p<0.01 and p<0.001, SD: Standard deviation, MDA: Malondialdehyde, SOD: Superoxide dismutase, CAT: Catalase

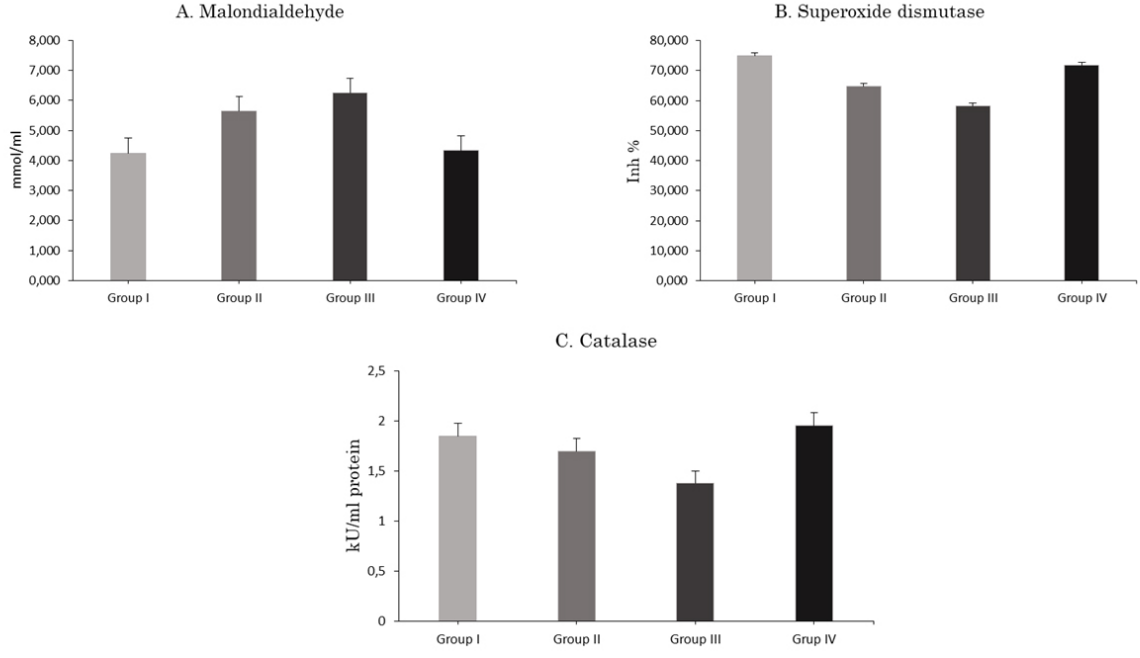

Figure 3. Mean MDA (A), SOD (B), CAT (C) activities of all groups. Data are presented as means ± SD. The significance was tested as n.s p>0.05, p<0.05, p<0.01 and p<0.001

showed decreased edema in the interstitial area and compared to the ischemia and IR group, decreased tubular damage and near-normal seminiferous tubules and spermatogenic cell lines in the tubule wall are seen (Figure 4.D1-D3).

Discussion

Testicular torsion is a urological emergency characterized by the rotation of the spermatic cord and the anatomical structures in it, which is frequently encountered especially during adolescence. Damage to testicular tissue varies depending on the degree and duration of torsion. While ischemic damage occurs during torsion, it has been observed that the main damage to the tissue occurs during detorsion. Many studies have shown that I/R damage can be improved with various antioxidant substances (ozone, diacerein, montelucast, curcumin, taurin etc.) (20,21). However, no study was found investigating the effect of CAP on I/R damage in testicular torsion. Therefore, the current study is first in the literature. The authors investigated the protective effect of CAP on testicles in I/R injury.

In the present study, CAP was given subcutaneously 30 min before reperfusion, showed effects on biochemical and histopathological levels in decreasing reperfusion injury in the testis. A previous study by Sarioglu-Buke et al. (22), demonstrated that CAP prevents apoptotic changes in the contralateral testis in ipsilateral testicular torsion. At another relevant study demonstrated that CAP has a strong neurotoxin effect on nerves and demonstrated this in her testicular ischemia study (23). However, Ilhan and Erdost (24), proved the beneficial effects of CAP on the reproductive system by showing that CAP increases the synthesis of ghrelin and thus triggers testicular cell proliferation and increases the testosterone level. Another recent study indicated that the protective properties of CAP by reducing the formation of free radicals, inhibiting the active caspase-3 and antioxidant defense mechanism (25).

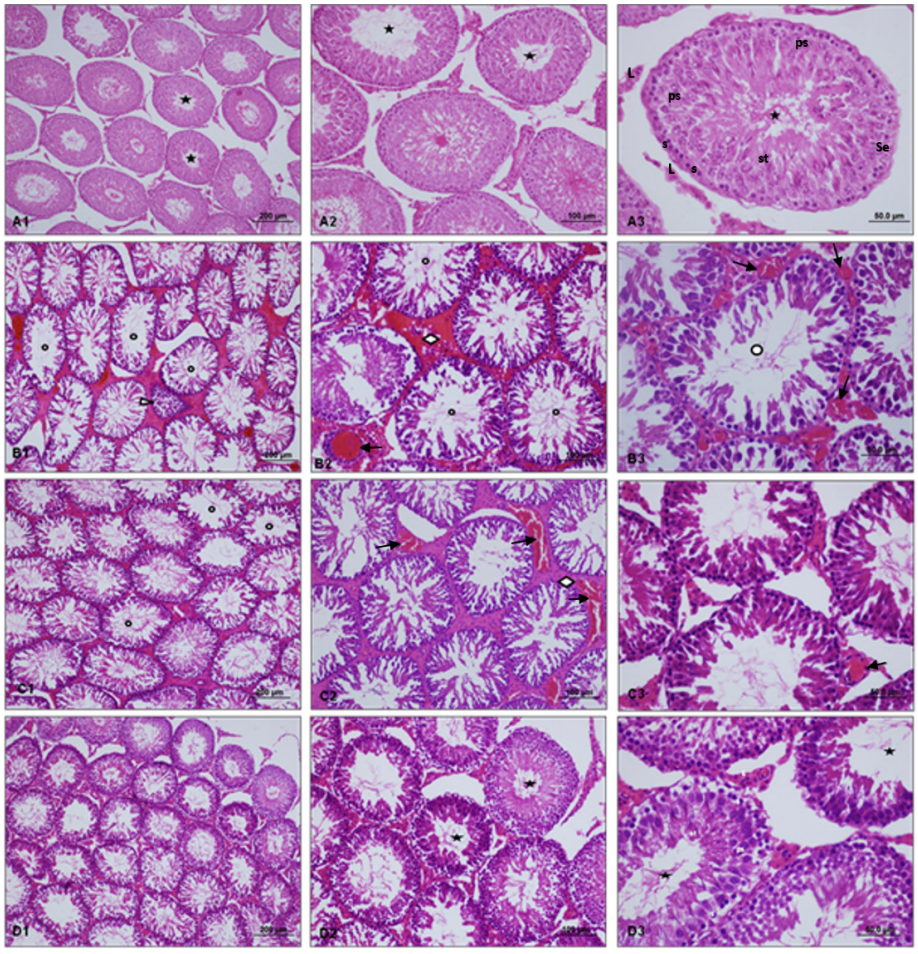

Figure 4. Light microscopic images of testes of rats in all groups at different magnifications. (HE, scale bar: 200 µm, 100 µm, 50 µm). (A1-A3) Sham group shows normal testicular structure. Regular seminiferous tubular morphology and spermatogenic cells in the tubule wall (*), interstitial area and Leydig cells (L) are seen in normal structure in this group. (B1-B3) In the ischemia group, it shows intense tubular damage (o), epithelial shedding, atrophic tubule structures (►), edema in the interstitial area ( ) and vascular congestion (→). (C1-C2) In I/R group, intense tubular damage (o), epithelial spills, edema in the interstitial area ( ) and vascular congestion (→) are observed. (D1-D3) CAP-treated group shows decreased edema in the interstitial area and compared to the ischemia and IR group, decreased tubular damage and near-normal seminiferous tubules (*) and spermatogenic cell lines in the tubule wall are seen

Studies have shown that one of the parameters that effective in the clinical importance of testicular torsion is the ischemia time, and the other is the degree of torsion. In this study, we complete ischemic damage at 720° counterclockwise and 2-hour ischemia period, which is sufficient time for the desired ischemic response to occur in the rat testis tissue. Both biochemical and histological evaluations showed that this time was sufficient to induce ischemia in the tissue. Turner and Brown (26) argued that even 1 h is sufficient for ischemic damage to the rat testis tissue. However, Gürdal et al. (27), showed that the testicular ischemia period, which was formed by 720° for 1 h, increased the level of lipid peroxidation with the increase in the malondialdehyde level in the tissue but they showed that the time for the formation of histopathological changes was insufficient. Another recent study emphasized that it is sufficient to torsion the testis tissue 360° and for 2 h for the formation of a moderate acute vascular response in the testis tissue (28). In another study, a counterclockwise 720° and 2-hour ischemia period was applied in the testicular torsion model and it was shown that the applied torsion period caused sufficient histopathological changes in the tissue and the desired ischemia table was formed because of MDA, SOD and CAT analyzes (3). The present results and studies have shown that the time we have planned in the experimental procedure is sufficient for the desired ischemic damage to the tissue.

The general idea in experimentally created ischemia models is that ischemia lasting longer than 4-6 hours will cause irreversible tissue damage. It has been shown that ischemia lasting longer than 4 h completely cuts off the blood flow in the testicular tissue and creates focal infarctions (29,2). Studies argue that the testicular tissue, which intervenes within 6 h, shows a recovery of 85-97% (28).

Biochemical and histopathological evaluations are the most reliable analysis to determine the degree of ischemic damage in ischemia and reperfusion studies. In the hypoxia caused by ischemia, the level of ROS in the tissue increases even more. Although the measurement of ROS for antioxidant activity is more reasonable, its short lifespan makes it impossible to measure ROS. For this reason, the measurement of MDA, a product of lipid peroxidation, gives more reliable results. MDA is the end product of lipid peroxidation and MDA levels in blood and tissue are an important indicator of oxidative damage in I/R studies. In our study, the level of MDA significantly increased in the T/D group compared with the sham group. Because of the increase in the I/R group, it shows that reperfusion has even more harmful effects on the tissues. Treatment with CAP significantly decreased MDA levels.

Endogenous antioxidant enzymes are used to eliminate free oxygen radicals formed during ischemia and reperfusion. We determined the levels of antioxidant enzymes such as SOD and CAT, which are frequently used in ischemia studies. It was observed that SOD and CAT values, which decreased in the ischemia and ischemia reperfusion group, compared with the control group, increased in the CAP group. It was stated that this increase in the CAP group approached the SOD and CAT values in the control group. In most of the experimental testicular torsion studies, SOD activity was decreased after reperfusion. Despite the decrease in SOD levels in I/R groups, there are also experimental studies in the literature studies to the contrary (20).

Histopathological examination in the I/R group revealed intense tubular damage, epithelial spills, edema in the interstitial area and vascular congestion in the rat testis. The present findings show that CAP-treated group had decreased tubular damage, near-normal seminiferous tubules and spermatogenic cell lines in the tubule wall.

Conclusion

The results of this study indicate that CAP treatment has a protective effect against I/R damage in testicular torsion with biochemical and histological examinations. Because of the meaningful data we have obtained, we suggest that CAP will be an alternative treatment method for eliminating the pathological conditions resulting from ischemia.

Ethics

Ethics Committee Approval: The treatment of the animals and experimental procedures were approved by the Experimental Animals Ethics Committee of Eskişehir Osmangazi University the decision no: 687/2018.

Informed Consent: Not necessary.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: H.G., S.Ö., İ.H., Concept: H.G., H.Ö., Ş.K., Design: H.G., H.Ö., Ş.K., Data Collection or Processing: H.G., S.Ö., D.B.D., M.C.Ü., Analysis or Interpretation: H.G., D.B.D., M.C.Ü., H.Ö., Literature Search: B.E., H.T., M.K., Writing: B.E.

Conflict of Interest: No conflict of interest was declared by the authors.

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