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Research Article

Effects of Meningic Extract on Testicular Torsion/Detorsion

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Abstract

Introduction: Testicular torsion leads to impaired blood supply to the testicles and ischaemia. The incidence is higher especially in newborns and adolescents. As in the treatment of many diseases, various plant combinations are used to prevent the damage caused by testicular torsion-detorsion. In this study, we aimed to investigate the protective effect of meningic (Pistacia terebinthus L.) extract on testicular torsion-detorsion (T/D) injury histopathologically.

Materials and Methods: The 24 animals used in our experiment were randomly divided into 3 groups. Testicular tissues were stained with hematoxylin-eosin and PAS stains to evaluate histological changes. Modified Johnsen scoring was then performed.

Results: In the sham group sections, seminiferous tubules were regular and germinal epithelium at various stages of spermatogenesis was seen in 4-5 rows on the basal lamina. In PAS stained sections of the sham group, the basement membrane was regular. In the sections of T/D group, destruction of seminiferous tubules, dilatation and congestion were observed in the vessels in the interstitial area. Thickening of the basal laminae of the seminiferous tubules was also detected in the sections of the T/D group stained with PAS. In the sections of meningic + T/D group, a partial recovery was observed in the seminiferous tubules. The basal lamina thickness in PAS-stained sections of meningic + T/D group was found to be close to normal. According to the results of modified Johnsen score analysis, there was a significant difference between the groups ($p<0.05$).

Conclusion: In our study, it was concluded that meningih extract partially protects the testis and its functions.

Keywords: Testicular injury, torsion, detorsion, meningic extract

INTRODUCTION

Testicular torsion is one of the urological emergencies that results in disruption of blood supply and ischaemia after the spermatic cords rotate around themselves. As a result of testicular torsion, various symptoms such as pain, nausea, vomiting and similar symptoms may develop in the lower quadrant of the abdomen. Approximately 20% of testicular torsion, which is generally idiopathic, occurs due to trauma. Testicular torsion is divided into two types as extravaginal and intravaginal. Intravaginal testicular torsion is generally seen in adults, while extravaginal testicular torsion is more common in the newborn period. In a study investigating the incidence of testicular torsion, it was reported that the incidence was approximately 3.5/100.000 [1].

The incidence of testicular torsion, which is mostly seen in neonates and adolescents, increases especially in adolescence. The reason for this has been shown to be the growth in the size of the testicle. However, the incidence of testicular torsion gradually decreases in individuals over the age of 18 [2]. Testicular torsion is one of the most important causes of acute scrotum in terms of its consequences. In the absence of urgent intervention, it is almost inevitable to develop obstruction in the spermatic vessels with torsion of the spermatic cords and subsequent necrosis of the testes [3]. Extravaginal and intravaginal testicular torsions are cases that require urgent surgical detorsion [4]. Surgical detorsion is a treatment method which has many side effects despite its benefits. The reperfusion that starts in the testes with the detorsion procedure causes injuries in the endothelial cells, disturbances in microcirculation in the testes and a serious loss in germ cells [5].

There are many scientific studies showing that seminiferous epithelium may be disrupted, and germ cells may be lost following ischaemia/reperfusion (I/R) injuries in the testis [6]. In these studies, reactive oxygen species (ROS) have been shown as one of the possible causes of ischaemia/reperfusion-induced damage [7]. This increase in ROS level leads to DNA damage and apoptosis in testicular germ cells [7,8].

Plants, which can be used in the treatment of many diseases, have many biological activities such as antioxidant, antimicrobial and anticarcinogenic due to phenolic acids, flavonoids, quinines, alkaloids, terpenoids and emetins [9]. Therefore, combinations of various enzymes, chemical drugs and plant extracts are used after testicular torsion/detorsion in order to prevent the damage that will occur after reperfusion [10].

Meningic (Pistacia terebinthus L.) is a plant native to the Mediterranean region and belongs to the gum tree family

(Anacardiaceae) [11]. In addition to the antioxidant properties of meningic, many studies have been conducted to investigate the phenolic substances, fixed fat content, fatty acid components and tocopherol content [12,13]. In addition, there are studies showing that the extracts obtained from the fruits of meningic, which has been used in alternative medicine for a long time, show antioxidant, anticarcinogenic, antimicrobial and antimutagenic properties [14,15].

In this study, we aimed to histopathologically investigate the protective effect of orally administered meningic extract on experimentally induced testicular torsion-detorsion (T/D) injury.

MATERIAL AND METHOD

Experimental Animals

All of our experimental work was carried out with the approval of Dicle University Animal Experiments Local Ethics Committee (DUAELEC) numbered 2022/09 (protocol number 2022/06). A total of 24 healthy Wistar Albino male rats weighing between 200 and 250 g were used in our study. Animals were kept in plastic cages in a room with a temperature of 24-25 C and a 12-hour light and dark cycle. Animals were provided with unlimited access to standard water and food.

Experimental Design

The 24 animals used in our experiment were randomly divided into 3 groups.

Sham group (Group 1): The scrotums of the animals in this group were opened with the appropriate surgical protocol and closed without any other intervention.

T/D group (Group 2): The scrotums of the animals in this group were opened and the testes, tunica vaginalis and spermatic cords were exposed. The testes were then rotated 720 degrees and fixed with disposable bulldog clamps. The animals were then detorsioned and the testes were placed in the scrotal sac. After 3 hours of detorsion, the testes of the sacrificed animals were removed.

Meningic + T/D group (Group 3): The animals in this group were orogastrically administered 2 ml/kg meningic extract for 15 days. Then, under general anaesthesia, the scrotums of the animals were opened and the testes were exposed, rotated 720 degrees and fixed with disposable bulldog clamps and torsion procedure was applied. After 3 hours, the animals were detorsioned and the testes were placed in the scrotum. After waiting for 3 hours, the testes of the sacrificed animals were taken for histopathological examination [16].

Histopathological Tissue Follow-up

Testicular tissues obtained from sacrificed animals were subjected to routine paraffin tissue tracing procedures. The tissues were kept in Zinc formalin fixative (catalogue no: Z2902-3.75L, Sigma, Germany) solution for 24 hours and then kept under running water overnight. The tissues were dehydrated by passing through increasing alcohol series and absolute ethyl alcohol. Then the tissues were kept in xylene for 3x30 minutes to remove the alcohol. The tissues removed from xylene were placed in paraffin incubation at 58°C and incubated for 3x45 minutes and embedded in paraffin blocks. Subsequently, 5 µm thick sections were taken on positively charged slides using a microtome (catalogue no: Leica RM2265, Wetzlar, Germany) and histochemical staining was performed.

Haematoxylin-Eosin Staining

Testicular tissue sections taken on 5 µm thick positively charged slides with the help of a microtome were kept in an oven at 58°C for 8 hours. The sections were then removed and allowed to cool at room temperature. The cooled sections were kept in xylene for 3x15 minutes for deparaffinisation. The sections were passed through decreasing alcohol series for 10 minutes each and kept in distilled water for 5 minutes. The sections stained with Harris Haematoxylin for 8 minutes were washed in running water for 5 minutes to remove excess haematoxylin. They were then stained with eosin for 8 minutes and then rapidly passed through increasing alcohol series. Finally, the sections were kept in xylene for 3x45 minutes, covered with entellan and examined under a light microscope.

PAS Staining

Sections of testes kept in xylol for deparaffinisation were then passed through a series of decreasing alcohols and then kept in distilled water. Ten drops of reagent A solution were added to the sections and waited for 30 minutes. Then, the solution on the sections that were not subjected to any washing process was poured and 15 drops of reagent B solution were poured and waited for 10 minutes. The sections were then placed under running tap water and kept there for 5 minutes and then kept in distilled water for 2 minutes. Sections taken from distilled water were poured 10 drops of reagent C solution and kept for 10 minutes. Then the sections were washed with distilled water and 10 drops of reagent D solution was poured into the sections and left for 20 minutes. The sections were washed again with distilled water and 10 drops of reagent E solution was poured on the sections and waited for 2 minutes. The solution on the sections was poured without any washing process and 10 drops of reactive F solution was dripped on them and kept for 2 minutes. Then, 10 drops of reactive G solution were added to the sections washed in distilled water, waited for 2 minutes and then washed under a running tap for 5 minutes. After the sections were passed through increasing alcohol series for dehydration, they were kept in xylol for 2x15 minutes and covered with entellan.

Johnsen Scoring

Johnsen testicular biopsy scoring was performed with 40 seminiferous tubules randomly selected for observational pathological evaluation from the sections stained with Haematoxylin & Eosin and PAS [17].

Statistical Analysis

All statistical analyses were performed with the help of SPSS software programme and the data obtained were determined as mean ± standard deviation. One-way analysis of variance was used to determine whether the means and the difference between these means were significant. Significant differences between groups were compared by TUKEY test. In comparisons, $p<0.05$ was accepted as significant.

RESULTS

Histopathological Findings

In the sections of the sham group, seminiferous tubules were smooth and germinal epithelium at various stages of spermatogenesis was observed in 4-5 rows on the basal lamina. It was observed that the germinal epithelium was composed of spermatocytes and spermatids together with spermatogonia. Leydig cells were smooth and there was no degeneration in the capillaries (Figure 1A and 1B). In PAS-stained sham group sections, the basement membrane was found to be regular and of appropriate thickness (Figure 1C).

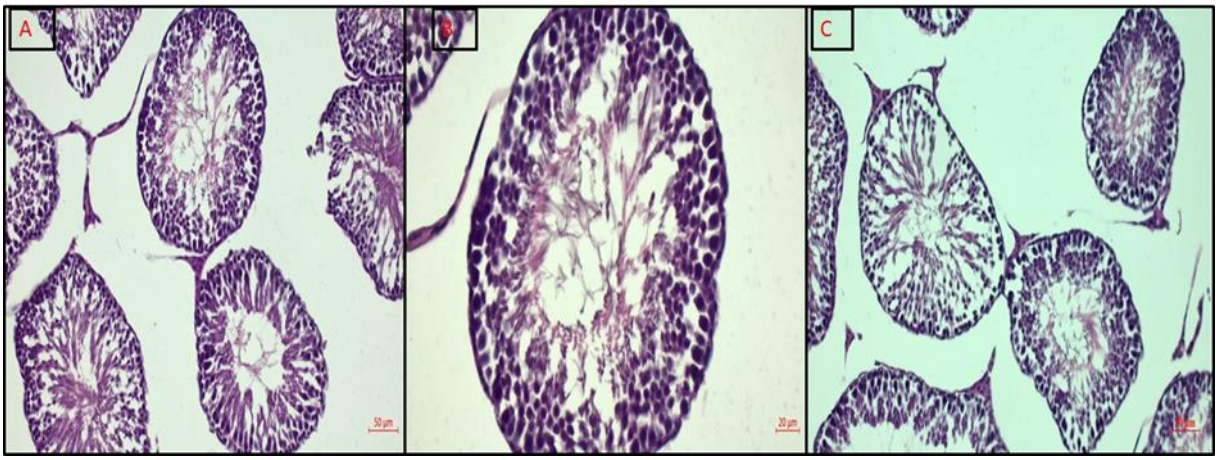


Figure 1: Histopathological appearances of testicular tissue of the sham group

In the sections of T/D group stained with Haematoxylin-Eosin, it was found that most of the seminiferous tubules were damaged and necrotic formations were observed in the germinal epithelial cells. Dilatation and congestion were observed in the vessels in the interstitial area. In addition, diffuse oedema and haemorrhages were detected in this area (Figure 2A and 2B). In PAS-stained sections of the T/D group, thickening and corrugation were observed in the basal laminae of the seminiferous tubules (Figure 2C).

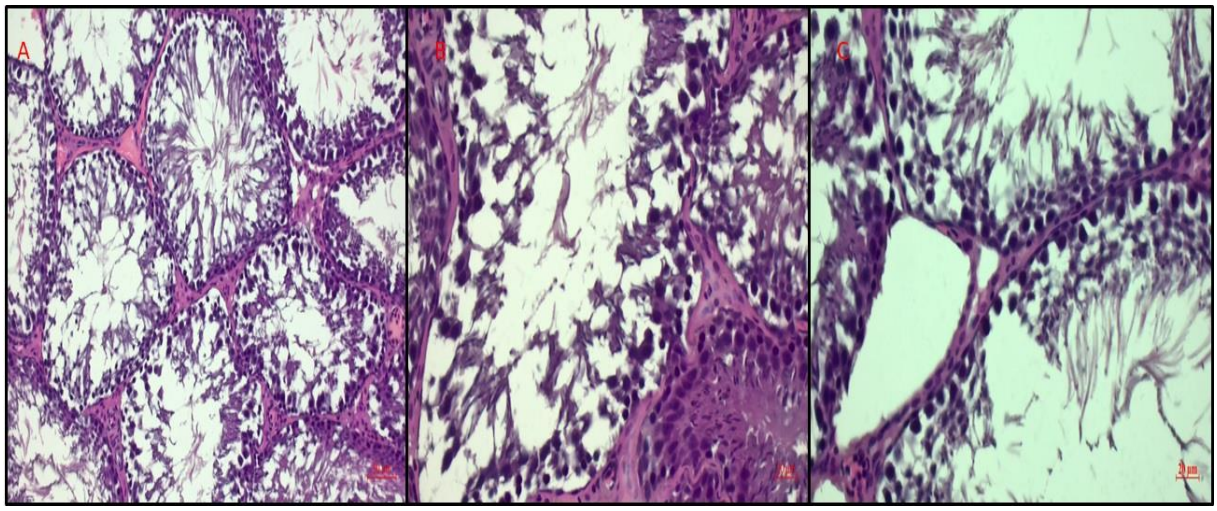


Figure 2: Histopathological appearances of testicular tissue of T/D group

In the sections of meningic + T/D group, it was observed that congestion and vascular proliferation continued, however, there was a partial recovery in seminiferous tubules and necrotic areas in germinal epithelial cells decreased (Figure 3A and 3B). It was observed that basal lamina thickening in PAS-stained meningic + T/D group sections decreased compared to T/D group (Figure 3C).

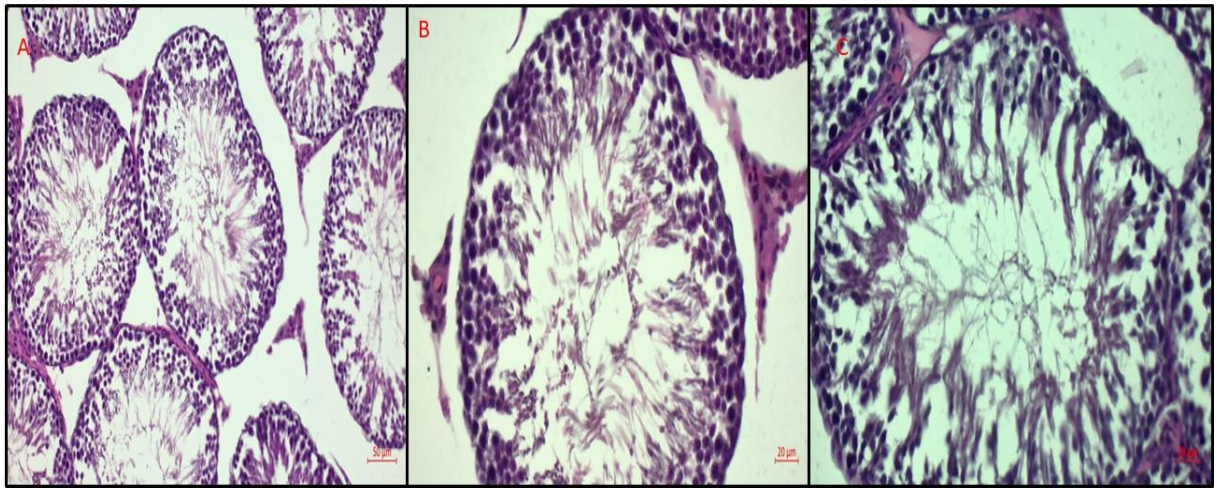


Figure 3: Histopathological appearances of testicular tissue of meningic + T/D group

Modified Johnsen Scoring Results

According to the results of the analysis of the modified Johnsen score, a significant difference was observed between the groups ($p<0.05$). According to the results of the TUKEY test performed to determine from which group the difference originated, the Johnson score of the Sham group was significantly higher than the Johnson score of the T/D and meningic + T/D groups. In addition, the Johnson score of the meningic + T/D group was significantly higher than that of the T/D group (Table 1 and Figure 4).

Table 1: Modified Johnsen scores of the groups

	N	Mean	Std. Deviation	F	p
Sham Group	40	8.60	0.98	294.345	0.000*
T/D Group	40	3.05	1.01		
Meningic + T/D Grubu	40	5.65	1.08		
Total	120	5.77	2.49		

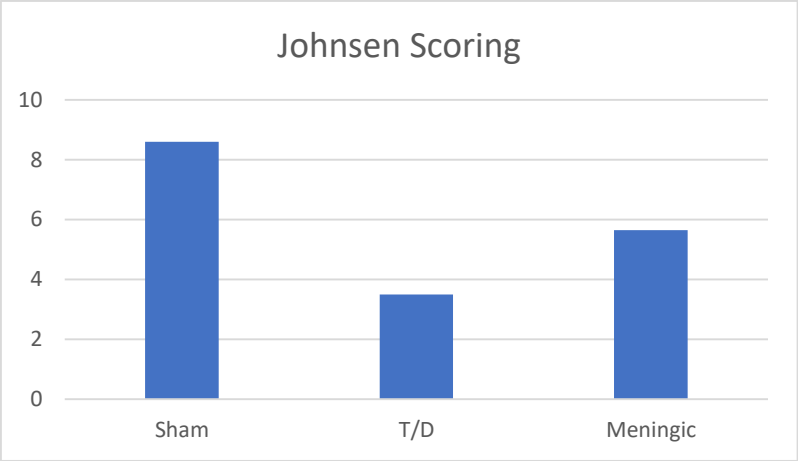


Figure 4: Graphical representation of modified Johnsen scoring

DISCUSSION

Testicular torsion is a urological condition that occurs as a result of disruption of blood flow as a result of rotation of the testicle around the spermatic cord and may lead to permanent ischaemic testicular damage and requires urgent intervention [18]. The age range of 12-18 years is the most common age group in which testicular torsion is observed. In addition, it may occur at almost any age. It mostly manifests itself with extremely severe scrotal pain which is felt acutely at rest. Time is of critical importance in order to save the testicle after torsion. In early surgical interventions, the probability of testicular rescue is much higher [19]. In cases of pain lasting longer than 4 to 8 hours, the time of presentation to the hospital is important since non-intervention most probably results in testicular death [20]. In approximately one third of the cases admitted to hospital, the testicle is considered dead and orchiectomy is performed. The first two most important factors in determining the damage that develops after testicular torsion are the time between the onset of symptoms and the reduction of torsion and the degree of twisting of the cord [19].

The ischaemic period during torsion and oxidative stress occurring at the end of detorsion have been shown as the source of long-term damage in the ipsilateral testis after testicular torsion [21]. With detorsion occurring after testicular torsion, oxygen-rich blood returns to the testis and this leads to the emergence of reactive oxygen species (ROS). Apoptosis develops in germinal cells as a result of damage to testicular DNA by ROS [22]. Following reperfusion, there is an increase in the release of superoxide anion, hydrogen peroxide-like ROS, reactive nitrogen species (RNS) including nitric oxide which is a source of various oxidative stress factors and proinflammatory cytokines. This results in metabolic acidosis, intracellular calcium overload, mitochondrial dysfunction and apoptosis in germ cells [23].

In our study, deterioration in the histology of the testes was observed in T/D group. It was observed that there were no spermatozoa in the lumen of some seminiferous tubules. In addition, it was observed that the epithelium of some of the seminiferous tubules was irregularly shaped, maturation of spermatogenic germ cells was retarded, germinal epithelium was damaged, some tubules were almost empty, atrophy developed in some tubules, intense haemorrhages occurred in the interstitial area, vessels were dilated and intense oedema developed. In addition, the extent of the damage in the T/D group was supported by Johnsen scoring.

Under normal conditions, sperm cells have a defence mechanism that includes antioxidants and thus resist ROS attacks. However, the excessive amount of ROS that occurs after T/D causes oxidative stress [24]. Studies have also revealed that various antioxidants may play a role in attenuating the damages caused by I/R in different organs including the testis [25, 26]. In addition, various antioxidants have been investigated according to their ability to directly counteract ROS and their ability to affect ROS toxicity in sperm of some mammalian species [27]. There are studies showing that antioxidants are used in the treatment of organs damaged by various diseases other than I/R injuries [28-29].

In a study conducted by Jafari et al., the protective effects of topiramate, which is known to have antioxidant properties and used in the treatment of epilepsy and prevention of migraine, were investigated. The study showed that topiramate administered 30 minutes before detorsion decreased MDA levels and increased the activities of antioxidant enzymes. In addition, it was found that anti-ischemic effects were decreased in the said study [30]. In another study, the protective effect of Ganoderma lucidum [31], which is known to have antioxidant properties, on ischaemia-reperfusion injury resulting from testicular T/D was investigated. In the study, it was revealed that G. Lucidum was able to reduce the histopathological damages occurring in the tissue with oxidative stress. This suggests that G. Lucidum has an important protective effect in preventing testicular T/D damage [32]. In another study, the protective effects of the leaves of Plantago major (PM), which belongs to the Plantaginaceae family and has antioxidant and anti-inflammatory properties, on testicular torsion were investigated. It was observed that PM provided protection of spermatogenesis to a great extent and reduced oedema, haemorrhage and coagulative necrosis caused by I/R. Therefore, it was stated that PM may have a protective effect in TD [33].

Extracts of various plants with known antioxidant properties have been investigated for their protective effects in torsion/detorsion models in different organs. Aşır et al. examined the protective effect of Momordica charantia (MC) against ischaemia-reperfusion damage in the ovary and found that MC extract given for a certain period of time before ovarian torsion reduced the pathologies occurring in the ovaries as a result of torsion [34].

Pistacia terebinthus L. fruit extracts which have anticarcinogenic, antioxidant, antimicrobial and antimutagenic properties are frequently used in alternative medicine [15, 35]. Due to these properties, they are also used in scientific studies to understand their protective and therapeutic effects in various diseases. In a study, the effects of Rhus coriaria and P. terebinthus on some biochemical parameters of brain tissues of rats with breast cancer were investigated. Although it was thought that they were not sufficiently effective on enzymatic activities at the molecular level, it was concluded that plant suspensions can exhibit anti-cancer effects. Researchers reported that Pistacia terebinthus and Rhus coriaria plants can be used in breast cancer to combat oxidative stress [36].

In another study in which the antidiabetic activity of meningic was investigated, it was observed that insulin immunoreactivity in pancreatic β cells increased significantly when diabetic animals were treated with meningic [37]. In another study, it was observed that meningic oil used topically in quails accelerated the wound healing process [38]. In our study, it was observed that seminiferous tubules partially recovered in the testicular sections of the animals given meningic extract for 15 days before the T/D procedure. In addition, a decrease in necrotic areas, limited oedema due to ischaemia and significant preservation of spermatogenesis were observed in germinal epithelial cells compared to the torsion group. We think that these results are important in terms of showing the protective effect of meningic against testicular torsion cases.

CONCLUSION

In our study, the effects of meningic extract applied before T/D in the testis were examined histopathologically and it was concluded that meningiç extract partially protected the testis and its functions. However, there is a need for comprehensive scientific studies to be carried out in order to reveal more clearly the effectiveness of T/D in both testis and other organs of meningic, whose antioxidant properties are known and both protective and therapeutic effects have been examined in many diseases.

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