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# The Protective Roles of Mito-TEMPO on Testicular Ischemia-Reperfusion Injury Based on Biochemical and Histopathological Evidences in Mice

Zohreh Mostahsan[1], Saeed Azizi ✉[1], Ali Soleimanzadeh[2], Ali Shalizar-Jalali[3]

*[1] Department of Surgery and Diagnostic Imaging, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran. [2] Department of Theriogenology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran. 3 Department of Basic Sciences, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.*

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**ABSTRACT**

Accumulation of reactive oxygen species during testicular torsion causes oxidative stress, which in turn causes ischemia-reperfusion (I/R) injury to the testis. In testicular torsion/detorsion (T/D) in male mice, the purpose of this study was to investigate the influence of Mito-TEMPO (MT) on I/R injury. Forty-two male mice were divided into seven groups, including a control group and six treatment groups (360° T/D, 720° T/D, 360° T/D + 0.70 mg/kg MT, 360° T/D + 1 mg/kg MT, 720° T/D + 0.70 mg/kg MT and 720° T/D + 1 mg/kg MT). After inducing testicular torsion, oxidative enzymes, and testicular histopathology were evaluated. The results showed that 720° T/D resulted in increased testicular malondialdehyde levels and histological damage, along with reduced activities of catalase, superoxide dismutase, and glutathione peroxidase. Treatment with MT reduced tissue malondialdehyde levels and increased the activities of catalase, superoxide dismutase, and glutathione peroxidase. These findings suggest that MT administration protects against acute testicular T/D injury in mice.

## Introduction

Testicular torsion is a urological surgical emergency in which the testicle and the spermatic cord twist. Delayed diagnosis and treatment can lead to testicular necrosis and atrophy. Testicular torsion is the most common cause of testicular loss in newborns, children and adolescent boys.[1,2] About 26% of people with acute testicular problems suffer from testicular torsion.[3,4] The extent and duration of torsion are crucial factors in determining testicular injury. Timely treatment within 6 h of pain onset offers a significant chance of saving the affected testicle, with a survival rate of 90-100%.[5] Treatment within 6-12 h can save 20-50% of the testicles depending on the degree of torsion, while treatment after 12-24 h has little chance of success.[5,6] Urgent surgery is required to reverse the twisted spermatic cord and restore blood flow, but reperfusion of the ischemic tissue can cause severe testicular damage.[7]

Ischemia-reperfusion (I/R) injury can result in long-lasting testicular degeneration even after the initial ischemic damage.[8,9] During both the ischemia and reperfusion phases, an overproduction of proinflammatory cytokines occurs, promoting leukocyte migration to the testicular tissue. This triggers the production of reactive oxygen species (ROS) by neutrophils, causing oxidative damage to testicular cells.[10-12] These changes, including lipid peroxidation, excessive pro-inflammatory cytokines, and intracellular calcium release, can contribute to infertility.[4] Oxidative stress impairs the ability of biological systems to detoxify reactive mediators and repair ROS-induced damage, affecting proteins, lipids, DNA, and overall cellular performance.[13] Oxidative stress plays a role in the development and progression of various diseases.[14]

Previous research has demonstrated the efficacy of antioxidants with free radical scavenging properties in

✉ Corresponding author. Email: s.azizi@urmia.ac.ir

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reducing I/R damage in different organs, including the testis.[15] Antioxidants prevent the oxidation of molecules and the formation of free radicals, which can initiate harmful chain reactions.[16] Combinations of enzymes and drugs have been used to support tissue repair and prevent post-I/R testicular damage.[4,17,18] Zinc aspartate, curcumin, and dexamethasone have been effective in reducing oxidative stress and enhancing antioxidant enzyme activity.[19]

Plants and animals naturally contain antioxidants like glutathione, vitamin C, vitamin E, catalase, and superoxide dismutase. Mito-TEMPO (MT) is a lipophilic antioxidant compound composed of piperidine nitroxide and triphenylphosphonium cation.[16] This cation easily crosses lipid bilayers and accumulates in mitochondria.[20] MT is a targeted antioxidant that protects cells from oxidative damage in various conditions, including sepsis-induced acute kidney injury, colitis, and endotoxin-induced liver injury.[21] Multiple studies have demonstrated the protective effects of MT against oxidative damage in diverse diseases.[22] To our knowledge, no prior studies have investigated the impact of Mito-TEMPO on testicular ischemia-reperfusion (I/R) injury. The objective of this study was to investigate the effects of intraperitoneally (IP) administered Mito-TEMPO on testicular tissue and oxidative stress markers in adult male mice following experimental testicular ischemia-reperfusion (I/R) injury.

## Materials and Methods

### Chemicals

All necessary chemical substances were sourced from reliable suppliers, including Sigma (St. Louis, MO, USA).

### Animals

The study involved forty-two adult male mice, weighing between 20 and 25 g and aged 6 to 8 weeks, obtained from the Animal Resource Center of Urmia University, Iran. The mice were maintained and observed in a controlled environment that was carefully regulated to maintain a stable temperature range of 20 to 22 °C, sufficient ventilation for optimal air quality, a precisely regulated 12 h light-dark cycle, and a relative humidity level of 50 ± 10%. They had unrestricted access to food and water and were acclimatized for one week before the experiment. The study was conducted in accordance with the regulations of the Animal Ethics Committee of Urmia University, Iran (IR-UU-AEC-3/45).

### Experimental Protocol

Following one week of acclimatization the mice were divided randomly into 7 groups each having six animals as follows: Group 1 (sham-operated control group) underwent a sham operation without testicular I/R injury; Group 2 (360° torsion/detorsion [T/D]) experienced 2 h of 360° torsion-induced ischemia; Group 3 (720° T/D) experienced 2 h of 720° torsion-induced ischemia; Group 4 (360° T/D + 0.7 mg/kg MT) experienced 2 h of 360° torsion-induced ischemia and 30 min,[23] before testicular detorsion mice received MT at a dose of 0.7 mg/kg; intraperitoneally (IP);[24] Group 5 (360° T/D + 1 mg/kg MT) experienced 2 h of 360° torsion-induced ischemia and 30 min,[23] before testicular detorsion mice received MT at a dose of 1 mg/kg; IP;[25] Group 6 (720° T/D + 0.70 mg/kg MT) experienced 2 h of 720° torsion-induced ischemia and 30 min,[23] before testicular detorsion mice received MT at a dose of 0.7 mg/kg; IP;[24] Group 7 (720° T/D + 1 mg/kg MT) experienced 2 h of 720° torsion-induced ischemia and 30 min,[23] before testicular detorsion mice received MT at a dose of 1 mg/kg; IP.[25]

### Surgical Procedure

The surgical procedure was performed under sterile conditions. The mice were anesthetized using 10% ketamine 80 mg/kg and 2% xylazine 10 mg/kg; (both from Alfasan, Netherlands) intraperitoneally. The testes were exposed through ventral midline laparotomy and rotated clockwise 360° (groups 2, 4 and 5) or 720° (groups 3, 6, and 7) following the surgical preparation of the testicular region, shaving and cleaning with a 10 % povidone-iodine solution. The testis was then fixed in the torsion position with three simple single stitches (5-0 silk, nonabsorbable; SUPA, Iran) and detorsion was performed after 2 hr. The incision was then closed with a simple running suture technique (4-0 nylon, nonabsorbable; SUPA, Iran). Orchiectomy were done as follow: The left testis was removed surgically after 24 h to determine oxidative stress parameters. The right testis and its epididymis were removed as well after 30 days to assess the sperm parameters and histological examination.[26]

### Enzymatic Antioxidant Activity Assessment

To assess enzymatic antioxidant activity, 20-30 mg of testicular tissue was homogenized in 1000 µl of lysis buffer and centrifuged at 9000 rpm for 15 min, and the supernatant was collected for biochemical analyses and subsequently stored at a temperature of -20 °C until the tests were performed.[27]

### Malondialdehyde (MDA) Level

The MDA test kit (Nalondi, Lipid Peroxidation Assay Kit; Navand Salamat Co., Urmia, Iran) was used. The MDA concentration was determined at a wavelength of 523 nm using a spectrophotometer (Thermo Fisher Scientific; Waltham, MA) and a standard curve and expressed as nmol/mg protein.[28]

### Total Antioxidant Capacity (TAC) Determination

The TAC levels in the testis were quantified using a TAC assay kit (Naxifer; Navand Salamat Company, Urmia, Iran). For the testicular TAC assay, the reaction was conducted in 1000 µl of reaction buffer containing 100 µl of supernatant, 400 µl of distilled water, and 500 µl of $ABTS^{+}$ buffer (containing 100 µl of $ABTS^{+}$ and 800 µl of distilled water, with 100 µl of potassium persulfate (10×). The absorbance was 1.14. Following a 5-min incubation period at room temperature, the absorbance was monitored at 414 nm. The results were reported as nmol/mg protein.[28]

### Glutathione Peroxidase (GPx) Activity

The GPx level in the testis was assessed using a GPx kit (Nagpix™; Navand Salamat Co., Urmia, Iran). GPx activity was determined with tert-butyl-hydroperoxide as a substrate. The assay mixture included 2 mM glutathione, 0.15 U/mL glutathione reductase, 0.4 mM azide, 0.5 mM tert-butyl-hydroperoxide, and 0.1 mM NADPH. Absorbance was measured at 340 nm, and outcomes were expressed as mU/mg protein.[28]

### Superoxide Dismutase (SOD) Level

A SOD test kit (Nasdox; Navand Salamat Co., Urmia, Iran) was used to determine the SOD content in the testis. SOD activity was measured by quantifying the reduction in color development at 405 nm. The SOD activity in the testis was expressed as U/mg protein.[28,29]

### Catalase (CAT) Level

To measure CAT activity, a two-step procedure using a commercially available CAT kit (Nactaz™ Catalase Activity Assay Kit; Navand Salamat Co., Urmia, Iran) was followed. After 10 min of incubation at room temperature, CAT activity was calculated using an absorbance rate of 550 nm. The results are reported as U/mg protein.

### Testicular Histopathology and Histomorphometry

The right testes of mice were preserved in a 10 % formalin solution. They were dehydrated with a series of ethanol and then embedded in paraffin. Thin layers with a thickness of 7 µm were created using a microtome. The resulting sections were then stained by hematoxylin and eosin (H&E) staining.[30–33] The stained sections were examined under a light microscope (Olympus Model BH-2, Tokyo, Japan).

Johnsen's score was used to assess seminiferous tubules in each cross section (Table 1). To monitor spermatogenesis, 200 seminiferous tubules were examined under a light microscope (CHT model, Olympus Optical Co. Ltd., Tokyo, Japan). To determine the seminiferous tubule diameter (STsD), 200 randomly selected round or nearly round cross-sections of seminiferous tubules (100 from each testis) were examined. Two vertical diameters of each seminiferous tubule cross-section were measured with a light microscopic eye micrometer (Model CHT, Olympus Optical Co. Ltd., Tokyo, Japan) and their average values were calculated.[34] The Sertoli cell index (SCI), repopulation index (RI) and mitotic index (MI) were calculated by randomly selecting sixty seminiferous tubules per group. SCI is the ratio of Sertoli cells with a distinct nucleus and nucleolus being present in seminiferous tubules, to the number of germ cells. [35] The RI calculates the proportion of tubules populated with germ cells that have reached at least the middle spermatogonial stage or later.[36]

To determine the proportion of cells lost during cell division, the MI (number of round spermatids for each pachytene primary spermatocyte) was used.[37] The Leydig cell nuclear diameter (LCND) was determined using a calibrated ocular micrometer as described by Elias and Hyde.[38] Two hundred transverse sections of the seminiferous tubules of each animal (100 *per* testis) were randomly examined to determine the tubular differentiation index (TDI) and spermiogenesis index (SPI). The TDI refers to the proportion of seminiferous tubules having at least three fully developed germ cells;[39] while, the SPI calculates the proportion of seminiferous tubules normally containing sperm cells.[40] The degree of testicular injury was assessed using the Cosentino scoring system.[41] This system divides the testicle into 4 grades. Grade 1 represents normal testicular architecture, grade 2 indicates less ordered, noncohesive germ cells and closely packed seminiferous tubules, grade 3 represents disordered, sloughed germ cells with shrunken, pyknotic nuclei and less distinct seminiferous tubule borders and grade 4 shows seminiferous tubules being densely packed along with germ cell coagulative necrosis.[41,42]

### Statistical Analysis

The study data were analyzed using SPSS software (version 26.0, IBM Corporation, Chicago, USA). A one-way ANOVA was performed to determine significant differences between the groups. Tukey's post hoc analysis was used to identify specific groups that differed significantly. A $p$-value of $\leq 0.05$ was considered statistically significant.

## Results

### Testicular Oxidant/Antioxidant Status

As shown in Figures. 1A and 2B, tissue TAC and CAT values were significantly lower in groups 2, 3, 4, 6 and 7 than in control and group 5 ($p \leq 0.05$). Group 3 had the lowest TAC values, while groups 6 and 7 (0.70 and 1

***Table 1.*** Johnsen scoring system used for testicular damage evaluation.

| Johnsen score | Description of histological criteria |
|---|---|
| **10** | Full spermatogenesis |
| **9** | Slightly impaired spermatogenesis, many late spermatids, disorganized epithelium |
| **8** | Less than five spermatozoa per tubule, few late spermatids |
| **7** | No spermatozoa, no late spermatids, many early spermatids |
| **6** | No spermatozoa, no late spermatids, few early spermatids |
| **5** | No spermatozoa or spermatids, many spermatocytes |
| **4** | No spermatozoa or spermatids, few spermatocytes |
| **3** | Spermatogonia only |
| **2** | No germinal cells, Sertoli cells only |
| **1** | No seminiferous epithelium |

mg/kg MT) were able to increase TAC values compared to group 3 (Figure 1A). Furthermore, tissue GPx values in group 3 were significantly lower ($p \leq 0.05$) than those in all other groups (Figure 1C). Although there was no significant difference in GPx values between groups 6 and 7, their GPx values were significantly lower than those of groups 1, 2, 4 and 5 ($p \leq 0.05$; Figure 1C). Finally, group 3 had significantly lower ($p \leq 0.05$) tissue SOD values than the other groups, while groups 1, 4 and 5 had significantly higher ($p \leq 0.05$) tissue SOD values than all other groups (Figure 1D). The results of the analyses showed that the MDA levels in group 3 increased significantly compared to those in the other groups ($p \leq 0.05$; Figure 1D). It is worth noting that the administration of 0.70 and 1 mg/kg MT in groups 6 and 7 had a positive impact on MDA levels, being better than that of group 3 ($p \leq 0.05$; Figure 1D).

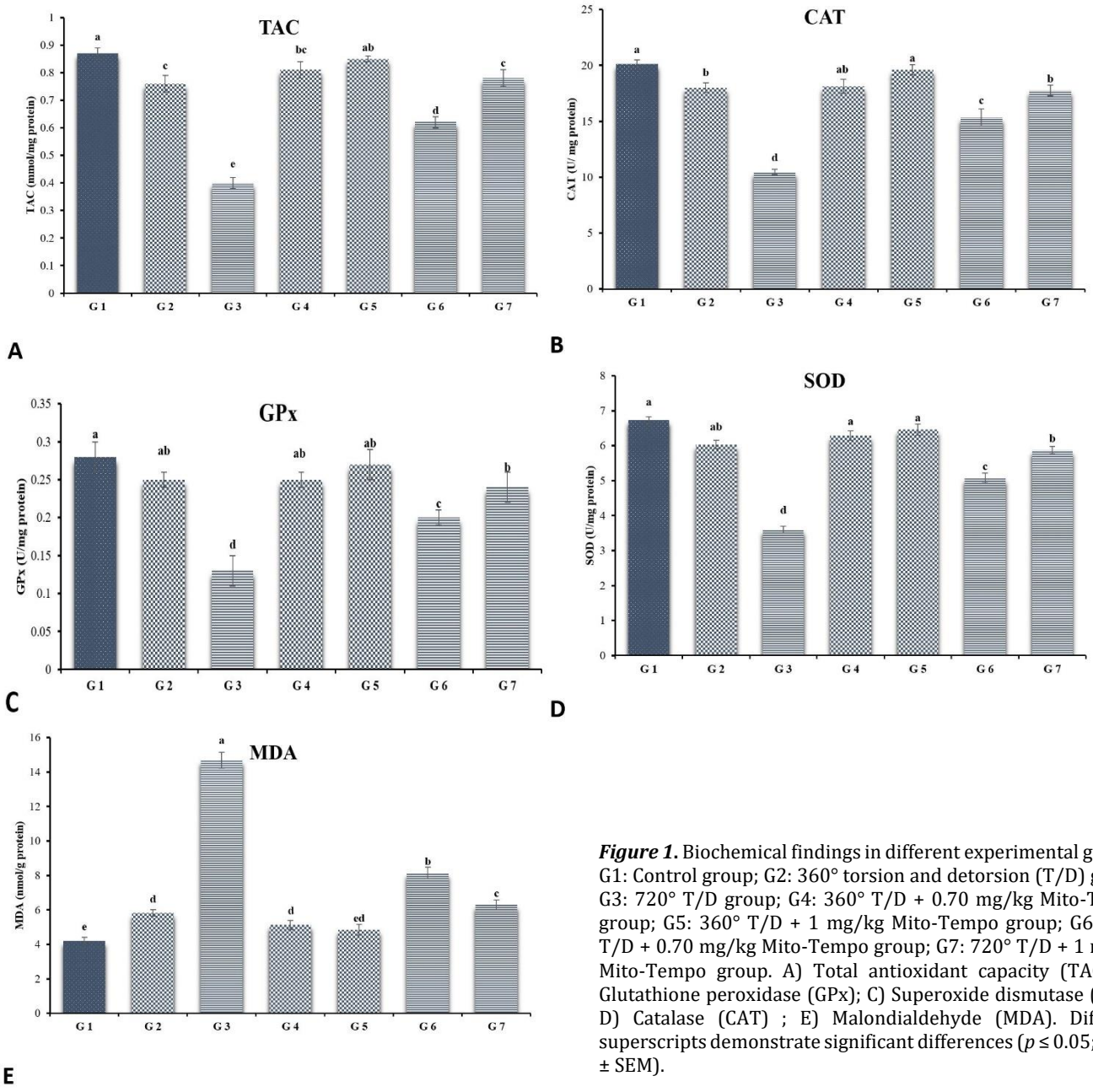


***Figure 1.*** Biochemical findings in different experimental groups. G1: Control group; G2: 360° torsion and detorsion (T/D) group; G3: 720° T/D group; G4: 360° T/D + 0.70 mg/kg Mito-Tempo group; G5: 360° T/D + 1 mg/kg Mito-Tempo group; G6: 720° T/D + 0.70 mg/kg Mito-Tempo group; G7: 720° T/D + 1 mg/kg Mito-Tempo group. A) Total antioxidant capacity (TAC); B) Glutathione peroxidase (GPx); C) Superoxide dismutase (SOD); D) Catalase (CAT) ; E) Malondialdehyde (MDA). Different superscripts demonstrate significant differences ($p \leq 0.05$; Mean ± SEM).

### Animal Weight

Table 2 shows the weight and size of the testis and epididymis. A nonsignificant ($p > 0.05$) decrease in the testis-to-body weight ratio was found between the test groups and the control group.

### Histological Findings

The testis underwent significant morphological changes due to testicular torsion, as shown in Figure 2. The atrophied seminiferous tubules showed intraepithelial vacuolation and severe hypocellularity (reduction in the number of germ cells). The intertubular connective tissue showed cracks, vacuolation, vascular occlusion, inflammatory cell infiltration, edematous fluid accumulation and interstitial space dilation. The Leydig cells were degenerated and exhibited pyknotic nuclei, while the Sertoli cells were detached from the germ cells and developed amorphous, irregular and smaller nuclei.

Johnsen's score (Figure 3 A) determination confirmed germ cell degeneration, desquamation and disorganization in torsioned testes. The mean Cosentino's score (Figure 3 B) showed that group 3 had significantly more histopathological changes than the control group ($p \leq 0.05$), but the differences between the other groups were not statistically significant ($p > 0.05$). The loss of germ cells during spermatogenesis due to the 720° T/D resulted in a significant reduction in SCI, RI and STsD in groups 3, 6 and 7 compared to the other groups ($p \leq 0.05$; Table 2). This also led to a significant decrease in the MI. However, no significant difference in these characteristics was observed between the control group and group 1 ($p > 0.05$; Table 2). Analysis of histological parameters revealed a reduction in testicular biopsy score, SPI, LCND and TDI of 720° T/D compared to the other groups. However, administration of 0.70 and 1 mg/kg MT (groups 6 and 7) improved the aforementioned parameters compared to group 3 ($p \leq 0.05$; Table 2).

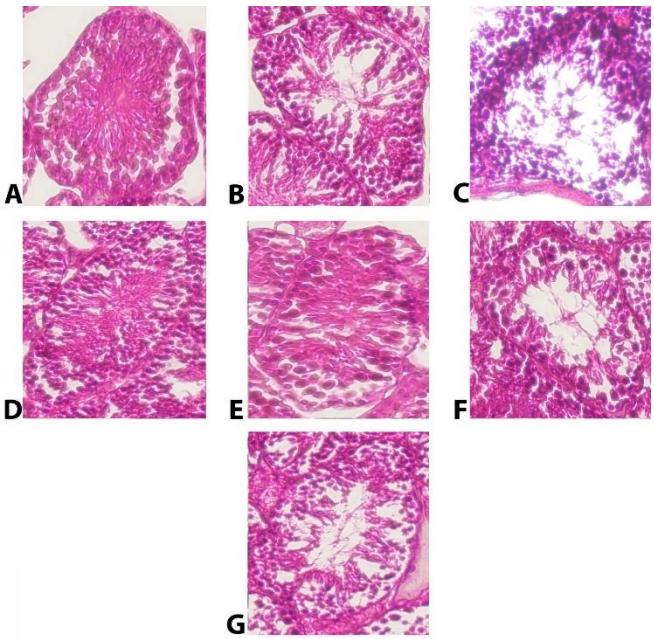


***Figure 2.*** Testicular histo-architecture in different experimental groups. A) Control group; B) 360° torsion and detorsion (T/D) group; C) 720° T/D group; D) 360° T/D + 0.70 mg/kg Mito-Tempo group; E) 360° T/D + 1 mg/kg Mito-Tempo group; F) 720° T/D + 0.70 mg/kg Mito-Tempo group; G) 720° T/D + 1 mg/kg Mito-Tempo group (Hematoxylin & Eosin staining, 400×).

## Discussion

Testicular atrophy and degeneration are caused by a significant disruption in the blood supply to the testicles as a result of testicular torsion.[43] After the damage is done, surgery is the only effective treatment method.[44] Delays in detorsion result in germ cell death because reperfusion is insufficient to maintain tissue function, which is the main cause of the pathophysiological effects of testicular torsion. In addition, the accumulation of toxic substances such as ROS and low oxygen levels during ischemia contribute to germ cell death.[45] Intestinal torsion-related tissue damage and oxidative stress may be less severe with the use of exogenous antioxidants.[46] The testes can be effectively protected from I/R damage by medications such as diclofenac, omeprazole, and hesperidin. To protect mice from testicular torsion, this study examined how well MT was administered.[47,48] The findings of this study indicate that the administration of MT intraperitoneally can reduce testicular tissue damage following testicular torsion and improve the oxidant/antioxidant balance.

Antioxidant defense mechanisms in testicular tissue including SOD, CAT and GPx decrease during testicular T/D.[49–52] It was found in this study that 720° T/D reduced TAC, GPx, SOD and CAT levels in testicular tissue and increased MDA levels; while, administration of MT following T/D could reinforce antioxidant defense machinery in testicular tissue. Reportedly, it has been shown that MT can scavenge ROS and improve the sperm antioxidant enzymes activity, causing oxidative stress reduction. These findings supported former reports on MT,[53,54] highlighting the ability of MT to scavenge ROS.[55] Accordingly, it has been revealed that testicular I/R increases oxidative stress while reducing antioxidant enzyme concentrations.[15] The simplest method to demonstrate that tissue lipid peroxide concentration is an important predictor of I/R is to measure MDA levels. Correspondingly, it has been reported that MDA levels in testicular tissue increase significantly with increasing testicular T/D duration, leading to oxidative damage.[56,57] It was also found that MDA values following testicular torsion increased significantly compared to the control group.[57] Furthermore, it was reported that MDA levels in experimentally-induced testicular T/D groups were higher than those of control and treatment groups.[56]

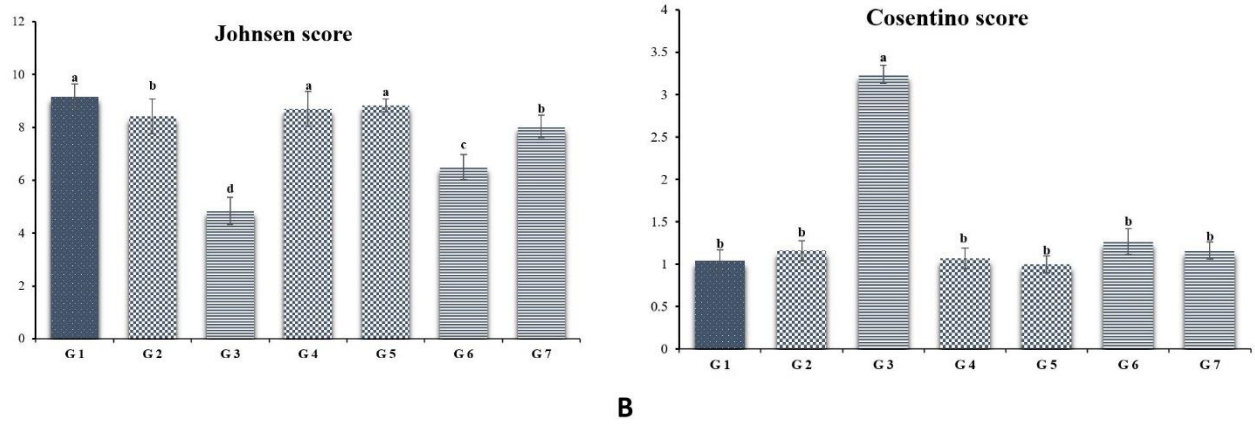


***Figure 3***. Testicular A) Johnsen's score, and B) Cosentino's score in different experimental groups.

***Table 2***. Histological parameters and reproductive organ weights in different experimental groups. Values are expressed as mean ± SEM.

| Groups / Analysis | 1 Control | 2 360° T/D | 3 720° T/D | 4 360° T/D + 0.70 mg/kg MT | 5 360° T/D + 1 mg/kg MT | 6 720° T/D + 0.70 mg/kg MT | 7 720° T/D + 1 mg/kg MT |
|---|---|---|---|---|---|---|---|
| **Testis weight (g)** | 0.65±0.02 $^{a}$ | 0.64±0.01 $^{a}$ | 0.62±0.02 $^{a}$ | 0.65±0.03 $^{a}$ | 0.65±0.02 $^{a}$ | 0.63±0.01 $^{a}$ | 0.63±0.01 $^{a}$ |
| **Epididymis weight (g)** | 0.27±0.01 $^{a}$ | 0.27±0.01 $^{a}$ | 0.27±0.02 $^{a}$ | 0.27±0.01 $^{a}$ | 0.27±0.02 $^{a}$ | 0.27±0.02 $^{a}$ | 0.27±0.02 $^{a}$ |
| **Testis/body weight (%)** | 0.296±0.004 $^{a}$ | 0.290±0.001 $^{a}$ | 0.277±0.003 $^{a}$ | 0.292±0.004 $^{a}$ | 0.296±0.002 $^{a}$ | 0.280±0.001 $^{a}$ | 0.284±0.003 $^{a}$ |
| **Johnsen score** | 9.17±0.47 $^{a}$ | 8.42±0.65 $^{b}$ | 4.84±0.51 $^{d}$ | 8.70±0.66 $^{a}$ | 8.83±0.24 $^{a}$ | 6.51±0.47 $^{c}$ | 8.04±0.42 $^{b}$ |
| **Cosentino score** | 1.04±0.04 $^{a}$ | 1.16±0.03 $^{a}$ | 3.24±0.09 $^{b}$ | 1.07±0.05 $^{a}$ | 1.00±0.03 $^{a}$ | 1.47±0.06 $^{a}$ | 1.16±0.05 $^{a}$ |
| **Seminiferous tubule diameter (µm)** | 52.84±1.85 $^{a}$ | 49.92±1.49 $^{a}$ | 27.91±1.31 $^{d}$ | 49.85±1.29 $^{a}$ | 51.47±1.64 $^{a}$ | 39.48±1.62 $^{c}$ | 44.62±1.53 $^{b}$ |
| **Sertoli cell index** | 87.86±2.70 $^{a}$ | 80.17±2.72 $^{b}$ | 53.89±1.80 $^{d}$ | 85.35±3.49 $^{a}$ | 86.49±2.58 $^{a}$ | 69.35±1.50 $^{c}$ | 79.74±3.57 $^{b}$ |
| **Repopulation index** | 74.65±2.91 $^{a}$ | 68.43±2.17 $^{cd}$ | 40.11±1.94 $^{f}$ | 70.17±2.61 $^{bc}$ | 72.85±2.25 $^{ab}$ | 53.57±1.30 $^{e}$ | 66.29±2.95 $^{d}$ |
| **Miotic index** | 2.30±0.12 $^{a}$ | 2.01±0.06 $^{b}$ | 1.04±0.03 $^{d}$ | 2.17±0.05 $^{a}$ | 2.19±0.06 $^{a}$ | 1.76±0.04 $^{c}$ | 2.01±0.03 $^{b}$ |
| **Leydig cell nuclear diameter (µm)** | 6.19±0.14 $^{c}$ | 6.95±0.53 $^{c}$ | 8.47±0.65 $^{a}$ | 6.42±0.53 $^{c}$ | 6.17±0.69 $^{c}$ | 7.23±0.41 $^{b}$ | 6.81±0.36 $^{c}$ |
| **Tubular differentiation index (%)** | 87.16±2.73 $^{a}$ | 79.87±2.81 $^{cd}$ | 50.64±0.71 $^{f}$ | 82.70±2.78 $^{bc}$ | 84.21±3.95 $^{ab}$ | 67.41±2.38 $^{e}$ | 77.69±2.78 $^{d}$ |
| **Spermiogenesis index (%)** | 85.49±2.49 $^{a}$ | 77.65±2.98 $^{cd}$ | 48.10±1.70 $^{f}$ | 80.34±3.74 $^{bc}$ | 81.96±2.80 $^{b}$ | 65.62±2.93 $^{e}$ | 75.27±2.57 $^{d}$ |

T/D: Torsion and detorsion; MT: Mito-TEMPO. $^{a-f}$ Different superscripts within the same row demonstrate significant differences ($p \leq 0.05$).

It has been indicated that reperfusion can lead to increased apoptosis and tissue damage.[58] Previous studies have shown that oxidative stress caused by ischemia can lead to tissue damage and that the testes are particularly vulnerable to this type of stress due to their physiological and anatomical nature. Adolescents experiencing torsion may suffer from reduced reproductive function and efficiency due to significant oxidative damage.[48] Venous drainage disruption during testicular T/D results in edema, hemorrhage, arterial obstruction and tissue ischemia. It has been documented that at a torsion of 720°, complete ischemia occurs when blood flow stops irreversibly.[59] In their rat model of 720° unilateral torsion of the left testis, Ganjiani *et al*. (2021) discovered that reperfusion injury develops biochemically after 2 hr of detorsion.[23] In our study, the testes were subjected to 2 hr of T/D and histological findings disclosed testicular tissue damage. After detorsion, tissue is exposed to excessive oxygen, resulting in ROS overproduction. Increased neutrophil levels in the testicular circulation and excessive ROS generation damage cell membranes and lead to tissue damage.[60] The imbalance between ROS and endogenous antioxidants leads to cell damage after I/R.[60] Due to the high concentration of highly unsaturated fatty acids in the testicles, they are particularly susceptible to oxidative stress damage. Oxidative stress has been reported to impair testicular activities after testicular detorsion through seminiferous tubule normal histostructure alteration and germ cell population reduction. According to Cvetkovic *et al*. (2015) biochemical markers of oxidative stress are much more reliable than histological markers regarding tissue damage detection.[61] To prevent I/R injury, various chemicals have been used, including anti-inflammatory and antioxidant drugs.[60] Verapamil[62] was reported to have a protective effect against testicular tissue I/R injury. Similarly, tadalafil could promote testicular histoarchitecture as well as spermatogenesis. Our research revealed that administration of MT following 720° T/D could improve histological damage in

mouse testes.[63] The amount of glucose-6-phosphate isomerase, an essential enzyme in the glycolytic pathway that is closely related to cell quality, is controlled by MT.[64] Because of its positive charge, MT builds up in the mitochondria. By reducing or inhibiting the mitochondrial free radical formation and lipid peroxidation, it has the effect of a targeted antioxidant. In addition, it controls the antioxidant enzyme activity of cells.[65]

In conclusion, this work is the first to investigate how MT, an agent with anti-inflammatory and antioxidant properties, affects I/R damage caused by testicular torsion. The results suggest that MT can improve oxidative parameters and reduce testicular tissue damage after I/R injury. This theory is supported by our results, which include monitoring of oxidative indicators and histopathological analysis. Consequently, MT may represent a viable new treatment option for I/R injury caused by testicular torsion.

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## Conflict of Interest

Authors disclose no potential conflict of interests.

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