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Original Article

Ferulago angulata; A Complementary Therapy in Testicular Torsion-Detorsion Damage

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ABSTRACT

The present study was conducted to determine the protective influences of *Ferulago angulata* aqueous extract against testicular ischemia/reperfusion (I/R) injury in rats. Twenty-eight subjects were assigned randomly into four groups: (I) sham group, (II) torsion/detorsion (T/D) group, (III) T/D followed by treatment with *F. angulata* (200 mg/kg, IP), (IV) intact group with only 200 mg/kg *F. angulata*. T/D damage was induced by 2 hours of testis rotation 720° followed by 24 hours of reperfusion injury. After reperfusion period, biochemical and hormonal parameters were measured in the serum, epididymal sperms were collected for evaluation of sperm characteristics (count, motility, viability, and abnormal morphology rate), and testes were studied histopathologically. I/R injury was associated with significant reduction in count, viability, and all sperm kinematic parameters ($p < 0.05$), which could be reversed significantly by *F. angulata* ($p < 0.05$) (except for some kinematic parameters). Although the abnormal morphology rate of sperms was numerically higher in the T/D group in comparison to the sham and intact groups; the difference was not statistically significant. Histopathological assessments showed that F. angulata could significantly increase the Johnsen's score, mean seminiferous tubular diameter, and germinal epithelial cell thickness in the treatment group compared to the T/D group ($p < 0.05$). GPx, SOD, and testosterone were significantly reduced following torsion/detorsion compared to the sham group ($p < 0.05$); and the reductions were prevented significantly by *F. angulata* ($p < 0.05$) (except for SOD). MDA level was significantly increased in the T/D group in comparison to the sham and intact groups ($p < 0.05$), which *F. angulata* was not able to reverse peroxidation index. The overall results of the research suggest that *F. angulata* extract can be a good natural alternative to testicular I/R injury.

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Introduction

Torsion of testicle, one of most emergency disorders in male reproductive system, is twisting of the spermatic cord around its axis, which results in reduction of blood flow and ischemia.[1,2] This condition has more prevalence in the first years of life (neonates) and prepubertal ages (13-16 years old).[3] Testicular ischemic damages will be increased linearly with degree and duration of torsion.[4] Nowadays, rapid diagnose and surgical intervention is the only management route for the problem.[5] Even in cases which have been surgically treated within 4-6 h after pain emergence, involved testicle will often be dysfunctional permanently.[2] Apart from correction time category, even if the torsion is well treated surgically, a significant increase in germ cell apoptosis and spermatogenesis arrest will be expected due to reperfusion injury.[6] In this situation, exogenous antioxidants can be helpful.[7] In spite of much and significant progress in this area, ischemia/reperfusion (I/R) injury is still a challengeable problem clinically.[7] Although numerous pharmacological approaches have been developed for treatment of the diseases, but medicinal plants usage is still preferable.[8]

Ferulago angulata (Chavil) is an annual herbaceous shrub with 60-150 cm tall grown in mountainous regions (at altitudes of 1900-3200 m above sea level) of western Iran.[9] *F. angulata* is frequently found in northern Iraq, eastern Turkey, Macedonia, Serbia, and Greece.[10] It has a high antioxidant potential containing phenolic compounds, flavonoids, and certain monoterpenes such as α-pinene, β-pinene, cis-ocimene, and borneol acetate.[11-13] Ameliorated kidney function followed by gentamycin-induced nephrotoxic lesions,[14] improved the oxidative damage caused by Parkinson's disease,[15] and improved liver function parameters and antioxidant status in alloxan-induced diabetic rats[16] are some evidences which greatly prove the *in-vivo* antioxidant potential of *F. angulata*. Also, anti-inflammatory activity is another prominent effect of this shrub[17, 18] because of being a rich source of monoterpene compounds such as α-pinene, β-ocimene, terpinolene, Linalool and terpinene.[19]

With these in mind, it sounded *F. angulata* could be an excellent candidate to reduce testicular oxidative stress during I/R injury due to its anti-inflammatory and antioxidant features. On the other hand, the literature review revealed that there is no investigation about the preventive properties of *F. angulata* on testicular I/R injury; so, the current study was set out to examine the protective effects of *F. angulata* aqueous extract on testicular I/R injury in a rat model.

Materials and methods

Extract Preparation

Aerial parts of fresh *F. angulata* were collected from Kohgiluye-Boyerahmad province in the west of Iran and a herbarium specimen was deposited (No. 55128) at the Department of Biology, Faculty of Science, Shiraz University. It was washed with sterile water, dried in the shade, and pulverized into powder. About 50 g of the powder was macerated in 250 ml of distilled water. The blend was mixed for 24 h at room temperature (25° C) by an orbital shaker. Following filtration of the suspension by filter paper, the filtrate was concentrated in a rotary evaporator, and resultant extract was lyophilized.[20] The ultimate powder was kept in a labeled sterile falcon tube and stored at −20° C. A suitable value of extract powder was weighed, dissolved in sterile Sodium Chloride 0.9% (Normal Saline, USP, Sterile Grade), and filtered using syringe filters (0.22 µm pore size) just before the intraperitoneal (IP) injection.

Animals and Experimental Design

Twenty-eight intact adult male Sprague Dawley rats with an average weight of 250 ± 20 g and an average age of 8 weeks were selected to be used in the study. Subjects were housed at room temperature (23 ± 2° C) in a controlled air-conditioned laboratory with 12 h light/12 h dark cycle. All animals spent one week for acclimation. In the 7-day-preoperative-care period and also 24 hours of the reperfusion period, food availability was free for rats with *ad libitum* access to tap water. The recommendations of the European Council Directive (86/609/EC) regarding protection of animals for experimental purposes were employed. Also, the study was approved by the local committee on animal ethics, Shiraz University, Shiraz, Iran (IACUC No: 4687/63). Subjects were randomly allocated into four groups (seven rats/group) as follows:

Group I (sham-operated control group): sham-operated control group was considered for achieving the baselines of evaluation techniques used in the present study;

Group II (T/D group): 2 hours of torsion followed by 24 hours of detorsion was applied to induction of I/R injury;[21]

Group III (treatment group): concomitant with T/D induction, 200 mg/kg *F. angulata* aqueous extract was intraperitoneally injected;[22]
Group IV (intact group): rats received only 200 mg/kg *F. angulata* aqueous extract for 24 hours by one IP injection (without T/D induction).

Surgical Procedure

All IP injections (anesthetics and extracts) were applied in the lower left abdominal quadrant. In order to prevent visceral penetration during injections, rats were kept in the head-down position. A blend of ketamine (80 mg/kg, Alfasan, The Netherlands) and xylazine (5 mg/kg, Alfasan, The Netherlands) was utilized to anesthetize rats.[23] After anesthesia induction, rats were placed in dorsal recumbent position. The surgical site was shaved and disinfected by povidone-iodine 10% solution. In groups I to III, the scrotum was entered via a 2-cm longitudinal incision. In group I, no torsion was created and the incision was closed routinely. After delivering the left testicle to the surgical site in order to induction of I/R injury in groups II and III, it was rotated 720° in the clockwise direction and held in this posture for 2 hours by suturing the tunica albuginea to the scrotal wall. After 2 hours of ischemia, the testicle was untwisted to its normal position and allowed to reperfuse for 24 hours. Group III (treatment group) received 200 mg/kg *F. angulata* aqueous extract 30 min before initiation of reperfusion period. Group IV received 200 mg/kg *F. angulata* aqueous extract as much as reperfusion period time (24 hours) in order to detect any possible side effects on testicular tissue. All steps were performed under aseptic conditions by two veterinary surgeons. Finally, approximately 5 mL blood was taken from the heart under anesthesia for serum preparation and assessment of antioxidant biomarkers and testosterone hormone. Next, all rats were sacrificed and epididymides were removed for evaluation of sperm characteristics. Also, testicular tissues were examined for appearance and weight, and then transferred into fixative for histopathological examination.

Epididymal Sperm Harvest

Rapidly after sacrifice, the involved testis was exposed, and then the epididymis tail was carefully removed and sliced sharply into several pieces in a sterile multi-well cell culture plate with 3 mL of pre-warmed semen analysis medium (37° C) in each well (Tris: 3.025 g, Citrate 1.7 g, Glucose: 1.25 g, Crystalline Penicillin G: 150,000 U, Crystalline Streptomycin Sulfate: 150 mgr, Distilled Water q.s. 100 ml, pH=6.76). Sperms were counted after the plates had left undisturbed on a 38.5° C warm stage for 15 minutes.

Total Epididymal Sperm Counts

The sperm count was carried out with a Neubauer hemocytometer (TM brand, Germany) after dilution and devitalization of the sperm in a ratio of 1:5 in distilled water. The epididymal tissues were carefully collected and weighed on an advanced 0.0001 g precision scale (Sartorius, Japan). Finally, the total number of epididymal sperm cell per gram of epididymal tissue was calculated for each.

Sperm Motility Parameters

Within one minute after slicing the tail of epididymides into the analysis medium, A computer-assisted semen analysis -HFTTM CASA System (HFT Co., Tehran, Iran) with Olympus bright-field microscope (Olympus, Tokyo, Japan)- beneath ×100 amplification was used to assess the motility parameters of gathered sperms. A 5 µl sample of analysis medium containing sperm cells set within the pre-warmed (37° C) counting chamber (Sperm 360 labs, India) and promptly evaluated. At least, five microscopic areas for each sample were assessed.

Sperm Viability and Morphology

To assess the viability of the sperms, an Eosin-Nigrosin staining method was used. A 5 µl sperm sample was mixed with an equal volume of commercially available dye (Minitube, Germany) on a preheated glass slide (37° C) and applied after 5 seconds. Sperm cells with colorless and reddish-pink heads were considered live and dead, respectively. The smears were also used to assess abnormal sperm morphology. The abnormal morphologies of sperm were classified according to anatomical location (head, mid-piece, and tail abnormalities). At least 200 cells were evaluated per sample.

Histopathological Evaluation

The testicular specimens were fixed immediately in modified Davidson's fluid for 48 h, and then immersed in 10% natural buffered formalin for 24 h.[21] For histopathology, routine tissue processing of dehydration in a graded ethanol series, clearance in xylene and embedding in paraffin wax was conducted. Finally, the tissue blocks were sectioned in 5 µm

thickness and stained with hematoxylin and eosin (H&E). The prepared tissue slides were evaluated under the light microscope (Olympus, Japan) by a blind pathologist for microscopic lesions such as interstitial edema, hemorrhage, inflammatory and degenerative processes, and the other histopathological changes. Johnsen's scoring system was applied on at least 50 seminiferous tubules to evaluate the mean testicular biopsy score (MTBS); assigning a score of 1 to 10 to each tubule.[24] Also, mean seminiferous tubular diameter (MSTD) and germinal epithelial cell thickness (GECT) were calculated in at least 20 completely round seminiferous tubules in each slide.

Superoxide Dismutase, Glutathione Peroxidase, and Malondialdehyde Assessments

The amounts of superoxide dismutase (SOD), glutathione peroxidase (GPx), and malondialdehyde (MDA) in the serum were determined using commercial kits (Kiazist Co, Iran) according to the manufacturer's instructions. SOD measuring is according to the Mn-SOD ability to inhibit the conversion of resazurin to resorufin along with reduction of superoxide radicals produced by the xanthine/xanthine oxidase system. Also, GPx measuring is according to the decrease of hydrogen peroxide to water along with oxidation of glutathione. Finally, MDA measuring is based on thiobarbituric acid reactive substance. The optical density value of samples was read using a microplate reader (BioTek Inc., USA) at 570, 340, and 532 nm for SOD, GPx, and MDA respectively. The level of SOD, GPx, and MDA was expressed in IU/ml, IU/ml, and nmol/mL respectively. The sensitivity of the utilized commercial kits for SOD, GPx, and MDA was reported as 0.5 mU/ml, 0.5 mU/ml, and 10 nmol/ml respectively.

Testosterone Assessment

A commercial kit (Biorexfars Co, Iran) was used to determine the amount of testosterone hormone based on the manufacturer's instructions. Finally, reading the absorbance of the sample was performed at 450 nm. The testosterone level was expressed in ng/ml. The commercial kit used had a sensitivity of 0.057 ng/ml for testosterone testing.

Statistical Analysis

SPSS software (v. 16.00) was utilized to analyze the data. All data were analyzed by one way ANOVA followed by LSD post hoc test. $p < 0.05$ was considered statistically significant, and data were expressed as Mean ± SEM.

Results

Testicular Gross Appearance and Weight

Macroscopically, severe congestion and hemorrhage was observed in testes related to the T/D group, while the rats in the sham and intact groups had normal testes with light pink color. In the therapeutic group, the color was dark pink. Testes weights in all four experimental groups were shown in Table 1.

Sperm Parameters

I/R injury caused a significant reduction in sperm count and viability versus the sham group ($P<0.05$), which could be significantly reversed by *F. angulata* administration ($p < 0.05$) (Figures 1a and 1b). The number of abnormal spermatozoa in the T/D group was higher than the sham group, and treatment with *F. angulata* reduced the abnormality; however, all these correlations were not significant ($p > 0.05$) (Figure 1c). Also, I/R injury was associated with a significant decrease in all sperm velocity parameters ($p < 0.05$), and *F. angulata* could significantly reverse some of them including TM, PM, LIN, and WOB ($p < 0.05$) (Table 2).

Histopathologic Characteristics

In the sham and intact groups, microscopic examination of tissue specimens revealed a normal histological architecture with organized seminiferous tubules. All germinal epithelial cells were in normal arrangement and no marked pathological change was observed (Figures 2a and 2d). Histopathological alterations such as coagulative necrosis, degeneration and desquamation of germinal epithelial cells, disorganization of the seminiferous tubules, hemorrhage, congestion, and interstitial edema were observed severely in the T/D group compared to the

Table 1. The mean weight of left testicles (g) at 24th hour post I/R injury

Groups	Mean testicular weight (g)
Sham group	1.33 ± 0.01[a]
T/D group	0.75 ± 0.02[b]
Treatment group	1.05 ± 0.02[c]
Intact group	1.43 ± 0.03[d]

Data are expressed as Mean ± SEM. The numbers with different superscript letters significantly differ from others ($p < 0.05$).

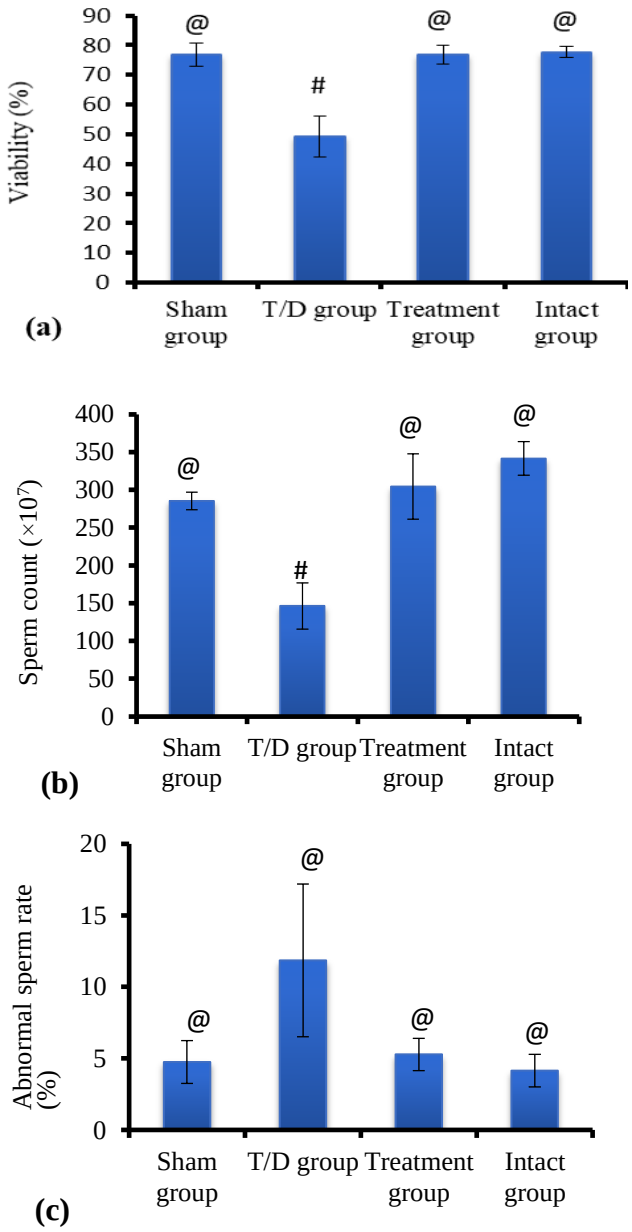

Figure 1. The level of sperm characteristics in experimental groups. (a) Sperm viability rate, (b) number of sperms, and (c) rate of abnormal sperms. Data were expressed as Mean ± SEM. The columns with different superscript letters significantly differ from others ($p < 0.05$).

sham and intact groups (Figure 2b). The abnormalities were remarkably attenuated in *F. angulata* extract treated rats (Figure 2c). Histomorphometrical assessments of all groups including MTBS according to Johnsen's score (JS) system, MSTD, and GECT were displayed in Figure 3.

Oxidative Stress Biomarkers

Table 3 shows the measured amounts of GPx, SOD, and MDA. I/R injury was associated with an increased level of MDA and also decreased levels of SOD and GPx compared to the sham and intact groups. However, only GPx activity was improved significantly in the treatment group compared to the T/D group ($p < 0.05$).

Testosterone Profile

Table 3 discloses the hormonal profile in each group. Briefly, testosterone was reduced significantly in the T/D group compared to the sham group, which could be reversed significantly by *F. angulata* treatment ($p < 0.05$).

Discussion

Testicular torsion is a prevalent serious urological urgency, which can lead to severe pain and swelling in the testicle because of spermatic cord rotation and vascular compromise.[25] About 25% of men having a history of testicular torsion will experience infertility at adult-onset.[26] Testicular salvage rate after surgical correction is 42 to 88%; however, it is not clear whether the testicular tissue will be preserved because further aggravation will be occurred during reperfusion period due to re-oxygenation.[27-29] Simultaneous with surgical correction, reperfusion injury should be prevented or treated with a complementary drug. Medicinal herbs are the main therapeutic resources in developing countries, so that 80% of people in Asian and African developing countries use these plants for prevention, treatment, and health protection.[30] A high antioxidant activity is the most prominent property of *Ferulago angulata*,[12] as well as, antibacterial and anti-inflammatory features.[18,31] Also, it is used in treating digestive pains, hemorrhoids, snake bites, ulcers, and as sedative.[32] Based on these evidences, the present study was performed to investigate the protective effects of *F. angulata* aqueous extract (200 mg/kg) on testicular I/R injury in a rat model.

I/R injury reduced the testicular weight significantly compared to the sham group ($p < 0.05$), which was prevented by *F. angulata* aqueous extract significantly in the treatment group ($p < 0.05$). Similar effect in increment of testicular weight was previously reported following the administration of *F. angulata* hydro-alcoholic extract.[33]

In the present study, I/R injury was associated with a significant decrease in count, viability, and all velocity parameters of epididymal sperms ($p < 0.05$), and *F. angulata* could show significant effects on count, viability, and some of velocity parameters ($p < 0.05$). Even more, the percentage of abnormal spermatozoa

in the T/D group was higher than other groups, and *F. angulata* aqueous extract could decrease this parameter in comparison to the T/D group; however, no significant difference was seen among groups ($p > 0.05$). Also, Naderi *et al*. in 2019 demonstrated that chronic exposure to lead acetate, diazinon, and their combination decreased the sperm quality, sperm chromatin maturity and integrity, and male fertility indices, which all were ameliorated upon treatment with hydro-alcoholic extract of *F. angulata*.[34]

Histopathologically, I/R injury was related to significant impaired spermatogenesis (MTBS diminishment) and mitigation of GECT and MSTD in comparison to the sham group ($p < 0.05$). In the present study, treatment with *F. angulata* could greatly reverse histopathological parameters (MTBS, MSTD, and GECT) in comparison to T/D group ($p < 0.05$) by promoting the seminiferous tubular recovery and germinal

Table 2. Descriptive statistics of sperm kinematic parameters in different groups of the current study.

Sperm kinematic parameters	Experimental Groups			
	Sham group	T/D group	Treatment group	Intact group
TM (%)	76.90 ± 2.33^{a}	27.60 ± 7.47^{b}	54.68 ± 9.40^{c}	76.60 ± 1.81^{a}
PM (%)	30.99 ± 0.74^{a}	8.09 ± 2.89^{b}	20.64 ± 3.50^{c}	29.72 ± 0.55^{a}
VCL (µm/s)	54.78 ± 2.90^{a}	23.54 ± 7.35^{b}	32.17 ± 8.93^{b}	53.30 ± 2.65^{a}
VAP (µm/s)	28.65 ± 1.38^{a}	12.06 ± 3.96^{b}	17.70 ± 4.23^{b}	28.44 ± 1.28^{a}
VSL (µm/s)	18.98 ± 1.68^{a}	8.24 ± 2.85^{b}	11.80 ± 2.74^{b}	18.61 ± 0.44^{a}
ALH (µm)	2.77 ± 0.08^{a}	1.32 ± 0.36^{b}	1.60 ± 0.38^{b}	2.66 ± 0.08^{a}
BCF (Hz)	2.74 ± 0.47^{a}	1.50 ± 0.34^{b}	1.98 ± 0.45^{ab}	2.70 ± 0.26^{a}
MAD (deg)	52.52 ± 2.15^{a}	23.48 ± 6.22^{b}	29.42 ± 8.74^{b}	52.43 ± 1.38^{a}
STR (%)	45.71 ± 0.80^{a}	25.36 ± 6.04^{b}	33.28 ± 4.33^{b}	45.92 ± 0.89^{a}
LIN (%)	26.57 ± 1.17^{ac}	13.93 ± 3.47^{b}	20.56 ± 1.69^{c}	26.97 ± 0.87^{a}
WOB (%)	41.17 ± 1.05^{a}	21.40 ± 5.15^{b}	32.34 ± 4.05^{a}	39.94 ± 1.19^{a}

TM: total motility, PM: progressive motility, VCL: velocity curved line, VAP: velocity average path, VSL: velocity straight line, ALH: amplitude of lateral head displacement, BCF: beat cross frequency, MAD: mean angular displacement, STR: straightness, LIN: linearity, and WOB: wobble. Data were expressed as Mean ± SEM. Data in each row were compared analytically. Different letters in superscripts indicate a statistically significant difference ($p < 0.05$) between current study groups within each parameter.

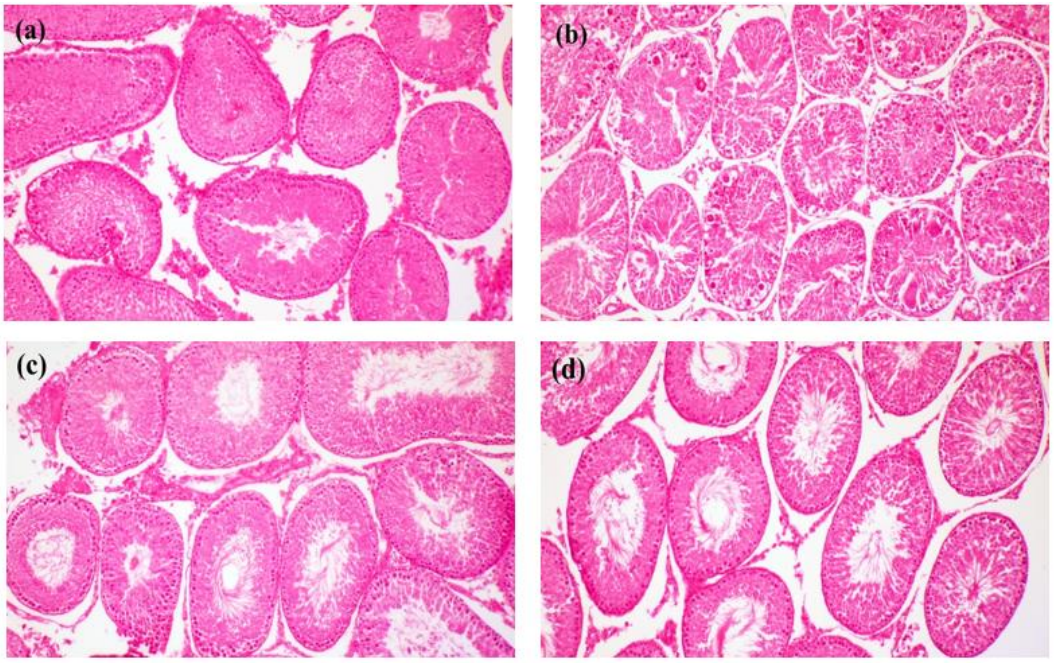

Figure 2. Light microscopy of testicular tissue sections (H&E, 100×). (a) Sham group exhibited normal well-arranged cell architecture. (b) Disorganization, desquamation in epithelial germ cells, formation of luminal multinucleated spermatid giant cells, and disruption of seminiferous tubules were observed in the T/D group. (c) T/D + 200 mg/kg *F. angulata* revealed almost normal seminiferous tubules. (d) The intact group (received only 200 mg/kg *F. angulata*) showed a normal architecture, similar to the sham group.

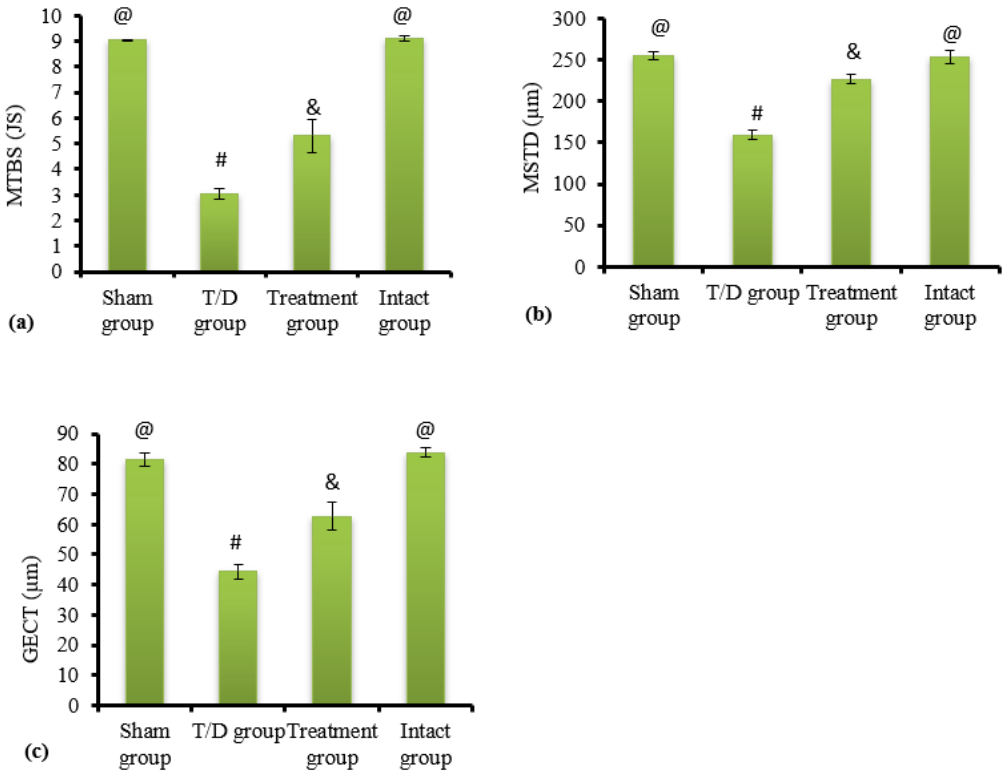

Figure 3. (a) Mean testicular biopsy score (MTBS) according to Johnsen's score (JS) system, (b) Mean seminiferous tubular diameter (MSTD), (c) Germinal epithelial cell thickness (GECT); Data were expressed as Mean ± SEM. The columns with different superscript letters significantly differ from others ($p < 0.05$).

Table 3. Measured amounts of superoxide dismutase (SOD), glutathione peroxidase (GPx), malondialdehyde (MDA), and testosterone in all four investigational groups

Groups	SOD (IU/mL)	GPx (IU/mL)	MDA (nM/mL)	Testosterone (ng/mL)
Sham	1.33 ± 0.12^{a}	14.98 ± 1.56^{a}	1.55 ± 0.22^{a}	3.17 ± 0.06^{a}
T/D	0.78 ± 0.12^{b}	8.25 ± 1.80^{b}	2.22 ± 0.12^{b}	2.90 ± 0.03^{b}
Treatment	0.88 ± 0.10^{b}	13.49 ± 0.73^{a}	1.83 ± 0.05^{ab}	3.16 ± 0.04^{a}
Intact	1.39 ± 0.11^{a}	15.82 ± 0.59^{a}	1.47 ± 0.18^{a}	3.21 ± 0.09^{a}

Data are expressed as Mean ± SEM. Data in each column were compared analytically. The numbers with different superscript letters significantly differ from others ($p < 0.05$).

epithelial cells arrangement. In consistent to our data, Nassab *et al*. in 2018 declared that using *F. angulata* hydro-alcoholic extract could significantly increase the MTBS and MSTD in the STZ-diabetic rats (which is a condition of oxidative stress) versus the diabetic control group.[22]

According to previous literature studies, it is well proven that I/R injury is relevant to decreased level of SOD and GPx activities;[25, 35] however, there are always notable contradictories such as Güzel *et al*. (2015) and Erdemir *et al*. (2008) investigations.[36, 37] In the present study, GPx and SOD antioxidant defense enzymes had a significant reduction in the T/D group in comparison to the sham and intact groups ($p < 0.05$). Moreover, treatment with *F. angulata* increased the GPx and SOD activity levels compared to the T/D group (only GPx increment was significant ($p < 0.05$)). In one study by Mousavi-Ezmareh *et al*. in 2017, increment of CAT, SOD, and GPx was observed in alloxan-induced diabetic rats that received three different doses of 200, 400, and 800 mg/kg *F. angulata* hydro-alcoholic extract in comparison to diabetic control group.[16] In another investigation by Valipour *et al*. in 2016, treatment with 200 and 400 mg/kg of *F. angulata* hydro-alcoholic extract increased significantly the CAT and SOD activities after their reductions in renal tissue following gentamycin-induced-nephrotoxicity (200 mg/kg was not significant effective on SOD, which is similar to

current study).[14] Also, MDA, an index of lipid peroxidation extent, was significantly increased in the T/D group compared to the sham and intact groups ($p < 0.05$), which *F. angulata* was not able to reverse this index ($p = 0.075$). while, Rafieian-Kopaei *et al.* in 2014 showed that hydro-alcoholic extract of *F. angulata* could effectively reduce plasma levels of MDA (as an oxidant agent), and also increase antioxidant capacity.[38]

Oxidative stress can damage to interstitial Leydig cells, and as a result, reduce the level of testosterone hormone, which has a key role in spermatogenesis support.[39] The question here is whether testosterone is released after the T/D correction as before; because it is unclear if interstitial Leydig cells are still active.[40, 41] So, we aimed to determine the functionality of these cells by testosterone measurement in the serum. I/R injury significantly mitigated this hormone in the T/D group in comparison to the sham and intact groups ($p < 0.05$). The finding is also approved by Abdel-Gaber *et al*. in 2018, which reported that a significant reduction in the amount of testosterone hormone level was observed in the T/D group in comparison to the sham group.[42] In the current study, using *F. angulata* significantly prevented the testosterone reduction in the treatment group in comparison to the T/D group ($p < 0.05$). In line with our results, Bohlouli *et al*. in 2019 demonstrated the high and enough potential of *F. angulata* to help the interstitial Leydig cells to secrete more testosterone hormone.[33]

Finally, the existence of compounds such as 9-octadecenoic acid, methyl ester, pentadecanoic acid, 14-methyl-, methyl ester; benzenesulfonothioic acid, S-(4-nitrophenyl) ester; 1,2,3-benzenetriol (pyrogallol); hexadecanoic acid, ethyl ester; myristic acid, methyl ester, and thymol seems to be responsible for the high antioxidant capacity of *F. angulata*, and by giving electron to ROS, can reduce adverse effects of I/R injury on testicular histology, epididymal sperm quality, oxidative condition, and hormone secretion.[34] Although the results of the current investigation uncovered *F. angulata* defensive impacts against oxidative stress and I/R injury, but other extract types (like alcoholic), other different concentrations, and bioactive components of *F. angulata* are of great values to be examined. Other than, the impact of *F. angulata* can be examined on testicular I/R injury at different administration routes or administration times.

In conclusion, *Ferulago angulata* is utilized due to its various remedial properties. Here, this component was explored against I/R injury. The outcomes of the current study showed that *F. angulata* could be a promising agent to secure the testicular tissue from I/R injury. The researchers propose *F. angulata*, as a novel approach to the prophylaxis and/or treatment for I/R injury, is of potential appropriateness in patients with testicular torsion, in any case, more detailed *in-vivo* studies are justified.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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