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Original Article

The effect of Fraxin against lung and testis damage induced by testicular torsion/detorsion in rats

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Abstract

Aim: This study was planned to determine the effect of Fraxin against damage of lung and testis tissues induced by testicular torsion detorsion in the experimental animals.

Material and Methods: For this aim, in this study, experimental animals were weighed and randomize grouped. Groups were designed as sham, testicular torsion/detorsion (TTD), 10 mg/kg Fraxin + TTD (10 mg/kg for 15 days i.p.+2 h torsion;2 h detorsion) and 50 mg/kg Fraxin + TTD (50 mg/kg for 15 days i.p.+2 h torsion;2 h detorsion) groups. After the detorsion period was completed, all subjects were sacrificed by high-dose anaesthesia. At the end of the experiment, testis and lung tissues of rats were taken rapidly for biochemical analyzes.

Results: When evaluating current biochemical data, Total oxidant status and malondialdehyde level were significantly elevated, and total antioxidant status and superoxide dismutase activity decreased significantly in the TTD group compared to the sham group ($p<0.001$). On the contrary, in the groups applied the doses of 10 and 50 mg/kg of fraxin, the level of oxidant were decreased and the level of antioxidant was increased ($p<0.001$).

Conclusion: These results demonstrate that the applications of fraxin at 10 and 50 mg/kg doses show the protective effect on lung and testicular tissue, demonstrating a positive effect on experimental TTD injury in rats.

Keywords: Fraxin; lung; rats; testis; torsion detorsion

INTRODUCTION

Testicular torsion is actually a urologic surgical case that is encountered more frequently in newborn male babies and requires urgent treatment that can be seen in adolescent boys (1,2). To reduce or prevent irreversible damage and infertility that may occur in the testicular tissue depends on ensuring detorsion as soon as possible. As a matter of fact, the studies conducted on this subject reveal that there is a direct relationship between testicular torsion duration and testicular function (1,3-5). Testicular damage due to spermatic cord torsion detorsion (TD) is similar to ischemia reperfusion (IR) phenomenon. It has been reported to result in irreversible aspermatogenesis due to spermatic cord torsion in experimental animals (6). IR directly leads to an increase in the production of various pro-inflammatory cytokines that stimulates the migration and chemotaxis of neutrophils and other leukocytes into the testicular tissue. Leukocytes are a critical resource for the production of reactive oxygen species (ROS) that cause peroxidation and damage of cellular molecules (fat, protein and even DNA) for the cells (2, 7-10). ROS ultimately creates the core of the damage mechanism by causing loss of cellular function. ROS produced in physiological conditions in healthy normal cells is modulated by the intracellular antioxidant defense system. However, the amount of ROS whose production is increased due to IR is too much to be eliminated by anti-oxidant system elements. Therefore, an imbalance occurs between the ROS and the anti-oxidant system. This situation is defined as the oxidative stress that causes cellular injury (7,11). In this regard, many experimental studies have been carried out today in order to alleviate oxidative damage caused by TTD (4,5,12,13).

Fraxin (8- (beta-d- (beta-glucoxygenyl) -7-hydroxyl-6-methoxy coumarin) (Figure 1), which is known to be the main active ingredient of Cortex Fraxini used in Chinese traditional medicine, has various biological properties (14). In studies made on fraxin, it has been identified that it has different bioactivities such as antiviral, anti-inflammatory, antibacterial, antioxidant and analgesic effects (15-17). However, it is uncertain whether there is a positive effect on the damage of testicular and lung tissues due to TTD.

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Therefore, this study was planned to determine the effect of Fraxin against damage of lung and testis tissues induced by testicular torsion detorsion in the experimantal animals.

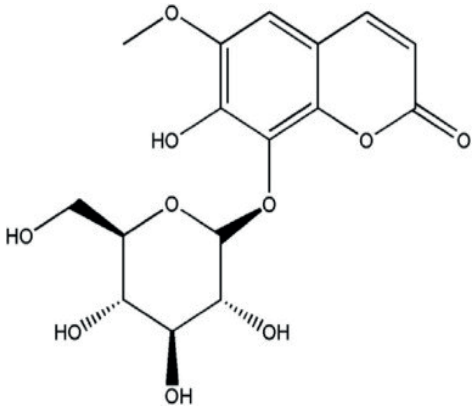

Figure 1. The Chemical Structure of Fraxin (16)

MATERIAL and METHODS

Laboratory Conditions, Ethical Approval and Drugs

This study was approved by Atatürk University Experimental Animals Local Ethics Committee (28.06.2018-139). This experimental study was carried out at Ataturk University Experimental Animals Research and Application Center and also, experimental animals were provided from the same center. All subjects were stored and experiments were performed in international laboratory conditions (12 h; 12h night/day, 55% humidity, 25 oC temperature). Fraxin used in this study was provided from Sigma-Aldrich, USA, ketamine (Ketalar 50 mg / ml injection) and xylazine (Rompun 2%) were bought from Pfizer Limited and from Bayer, Istanbul, Turkey.

Design of Groups and Experimental Procedure

In this study, thirty-two Wistar albino male rats (220-230 g) were weighed and distributed in four groups. Subjects were fed daily with standard laboratory feed and tap water. All experimental procedures were performed under the conditions of anesthesia (ketamine / xylazine mixture 60/10 mg/kg dose i.p) without pain. The experimental groups are designed as presented in the Table 1.

For the torsion process, the scrotum was cleaned with povidone iodine solution and opened by making a small incision. Later the testes were fixed with a clamp by twisting the spermatic cord 720 degrees for 2 hours and the scrotal opening was simply closed. When the torsion period expired, the clamps were opened so that the blood flow was provided again to testes for 2 hours, the spermatic cord was reinstated. Fraxin given to the treatment groups was prepared fresh daily in normal saline and administered intraperioneally. The last fraxin doses were administered 30 minutes before detorsion. The doses of fraxin (17) and anesthesia (13,18) were selected in accordance with the doses used in the previous studies. In this study, the TTD model was used the method used in the studies previously made (12).

At the end of the 15th day of the experiment, all animals were sacrificed by high dose anesthesia after the TTD period ended. The testis and lung tissues taken were stored under suitable conditions at -80°C until biochemical measurements made.

Biochemical Analysis

In current study, superoxide dismutase (SOD) activity measurement was mainly based on the inhibition of the nitroblue tetrazolium reduction developed by Sun et al (19). SOD activity shown as U/mg tissue. Myeloperoxidase (MPO) activity was quantified according to the method defined by Bradley et al (20). The MPO activity was presented as U/g tissue. Malondialdehyde (MDA) level determination was done according to the method based on reaction with thiobarbituric acid (21). MDA level was expressed in nmol/g tissue unit. Oxidative stress biomarkers (Total Antioxidant Status/TAS and Total oxidant Status/TOS) were measured using kits (Rel Assay

Table 1. Design of experimental groups

Groups	n	Anesthesia	Operation	Treatment	Experimental model	Testis and lung tissues were removed
Sham	8	Yes	Only an scrotal incision was made and closed again.	No	No	Yes
TTD	8	Yes	Scrotal area was opened and testicular torsion and detorsion model applied	No	2h torsion;2 h detorsion	Yes
10 mg/kg Fraxin + TTD	8	Yes	Scrotal area was opened and testicular torsion and detorsion model applied	10 mg/kg Fraxin i.p. for 15 days	2h torsion;2 h detorsion	Yes
50 mg/kg Fraxin + TTD	8	Yes	Scrotal area was opened and testicular torsion and detorsion model applied	10 mg/kg Fraxin i.p. for 15 days	2h torsion;2 h detorsion	Yes

Diagnostics, Turkey) according to the manufacturer's instructions. Oxidative stress index (OSI) can be obtained by the ratio of TOS to TAS. We determined the OSI value as calculated in previous studies: OSI = [(TOS, µmol H2O2 equivalent L) / (TAS, mmol Trolox equivalent / L) x 10] (13,18).

Statistical Analysis

In this study, One-Way variance analysis (ANOVA) was performed primarily for all data, and then Tukey HSD test, which is one of the post hoc tests, was applied for comparisons between groups. All of our results are presented as mean, minimum, maximum and standard error mean and p value below 0.05 is accepted as significance level.

RESULTS

Results of SOD, MPO activities and MDA level for testicular and lung tissues

At the end of present study, when the data we obtained from the testis and lung tissues were carefully evaluated, In the TTD group, MPO activity and MDA level were significantly increased, but the SOD activity was decreased compared to the sham group ($p<0.01$). However, it was determined that SOD activity increased, MPO activity and MDA level decreased in the treatment groups given 10 and 50 mg/kg doses of fraxin. In addition, when the treatment groups that received 10 and 50 mg/kg doses of fraxin were compared, it was observed that the administration of 50 mg/kg dose of fraxin was more effective in terms of numerical data

Table 2. Testicular tissue mean, minimum, maximum and standard error mean results for SOD, MPO activities and MDA level

Groups		MDA (µmol/g tissue)	SOD (U/mg protein)	MPO(U/g protein)
Sham	Mean	217.86*	394.70*	33496.79*
	Min.	160.81	282.75	17589.12
	Max.	254.79	569.35	53789.80
	SEM	13.51	37.80	4192.98
TTD	Mean	398.48*,**,#	175.40*,**,#	88732.38*,**,#
	Min.	267.62	104.74	76777.37
	Max.	542.97	219.86	98924.95
	SEM	33.77	11.94	2968.82
10 mg/kg Fraxin + TTD	Mean	257.46**	335.44**	39180.11**
	Min.	203.10	213.11	32389.80
	Max.	303.63	415.37	46745.94
	SEM	11.89	26.49	1886.07
50 mg/kg Fraxin + TTD	Mean	220.13#	389.45#	33573.86#
	Min.	183.46	230.35	22348.95
	Max.	265.85	484.04	49032.94
	SEM	9.62	27.04	3586.05

*,**,#: The same symbols in different groups indicate statistical significance among those groups. P<0.001; TTD; Testicular Torsion Detorsion, SEM; Standart Error of Mean, Min; Minimum, Max; Maximum, SOD; superoxide dismutase, MPO; myeloperoxidase and MDA; malondialdehyde

Table 3. Testicular tissue mean, minimum, maximum and standard error mean results for TAS, TOS and OSI value

Groups		TAS (mmol/L)	TOS (µmol/L)	OSI
Sham	Mean	1.23*	7.63*	0.61*
	Min.	1.13	6.67	0.55
	Max.	1.32	8.32	0.67
	SEM	0.02	0.21	0.01
TTD	Mean	0.67*,**,#	12.93*,**,#	2.02*,**,#
	Min.	0.45	11.28	1.60
	Max.	0.86	14.21	3.19
	SEM	0.05	0.38	0.19
10 mg/kg Fraxin + TTD	Mean	1.09**	9.05**	0.83**
	Min.	0.95	7.38	0.57
	Max.	1.31	10.53	1.05
	SEM	0.04	0.41	0.05
50 mg/kg Fraxin + TTD	Mean	1.18#	7.67#	0.65#
	Min.	1.00	6.44	0.48
	Max.	1.34	8.79	0.88
	SEM	0.04	0.26	0.04

*,**,#: The same symbols in different groups indicate statistical significance among those groups. P<0.001; TTD; Testicular Torsion Detorsion, SEM; Standart Error of Mean, Min; Minimum, Max; Maximum, TAS; total antioxidant status, TOS; total oxidant status and OSI; oxidative stress index

than the 10 mg/kg dose of fraxin. However, this situation was not statistically significant (See Table 2 and 4).

Results of TAS, TOS and OSI value for testicular and lung tissues

In the present study, TOS and OSI values were increased as meaningful, but the TAS value of testis and lung tissues were reduced compared to the sham group in the TTD group ($p<0.01$). Moreover, it was determined that TAS value increased, TOS and OSI values decreased in the treatment groups given 10 and 50 mg/kg doses of fraxin. Also, when the treatment groups that received 10 and 50 mg/kg doses of fraxin were compared, it was observed that the administration of 50 mg/kg dose of fraxin was more effective in terms of numerical data than the 10 mg/kg dose of fraxin. Nevertheless, this situation was not statistically significant (See Table 3 and 5).

Table 4. Lung tissue mean, minimum, maximum and standard error mean results for SOD, MPO activities and MDA level

Groups		MDA (µmol/g tissue)	SOD (U/mg protein)	MPO (U/g protein)
Sham	Mean	128.06*	311.18*	458444.83*
	Min.	88.52	225.88	332674.14
	Max.	164.11	376.34	618492.29
	SEM	10.46	23.19	42340.33
TTD	Mean	248.51*,**,#	162.92*,**,#	747590.80*,**,#
	Min.	177.22	129.72	653674.22
	Max.	273.28	186.84	921674.35
	SEM	11.11	7.86	32916.61
10 mg/kg Fraxin + TTD	Mean	158.34**	257.52**	530099.21**
	Min.	144.47	219.90	443728.50
	Max.	173.96	301.19	603265.43
	SEM	3.47	9.02	22493.90
50 mg/kg Fraxin + TTD	Mean	132.72#	304.22#	467599.21#
	Min.	104.33	248.44	398332.44
	Max.	148.83	350.33	523647.63
	SEM	5.24	13.41	14353.26

*,**,#: The same symbols in different groups indicate statistical significance among those groups. P<0.001; TTD; Testicular Torsion Detorsion, SEM; Standart Error of Mean, Min; Minimum, Max; Maximum, SOD; superoxide dismutase, MPO; myeloperoxidase and MDA; malondialdehyde

Table 5. Lung tissue mean, minimum, maximum and standard error mean results for TAS, TOS and OSI value

Groups		TAS (mmol/L)	TOS (µmol/L)	OSI
Sham	Mean	0.85*	11.42*	1.35*
	Min.	0.64	9.69	1.08
	Max.	1.02	13,15	1.91
	SEM	0.03	0.40	0.08
TTD	Mean	0.44*,**,#	17.61*,**,#	4.08*,**,#
	Min.	0.34	14.68	2,97
	Max.	0.61	19.60	5,53
	SEM	0.03	0.57	0.26
10 mg/kg Fraxin + TTD	Mean	0.66**	12.91**	2.07**
	Min.	0.46	12.22	1.23
	Max.	1.02	14.27	3.10
	SEM	0.06	0.22	0.21
50 mg/kg Fraxin + TTD	Mean	0.84#	11.59#	1.39#
	Min.	0.63	10.00	1.14
	Max.	1,07	13.44	1.66
	SEM	0.05	0.44	0.06

*,**,#: The same symbols in different groups indicate statistical significance among those groups. P<0.001; TTD; Testicular Torsion Detorsion, SEM; Standart Error of Mean, Min; Minimum, Max; Maximum, SOD; superoxide dismutase, MPO; myeloperoxidase and MDA; malondialdehyde

DISCUSSION

Testicular torsion caused by the rotation of the testicle around the spermatic cord axis, which requires urgent diagnosis and treatment, is among the most common causes of infertility in men (8,22). Although it is thought that testicular tissue can be protected from damage by rapid surgical intervention, despite successful surgical intervention unfortunately, infertility has been expected to develop in a part of men with a history of torsion (23). Torsion in the testis is the trigger and the beginning of ischemic damage. The main purpose of the treatment here is to correct the blood flow in ischemia and provide tissue perfusion. After detorsion, the irreversible damage of the testicular tissue is formed at this stage, which leads to overproduction of ROS due to given an excessive amount of oxygen into the tissue (24). In case the ROS produced in the tissue by the cellular antioxidant defense system cannot be cleaned, oxidative stress is formed in the tissue. As a result, ROS leads to damage by directly attacking cellular structures (8-11). As a result, ROS causes events such as peroxidation of membrane lipids, inhibition of protein synthesis, disruption of the sperm formation cycle and DNA damage in the cell (11). We have previously reported that torsion and detorsion-induced testicular injuries directly lead to leukocyte and neutrophil infiltration, leading to production excessive amounts of ROS. SOD is among important enzymes that scavenge the detrimental ROS in male reproductive organs. In addition, when we look at the literature, it is clearly demonstrated that the TTD mechanism reduces the SOD activity of the testicular tissue (4,25,26). MDA is the end product of lipid peroxidation, as a well-known and frequently used parameter to detect increased ROS formation in ischemic tissues (10,27). Many previous studies investigating oxidative stress in experimental animal models of testicular torsion have assessed the level of the lipid peroxidation by measuring MDA, the level of antioxidant defense by measuring SOD activity and TAS level and the level of the oxidative damage via MPO, TOS, OSI analyses. In these studies, it was determined that with the application of TTD, lipid peroxidation increases in tissue, on the contrary, antioxidant defense is insufficient and oxidative stress response occurs. As a result, oxidative tissue damage has been seen (3,12,28,29).

Until today, many drugs and chemical agents (11-13,30) have been used to protect the testes and secondary organs against experimental TTD-induced tissue damage, and of course some have shown strong properties in preventing testicular or secondary organ damage, but some have not been concerted for clinical use. In present experimental TTD study, there are some literature studies with fraxin that we selected to protect the testicular and lung tissues. If we mention a few of them as following; it has been reported that in a renal ischemia reperfusion model, fraxin reduces kidney damage thanks to its antioxidant activity. Additionally, it has been reported by researchers that fraxin protects tissue or organ from damage against acute organ damage in polymicrobial sepsis model induced by cecal ligation and puncture. Moreover, it has been suggested that fraxin ameliorates lipopolysaccharide-induced acute lung injury in mice by inhibiting the nuclear factor kappa B (NF-κB) and NLRP3 (NOD-, LRR- and pyrin domain-containing protein 3) signalling pathways (16-18).

The results of present study stated that treatments with fraxin caused improvement on oxidative damage arising by TTD. Regarding our biochemical results, in testis and lung tissues, TOS, OSI and MPO were increased after TTD, while it was associated a significant decrease in TOS, OSI and MPO via the administration of fraxin (10 and 50 mg/kg). In addition, SOD activity and TAS value decreased after TD induction in bilateral testes, while treatment with fraxin (10 and 50 mg/kg) resulted in a significant increase in SOD activity and TAS value.

Another important point that we have to express is that, until today, in the IR studies, the remote organ damages has been researched in next to primary organ damage depending on IR created in various organs such as intestine, lower extremity, liver and heart. It has been reported to cause oxidative damage even in structures remote from the organ from which IR was created (31-34). However, we could not reach studies on whether oxidative damage occurred in remote organs originating from TTD. In this regard, in current study, we investigated ROS, which occurs intensely in damage caused by TTD how can effect antioxidant defense by causing a lipid peroxidation in lung tissue. As a result, we have seen that TTD causes MDA production in the lungs and makes antioxidant defense insufficient. For this reason, present study has made a valuable contribution to the literature in this respect.

CONCLUSION

Fraxin, used in two different doses, has been shown to be effective in relieving the oxidative stress response and beneficial in protecting against testis and lung damage arising from TTD. However, comprehensive studies are needed to realize the use of fraxin in the clinic. We believe that this study presented in this context may guide future studies.

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Competing interests: The authors declare that they have no competing interest.

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Ethical approval: This study was approved by Atatürk University Experimental Animals Local Ethics Committee (28.06.2018-139).

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