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Protective Effect of *Fumaria parviflora* Extract on Oxidative Stress and Testis Tissue Damage in Diabetic Rats

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Abstract

Objectives: In this experimental research, a rat model was used to investigate the protective effect of *Fumaria parviflora* on oxidative stress and testis tissue damage in diabetic rats.

Materials and Methods: To this end, a total of 28 male Wistar rats were utilized and randomly divided into 4 groups including control group (G1), diabetic control group (G2, DC), diabetic treated with *F. parviflora* extract (G3), and healthy group (G4) that received *F. parviflora* extract. In addition, the therapy lasted for 56 days. Then, the blood levels of some markers of oxidative stress and antioxidant enzymes, as well as sperm parameters such as concentration, motility, and morphology were assayed based on the aim of the study.

Results: The histological examination showed a negative change in the DC group, and they were included in the treated groups. The plasma levels of glutathione peroxidase (GPx) and superoxide dismutase (SOD) significantly reduced in the DC group while the malondialdehyde (MDA) level enhanced the duration of diabetes. As regards the sperm parameters, the results demonstrated a significant reduction in the DC group whereas treatment with FP extract led to an increase in the quality of sperm.

Conclusions: Based on the results of the present research, the *F. parviflora* extract has a positive role in protecting the testis tissue and sperm quality against oxidative stress in diabetic rats.

Keywords: Diabetes, Fumaria parviflora, Testis, Sperm parameters, Oxidative stress

Introduction

According to an estimation in 2010, there are 48.5 million infertile couples in the world among whom males include 40%-50% of the cases. For example, 2% of men exhibit unusual sperm parameters (1,2). Diabetes is considered as one of the reasons which causes fertility problems among this population. In addition, diabetes results in hyperglycemia because of the problems in insulin function and/or production. Further, hyperglycemia disrupts homeostasis in the body and oxidative stress is one of the complications created accordingly (3). Then, oxidation-reduction balance disrupts due to an increase in reactive oxygen species levels and free radicals and thus cellular activities, especially sperm production disrupt as well (4-6). In addition, diabetes makes failures in gonadal hormones production such as luteinizing, follicle-stimulating, and testosterone hormones, and eventually, the spermatogenesis process will be ruined (7). Using antioxidant and free radical scavenger supplementations can have positive effects on spermatogenesis (8, 9). Fumaria parviflora lam (Fumarioideae) is one of the plants with different antioxidant components. It is an annual plant which grows in a variety of areas such as Iran, Turkey, Pakistan, India, as well as different parts of South Asia, the Middle East, and Africa. "Shahtareh" is another name of this plant in Iran. In Iranian traditional medicine, *Fumaria parviflora* is used as an agent against liver diseases, dermatological disorders, including diuretic, expectorant, acne, anti-bronchite, scabies, antipyretic, antiscorbite, eczema, diaphoretic and the like (10-12). Studies about *F. parviflora* and other species of fumaria plants revealed protective and antioxidative effects of these plants against CCl4 model of hepatotoxicity. For example, fumaria parviflora exhibited positive effects in paracetamol hepatotoxicity (13). Another study in 2007 demonstrated that *F. parviflora* ethanolic extract has anti-inflammatory, antinociceptive, and protective effects (14). Furthermore, *F. parviflora* is confirmed to have positive and favorable effects on the sperm production process in male rats (15). Phytochemical assess for different fumaria plants shows that these plants, especially *F. parviflora* have various components such as protropine, cryptopine, sinactine, stylopine, bicuculline, adlumine, parfumine, fumariline, fumarophycine, fumaritine, dihydrofumariline, perfumidine, and dihydrosanguirine (16). Generally, this study was conducted to survey the

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effect of *F. parviflora* extract on oxidative stress and spermatogenesis failure in diabetic male rats which were induced with streptozotocin (STZ).

Materials and Methods

Twenty-eight male Wistar rats with an average weight of 200-250 g were evaluated based on the purpose of the study. All animals were purchased from Tabriz University of Medical Sciences and then were kept under standard conditions (i.e., the temperature of 25°C and a 12 h/12 h light/dark cycle). Moreover, all rats had free access to water and food during the experimental period.

Animal Preparation

In this experimental study, all of the rats were distributed into 4 groups as follows.

- Control group (G1): Seven rats were included in a group and received the normal saline for 20 days (control);
- Diabetic control group (G2): Diabetes was induced by STZ and the rats took the normal saline for 20 days (DC);
- Diabetic treated group (G3): Diabetes was induced by STZ and the animals received 250 mg/kg of *F. parviflora* extract for 20 days (DFP);
- Sham group (G4): The rats took 250 mg/kg of *F. parviflora* extract for 20 days without diabetes induction (FP).

In the diabetic groups, diabetes was induced by a single intraperitoneal injection dose (50 mg/kg) of STZ (Sigma) dissolved in 5 mL citrate buffer with a pH of 4.5 (17, 18). After 72 hours of STZ injection, the blood level of glucose was assessed by a glucometer. Then, a higher blood sugar level of 250 mg/dL was considered as the verification of diabetes (18). After the treatment, all animals were anesthetized by ketamine/xylazine (50/10 mg/kg). Next, their blood samples were derived from the heart of the rats in order to measure the plasma levels of testosterone and some oxidative stress markers such as malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GPx) and then the samples were frozen at -70°C for subsequent assessments. Moreover, the testicles were isolated and fixed by Bouin's solution for histological study.

Hydroalcoholic Extract Preparation of *Fumaria parviflora*

In order to prepare a hydroalcoholic extract of *F. parviflora*, 500 g of *F. parviflora* was dried at room temperature. Then, the dried plants were powdered and dissolved in one L of ethanol 96% and kept at 25°C for 2 days. After repeated shaking, the solution was filtered during this period, followed by centrifuging at 1008 × g for 5 minutes. At the end of this procedure, the produced solution was poured into an open-top dish and the solvent was volatilized as well. Next, 100 g of a semisolid extract was taken from 500 g of *F. parviflora* powder. Finally, the *F. parviflora* extract was dissolved in normal saline to reach an adequate concentration (19).

Tissue Fixation and Preparation of Specimens

The testicular specimens were fixed by Bouin's solution. Then, the tissue of the testicles was dehydrated by incremental in ethanol and added to liquid paraffin. Next, the sections were cut into five µm thick, deparaffinized and stained by hematoxylin-eosin (H & E) method, and eventually, were assessed by an optical microscope (NIKON) at a final magnification of 400x.

Histological Evaluation and Maturation of Seminiferous Tubules

The spermatogenesis in seminiferous tubules was evaluated by Johnson's score. Thus, in each cross-section, 50 seminiferous tubules were examined and a score of 1–10 was assigned to each tubule according to Johnson's criteria (20). The seminiferous tubules morphometry was randomly confiscated by measuring twenty cross-sections of "seminiferous tubules" that made as round as possible or nearly terete cross-sections. In the same sections, the mensuration of the seminiferous epithelium height was also performed from the basal membrane on one side to the luminal edge of the tubule. This mensuration was taken by the linear eyepiece grids on the light microscope at 400x magnification (21, 22).

Evaluation of Oxidative Stress Markers in the Blood Serum

Evaluation of the levels of MDA, SOD, and GPx was explained in our previous study. Briefly, the MDA level was assessed by locating 0.20 mL of the rat's serum into a tube which included 3.0 mL of glacial acetic acid. Then, one percent thiobarbituric acid in 2% NaOH was added to the tube and the solution was located in the boiling water for fifteen minutes. After cooling, the absorbance of the product was read at 532 nm by a spectrophotometer (Biospect Inc., USA). The calibration curve was manufactured by MDA tetrabutylammonium salt obtained from Sigma Company (23, 24). Finally, the SOD and GPx levels were evaluated in the plasma by an Elisa reader (Anthos) according to the protocols of Randox and Ransod kits (UK).

The Sperm Parameters Assessment

First, the left epididymis was removed from the left testis and was sliced into 5 mL phosphate buffered saline (PBS) with a pH of 7.2. Next, it was transferred into the incubator with 37°C for 20 minutes. Then, 100 lambdas of this solution were removed and added to 900 lambdas of PBS and again this was repeated and one drop of this dilution was added to Neubauer's chamber for the sperm count. The counting of sperm was managed according to the standard protocol in 8 squares of 0.1 cm^2 each except for the central area (8, 9). In addition, each slide was dried exposed to the air and then fixed with ethanol 96% in

order to assess the morphology of the sperm. Next, the slides were stained with H and E method. In each slide, 100 sperm were assessed and the percentage of normal and abnormal sperms was determined as well (19, 25).

Statistical Analysis

The obtained data were analyzed with SPSS software, version 19 (USA). All data were presented as mean ± SE and compared using one-way ANOVA and Tukey post hoc tests. $P<0.05$ was considered statistically significant.

Results

Histological Parameters of Testis

The results related to the histological examination of testis tissue in the study group are shown in Figure 1 and Table 1. As represented, the mean Johnson's score (MJS) was significantly increased in the control group compared to the DC group ($P=0.001$). Further, the MJS was significantly higher in all groups treated with hydroalcoholic extract of *F. parviflora*, compared to the DC group ($P=0.001$). Conversely, the mean diameter of seminiferous tubule (MDST) demonstrates a significant declined in the DC group compared to the control group ($P<0.001$). However, the MDST was significantly enhanced in all treated groups (FP and DFP) when compared with the DC group ($P<0.001$). In addition, the height of seminiferous epithelium (HSE) was significantly lower in the DC group compared to the control group ($P<0.001$). On the other hand, the HSE showed a significant increase in FP and DFP groups compared to the DC group ($P<0.001$).

The Level of Oxidative Stress Marker in Plasma

Further, the level of MDA, SOD, and GPx in the study groups are presented in Table 2. As demonstrated, the plasma GPx levels are significantly decreased in the diabetic control group compared to the control group ($P<0.001$). Moreover, in all treated groups it is significantly higher than the DC group ($P<0.001$). On the other hand, the control group represents a significantly higher plasma level of SOD compared to the DC group ($P<0.001$). A comparison between the DC group and treated groups (FP and DFP) shows a significant difference regarding plasma SOD level as well ($P<0.001$). In the diabetic control group, the plasma level of MDA is significantly enhanced compared to the control group ($P<0.001$). In this regard, there is a significant reduction in FP and DFP groups compared to the DC group ($P<0.001$).

The Sperm Parameters

Regarding the sperm parameters, the analysis shows a significant decrease in the count of sperm in the DC group compared to the control group ($P\leq0.01$) while there is a significant increase in the DFP and FP groups in this respect ($P<0.001$). The assessment of sperm morphology in all studied groups indicates that the percentage of normal sperm in all diabetic groups is significantly lower than that of the control group ($P\leq0.001$) whereas a significant increase is observed in the DFP and FP groups compared to the DC group ($P\leq0.001$) in this regard. The evaluation of sperm motility shows a significant reduction in the percentage of motile sperm in all diabetic groups (DC and DFP) compared to the control group ($P\leq0.001$)

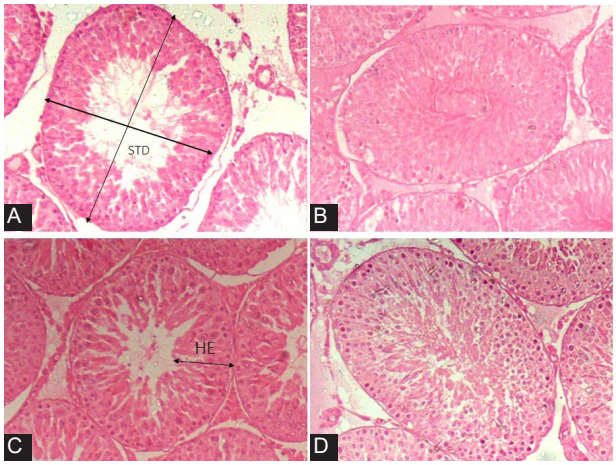

Figure 1. Histological image of testis stained with hematoxylin and eosin method in different study groups. (A) Diabetic control group in which severe testicular injury was noted; (B) Control group in which normal testicular architecture was observed; (C) Diabetic group treated with Fumaria parviflora extract where there was an improvement in the structure of seminiferous tubule; (D) Control group treated with Fumaria parviflora extract in which normal testicular architecture was detected; Scale bar: x400.

Table 1. Histological Examination of Testis Tissue in the Study Groups

Groups	MJS	MSTD	HE
Control	9.65 ± 0.021	248.12 ± 3.69	71.82 ± 2.44
DC	4.88 ± 0.018[a]	167.70 ± 4.34[a]	37.5 ± 2.54[a]
DFP	7.25 ± 0.032[ba]	203.35 ± 6.42[ba]	58.87 ± 3.11[ba]
FP	9.78 ± 0.011[b]	259.42 ± 5.30[b]	76.5 ± 2.64

MJS: Mean Johnson's score; MSTD: Mean seminiferous tubule diameter; HE: The height of seminiferous epithelium (HE); Control: Healthy control group; DC: Diabetic control group; DFP: Diabetic group treated with 250 mg/kg of *Fumaria parviflora* extract; FP: Healthy group which received 250 mg/kg of *Fumaria parviflora* extract.
Note. Data are shown as Mean ± SD.
[a] In comparison with control group ($P = 0.001$).
[b] In comparison with diabetic group ($P = 0.001$).

Table 2. The Level of MDA, SOD, and GPx in Study Groups

Groups	SOD (U/mL)	GPx (U/mL)	MDA (mL)
Control	1.64 ± 0.04	2.75 ± 0.07	79 ± 0.75
DC	0.67 ± 0.04[a]	1.51 ± 0.09[a]	141 ± 2.84[a]
DFP	1.32 ± 0.07[ab]	2.03 ± 0.03[ab]	100 ± 1.45[ab]
FP	1.68 ± 0.04	2.93 ± 0.02	0.54 ± 1.63

DC: Diabetic control group; DFP: Diabetic group treated with 250 mg/kg of *Fumaria parviflora* extract; FP: Healthy group which received 250 mg/kg of *Fumaria parviflora* extract.
Note. Data are shown as Mean ± SD.
[a] In comparison with control group ($P = 0.001$).
[b] In comparison with diabetic group ($P = 0.001$).

Table 3. The Comparison of Sperm Parameters After Treatment Period

Sperm Parameters Groups	Sperm Count x 10^6	Morphology		Sperm Motility	
		Normal	Abnormal	Motile	Immotile
Control	67.3 ± 5.48	72.4% ± 1.07	27.6% ± 1.07	70.2%± 1.03	29.8 ± 1.03
DC	18.45 ± 3.98[a]	28.5% ± 0.50[a]	71.5% ± 1.17[a]	25.4%± 1.15[a]	76.6± 1.15[a]
DFP	48.5 ± 6.34[ab]	58.2% ± 0.70[ab]	41.8% ± 0.70[ab]	56.5 ± 0.84[ab]	46.65± 0.84[ab]
FP	72.35 ± 4.52[ab]	76.3% ± 1.3[ab]	23.7% ± 1.3[ab]	75.05 ± 1.04[ab]	24.95± 1.04[ab]

[a] Shows a significant difference between control and diabetic groups.
[b] Displays a significant difference between the treated group and the DC group.
Data are indicated as Mean ± (SD).
Control: Healthy control group; DC: Diabetic control group; DFP: Diabetic group treated with 250 mg/kg of *Fumaria parviflora* extract; FP: Healthy group received 250 mg/kg of *Fumaria parviflora* extract.

while it is higher in the treated groups (DFP and FP) as compared to the DC group ($P \leq 0.001$) (Table 3).

Discussion

Fumaria parviflora in different societies including Iran is associated with anti-oxidant and fertility positive properties (10). The present study investigated the reproductive effects of *F. parviflora* extract in male rats. Generally, the results of the study represented that using *F. parviflora* increases sperm counts, normal sperm, testosterone hormone levels, and anti-oxidant enzymes levels in Wistar rats.

Based on the results of different studies, sperm production from germinal cells correlated with protection against cytotoxicity damages is related to spermatogenesis process. In addition, free radicals are cytotoxicity agents which damage the fatty acids in cell membranes and oxidize these molecules. Therefore, membrane structure and function damage as well. However, antioxidants protect membrane molecules from such damages. The findings of various studies showed that antioxidant compositions exist in *F. parviflora* plant (26-28).

Similar to the results of the present study, the results of Heydari Nasrabadi et al demonstrated that *F. parviflora* positively affects the reproductive system of the male rat. In the above-mentioned research, *F. parviflora* was utilized with various doses including 750 mg/kg, 1050 mg/kg (3 days), and 250 mg/kg (5 days) by using gavage and the results revealed the positive effects of *F. parviflora* including increasing the Leydig cells, spermatocytes, and spermatogonium (15). Further, an increase in the number of germinal, Leydig, and spermatozoid cells is possible due to the antioxidant components of *F. parviflora*. Therefore, the increased number of Leydig cells may lead to an increase in the level of testosterone hormone. Accordingly, spermatogenesis is stimulated with an increase in testosterone (15). The positive effects of antioxidant compositions on testis tissue were expressed in many studies, which is in line with the findings of the current study (29-31).

Androgen hormones are necessary during the development and growth of the testis. Furthermore, these hormones are important for normal functions of the male reproductive system. The previous investigation indicates that the weight and size of the testis has a direct relationship with the level of testosterone hormone (32). In 2 different studies, applying multiple doses of *F. parviflora* represented positive curative effects such as sperm normality and testosterone level enhancement. These results corroborate with the results of the present research (15,33).

In the present study, the oxidative stress levels of blood serum, as well as SOD, and GPx enzymes decreased while the levels of MDA enzyme increased. By using *F. parviflora*, the positive effect of this antioxidant plant was observed in enzymatic levels, including an increase in SOD and GPx enzymes whereas a decrease in MDA. Moreover, based on the observations, *F. parviflora* extract improved testicular anti-oxidative properties. In several studies, including the study by Agarwal et al, sperm motility was found to be correlated with reactive oxygen species (ROS) production. In *Fumaria* samples, the types of antioxidants such as flavonoids, isoquinoline alkaloids, and phenolic compounds were observed (34,35). In another study, Heydari Nasrabadi et al represented that fertility increases in male rats by using *F. parviflora*. This fertility increase can be due to the antioxidant effects of *F. parviflora* (15). Additionally, Sousek et al observed the antioxidative properties of *F. parviflora* (36).

In addition, Heydari Nasrabadi et al found that using *F. parviflora* extract caused an increase in the number of Leydig cells in the rats (15). In the present study, the number of sperms increased in male rats by using the *F. parviflora* extract. Sperm count index is used for the amount of produced sperm and a positive function of the male reproductive system. Thus, a high sperm count and a low percentage of abnormal sperms are associated with increased fertility (37). This functional chain affects Sertoli cells as one of the spermatogenesis regulators, and thus increases germinal cells. In many studies, the destructive effects of ROS were confirmed in the male reproductive system, including a decrease in function,

motility, and normality related to the sperm. In addition, antioxidant capacity was found to be lower in infertile men while ROS levels were higher in these patients. In general, positive results in the reproductive system of male rats may result from the antioxidant components of the *F. parviflora* extract (38-43).

Conclusions

Generally, the findings of the current study revealed that hydroalcoholic extract of *F. parviflora* protects from damages to the male reproductive system, including lipid membrane peroxidation, the production of abnormal sperms, and probably apoptotic effects related to the types of cells in the testis.

Conflict of Interests

Authors have no conflict of interests.

Ethical Issues

All the research processes of this study were in accordance with the guidelines of Tabriz University of Medical Science for the Maintenance and Use of Laboratory Animals (under the ethical code of IR.TBZMED.VCR.REC.1397.033).

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References

1. Mascarenhas MN, Flaxman SR, Boerma T, Vanderpoel S, Stevens GA. National, regional, and global trends in infertility prevalence since 1990: a systematic analysis of 277 health surveys. PLoS Med. 2012;9(12):e1001356. doi:10.1371/journal.pmed.1001356
2. Kumar N, Singh AK. Trends of male factor infertility, an important cause of infertility: A review of literature. J Hum Reprod Sci. 2015;8(4):191-196. doi:10.4103/0974-1208.170370
3. Maritim AC, Sanders RA, Watkins JB 3rd. Diabetes, oxidative stress, and antioxidants: a review. J Biochem Mol Toxicol. 2003;17(1):24-38. doi:10.1002/jbt.10058
4. Dowling DK, Simmons LW. Reactive oxygen species as universal constraints in life-history evolution. Proc Biol Sci. 2009;276(1663):1737-1745. doi:10.1098/rspb.2008.1791
5. Cansian RL, Kubiak GB, Borsatti L, et al. Antioxidant and genotoxic properties of *Maytenus dasyclada*: a comparative study in relation to Maytenus reference species. Braz J Biol. 2015;75(2):471-476. doi:10.1590/1519-6984.18113
6. Takao LK, Imatomi M, Gualtieri SC. Antioxidant activity and phenolic content of leaf infusions of *Myrtaceae* species from Cerrado (Brazilian Savanna). Braz J Biol. 2015;75(4):948-952. doi:10.1590/1519-6984.03314
7. Ballester J, Munoz MC, Dominguez J, Rigau T, Guinovart JJ, Rodriguez-Gil JE. Insulin-dependent diabetes affects testicular function by FSH- and LH-linked mechanisms. J Androl. 2004;25(5):706-719.
8. Agarwal A, Gupta S, Sikka S. The role of free radicals and antioxidants in reproduction. Curr Opin Obstet Gynecol. 2006;18(3):325-332.
9. Menezo YJ, Hazout A, Panteix G, et al. Antioxidants to reduce sperm DNA fragmentation: an unexpected adverse effect. Reprod Biomed Online. 2007;14(4):418-421.
10. Amin GR. Popular medical plants of Iran. Tehran: Iranian Research Institute of Medicinal Plants; 1991:51-52.
11. Mossa JS, Al-Yahya MA, Al-Meshal IA. Medicinal Plants of Saudi Arabia. Riyadh: King Saud University; 1987.
12. Baquar SR. Medicinal and poisonous plants of Pakistan. Pakistan: Printas; 1989.
13. Gilani AH, Janbaz KH, Akhtar MS. Selective protective effect of an extract from *Fumaria parviflora* on paracetamol-induced hepatotoxicity. Gen Pharmacol. 1996;27(6):979-983.
14. Rao CV, Verma AR, Gupta PK, Vijayakumar M. Anti-inflammatory and anti-nociceptive activities of Fumaria indica whole plant extract in experimental animals. Acta Pharm. 2007;57(4):491-498. doi:10.2478/v10007-007-0039-z
15. Heydari Nasrabadi M, Aboutalebi H, Naseri M. Effect of *Fumaria parviflora* alcoholic extract on male rat reproductive system. J Med Plant Res. 2012;6(10):2004-2010.
16. Suau R, Cabezudo B, Rico R, Najera F, Lopez-Romero JM. Direct determination of alkaloid contents in Fumaria species by GC-MS. Phytochem Anal. 2002;13(6):363-367. doi:10.1002/pca.669
17. Abtahi-Evari SH, Shokoohi M, Abbasi A, Rajabzade A, Shoorei H, Kalarestaghi H. Protective effect of *Galega officinalis* extract on streptozotocin-induced kidney damage and biochemical factor in diabetic rats. Crescent Journal of Medical and Biological Sciences. 2017;4(3):108-114.
18. Gholami Farashah MS, Pasbakhsh P, Omidi A, Nekoonam S, Aryanpour R, Regardi Kashani I. Preconditioning with SDF-1 Improves Therapeutic Outcomes of Bone marrow-derived Mesenchymal Stromal Cells in a Mouse Model of STZ-induced Diabetes. Avicenna J Med Biotechnol. 2019;11(1):35-42.
19. Shokoohi M, Shoorei H, Soltani M, Abtahi-Eivari SH, Salimnejad R, Moghimian M. Protective effects of the hydroalcoholic extract of *Fumaria parviflora* on testicular injury induced by torsion/detorsion in adult rats. Andrologia. 2018;50(7):e13047. doi:10.1111/and.13047
20. Johnsen SG. Testicular biopsy score count--a method for registration of spermatogenesis in human testes: normal values and results in 335 hypogonadal males. Hormones. 1970;1(1):2-25.
21. Filho DW, Torres MA, Bordin AL, Crezcynski-Pasa TB, Boveris A. Spermatic cord torsion, reactive oxygen and nitrogen species and ischemia-reperfusion injury. Mol Aspects Med. 2004;25(1-2):199-210. doi:10.1016/j.mam.2004.02.020

22. Soltani M, Moghimian M, Abtahi-Eivari SH, Shokoohi M. The protective effect of *Matricaria chamomilla* extract on histological damage and oxidative stress induced by torsion/detorsion in adult rat ovary. International Journal of Women's Health and Reproduction Sciences. 2017;5(3):187-192. doi:10.15296/ijwhr.2017.34
23. Gwarzo MY, Ahmadu JH, Ahmad MB, Dikko AU. Serum glucose and malondialdehyde levels in alloxan induced diabetic rats supplemented with methanolic extract of *Tacazzea apiculata*. Int J Biomed Sci. 2014;10(4):236-242.
24. Ameli M, Hashemi MS, Moghimian M, Shokoohi M. Protective effect of tadalafil and verapamil on testicular function and oxidative stress after torsion/detorsion in adult male rat. Andrologia. 2018;50(8):e13068. doi:10.1111/and.13068
25. Benny B, George Priya Doss C, Thirumal Kumar D, Asha Devi S. Assessing reproductive toxicity and antioxidant enzymes on beta asarone induced male Wistar albino rats: In vivo and computational analysis. Life Sci. 2017;173:150-160. doi:10.1016/j.lfs.2016.08.021
26. Modaresi M, Mesripour M, Rajaei RA. Effect of cinnamon extract on the number of spermatocyte and spermatozoa cells in mice. Iranian Journal of Medicinal and Aromatic Plants. 2010;26(1):83-90.
27. Nikravesh MR, Jalali M, Mohammadi SH. Study the effect of raw extract onion on rat's testis. Effects of crude onion extract on murine testis J Reprod Infertil. 2010;10(4):239-244.
28. Haq I, Hussain M. Medicinal plants of Mansehra. Hamdard Med. 1993;36(3):63-100.
29. Fouad AA, Qutub HO, Jresat I. Dose-dependent protective effect of baicalin against testicular torsion-detorsion in rats. Andrologia. 2017;49(1). doi:10.1111/and.12580
30. Mestrovic J, Pogorelic Z, Drmic-Hofman I, Vilovic K, Todoric D, Popovic M. Protective effect of urapidil on testicular torsion-detorsion injury in rats. Surg Today. 2017;47(3):393-398. doi:10.1007/s00595-016-1388-3
31. Ozbek O, Altintas R, Polat A, et al. The protective effect of apocynin on testicular ischemia-reperfusion injury. J Urol. 2015;193(4):1417-1422. doi:10.1016/j.juro.2014.11.086
32. Prins GS, Birch L, Greene GL. Androgen receptor localization in different cell types of the adult rat prostate. Endocrinology. 1991;129(6):3187-3199. doi:10.1210/endo-129-6-3187
33. Dorostghoal M, Seyyednejad SM, Khajehpour L, Jabari A. Effects of *Fumaria parviflora* leaves extract on reproductive parameters in adult male rats. Iran J Reprod Med. 2013;11(11):891-898.
34. Agarwal A, Ikemoto I, Loughlin KR. Relationship of sperm parameters with levels of reactive oxygen species in semen specimens. J Urol. 1994;152(1):107-110.
35. Naz I, Palomares-Rius JE, Saifullah S, et al. In vitro and in planta nematicidal activity of *Fumaria parviflora* (Fumariaceae) against the southern root-knot nematode Meloidogyne incognita. Plant Pathol. 2013;62(4):943-952. doi:10.1111/j.1365-3059.2012.02682.x
36. Sousek J, Vavreckova C, Psotova J, Ulrichova J, Simanek V, editors. Antioxidant and antilipoperoxidant activities of alkaloid and phenolic extracts of eight *Fumaria* species. Acta Hortic. 1997;501(501):239-244. doi:10.17660/ActaHortic.1999.501.38
37. Raji Y, Udoh US, Mewoyeka OO, Ononye FC, Bolarinwa AF. Implication of reproductive endocrine malfunction in male antifertility efficacy of *Azadirachta indica* extract in rats. Afr J Med Med Sci. 2003;32(2):159-165.
38. Aitken RJ, Clarkson JS. Cellular basis of defective sperm function and its association with the genesis of reactive oxygen species by human spermatozoa. J Reprod Fertil. 1987;81(2):459-469.
39. Iwasaki A, Gagnon C. Formation of reactive oxygen species in spermatozoa of infertile patients. Fertil Steril. 1992;57(2):409-416.
40. Mazzilli F, Rossi T, Marchesini M, Ronconi C, Dondero F. Superoxide anion in human semen related to seminal parameters and clinical aspects. Fertil Steril. 1994;62(4):862-868.
41. Smith R, Vantman D, Ponce J, Escobar J, Lissi E. Total antioxidant capacity of human seminal plasma. Hum Reprod. 1996;11(8):1655-1660. doi:10.1093/oxfordjournals.humrep.a019465
42. Lewis SE, Sterling ES, Young IS, Thompson W. Comparison of individual antioxidants of sperm and seminal plasma in fertile and infertile men. Fertil Steril. 1997;67(1):142-147.
43. Shokoohi M, Shoorei H, Soltani M, Abtahi-Eivari SH, Salimnejad R, Moghimian M. Protective effects of the hydroalcoholic extract of *Fumaria parviflora* on testicular injury induced by torsion/detorsion in adult rats. Andrologia. 2018;50(7):e13047. doi:10.1111/and.13047