





*Antioxidant and Antiapoptotic Effects of Primula vulgaris L. Against
Methotrexate-Induced Testicular Damage in Rats*

*Primula vulgaris L.'nin Sıçanlarda Metotreksat Kaynaklı Testis Hasarına
Karşı Antioksidan ve Antiapoptotik Etkileri*

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Abstract

The aim of this study was to investigate the antioxidant and antiapoptotic effects of *Primula vulgaris* extract against methotrexate (MTX)-induced testicular damage. In this study, 4 groups were formed with 8 rats in each group. Rats in group 1 were given 0.8 mg/kg physiological serum via gavage for 7 days. The rats in group 2 were administered a single dose (30 mg/kg) of MTX intraperitoneally on the first day of the study. The rats in group 3 were administered a single dose (30 mg/kg) of MTX on the first day of the study and then 100 mg/kg of aqueous extract via gavage for 7 days starting from the first day. The rats in group 4 were given 100

mg/kg of aqueous extract via gavage for 7 days. On the 8th day, the testicles and epididymis of the rats were removed under anesthesia and their blood was collected. The removed testicles were used for histological and biochemical analyses. When group 2 was compared with group 1, it was determined that seminiferous tubule diameter, epithelial thickness, sperm count, motility, vitality, and Johnsen scoring values decreased; tubule number that immature cells sloughed into the lumen and apoptotic index (AI) increased. In group 3, it was observed that seminiferous tubule diameter, epithelial thickness, sperm count, motility, vitality, and Johnsen scoring values increased; tubule number that immature cells sloughed into the lumen and AI decreased compared to group 2. When group 2 was compared with group 1, it was found that MDA values increased, and SOD and CAT values decreased in blood plasma and testicular tissue. According to the study results, it was determined that MTX caused damage to the testicle by creating oxidative stress, while *Primula vulgaris* reduced this damage thanks to its antioxidant effects.

Keywords: Antioxidant, Apoptosis, *Primula vulgaris*, Testes

Özet

Bu çalışmanın amacı metotreksat (MTX) kaynaklı testis hasarına karşı *Primula vulgaris* ekstraktının antioksidan ve antiapoptotik etkilerini araştırmaktır. Bu çalışmada her grupta 8 adet sıçan olacak şekilde 4 grup oluşturulmuştur. Grup 1'deki sıçanlara 7 gün boyunca 0.8 mg/kg serum fizyolojik gavaj ile verilmiştir. Grup 2'deki sıçanlara çalışmanın ilk günü tek doz (30 mg/kg) MTX intraperitoneal olarak uygulanmıştır. Grup 3'deki sıçanlara çalışmanın ilk günü tek doz (30 mg/kg) MTX uygulanmış ve daha sonra ilk günden başlayarak 7 gün boyunca 100 mg/kg sulu ekstrakt gavaj yoluyla verilmiştir. Grup 4'deki sıçanlara 7 gün boyunca 100 mg/kg sulu ekstrakt gavaj yoluyla verilmiştir. 8. gün anestezi altında sıçanların testisleri ve epididimisi çıkarılmış, kanları alınmıştır. Çıkarılan testisler histolojik ve biyokimyasal analizler için kullanılmıştır. Grup 2, grup 1 ile kıyaslandığında seminifer tübül çapı, epitel kalınlığı, sperm sayısı, motilite, vitalite ve Johnsen skorlama değerlerinin azaldığı; lümenine immatür hücre dökülen tübül sayısı ve apoptotik indeksin arttığı belirlenmiştir. Grup 3, grup 2'ye göre seminifer tübül çapı, epitel kalınlığı, sperm sayısı, motilite, vitalite ve Johnsen skorlama değerlerinin arttığı; lümenine immatür hücre dökülen seminifer tübül sayısı ve AI'nın azaldığı gözlenmiştir. Grup 2, grup 1 ile kıyaslandığında kan plazmasında ve testis dokusunda MDA değerinin arttığı, SOD ve CAT değerlerinin ise azaldığı bulunmuştur. Çalışma sonuçlarına göre MTX'in testiste oksidatif stres oluşturarak testise zarar verdiği, *Primula vulgaris*'in ise antioksidan etkileri sayesinde bu hasarı azalttığı belirlenmiştir.

Anahtar Kelimeler: Antioksidan, Apoptozis, *Primula vulgaris*, Testis

Abbreviations: AI, Apoptotic index; CAT, Catalase; SOD, Süperoxide dismutase; MDA, Malondialdehyde; MTX, Methotrexate

1. INTRODUCTION

Chemotherapeutics can produce acute toxic outcomes in multiorgan systems (Kim et al., 1999). The side effects of such drugs include azoospermia and infertility in males (Schilsky et al., 1980). Methotrexate (MTX) is a folic acid antagonist agent that is widely used in the treatment of malignant tumors (including acute lymphoblastic leukemia, non-Hodgkin lymphoma, breast cancer, and malignancies of the head and neck) and non-neoplastic conditions (particularly rheumatoid arthritis) (Nouri et al., 2009). Side effects resulting from the administration of MTX observed in previous studies include injury (such as disorganization and vacuolization) to the testicular seminiferous tubules, a reduced sperm count, and impairment of sperm DNA (Padmanabhan et al., 2009; Vardi et al., 2009). Oxidative stress has been implicated in the pathogenesis of MTX-induced testicular injury (Armagan et al., 2008). It has been suggested that reactive oxygen radicals (ROS) can lead to atrophy in the testicular seminiferous tubules and apoptosis in spermatocytes (Nouri et al., 2009; Padmanabhan et al., 2009; Vardi et al., 2009). More recent research has investigated the use of antioxidants for the purpose of minimizing side effects caused by the application of MTX (Armagan et al., 2008; Vardi et al., 2009; Gulgun et al., 2010).

Primula, a medicinal, flowering plant, is a member of the family Primulaceae with some 400-500 known species. *Primula* herbs are widely employed in traditional medicine for their antispasmodic, vermifuge, emetic, and astringent effects. Folk doctors use various *Primula* species in the treatment of a range of conditions, including bronchitis, epilepsy, convulsions, cramps, spasms, paralysis, and rheumatic pains (Jager et al., 2006; Basbulbul et al., 2008; Orhan et al., 2012; Majid et al., 2014). The principal compounds in the genus are phenolic glycosides and saponins (Basbulbul et al., 2008). Various studies have reported that different *Primula* species exhibit cytotoxic, antibacterial, antiviral, antioxidant, antiangiogenic, anti-inflammatory and antimetabolic activities. Studies have ascribed these effects to their phenolic contents (Kati et al., 2001; Tokalov et al., 2004; Buruk et al., 2006; Basbulbul et al., 2008; Orhan et al., 2012; El-Sayed et al., 2014).

This study was intended to investigate the contribution of oxidative stress to testicular injury deriving from the use of MTX, and to determine the protective potential of *Primula vulgaris* against such injury by means of histopathological and biochemical analyses.

2. MATERIALS and METHODS

2.1. Plant Material and Aqueous Extract Preparation

Primula vulgaris subsp. *sibthorpii* was collected from Trabzon, Turkey, in May 2014. The plant was identified by Prof. Dr. Ufuk OZGEN. The dried powder obtained from blossom parts of *P. vulgaris* (1 g) was weighed and mixed with 20 mL methanol. This mixture was stirred on a continuous basis at room temperature for 24 hours. The suspension was then removed by centrifugation at a speed of 10,000 g for 15 mins. The supernatant was subsequently concentrated at 40 °C inside a rotary evaporator (IKA-Werke RV05 Basic, Staufen, Germany). Finally, the dry residue was resolved with distilled aqueous and filtered using a 0.45 µm filter before being stored at 4 °C until further experiments (Kaynar et al., 2023).

2.2. Animals

The rats in this randomized, controlled animal study were housed at room temperature in a 12/12 h light/dark cycle with ad libitum access to standard laboratory chow and water. All animals were treated in line with the principles of the “Guide for the Care and Use of Laboratory Animals” issued by the National Institutes of Health. The study was approved by the Karadeniz Technical University Animal Care and Ethical Committee (Ethics Board Number: 3)

2.3. Experimental Protocol

Thirty-two adult male Sprague Dawley rats (8 weeks old) were used. MTX was administered intraperitoneally (i.p.) and *P. vulgaris* extract (PVE) by gavage. The rats were divided at random into 4 groups of 8 animals each. Group 1 (control group) received 0.8 mL/kg saline intraperitoneally (ip) for 7 days (Berber, 2017). Group 2 (MTX group) was given 30 mg/kg methotrexate (Kocak Farma, Tekirdag, Turkey) intraperitoneally (i.p.) on the first day of the experiment (Yulug et. al., 2013; Ayan, 2016; Berber, 2017). Testicular damage was induced by giving MTX to rats in Group 2 (Yulug et. al., 2013; Ayan, 2016; Berber, 2017). Group 3 (MTX + PVE group) was given 30 mg/kg methotrexate (Kocak Farma, Tekirdag, Turkey) intraperitoneally (i.p.) on the first day of the experiment, following 100 mg/kg of *P. vulgaris* extract by gavage for 7 days (Berber, 2017). Group 4 (PVE group) was administered 100 mg/kg *P. vulgaris* extract by gavage for 7 days (Berber, 2017). All rats were subjected to laparotomy on the 8th day of the experiment. The abdominal cavity was incised, and the bilateral testes and epididymis were removed. At the end of the procedure, all animals were sacrificed by exsanguination (Berber, 2017).

2.4. Biochemical Analysis

Plasma malondialdehyde (MDA) levels were calculated based on the technique previously described by Yagi (Yagi, 1994). Briefly, 2.4 mL of 0.08N H₂SO₄ and 0.3 mL of 10% phosphotungstic acid were added to 0.3 mL of serum. This mixture was allowed to stand at room temperature for 5 min before being centrifuged at 1600 g for a further 10 min. Discard supernatant and sediment were suspended in 4 mL of distilled water. In the next stage, 1 mL of 0.67% thiobarbituric acid was added to the mixture, which was placed in boiling water for 1 hour. The resulting color was extracted into n-butanol. The mixture was again centrifuged at 1600 g for another 10 min. The absorbance of the organic layer was read at 532 nm. Tetramethoxypropane was adopted as a standard, and MDA levels were expressed as nmol/mL.

MDA levels in testis specimens were calculated according to Uchiyama and Mihara's technique (Uchiyama & Mihara, 1978). Briefly, in the initial stage, a piece of testicular tissue was minced before being homogenized in an ice-cold 1.15% KCl solution containing 0.50 mL/L Triton X-100 with the assistance of an Ultra-Turrax T25 homogenizer. To the resulting homogenate (0.5 mL) was added 3 mL of 1% H₃PO₄, followed by 1 mL of 0.67% thiobarbituric acid. This mixture was placed into boiling water for 45 min. The color phase was subsequently extracted into n-butanol. Following further centrifugation, the absorbance of the resulting organic layer was read at 532 nm. Tetramethoxypropane was employed as a standard for this procedure, and MDA levels were expressed as nmol per milligram protein.

Both plasma and testis tissue specimens were used in the measurement of superoxide dismutase (SOD) and catalase (CAT) levels. Specimens were first homogenized in an ice-cold Tris-HCL buffer (50 mM, pH 7.4) containing 0.50 mL/L Triton X-100. SOD activities were determined based on the reduction of nitroblue tetrazolium by the xanthine-xanthine oxidase system (Sun et al., 1988). The formation of formazon formation was evaluated using spectrophotometric methods at 560 nm. Enzyme activity resulting in an inhibition level of 50% was adopted as one unit. Bovine erythrocyte SOD was employed as standard. The results were variously expressed as U/mg protein in testis tissue and as U/mL in plasma. CAT activity was determined using the method previously described by Aebi (Aebi, 1974). This relies on the principle that absorbance at 240 nm decreases on account of the dismutation of H₂O₂. The results were again expressed in the form of U/mg protein in testicular tissue and U/mL in plasma. Protein concentrations were calculated based on the method described by Lowry (Lowry et al., 1951).

2.5. Histopathological Staining and Analysis

The right testis and epididymis tissues were fixed and dehydrated before being embedded in paraffin. Tissues were then stained with hematoxylin and eosin (H&E). All testicular histology assessments were performed by a histologist blinded to the various experimental groups. Light microscopy (Olympus BX-51; Olympus, Tokyo, Japan) was used for evaluations. Testis sections from all experimental groups were assessed in terms of structural alterations. Johnsen's tubular biopsy score (JTBS) was adopted as a semiquantitative technique for assessing spermatogenesis in 20 seminiferous tubules from each testicular section (Kaltsonoudis et al., 2005). Testicular tubule sections were classified from 1 to 10 based on the following definitions; 10 was equivalent to full spermatogenesis and a normal structure; 9 indicated the presence of numerous spermatozoa and disorganization in tubules; 8 indicated a low number of spermatozoa; 7 indicated a complete absence of spermatozoa, but numerous spermatids; 6 indicated an absence of spermatozoa and the presence of a small number of spermatids; 5 described an absence of spermatozoa and spermatids, but the presence of numerous spermatocytes; 4 indicated the presence of a low number of spermatocytes; 3 indicated the presence of spermatogonia only; 2 indicated that no germ cells were observed, only Sertoli cells; and 1 indicated total absence of germ cells and spermatogenesis. We divided the sum of all scores by the total number of seminiferous tubules observed in order to elicit the JTBS.

2.6. TUNEL Analysis

Apoptosis in testicular tissue was determined using the terminal deoxynucleotidyl transferase (TdT) deoxyuridine triphosphate nick end labeling assay (TUNEL). This was performed with an in situ cell death detection kit, POD, (ROCHE, Mannheim, Germany) in accordance with the manufacturer's recommendations. Color was subsequently analyzed using a kit containing 3,3-diaminobenzidine (DAB, Sigma, St. Louis, MO, USA). The presence of DNA fragmentations was investigated in seminiferous tubule germinal cells and in epithelial cells from the epididymal canal. TUNEL (+) cells staining brown were considered apoptotic. TUNEL (+) cells were counted from 20 seminiferous tubules and 20 epididymal canal sections from each testis at $\times 400$ magnification with the assistance of Analysis 5 Research software (Olympus Soft Imaging Solutions, Münster, Germany). The proportion of TUNEL (+) apoptotic testicular cells to the total number of cells was calculated as the testis apoptotic index (TAI) (Patel et al., 2014), while the epididymal apoptotic index (EAI) was calculated as the proportion of TUNEL (+) apoptotic cells in the epididymis to the total number of cells.

2.7. Statistical Analysis

Freidman variance analysis was performed on the seminiferous tubule diameter, epithelial thickness, number of tubules with immature cells shed into the lumen, Johnsen scoring, AI, sperm count, motility, and vitality parameters of the study groups. Holm test was applied for post-hoc evaluation. $p < 0.05$ was considered significant in the Freidman test. Kruskal-Wallis analysis of variance was used to compare differences between group parameters (MDA, SOD, CAT). Dual comparisons between groups exhibiting significant values were evaluated using the Mann-Whitney U test. $p < 0.05$ was considered statistically significant. All results were expressed as means (\pm) standard deviation (SD).

3. RESULTS and DISCUSSION

3.1. Biochemical Analysis

Biochemical results in testicular tissue and plasma for the experimental groups are given in Table 1 and Table 2, respectively. Tissue and plasma MDA concentrations in the group 2 were higher compared to the group 1 and group 4, while SOD and CAT activity were lower in testicular tissue. Tissue and plasma MDA concentrations in the group 3 were lower compared to the group 2, while there was a rise in SOD and CAT activities. MTX-induced sperm injury has been linked to oxidative stress. Malondialdehyde levels are frequently measured as a marker of oxidative stress. Serum and tissue MDA concentrations increased in the group 2 in this study. This finding is compatible with the results of various previous studies to the effect that MTX leads to oxidative stress in tissues by raising MDA levels (Oktem et al., 2006; Armagan et al., 2008; Cetin et al., 2008; Vardi et al., 2010).

Table 1. Biochemical results in testicular tissue

Groups	MDA (nmol/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)
1	12.27 \pm 1.89	1.86 \pm 0.13	29.77 \pm 4.60
2	15.381 ^a \pm 1.97	1.24 ^b \pm 0.12	19.10 ^b \pm 5.08
3	14.08 \pm 2.65	1.41 ^c \pm 0.29	25.22 \pm 9.72
4	12.60 \pm 1.53	1.56 ^d \pm 0.54	26.79 \pm 8.40

MDA, malondialdehyde; SOD, superoxide dismutase; CAT, catalase; MTX, methotrexate

^aThe value in the group 2 increased significantly compared to the group 1 ($p < 0.05$).

^bThe value in the group 2 was significantly lower than in the group 1 ($p < 0.05$).

^cThe value of group 3 was significantly lower than group 1 ($p < 0.05$).

^dThe value of the group 4 was significantly lower than the group 1 ($p < 0.05$).

Antioxidant defense mechanisms in the testis play a significant role in protecting sperm against ROS. Protective biomolecules including a range of antioxidants, vitamins, and glutathione enable the spermatozoa to combat ROS (Prahalathan et al., 2004). SOD is one of the most important antioxidant enzymes that protect the male reproductive organs against the deleterious effects of ROS (Fujii et al., 2003). Hydrogen peroxide (H₂O₂) is less effective than the superoxide group. Its effect is weakened through conversion by enzymes in tissue, including catalase and glutathione, into products with a lesser effect, such as water and oxygen (Callaghan et al., 2013). This study was also intended to determine whether the antioxidant enzymes SOD and CAT exhibit protective effects that eliminate free radicals emerging due to MTX. SOD is significantly involved in testis development and spermatogenesis. Alterations in SOD may result in a compromise of testicular functions and interrupted sperm development (Hung et al., 2002). The findings of this study show that MTX reduced SOD and CAT activity in testicular tissue. We would attribute the low levels of SOD and CAT observed to increased consumption and disequilibrium in resynthesis mechanisms.

Table 2. Biochemical results in plasma

Groups	MDA (nmol/mL)	SOD (U/mL)	CAT (U/mL)
1	0.81 ± 0.41	4.47 ± 3.30	167.77 ± 1.00
2	1.03 ^a ± 0.01	2.38 ± 1.29	163.89 ± 2.92
3	0.88 ± 0.39	2.91 ± 2.62	166.24 ± 2.03
4	0.89 ± 0.15	2.46 ± 0.87	164.62 ± 4.59

MDA, malondialdehyde; SOD, superoxide dismutase; CAT, catalase; MTX, methotrexate

^aThe value in the group 2 increased significantly compared to the group 1 (p < 0.05).

The administration of *P. vulgaris* before MTX partly or entirely neutralized these effects in this study. In Group 3, tissue and plasma MDA concentrations were lower than in Group 2, while SOD and CAT activities increased. Plasma and tissue MDA concentrations were significantly lower and SOD enzyme activity was significantly higher in group 3. Previous studies have reported that antioxidant substances reduce oxidative stress by lowering MDA concentrations in tissue (Sikka, 2001; Saral et al., 2016). Antioxidant enzymes including glutathione peroxidase (GP-x), SOD, and CAT, and other antioxidants, such as vitamins C and E protect seminal plasma and sperm against cellular injury (Smith et al., 1996). Yüncü et al. founded that MDA levels increased significantly and SOD activity decreased significantly in the MTX group (Yüncü et al., 2015). Sönmez et al. determined that tissue MDA levels increased insignificantly

in the MTX group (Sönmez et al., 2016). Dagguli et al. reported that MDA, total oxidant capacity and oxidative stress index increased by inducing testicular damage with MTX in rats (Daggulli et al., 2014). *P. vulgaris* may be a free radical scavenger and enzyme regulator and therefore protect against tissue damage caused by oxidative stress.

3.2. Histopathological Analysis

In testicular tissue, it was determined that seminiferous tubule diameter, epithelial thickness, sperm count, motility, vitality, and Johnsen scoring values decreased in group 2 compared to group 1; tubule number that immature cells sloughed into the lumen and apoptotic index increased (Table 3).

Table 3. In the testicular tissue of the groups; seminiferous tubule diameter, epithelial thickness, and tubule number that immature cells sloughed into the lumen

Groups	Seminiferous Tubule Diameter (µm)	Epithelial Thickness (µm)	Tubule Number that Immature Cells Sloughed into The Lumen (%)
1	293.64 ± 27.12	59.62 ± 7.93	16.52 ± 11.44
2	229.95 ^a ± 18.69	42.84 ± 4.13	45.33 ^a ± 17.43
3	285.53 ^b ± 7.99	62.59 ^b ± 3.60	26.10 ± 10.91
4	294.50 ^b ± 12.54	61.34 ± 1.82	19.57 ^b ± 15.55

^aThere is a significant difference compared to the group 1 (p < 0.05).

^bThere is a significant difference compared to the group 2 (p < 0.05).

In testicular tissue, it was determined that seminiferous tubule diameter, epithelial thickness, sperm count, motility, vitality, and Johnsen scoring values increased in group 3 compared to group 2; tubule number that immature cells sloughed into the lumen and apoptotic index decreased (Table 4).

Johnsen's score was found to decrease in group 2, group 3, and group 4 compared to group 1, and this decrease was significant only in group 2. A non-significant increase was found in group 3 and group 4 compared to the group 2. A non-significant increase was observed in the group 4 compared to the group 3 (Table 5).

Table 4. Sperm count, motility, and vitality values of the groups

Groups	Sperm count (x10 ⁶)	Motility (%)	Vitality (%)
1	19.12 ± 3.75	49.72 ± 9.60	61.50 ± 3.50
2	16.62 ± 3.92	30.95 ± 13.33	42.75 ^a ± 8.20
3	20.25 ± 3.91	34.41 ± 11.58	51.00 ± 7.55
4	21.25 ^b ± 1.90	49.87 ± 11.21	58.75 ^b ± 8.06

^aThere is a significant difference compared to the group 1 (p < 0.05).

^bThere is a significant difference compared to the group 2 (p < 0.05).

In the apoptotic index assessment; it was found that there was an increase in group 2, group 3, and group 4 compared to group 1, and this increase was significant in group 2. A significant decrease was found in group 3, and group 4 compared to group 2. A non-significant increase was observed in the group 4 compared to the group 3 (Table 5).

Table 5. Johnsen Scoring and apoptotic index (AI) of the groups

Groups	Johnsen Scoring	AI (%)
1	9.38 ± 0.11	12.03 ± 3.11
2	4.12 ^a ± 0.62	34.7 ^a ± 2.10
3	7.7 ± 0.37	5.16 ^b ± 3.33
4	8.08 ± 0.73	5.81 ^b ± 1.73

^aThere is a significant difference compared to the group 1 (p < 0.05).

^bThere is a significant difference compared to the group 2 (p < 0.05).

MTX is commonly employed in the treatment of testicular, bladder, head and neck, and breast cancer. In addition to its antineoplastic activity, it has also been used to treat psoriasis and as an immunosuppressive medication against various auto-immune diseases, including ankylosing spondylitis, Crohn's disease, dermatomyositis, Wegener's granulomatosis and rheumatoid arthritis (Kaltsonoudis et al., 2005; Patel et al., 2014; Specks, 2005; Roychowdhury et al., 2002). When MTX is employed during cancer chemotherapy normal cells begin to divide very quickly, a process that leads to various forms of toxicity. The therapeutic and toxic effects of MTX are delayed as a result of conversion to a polyglutamated form with a longer metabolic half-life. MTX also suppresses DHFR. The effect mechanism involves inhibition of DNA synthesis, which results in compromise of normal cellular processes (Kamen et al., 1981; Novakovic et al., 2003). Testicular toxicity is a particularly significant potential side-effect of

MTX, and one that may ultimately result in infertility. It is important that germinal cells be protected in the course of chemotherapeutic procedures. MTX has been reported to induce testicular toxicity through ROS generation (Yulug et al., 2013). The purpose of the present study was to investigate whether modification of oxidative stress status would occur in the rat testis following exposure to MTX and whether such effects can be attenuated through the use of *P. vulgaris*.

Sönmez et al., Yüncü et al., Yulug et al., Gökçe et al., Nouri et al., Vardi et al., Saxena et al. examined the effects of MTX on rat testes. These researchers examined the effects of MTX at different times and doses on seminiferous tubules, spermatogenic series cells and interstitial areas and determined the damages that occurred. Sönmez et al. determined significant damage in the seminiferous tubules and interstitial areas in the group to which they applied MTX. According to the study results, they detected vacuolization in the seminiferous epithelium, irregularity in germinal cells, immature germinal cell shedding into the seminiferous tubule lumen and atrophy in some seminiferous tubules (Sönmez et al., 2016).

Oxidative stress results in an imbalance between ROS and the antioxidant reserve system. ROS is the product of normal cellular metabolism. Sperm cells produce free oxygen radicals. Low levels of reactive oxygen radical production result in sperm cell capacitation, acrosome reaction, and sperm binding to the zona pellucida (de Lamirande et al., 1997). Overproduction of ROS results in sperm anomalies and infertility. The sperm membrane contains high levels of polyunsaturated fatty acids, resulting in increased oxygen-induced lipid peroxidation (Sikka, 2001). Peroxidative damage has been implicated as a significant factor in sperm function damage (Moustafa et al., 2004).

4. CONCLUSION

Our study shows that oxidative stress is important in MTX induced testicular damage. The administration of *P. vulgaris* reduced oxidative stress and apoptotic cell death and protected spermatogenesis in MTX-induced oxidative testicular damage. We think that this protective effect of *P. vulgaris* may be due to its antioxidant properties. Further experimental and clinical studies are now needed to confirm our findings.

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DECLARATIONS

There is no conflict of interest between the authors.

AUTHORS' CONTRIBUTIONS

The authors have equal contributions.

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