European Journal of Therapeutics

DOI: 10.5152/EurJTher.2018.790

Original Research

Protective Effect of Proanthocyanidin against Methotrexate-Induced Testicle Damage in Rats

Mehmet Yüncü[1], Gülşen Zengin[1], Hülya Birinci[1], Nuray Bostancıeri[1], Sait Polat[2]

[1]Department of Histology and Embryology, Gaziantep University, School of Medicine, Gaziantep, Turkey

[2]Department of Histology and Embryology, Çukurova University, School of Medicine, Adana, Turkey

ABSTRACT

Objective: Methotrexate (MTX) isawidely used chemotherapeutic agent showing side effects such as hepatotoxicity and also testicular damage due to an increase in the production of reactiveoxygen species. Proanthocyanidins (PACs) are known as natural antioxidants that have protective effects against oxidative stress. This study investigates the protective effect of PAC against the MTX-induced testicular damage.

Methods: Twenty-six male Wistar albinorats were divided into fourgroups. Saline (2 cc/kg) was administered to the control group, and PAC (200 mg/kg) was administered to the PACgroup for 7 days. The MTX group received a single dose of MTX (20 mg/kg) on Day 3. A total of 200 mg/kg of PACfor 6 days was administered to the PAC+MTX group, and the group also received a single dose of 20 mg/kg of MTX on Day 3. The animals were sacrificed on Day 8. Analyses were performed, in addition tolight and electron microscopic examinations on the testicular samples.

Results: Histopathological findings such as thinning of the seminiferous tubule epithelium and decrease in spermatogonial cells were detected in the MTX group. Electron microscopic findings indicated similar damage. The MDA levels increased in testicular tissues, while the SOD and GPX levels decreased significantly. The tissue damage and deterioration in biochemical parameters were decreased in the PAC+MTXgroup.

Conclusion: The results show that PAC does not completely prevent damage to the testicles caused by MTX but that it has an important protective effect.

Keywords: Methotrexate, proanthocyanidin, testis

INTRODUCTION

Methotrexate (MTX), a folic acid precursor, is widely used in the treatment of malignant tumors and some non-neoplastic diseases. Chemotherapeutic drugs such as MTX act on the S phase of the cell cycle by inhibiting the synthesis of DNA precursors. As a result, organs with high mitochondrial activity, such as the testicular tissue, become targets of these drugs (1). It has been reported that the drugs used in cancer treatment cause a decrease in the diameter of the lumen of the seminiferous tubule, and the degeneration and shedding of germ cells (2). Oxidative stress plays an important role in testicular damage and pathogenesis resulting from the use of MTX (2, 3).

Oxygen-derived free radicals or reactive oxygen species (ROS) are produced during normal metabolism and energy production of the body. However, if ROSs exceed the antioxidant capacity of the biological system, they cause an imbalance, known as oxidative stress, due to metabolic and other environmental factors. This can damage biological molecules, such as lipids, proteins, polysaccharides, and DNA. Scientific interest in antioxidant compounds, especially those from plants, has been increasing over recent years. The discovery of the protective function of phytonutrients against cancer and other neurodegenerative diseases, and the growing concern about synthetic antioxidants, have made natural antioxidants more attractive (4).

Proanthocyanidins (PACs) are natural antioxidants found in the seeds, flowers and shells of fruits, vegetables, nuts, and especially grapes (*Vitisvinifera*) (5). PACshave biological and pharmacological properties and therapeutic effects against free oxygen radicals and oxidative stress (6, 7). Abundant amounts of grape seed extract containing PAChave been shown to protect against cardiovascular diseases, nephropathy, atherosclerosis, and neuropathy (8-12). In addition, its anti-apoptotic, anti-inflammatory and antimicrobial effects have been demonstrated through animal culture studies, as well as its anticarcinogenic effects (13) through cell culture studies (9-11, 14).

In this study, the effects of PAC on the testicles of rats who were administered MTX were investigated through light microscopy, electron microscopy, and on a biochemical level.

ORCID IDs of the authors: M.Y. 0000-0002-2519-6834; G.Z. 0000-0002-0853-1362; H.B. 0000-0002-6749-9665; N.B. 0000-0002-3765-8274; S.P. 0000-0003-1646-8831.

Corresponding Author: Mehmet Yüncü **E-mail:** yuncu@gantep.edu.tr

Received: 11.09.2018 • **Accepted:** 09.10.2018

Table 1. Experiment schedule

Group	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control, n=5	Sa	Sa	Sa	Sa	Sa	Sa	Sa
PAC, n=7	PAC	PAC	PAC	PAC	PAC	PAC	PAC
MTX, n=7	Sa	Sa	MTX+Sa	Sa	Sa	Sa	Sa
PAC+MTX, n=7	PAC	PAC	MTX+PAC	PAC	PAC	PAC	PAC

PAC: proanthocyanidin, 200 mg/kg (gavage); MTX: methotrexate, 20 mg/kg (intraperitoneal); Sa: saline, 2 cc/kg (gavage)

METHODS

In our study, 26 young adult Wistar albino rats with a mean age of 5-6 months old were used. The animals were housed in wire cages in rooms where the ambient light was set to 12-hour darkness and 12-hour light, and the ambient temperature was fixed at 21±1°C. During the experiment, all animals were fed ad libitum. All animals were weighed, and the weights were recorded at the beginning of the study and before the sacrifice. All the procedures carried out in our study were approved by Animal Experiments Local Ethics Committee of Kahramanmaraş Sütçü İmam University (decision date 05.05.2015, decision number 07).

All chemicals used in our study were of the analytical grade and were from the Sigma Chemical Company (SigmaAldrich; St. Louis, Missouri, USA).

Experimental Groups

The experimental animals were divided into fourgroups: thecontrol group, PACgroup, MTXgroup, and MTX+PAC group, where the control group had 5, and other groups had seven rats each (Table 1).

Collecting the Tissues

All animals were sacrificed 24 hours after the last administration (day 8). The animals' testicles were removed, cleaned from the surrounding tissues, and weighed. For each animal, the right testicles were placed in 10% neutral formaldehyde for a follow-up with light microscopy. A small fraction of the left testicles was placed in a 5% glutaraldehyde solution for electron microscopy, and the remaining part was stored at −20°C for biochemical procedures.

Calculation of Testicle Weight Index

The testicle weight index (TWI) values for each animal were determined by calculating the body weight measured on the last day of the experiment and the sum of the right and left testicle weights of the same animal according to the following formula.

TWI= [(right+left testicle total weight)/body weight] × 100

Tissue Biochemical Analysis

The tissues stored in a cold environment (−20°C) were brought up to +4°C just before the experiment. The dissolving tissue samples were weighed in their frozen form and put in glass tubes. Superoxide dismutase (SOD), glutathione peroxidase (GPX), and malondialdehyde (MDA) analyses were performed on the tissues.

Determination of MDA

The MDAof the homogenates was determined spectrophotometrically by measuring the existence of thiobarbituric acid reactive substances (TBARS), as defined by Altintas et al. (15). Phosphoric acid (1%) at 3 mL and 0.6% thiobarbituric acid solutionat 1 mlwere added to 0.5 ml of homogenate. The mixture was heated in boiling water for 45 min. After the mixture was cooled, the colored part was extracted into 4 mL of n-butanol. The absorbance was measured by a spectrophotometer (UV-1601; Shimadzu, Kyoto, Japan) at 532 nm. The amount of lipid peroxides was calculated as the TBARS of lipid peroxidation.

Determination of the SOD Activity

Total SOD activity was determined as defined by Parlaktas et al. (16). Theprinciple of this method is the prevention ofnitrobluetetrazolium (NBT) reduction by the xanthine-xanthineoxidase system as asuperoxide generator. One unit of SOD was defined as the amount of enzyme to cause 50% inhibition in the NBT reduction rate. The results were given in Units per gram of protein.

Determination of the GPX Activity

The GPX activity was measured as defined by Parlaktas et al. (16). An enzymatic reaction in a tube containing nicotinamideadenine dinucleotide phosphate (NADPH), glutathione (GSH), sodium azide, and glutathione reductase was initiated by adding H_2O_2; the change in absorbance at 340 nm was observed using a spectrophotometer. The results were given in Units per milligram of protein.

Preparation of Tissues for Light Microscopy

After the 10% formaldehyde fixation, paraffin blocks were prepared by routine procedures on a tissue-monitoring device (TP 1010; Leica, Wetzlar, Germany).

Sections that were 5-6 µ thick were taken from the prepared paraffin blocks with a microtome (RM2245; Leica, Wetzlar, Germany) device. The preparations were stained with hematoxylin-eosin (HE), analyzed under a light microscope, and photographed. For assessing spermatogenesis in testicular tissues, the Johnsen score has determined. A minimum of 50 tubules were evaluated, and each tubule was given a score from 1 to 10.

Measurement of Tubular Diameter (TD) and Tubular Epithelial Thickness (TET)

Light microscopic sections were examined with the microimage software (cellSens Analysis Systems; Olympus, Tokyo, Japan) and

a light microscope (DM 750; Leica, Wetzlar, Germany). In each group, morphometric analyses were performed by measuring the diameters of ten seminiferous tubules of each animal, two measurements per tubule, and through germinal epithelial thickness measurement from four different regions of the same tubule with 10X magnification and averaging them.

Preparation of Tissues for Electron Microscopy

Tissue samples were fixed with 5% glutaraldehyde and 1% osmium tetraoxide solutions prepared with Millonig's phosphate buffer. After dehydration with the alcohol series, the tissues were immersed in the rotator overnight in the embedding material (resin), and the Epon blocks were prepared the next day. The thin sections obtained from the blocks were placed on a copper grid and after being contrasted with uranyl acetate and lead citrate, they were analyzed with Transmission Electron Microscopy (JEM 1400; Jeol, Massachusetts, USA), and photographs were taken.

Statistical Analysis

Biochemical data were analyzed using the Statistical Package for the Social Sciences 22.0 package program (SPSS IBM Corp.; Armonk, NY, USA). An analysis of variance (ANOVA) test was used to assess whether there was a significant difference between the groups' averages. Values with a p-value <0.05 were considered statistically significant. Post-hoc tests were performed to assess the difference between the groups in the parameters that resulted in a significant difference between the group averages. Taking the homogeneity of the variances of the groups into account, the Tukey test was applied for the parameters of SOD and GPX, which had a greater value than p=0.05. For the MDA with a p-value <0.05, the Games Howell test was applied to see among which groups the group averages differ significantly.

The statistical SPSS 22.0 package program was used forthestatistical evaluation of tubal diameter and epithelium thickness measurements. The normal distribution of the numerical data in this parameter was tested with the Shapiro-Wilk test. The ANOVA test and the Least Significant Difference (LSD) test were used for comparison of the variables that fit normal distribution.

RESULTS

Testis Weight Index

The difference between the TWI values of the groups was not statistically significant (p=0.447; Table 2).

Biochemical Findings

The levels of SOD, GPX, and MDA in the testicular tissues of the groups are shown in Table 3.

While SOD significantly decreased (p<0.001) in the MTX group compared with the control group, it increased (p=0.002) again in the PAC+MTX group, and its difference from the control group became insignificant (p=0.096). There was also a significant difference between the MTX group and the PAC group (p<0.001).

The results of GPX were foundsimilar to SOD. The level of GPX in the PAC+MTX group was high (p=0.002), while in the MTX group, there was a decrease in the GPX level compared to both the control group (p=0.015) and the PAC group (p=0.003). The difference between the PAC group and the PAC+MTX group was not significant (p=0.952).

Table 2. Testicle weight index according to groups of rats (%)

Group	Mean±SD
Control	1.2841±0.1304
PAC	1.2232±0.1090
MTX	1.3122±0.1071
PAC+MTX	1.2182±0.1354

PAC: Proanthocyanidin; MTX: Methotrexate

Table 3. Tissue biochemical values of the experimental groups (mean±standard deviation)

Group	SOD (IU/mg)	GPX (IU/mg)	MDA (nmol/mg)
Control	10.9076±1.3813	0.0555±0.0117	1.5715±0.3216
PAC	12.6453±1.8946	0.0506±0.0027	1.9864±0.5953
MTX	4.5552±1.0220	0.0289±0.0078	4.0179±0.4265
PAC+ MTX	8.4570±2.0908	0.0497±0.0039	2.7004±0.4797

PAC: Proanthocyanidin; MTX: Methotrexate

There was no significant MDA difference between the control and PAC groups (p=0.468), but it was significantly higher in the MTX group than the control group (p<0.001). In the PAC+MTX group, it decreased with respect to the MTX group (p<0.001). The difference between the PAC group and the PAC+MTX group was not significant (p=0.050). The increase in the PAC+MTX group was significant compared to the control group (p=0.003).

Light Microscopic Findings

Histological analysis

Samples from the control group and the PAC group showed a normal testicular histology (Figure 1). Thinning and vacuolization in the seminiferous tubule, degeneration in the spermatogonium cells and shrinkage of the nuclei, and interstitial edema and congestion in some vessels were observed in MTX group (Figure 2). In MTX+PAC group, there was reduced tubular damage, the spermatogenic cells were preserved and the spermatogenesis continued, while the change in the spermatogonia was not as severe as in the MTX group, although some tubular damage in several tubules persisted. In the interstitial area, although there was a small amount of edema, no congestion was seen (Figure 3).

Johnsen score analysis

According to the score results, in the PAC group, there was no difference according to the control group, statistically (p=0.116). The MTX group showed impaired spermatogenesis, and this difference was statistically significant (p<0.001). The MTX+PAC

Table 4. Johnsen scores of the experimental groups

Group	Johnsen Score (Mean±SD)	p
Control	9.529±0.13	
PAC	9.210±1.27	0.116
MTX	6.975±0.475**	<0.001
PAC+MTX	8.903±0.722	0.903

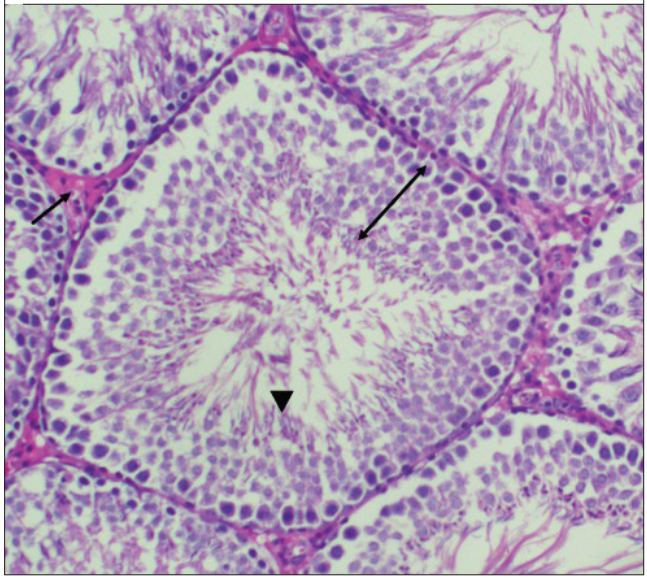
**: p<0.05 compared to control group by ANOVA test
PAC: Proanthocyanidin; MTX: Methotrexate

Table 5. Tubular diameter (TD) and tubular epithelial thickness (TET) measurements

Group	TD (Mean±SD)	TET (Mean±SD)
Control	317.0134±26.4338	38.6471±3.9864
PAC	309.8520±26.8232	35.3019±5.3264
MTX	236.6670±18.4465	25.2050±4.3498
PAC+MTX	334.3897±23.6394	32.9007±2.6315

PAC: Proanthocyanidin; MTX: Methotrexate

Figure 1. The control group light microscopic image revealed normal seminiferous tubules and spermatogenic cell lines of a normal structure (double-headed arrow) on its wall, a normal interstitial area (arrow), and normal spermatids (arrowhead). H&E



group was similar to the control and PAC groups, andthere was no difference statistically (p=0.093) (Table 4).

Tubule diameter and epithelium thickness measurements

While the diameter of the seminiferous tubules was significantly lower in the MTX group than in the control group (p=0.001), the PAC+MTX group maintained values close to the control group. Testicular epithelium thickness was again thinner in the MTX group than in the control group (p=0.001) and was normal again in the PAC+MTX group (Table 5).

Figure 2. The methotrexategroup light microscopic image revealed edema in the interstitial area (arrowhead), thinning in the seminiferous tubule wall (double-headed arrow), and occasional vacuolization (thin arrows) in the seminiferous tubule wall. H&E

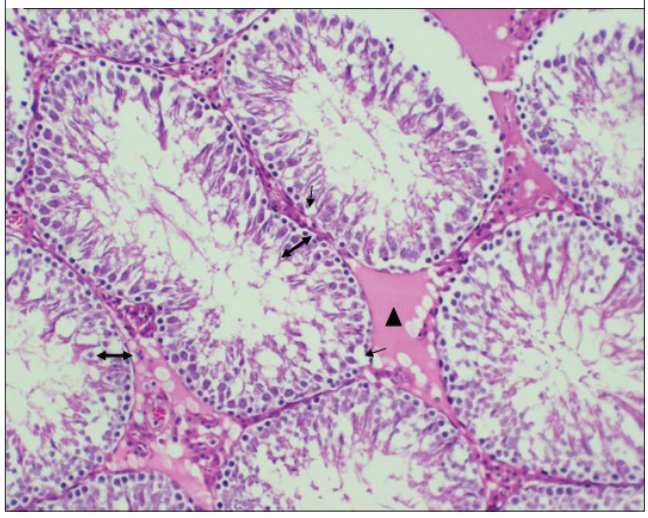
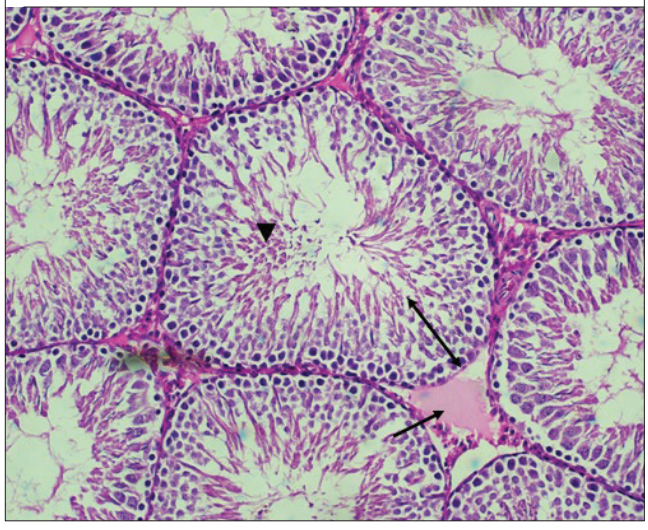


Figure 3. Methotrexate+proanthocyanidin group light microscopic image showed almost normal seminiferous tubule structures (double-headed arrow), preserved spermatogenic cells, continuing spermatogenesis and spermatozoon (arrowhead) in the tubule lumen, and a mild edema in the interstitial area (arrow). H&E



Electron Microscopic Findings

In the control group, normal testicular structures were observed in general (Figures 4, 5). In MTX group, the membranepropria surrounding the seminiferous tubule showed thickening, an increased amount of collagen fibers, and irregularity in the basal lamina (Figure 6). There were indentations between the spermatogonium cells in the tubule epithelium and degradation of the Sertoli cell nucleus (Figure 7). It was observed that the agranular endoplasmic reticulum cisternae in the Sertoli cells

Figure 4. Control group: Sertoli cells (SC) and Sertoli nuclei (SN), spermatogonium (Sg), and primary spermatocytes (PSt) were seen on the membrana propria (MP) surrounding the seminiferous tubule, on the myoid cells (My), and the seminiferous epithelium on the basal lamina (arrowhead). Nucleus (N), mitochondria (m).

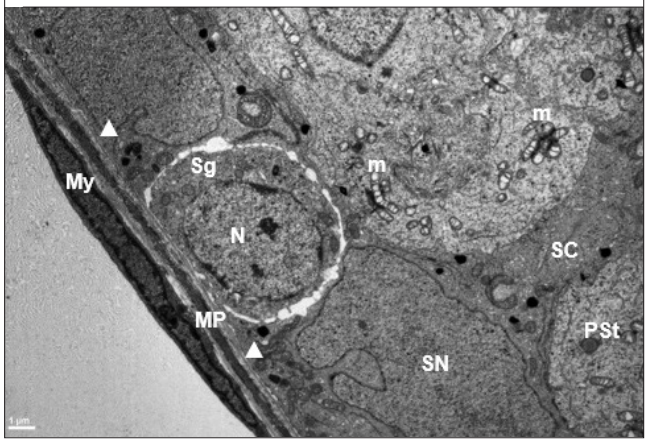


Figure 5. Control group: Normal looking spermatocytes (St) and nuclei (N) are seen located near the seminiferous tubule lumen. The acrosomal vesicle formation (arrows) and tail formation (arrowhead) developing around the nucleus were noteworthy.

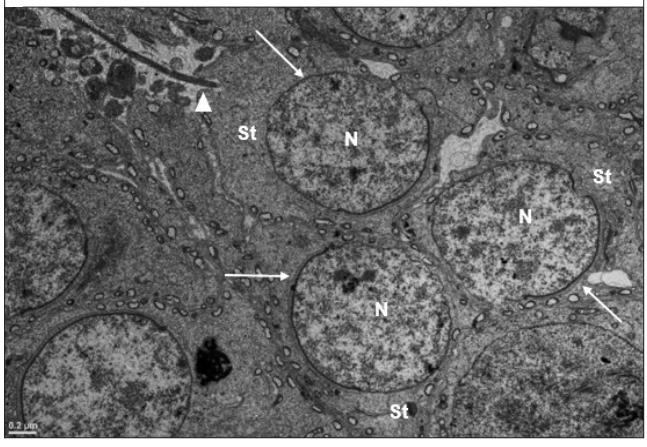


Figure 6. Methotrexategroup: Thickened membrane propria (MP) on the degraded basal lamina and a degenerated myoid cell (My) are observed. Occasional large lipid droplets (L) are seen.

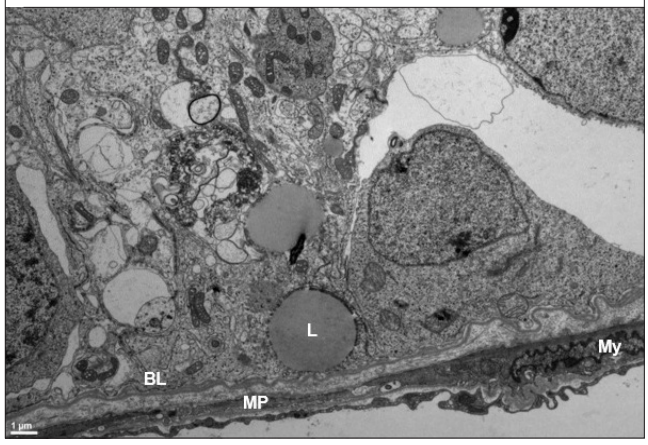
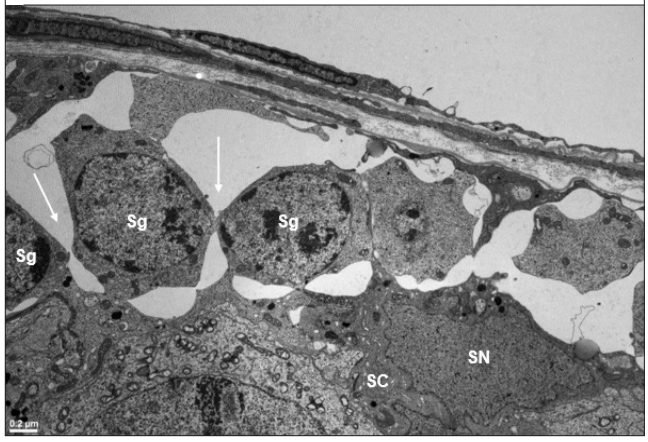


Figure 7. Methotrexategroup: Indentations (arrow) among the spermatogonium (Sg) cells in the seminiferous tubule epithelium were observed, as well as corruptions in the morphology of the Sertoli cell (SC) and the nucleus (SN).



expanded, while lysosomes and lipid droplets increased. Corruptions were observed in the structures of primitive spermatocytes in the seminiferous tubule epithelium, and apoptotic cell structures were seen in the mitochondria, along with some shrinking (Figure 8). Fragmented Leydig cells and nuclei in the interstitial area and small lipid droplets were worthy of note (Figure 9). The PAC group images were similar to those in the control group (Figure 10).When MTX and PAC were administered together in the MTX+PAC group, the pathological findings in the MTX group partially disappeared (Figure 11).

DISCUSSION

Medicines used in chemotherapy generally have adverse effects on all cells in division (17). MTX, a folic acid antagonist, is used in the treatment of diseases, such as lymphoma, osteosarcoma, neck tumors, lung and breast cancers, and testicular cancer (1, 18). Despite its therapeutic effects, MTX is known to cause thinning of the testicles in the walls of the seminiferous tubules, permanent azoospermia, damage to the sperm DNA, abnormalities in the sperm head morphology, and infertility (19-22).

Seminiferous tubule atrophy in the MTX treatment and apoptosis in spermatocytes are thought to be related to the increase of reactive oxygen radicals, and a number of studies have been conducted on the use of antioxidant substances to reduce these side effects (17, 19-21). MTX also reduces the NADPH in cells, leading to glutathione depletion, thereby weakening the cell against ROS and increasing oxidative stress (23). MTX can damage intercellular connection complexes and cause cells to spill into the lumen.

In our study, the light and electron microscopy findings for the MTX group were similar to those of previous studies, including

Figure 8. Methotrexategroup: Deteriorations in the primary spermatocyte (PSt) structures in the seminiferous tubule epithelium, and mitochondrial shrinkage (arrow) are seen. The apoptotic cells (A) stand out.

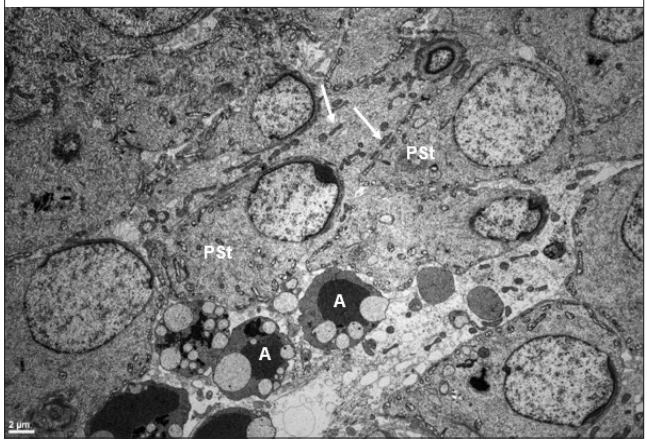


Figure 9. Methotrexategroup: The interstitial area shows lysed Leydig cells (LC), nuclei (LN), and small lipid droplets (arrowhead).

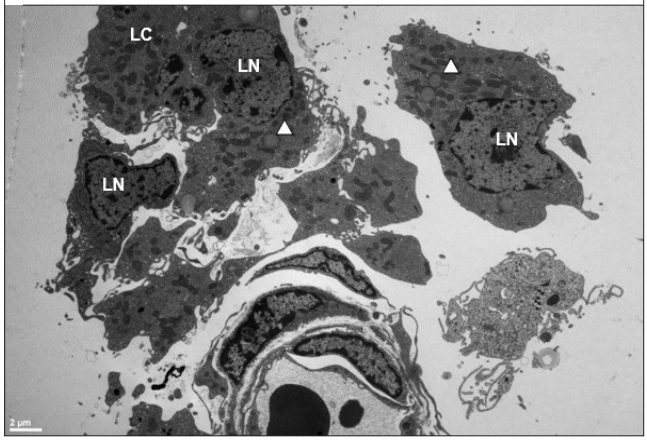


Figure 10. Proanthocyanidingroup:The interstitial area is observed with nearly normal Leydig cells (LC), nuclei (LN), and capillaries (arrow).

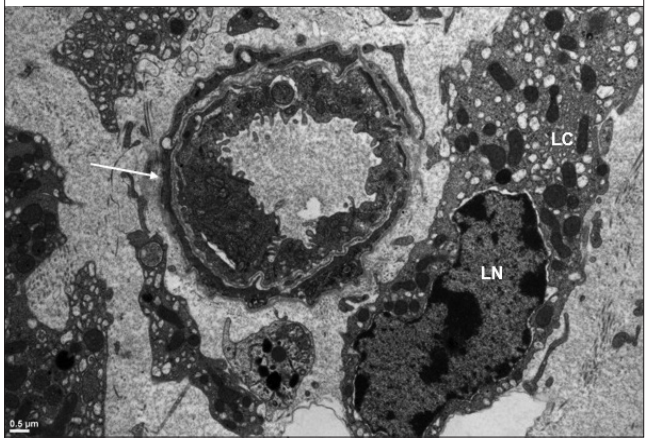
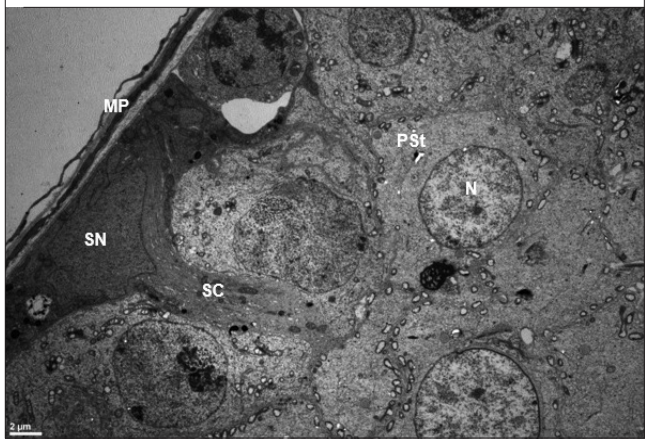


Figure 11. Proanthocyanidin+methotrexategroup. Membrane propria (MP), Sertoli cells (SC) and nuclei (SN), primary spermatocytes (PSt), and nuclei (N) are observed near the seminiferous tubule.



the damage and thinning of the seminiferous epithelium and interstitial edema and congestion (17, 19, 21, 24). Similar to previous studies, the MDA increase in the MTX group and the decreases in the SOD and GPX levels supported these findings.

It is now known that testicular oxidative stress usually causes male infertility in normal or pathological conditions. It has been reported that about 50% of male infertility is due to oxidative stress (25). For this reason, the use of natural antioxidants has become necessary.

In our study, the PAC treatment prevented histopathological findings caused by MTX and improved biochemical parameters that were deteriorated. The electron microscope findings also support these results. There are numerous studies in the literature that show such effects of PAC (5, 12, 14, 25).

Malondialdehyde is a stable end-product of lipid peroxidation generated by ROS, and grape extracts rich in PAC have been shown to reduce elevated MDA levels (9-12, 26).

CONCLUSION

Our study has shown that MTX significantly damages the testicles and that PACsignificantly reduces the severity of such damage, even if it does not completely reverse it.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Kahramanmaraş Sütçü İmam University (decision date 05.05.2015, decision number: 07).

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - M.Y., G.Z.; Design -M.Y., G.Z., S.P.; Supervision -M.Y., H.B., N.B.; Resources -H.B., N.B.; Materials -M.Y., G.Z., N.B.; Data Collection and/or Processing -S.P., M.Y., H.B.; Analysis and/or Interpretation -H.B., G.Z., N.B.; Literature Search -M.Y., H.B., S.P.; Writing Manuscript -M.Y., G.Z., H.B., N.B., S.P.; Critical Review -M.Y., G.Z., H.B., N.B., S.P.

Conflict of Interest: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES

1. Koc F, Erısgın Z, Tekelıoglu Y, Takır S. The effect of beta glucan on MTX induced testicular damage in rats. Biotech Histochem 2018; 93: 70-5.
2. Liu Z, Sun Y, Su L, Sun Y, Kong S, Chang X, et al. Effects of cisplatin on testicular enzymes and Sertoli cell function in rats. Fundam Toxicol Sci 2015; 2: 137-45.
3. Daggulli M, Dede O, Utangac MM, Bodakci MN, Hatipoglu N K, Penbegul N, et al. Protective effects of carvacrol against methotrexate-induced testicular toxicity in rats. Int J Clin Exp Med 2014; 7: 5511-6.
4. Aliyu AB, Ibrahim MA, Musa AM, Musa AO, Kiplimo JJ, Oyewale AO. Free radical scavenging and total antioxidant capacity of root extracts of Anchomanes difformis Engl. (Araceae). Acta Pol Pharm 2013; 70: 115-21.
5. Li SG, Ding YS, Qiang N, Xu SZ, Pang LJ, Lin R, et al. Grape seed proanthocyanidin extract alleviates arsenic-induced oxidative reproductive toxicity in male mice. Biomed Environ Sci 2015; 28: 272-80.
6. Mansouri E, Khorsandi L, Abedi HA. Antioxidant effects of proanthocyanidin from grape seed on hepatic tissue injury in diabetic rats. Iran J Basic Med Sci 2014; 17: 460-4.
7. Mansouri E, Khorsandi L, Zare Moaiedi M. Grape seed proanthocyanidin extract improved some of biochemical parameters and antioxidant disturbances of red blood cells in diabetic rats. Iran J Pharm Res 2015; 14: 329-34.
8. Ozkan G, Ulusoy S, Orem A, Ersoz S, Alkanat M, Yucesan FB, et al. Protective effect of the grape seed proanthocyanidin extract in a rat model of contrast-induced nephropathy. Kidney Blood Press Res 2012; 35: 445-53.
9. Günay E, Celik S, Sarinc-Ulasli S, Özyürek A, Hazman Ö, Günay S, et al. Comparison of the anti-inflammatory effects of proanthocyanidin, quercetin, and damnacanthal on benzo (a) pyrene exposed A549 alveolar cell line. Inflammation 2016; 39: 744-51.
10. Chen S, Zhu Y, Liu Z, Gao Z, Li B, Zhang D, et al. Grape seed proanthocyanidin extract ameliorates diabetic bladder dysfunction via the activation of the Nrf2 pathway. PLoS One 2015; 10: e0126457.
11. Pinent M, Castell-Auví A, Genovese MI, Serrano J, Casanova A, Blay M, et al. Antioxidant effects of proanthocyanidin-rich natural extracts from grape seed and cupuassu on gastrointestinal mucosa. J Sci Food Agric 2016; 96: 178-82.
12. Bayatli F, Akkuş D, Kilic E, Saraymen R, Sönmez MF. The protective effects of grape seed extract on MDA, AOPP, apoptosis and eNOS expression in testicular torsion: an experimental study. World J Urol 2013; 31: 615-22.
13. Yogalakshmi B, Sreeja S, Geetha R, Radika MK, Anuradha CV. Grape seed proanthocyanidin rescues rats from steatosis: a comparative and combination study with metformin. J Lipids 2013; 2013: 153897.
14. Yıldırım Ş, Topaloğlu N, Tekin M, Küçük A, Erdem H, Erbaş M, et al. Protective role of Proanthocyanidin in experimental ovarian torsion. Med J Islam Repub Iran 2015; 29: 185.
15. Altintas R, Polat A, Parlakpinar H, Vardi N, Beytur A, Oguz F, et al. The effect of melatonin on acetylsalicylic acid-induced kidney and testis damage. Hum Exp Toxicol 2014; 33: 383-95.
16. Parlaktas BS, Atilgan D, Ozyurt H, Gencten Y, Akbas A, Erdemir F, et al. The biochemical effects of ischemia-reperfusion injury in the ipsilateral and contralateral testes of rats and the protective role of melatonin. Asian J Androl 2014; 16: 314-8.
17. Oufi HG, Al-Shawi NN. The effects of different doses of silibinin in combination with methotrexate on testicular tissue of mice. Eur J Pharmacol 2014; 730: 36-40.
18. Makary S, Abdo M, Fekry E. Oxidative stress burden inhibits spermatogenesis in adult male rats: testosterone protective effect. Can J Physiol Pharmacol 2017; 96: 372-81.
19. Yuluğ E, Türedi S, Alver A, Türedi S, Kahraman C. Effects of resveratrol on methotrexate-induced testicular damage in rats. Sci World J 2013; 2013: 489659.
20. Yucel Y, Oguz E, Kocarslan S, Tatli F, Gozeneli O, Seker, A, et al. The effects of lycopene on methotrexate-induced liver injury in rats. Bratisl Lek Listy 2017; 118: 212-6.
21. Yüncü M, Bükücü N, Bayat N, Sencar L, Tarakçıoğlu M. The effect of vitamin E and L-carnitine against methotrexate-induced injury in rat testis. Turk J Med Sci 2015; 45: 517-25.
22. Maremanda KP, Jena GB. Methotrexate-induced germ cell toxicity and the important role of zinc and SOD1: Investigation of molecular mechanisms. Biochem Biophys Res Commun 2017; 483: 596-601.
23. Vardi N, Parlakpinar H, Ates B. Beneficial effects of chlorogenic acid on methotrexate-induced cerebellar Purkinje cell damage in rats. J Chem Neuroanat 2012; 43: 43-7.
24. Güvenç M, Aksakal M. Ameliorating effect of kisspeptin-10 on methotrexate-induced sperm damages and testicular oxidative; stress in rats. Andrologia 2018; 50: e13057.
25. Sönmez MF, Tascioglu S. Protective effects of grape seed extract on cadmium-induced testicular damage, apoptosis, and endothelial nitric oxide synthases expression in rats. Toxicol Ind Health 2016; 32: 1486-94.
26. Dogan A, Celik I. Hepatoprotective and antioxidant activities of grapeseeds against ethanol-induced oxidative stress in rats. Br J Nutr 2012; 107: 45-51.

How to cite:

Yüncü M, Zengin G, Birinci H, Bostancıeri N, Polat S. Protective Effect of Proanthocyanidin against Methotrexate–Induced Testicle Damage in Rats. Eur J Ther 2019; 25(1): 51–7.