**Thymus vulgaris Attenuates Myleran-induced Reproductive Damage by Decreasing Oxidative Stress and Lipid Peroxidation in Male Rats**

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**INTRODUCTION**

One of the most common side effects of anticancer drugs is the interruption of spermatogenesis, which leads to sterility in numerous cases.[1] Myleran (MYL) is a DNA-destructive chemotherapy agent.[2] MYL stops cell division and has opposing effects on the cells with a high division rate, thus applying its highest influence on spermatogonial stem cells.[3] MYL also causes sperm chromosomal anomalies and lethal mutation mostly in sperms.[4] Boujrad et al. stated that the use of MYL induces incomplete performance of gonad in pregnant females and decreases somatic and germinal cells in the testis of newborns.[5] The disrupted spermatogenesis following treatment of MYL seems to be associated with the antioxidant properties of MYL and its major effect on spermatogonial stem cells.[6] MYL as an appropriate pharmaceutical method to evacuate seminiferous tubules has long been taken into consideration in order to study the performance of germinal stem cells.[7] MYL induces the cell death via free radical production[8] which impairs lipids, proteins, and nucleic acids.[9] The oxidative stress related to the free radicals decreases the antioxidant enzyme activity, increases reactive oxygen species (ROS) level, and induces lipid oxidation consequently.[10] This phenomenon, in turn, causes DNA breakdown and inactivation of specific proteins and consequently loss of biologic membranes.[11] Many plants with antioxidant properties exert protective effects against damage to the male rats’ reproductive system. However, MYL stops cell division and has opposing effects on the cells with a high division rate, thus applying its highest influence on spermatogonial stem cells. It also causes sperm chromosomal anomalies and lethal mutation mostly in sperms. Boujrad et al. stated that the use of MYL induces incomplete performance of gonad in pregnant females and decreases somatic and germinal cells in the testis of newborns. The disrupted spermatogenesis following treatment of MYL seems to be associated with the antioxidant properties of MYL and its major effect.

**Context:** *Thymus vulgaris* is an herbal with potent antioxidant and it has been shown to have beneficial effects during short-term administration. Myleran (MYL) is used for treatment of certain types of tumors. MYL produces free radicals and induces disturbance in sperm parameters. **Aims:** This study is designed to assess the effects of *T. vulgaris* against damage to the male rats’ reproductive features induced by MYL. **Subjects and Methods:** Sixty-four male Wistar rats were randomly assigned into eight groups: control group; MYL (10 mg/kg) group; *T. vulgaris* groups (4.5, 9, and 18 mg/kg); and MYL (10 mg/kg) + *T. vulgaris* groups (4.5, 9, and 18 mg/kg; separately). Treatments were administered daily intraperitoneal injection for 60 days. Total antioxidant capacity, sperm factors, malondialdehyde (MDA), testosterone, and germinal layer height were analyzed. **Results:** Whole variables of MYL group decreased significantly compared to the control group (*P < 0.05*) except MDA level (which increased). The *T. vulgaris* and *T. vulgaris* + MYL treatments in all doses increased all parameters significantly except MDA level (which decreased) compared to the MYL group (*P < 0.05*). No significant modifications were observed in all *T. vulgaris* groups compared to the control group (*P > 0.05*). **Conclusions:** *T. vulgaris* reduces the poisonous properties of MYL on male reproductive factors. **Keywords:** Lipid peroxidation, Myleran, oxidative stress, reproductive parameters, *Thymus vulgaris*
against chemoprotective agents. One of these plants is *Nigella sativa* L., which has a medical and religious history. This herbal has been stated to have frequent pharmacologic effects such as attenuation of glucose, lipid, and hypertension; protection of kidney; and antimicrobial and antifungal effects due to its antioxidant and anti-inflammatory properties. *Thymus vulgaris* by antioxidant properties can protect the male reproductive factors against MYL-induced oxidative injury. Considering the antioxidant properties of *T. vulgaris*, it seems that extract of *T. vulgaris* is able to protect the male reproductive parameters contrary to MYL-induced oxidative stress. Furthermore, an evaluation of the collected works designated that there is no learning about the effects of *T. vulgaris* against MYL-induced oxidative stress on male reproductive factors of rats.

**SUBJECTS AND METHODS**

**Animals**

This study was done on 64 male Wistar rats (9-week-old, 230–240 g) in the Kermanshah University of Medical Sciences. The study was lead conferring to the Committee for the Purpose of Control and Supervision of Experiments on Animals guidelines after due approval of the experimental protocol from the Institutional Animal Ethics Committee, Research Deputy of Kermanshah University of Medical Sciences. The rats were maintained on a regular diet and water *ad libitum* with a 12:12 h light/dark cycle at 23°C ± 2°C with a relative humidity of 50% ± 5%, in the animal home by considering 1-week adaptation before the experiments.

**Study groups and treatment of animals**

The animals were randomly separated into eight groups: first group, the control group, which received intraperitoneal (IP) injection of saline equivalent to the amount of experimental groups; second group, the MYL group, in this crowd, the animals were given MYL (10 mg/kg, a single dose by IP injection) for 60 days; third to fifth groups, the *T. vulgaris* administration groups, in these groups, each animal, respectively, received doses of 4.5, 9, and 18 mg/kg of *T. vulgaris* IP injection for 60 days at 9 am; and sixth to eighth groups, *T. vulgaris* + MYL groups, in this group, every rats first received MYL (10 mg/kg) in order to make reproductive parameters destruction and then they respectively received diverse dose (4.5, 9, and 18 mg/kg, IP injection) of *T. vulgaris* for 60 days at 9 am.

**Animals’ dissection and sampling**

At the finale of the treatment dated, wholly rats were deeply anesthetized by IP injection of ketamine HCl (70 mg/kg) and xylazine (30 mg/kg). Blood sample was taken from the heart without cutting the chest. The samples were centrifuged at 255 g for 15 min. The blood serum was isolated and part of this was kept at −70°C. Then, the abdomen of the rats was cut. The epididymis tail was remote from the testes and placed in DMEMF12 medium. The left testis was fixed in a 10% formalin for morphometrical checks and the right testis for the malondialdehyde (MDA) level valuations.

**Sperm collection**

Both cauda epididymides from each animal were crushed and conserved in a warmed Petri dish containing 10 ml Hank’s Balanced Salt Solution at 37°C. The spermatozoa were allowed to disperse into the buffer. Fifteen minutes later, the cauda was removed and the suspension was slightly shaken to be normalized. It was then observed by a light microscope at a magnification of ×400.

**Motility**

In this method, four degrees of sperm motility were studied based on the WHO methods, Class A: Progressive motility. Progressive motility of the sperm of each sample was examined by an optical microscope with a magnification of ×40 in ten fields of view. For this purpose, at first, about 50 μl of semen liquid culture medium was taken and placed on a slide culture that was previously cleaned and dried with alcohol. Then, the slide culture was placed on it and examined by a microscope. Sperm counting was performed through a cell count device, and about 100 sperms were counted in each sample. In all the experimental and control groups, sperm parameter assessment was done by two qualified expert persons to minimize subjectivity.

**Viability**

In this method, eosin staining was used to recognize living sperms from dead sperms. The basis of this staining is the absorption of stain by the membrane of dead cells and its disposal by the membrane of living cells. At the end of the given time, about 10 μl of the medium containing semen fluid was collected from each dish and then mixed with an equal volume of eosin stain solution (about 10 μl). After 5 min, part of the mixture was poured on a Neubauer’s slide culture. Then, living sperms lack stain and dead sperms become pink. The prepared slide culture was examined with the magnification of ×40. At least 100 sperms were calculated from each random sample from the 10 fields of imaging, and the percentage of live sperm cell was documented.

**Morphology**

The normal sperm cell morphology was evaluated through the investigation of smears from the right
epididymis. An aliquot of the sample was used to make the smears to appraise the deformities in the spermatozoa. Eosin/nigrosin staining was used to estimate the normal morphology. One drop of eosin was added to the suspension. The slides were then observed by a light microscope at × 400 magnification. A total of 500 spermatozoa were studied on the respective slide (5000 cells in each group) for irregularities of the head and tail.[17]

Sperm calculation
To investigate the number of sperms, 500 µL of the sperm suspension was diluted through the formaldehyde fixative (Sigma, USA). Approximately 20 µL was removed from the diluted solution into a hemocytometer by a Pasteur pipette. The hemocytometer was located into a Petri dish with dampened filter paper and allowed to stand for 20 min. The stable sperms were counted and assessed per 250 small squares of the hemocytometer using a × 40 objective lens. The number of sperm per mm² equated the number of sperm counted × the dilution/number counted in mm² × the depth of the chamber.[10]

Morphometric examination
The nonparenchymal tissues (fat, fascia, and vessels) of the removed left testis were dissected, and paraffin-entrenched blocks were prepared by an automatic tissue processor. The steps of this process consequently included fixation with 10% formal saline (48 h), washing under running water, dehydrating by raised doses of ethanol, clearing by xylene (10 min for each one), and embedding in soft paraffin (three times). At this stage, 5-µm histological thin sections were cut from the blocks, undertaken by a microtome instrument (Leica RM 4327, Leica Microsystems Nussloch GmbH, Germany), and five sections. For the confederation of the section choice, the first slice was the 4th and the last was the 24th (5 sections interval), and finally, the routine protocol for H and E staining was implemented. At the end of tissue processing, the stained sections were mounted by Entellus glue and assessed by an Olympus BX-42D-76P80 research microscope connected to a DP14 Camera with a 5.45-million pixel resolution and Olysia Bio-software (Olympus Optical Co., Ltd., Tokyo, Japan).[11]

Testosterone
The collected blood sample was centrifuged (5000 g) at 23°C for 15 min to get the serum. The serum samples were then kept in a deep freezer (−180°C). The serum testosterone level was examined through ELISA (Abcam 108666, USA) technique.[11]

Testis malondialdehyde
MDA levels were assessed as a marker of lipid peroxidation. In this regard, standardizing of the samples were carried out by homogenization buffer containing 5.55% KCl solution and the specimens centrifuged at 1500 g for 10 min, respectively. Then, the homogenated subjects were added to a reaction mixture containing sodium dodecyl sulfate, acetic acid (pH 4.5), and thiobarbituric acid. Following boiling the mixture for 30 min at 85°C, the absorbency of the supernatant was measured by spectrophotometry at 450 nm.[18]

Total antioxidant capacity
To measure the total antioxidant capacity (TAC), an acquisition kit (Cat No: TAC-96A, ZellBio GmbH, Germany) was purchased. The kit contains one reagent ready to use, buffer (200×), dye powder, reaction suspension solution, and standard and a microplate of 96 wells. In this assay, the TAC was equivalent to some antioxidant in the sample that was compared with ascorbic acid as standard. The kit’s sensitivity was equal to 0.2 mM and final absorbance was read at 540 nm, and unit conversion was performed.[18]

Statistical analysis
The data were analyzed by SPSS software (SPSS, New York: IBM, SPSS version 16.0) using one-way ANOVA postulation followed by Tukey’s post hoc test, and P < 0.05 was considered statistically significant. The variables were represented as the mean ± standard error of mean.

RESULTS
Motility and viability
MYL caused a significant decline in viability and progressive motility compared to the control group (P < 0.05). No significant variations were detected in the *T. vulgaris* groups in comparison with the control group (P > 0.05). Furthermore, viability and progressive motility in completely treated *T. vulgaris* and MYL + *T. vulgaris* groups improved significantly in comparison with the MYL group [P < 0.05, Table 1].

Count and morphology
The sperm count and morphological normality reduced significantly in the MYL group equated to the control group (P < 0.05). No significant deviances were realized in the *T. vulgaris* groups in comparison with the control group (P > 0.05). However, the sperm count and normal morphology were improved significantly in all treated *T. vulgaris* and MYL + *T. vulgaris* groups compared with the MYL group (P < 0.05) [Figure 1 and Table 1].
Seminiferous tubules
MYL caused a significant decline in the germinal layer of seminiferous tubule height in comparison with the control group \( (P < 0.05) \). No significant changes were observed in comparison with the control group \( (P > 0.05) \). Germinal layer of seminiferous tubule height in completely treated \( T. vulgaris \) and MYL + \( T. vulgaris \) groups improved significantly compared to the MYL group \( [P < 0.05, \text{Figures 2 and 3}] \).

Testosterone level
MYL affected a significant reduction in the testosterone hormone level compared to the control group \( (P < 0.05) \). No significant alterations were detected in the \( T. vulgaris \) groups in comparison with the control group \( (P > 0.05) \). In addition, the level of testosterone hormone in all treated \( T. vulgaris \) and MYL + \( T. vulgaris \) groups improved significantly compared to the MYL group \( [P < 0.01, \text{Figure 4}] \).

Malondialdehyde
Levels of MDA revealed a significant growth in the MYL group compared to the control group \( (P < 0.05) \). Correspondingly, a significant reduction in MDA levels was shown in all THYM and \( T. vulgaris + MYL \) groups compared to the MYL group \( (P < 0.05) \), although had no significant effect on the levels of MDA in all \( T. vulgaris \) groups compared to the control group \( [P > 0.05, \text{Figure 5}] \).

Total antioxidant capacity
The consequences of measured TAC levels in the study groups displayed a significant reduction in the MYL group compared to the control group \( (P < 0.05) \). Furthermore, a significant rise in TAC levels was displayed in the completely treated \( T. vulgaris \) and \( T. vulgaris + MYL \) groups equated to the MYL group \( (P < 0.05) \), although had no significant effect on the levels of TAC in all \( T. vulgaris \) groups compared to the control group \( [P > 0.05, \text{Figure 6}] \).

Discussion
Chemotherapy as an agent of oxidative stress production in the body is able to disrupt the spermatogenesis

![Figure 1: Comparison of normal sperm cell morphology in the treatment groups. *Significant different compared to the normal control group \( (P < 0.05) \). †Significant different compared to the MYL control group \( (P < 0.05) \). ‡Significant different compared to the MYL control group \( (P < 0.01) \). MYL = Myleran](image1)

![Figure 2: Comparison of germinal layer seminiferous tubule height in the treatment groups. *Significant different compared to the control group \( (P < 0.05) \). †Significant different compared to the MYL group \( (P < 0.05) \). ‡Significant different compared to the MYL group \( (P < 0.01) \). MYL = Myleran](image2)

Table 1: Effect of MYL and \( T. vulgaris \) on sperm parameters in male rats \( (n=8 \text{ for each group}) \)

| Groups                | Mean of sperm count \( (106) \) | Sperm progressive motility (%) | Sperm viability (%) |
|-----------------------|----------------------------------|--------------------------------|---------------------|
| Control               | 83.17±2.16                       | 16.9±1.02                      | 73.35±1.24          |
| MYL                   | 32.11±1.05*                      | 2.23±0.41*                     | 37.03±1.55*         |
| THYM 4.5 mg/kg        | 82.75±2.63†                      | 20.12±1.04†                    | 77.61±4.09†         |
| THYM 9 mg/kg          | 85.12±4.17†                      | 22.07±1.54†                    | 75.05±3.74†         |
| THYM 18 mg/kg         | 86.25±4.09†                      | 23.11±0.61†                    | 78.22±1.47†         |
| THYM+MYL 4.5mg/kg     | 48.50±2.51†                      | 8.14±1.63†                     | 52.05±4.18†         |
| THYM+MYL 9 mg/kg      | 52.36±3.17†                      | 7.27±0.91†                     | 53.32±2.13†         |
| THYM+MYL 18mg/kg      | 57.25±4.23†                      | 9.15±1.10†                     | 58.51±3.34†         |

Data are presented as mean±SEM. * \( P<0.05 \) compared to the control group. † \( P<0.05 \) compared to MYL group. ‡ \( P<0.05 \) compared to the MYL group. THYM: Thymus vulgaris, MYL: Myleran
Salahshoor, et al.: Thymus vulgaris and Myleran effect on reproductive process, reduce the production of spermatozoids, increase the number of abnormal spermatozoids, and decrease fertility rate.\cite{19} Thus, the simultaneous use of potential antioxidant compounds and chemotherapy drugs has dramatically increase the protective effects for cells against the destructive side effects of free radicals.\cite{20} The findings of this study proposed that the MYL had destructive testicular effects and sperm parameters, oxidant–antioxidant disorganization, and enhancement of testosterone hormone level. On the other hand, *T. vulgaris* as a phytoestrogen relief destructive effects of MYL administration. It also restores the cell damage caused by decreased level of MDA. The results of the current study also showed that *T. vulgaris* is able to reduce the lipid peroxidation (decreased MDA) and increase antioxidant capacity (increased TAC) of testis tissue; thus, it is reducing oxidative stress. Consistent with these findings, a large body of studies has shown antioxidant properties of *T. vulgaris*.\cite{12-14} Seemingly, *T. vulgaris* inhibits tert-butyl-hydroperoxide-induced lipid peroxidation in sperms. *T. vulgaris* is also a lipophilic molecule that is able to prevent lipid peroxidation through Fenton reaction.\cite{13} Thus, it appears that *T. vulgaris* with its antioxidant properties could reduce MDA and increase TAC in the treatment groups by inhibiting the production of ROS. The present study also indicated the recovery effects of *T. vulgaris* on some male reproductive parameters as well as decreasing the oxidative stress by showing declining of MDA level. Because sperms lose a large amount of their cytoplasm during spermatogenesis (lack of antioxidant systems), they apparently have a higher sensitivity to increased ROS than somatic cells.\cite{11} The first outcome of the ROS attack to the membrane structures can be cellular peroxidation within the membrane of cells and organelles.\cite{10} Antioxidants such as *T. vulgaris* eliminate toxins and free radicals from the cell surroundings, and inhibit lipid peroxidation, which results in the maintenance of the cellular biochemical structure.\cite{11} The results of the present study showed that all sperm parameters in the MYL control group reduced significantly compared to the control group. In the *T. vulgaris* and MYL + *T. vulgaris* groups, a significant increase was
This study concluded that the decrease of sperm parameters in the MYL group compared to the control group is due to oxidative stress induced by MYL administration. This oxidative stress induces mitochondrial damage and causes release of pro-apoptotic factors within intermembrane space. The antioxidant actions of *T. vulgaris* seem to play a role in elevating sperm motility and viability in diabetic rats, which is in line with the results of the present study.

In fact, *T. vulgaris* prevents the formation of free radicals and lipid peroxidation. *T. vulgaris* can inhibit the expression of MMP and proinflammatory factors such as tumor necrosis factor (TNF)-α, NF-κB, interleukin-1 β, and interleukin-6. It may also exert its antiapoptotic and cytoprotective effects by suppressing the expression of caspase-3 and TNF-α. *T. vulgaris* seems to play a role in elevating sperm motility and viability by promoting the sperm antioxidant defense system, including superoxide dismutase, glutathione peroxidase, and catalase.

This study concluded that serum testosterone level and germinal layer thickness of seminiferous tubules was significantly attenuated in the MYL group than the normal control group. There was also a significant increase in the level of testosterone hormone and germinal layer height of seminiferous tubules in all *T. vulgaris* and *T. vulgaris + MYL* groups than MYL. Further, histological assessments showed loss of natural form, order, and consistency of cells of seminiferous tubule walls, creating vacuoles in them after all. Development of vacuole in the testis can be indicative of the effect of the oxidative stress.
mechanism. It seems that MYL as an alkylating factor causes cell and DNA destruction, thus decreasing the thickness of the germinal layer in the testis.[29] The results of Vahdati et al. were in agreement with the findings of this research, indicating that MYL reduced the diameter of seminiferous tubules and sperm count, motility, and viability, increased the abnormal sperms, and decreased the epithelial thickness of seminiferous tubules.[30] As an antioxidant, T. vulgaris not only inhibits peroxidation of lipid and testicular oxidative stress but also plays a key role in the production of steroids in testis.[31] In addition, T. vulgaris reduces the effects of the oxidative stress induced under various conditions and empowers the cells to cope with these conditions by preventing the reduction of glutathione and increasing antioxidant capacity.[31] The findings of Walf et al. were in line with our study, indicating that the phytoestrogens, including T. vulgaris attaches to the estrogen receptors in testis and stimulates spermatogenesis.[32] The present study showed that MYL-induced male reproductice damage in rats could be reduced by plant antioxidants such as T. vulgaris. Therefore, according to the previous data, THYM can improve some male reproductive dysfunctions, which has been caused by MYL-induced toxicity considering its antioxidant properties.

**CONCLUSION**

The results showed that MYL can produce damage to various reproduction values of male. Moreover, it was shown that a T. vulgaris scavenges the oxidation agents. It was concluded that T. vulgaris increases spermatozoa quality, normal morphology, viability, height of germinal layer seminiferous tubules, TAC, motility, and count. This also reduces MDA level in the testes. T. vulgaris can be used for subfertile men. Antioxidant feature of T. vulgaris is known as the crucial cause of reproductive parameter enhancement. More investigations are needed to explore the exact mechanism of molecular action.

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**Conflicts of interest**

There are no conflicts of interest.

**REFERENCES**

1. Solomon R, Shvartsur R, Azab AN. The association between psychotropic drug use and fertility problems among male subjects. J Psychiatr Pract 2019;25:22-33.
2. Liu FJ, Dong WY, Zhao H, Shi XH, Zhang YL. Effect of molybdenum on reproductive function of male mice treated with busulfan. Theriogenology 2019;126:49-54.
3. Ganjali&khan Hakemi S, Sharififar F, Haghpanah T, Babaea E, Eftekhar-Vaghefi SH. The effects of olive leaf extract on the tests, sperm quality and testicular germ cell apoptosis in male rats exposed to busulfan. Int J Fertil Steril 2019;13:57-65.
4. Abofoul-Azab M, Lunenfeld E, Levitas E, Zeadna A, Younis JS, Bar-Ami S, et al. Identification of premeiotic, meiotic, and postmeiotic cells in testicular biopsies without sperm from sertoli cell-only syndrome patients. Int J Mol Sci 2019;20: pii: E470.
5. Boujrad N, Hochereau-de Reviers MT, Kamitchouing P, Perreau C, Carreau S. Evolution of somatic and germ cell populations after Busulfan treatment in uto or neonatal cryptorchidism in the rat. Andrologia 1995;27:223-8.
6. Jalilvand N, Hosseini M, Beheshti F, Ebrahimzadeh-Bideskan A. Protective effect of PPAR? Agonist pioglitazone, on testicular tissue and sperm parameters in hypothyroid rats. Toxin Rev 2019;24:1-10.
7. Honaramooz A, Behbodui E, Hauser Cl, Blash S, Ayres S, Azuma C, et al. Depletion of endogenous germ cells in male pigs and goats in preparation for germ cell transplantation. J Androl 2005;26:698-705.
8. Li B, He X, Zhuang M, Niu B, Wu C, Mu H, et al. Melatonin ameliorates Busulfan-induced spermatogonial stem cell oxidative apoptosis in mouse testes. Antioxid Redox Signal 2018;28:385-400.
9. Vafaeri A, Mohammadi S, Fazel A, Soukhtanloo M, Pour AM, Beheshti F. Effects of carob (Ceratonia siliqua) on sperm quality, testicular structure, testosterone level and oxidative stress in Busulfan-induced infertile mice. Pharm Sci 2018;24:104-11.
10. Jalili C, Kamani M, Roshankhah S, Sadeghi H, Salahshoor MR. Effect of Faucaria vulgaris extracts on sperm parameters in diabetic rats. Andrologia 2018;50:e13130.
11. Salahshoor MR, Roshankhah S, Jalili C. Antioxidative properties of Thymus vulgaris on liver rats induced by palitaxel. Phcog Res 2019;11:315-20.
12. Mazaheri Y, Torbati M, Azadmand-Damirchi S, Savage GP. Effect of roasting and microwave pre-treatments of Nigella sativa L. seeds on lipase activity and the quality of the oil. Food Chem 2019;274:480-6.
13. Maia JG, La Corte R, Martinez J, Ubbink J, Prata AS. Improved activity of thyme essential oil (Thymus vulgaris) against Aedes aegypti larvae using a biodegradable controlled release system. Ind Crop Prod 2019;136:110-20.
14. Gedikoglu A, Sökmen M, Civiit A. Evaluation of Thymus vulgaris and Thymbra spicata essential oils and plant extracts for chemical composition, antioxidant, and antimicrobial properties. Food Sci Nutr 2019;7:1704-14.
15. Bistgani ZE, Hashemi M, DaCosta M, Craker L, Maggi F, Morshedloo MR. Effect of salinity stress on the physiological characteristics, phenolic compounds and antioxidant activity of Thymus vulgaris L. and thymus daenensis Celak. Ind Crop Prod 2019;135:311-20.
16. Banerjee P, Mukherjee S, Bera K, Ghosh K, Ali I, Khawas S, et al. Polysaccharides from Thymus vulgaris leaf: Structural features, antioxidant activity and interaction with bovine serum albumin. Int J Biol Macromol 2019;125:580-7.
17. Roshankhah S, Jalili C, Salahshoor MR. Effects of crocin on sperm parameters and seminiferous tubules in diabetic rats. Adv Biomed Res 2019;8:4.
18. Jalili C, Roshankhah S, Moradi Y, Salahshoor MR. Resveratrol
Salahshoor, et al.: *Thymus vulgaris* and Myleran effect on reproductive attenuates malathion-induced renal damage by declining oxidative stress in rats. Int J Pharma Investig 2018;8:192-9.

19. Howell S, Shalet S. Gonadal damage from chemotherapy and radiotherapy. Endocrinol Metab Clin North Am 1998;27:927-43.

20. Selvakumar E, Prahalathan C, Mythili Y, Varalakshmi P. Protective effect of DL-alpha-lipoic acid in cyclophosphamide induced oxidative injury in rat testis. Reprod Toxicol 2004;19:163-7.

21. Olejnik J, Suchowerska N, Herrid M, Jackson M, Hinch G, Hill J. Spermatogenesis survival in young ram lambs following irradiation, Busulfan or thermal treatment. Small Ruminant Res 2018;166:22-7.

22. Aitken RJ, Baker MA. Oxidative stress, sperm survival and fertility control. Mol Cell Endocrinol 2006;250:66-9.

23. Molooody M, Shariooz R, Razi M, Zarei L, Mohammadi V. The effect of CoQ10 on testicular tissue in rats treating with Busulfan: Sperm quality and histological changes. Iran J Vet Med 2018;13:29-38.

24. Bahmanpour S, Jahromi BN, Kooheymya F, Keshavarz M, Bakhtari A. Effects of different doses and time-dependency of Busulfan on testis parameters and spermatogenesis in a rat model: A quantitative stereological study. J Adv Med Sci Appl Technol 2017;3:155-62.

25. Bar-Shira Maymon B, Yogeve L, Marks A, Hauser R, Botchan A, Yavetz H. Sertoli cell inactivation by cytotoxic damage to the testis after cancer chemotherapy. Fertil Steril 2004;81:1391-4.

26. Dera A, Rajagopalan P. Thymoquinone attenuates phosphorylation of AKT to inhibit kidney cancer cell proliferation. J Food Biochem 2019;43:e12793.

27. Nagi MN, Almakki HA, Sayed-Ahmed MM, Al-Bekairi AM. Thymoquinone supplementation reverses acetaminophen-induced oxidative stress, nitric oxide production and energy decline in mice liver. Food Chem Toxicol 2010;48:2361-5.

28. Hassan E, El-Neweshy M, Hassan M, Noreldin A. Thymoquinone attenuates testicular and spermotoxicity following subchronic lead exposure in male rats: Possible mechanisms are involved. Life Sci 2019;230:132-40.

29. Bucci LR, Meistrich ML. Effects of busulfan on murine spermatogenesis: Cytotoxicity, sterility, sperm abnormalities, and dominant lethal mutations. Mutat Res 1987;176:259-68.

30. Vahdati A, Fathi AR, Nasimi P, Saki G. Busulfan induces apoptotic and cytotoxic effects on testis and epididymal sperm of adult male mouse following low dose treatment. Int J Bio 2015;5:70-8.

31. Meydan S, Esrefoglu M, Selek S, Akbas Tosunoglu E, Ozturk O, Kurbetli N, et al. Protective effects of caffeic acid phenethyl ester and thymoquinone on toluene induced liver toxicity. Biotech Histochem 2019;94:277-82.

32. Waly H, Ragab SM, Hassanein KM, Abou Khalil NS, Ahmed EA. Uranium exposure increases spermatocytes metaphase apoptosis in rats: Inhibitory effect of thymoquinone and N-acetylcysteine. Gen Physiol Biophys 2019;38:145-55.