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# IMPACTS OF QUERCETIN AND MERCAPTOTHION ON MALE REPRODUCTIVE PARAMETERS OF RATS

## Impactos de la Quercetin y Mercaptothion en los parámetros reproductivos de rata macho

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**ABSTRACT**

Quercetin is an herbal polyphenol with valuable antioxidant properties. Mercaptothion is categorized as organophosphates which can generate free radicals and induce male fertility disorders. This study was aimed to assess the impacts of Quercetin against destruction of male fertility parameters induced by Mercaptothion. 64 male rats were randomly assigned into 8 groups; control, and Mercaptothion (250 mg/kg) groups; Quercetin groups (7.5, 15, and 30 mg/kg) and Mercaptothion + Quercetin (7.5, 15, and 30 mg/kg). Treatments were administered intraperitoneally (Mercaptothion) and orally (Quercetin) daily for 65 days. The sperm parameters, testis malondialdehyde (MDA), total antioxidant capacity (TAC), testosterone level and height of germinal layer were evaluated. Expressions of p53, caspase-3, Bax, and Bcl-2 were measured through real-time quantitative PCR. Values of all factors were reduced significantly except the MDA level (which increased) in Mercaptothion group compared to the control group ($p<0.001$). Studied criteria in groups of Quercetin and Quercetin + Mercaptothion in whole doses increased significantly except MDA level (which reduced) compared to the Mercaptothion group ($p<0.001$). Also, downregulated levels of p53, caspase-3, and Bax genes and unregulated levels of Bcl-2 gene expression were detected in control and the sixth treatment groups significantly in Quercetin group compared to the Mercaptothion group ($p<0.001$). No significant alterations were detected in Quercetin groups compared to the control group ($p>0.05$). Quercetin reduced toxic effects of Mercaptothion on male fertility parameters.

**Keywords:** Quercetin, Fertility, Mercaptothion, Rat.

**RESUMEN**

La quercetina es un herbal polifenol con valiosas propiedades antioxidantes. El mercaptothion se clasifica como organofosforados que pueden generar radicales libres e inducir trastornos de la fertilidad masculina. Este estudio tiene como objetivo evaluar el impacto de la quercetina frente a la destrucción de los parámetros de fertilidad masculina inducidos por Mercaptothion. 64 ratas macho se asignaron aleatoriamente en 8 grupos; control y grupos de Mercaptotión (250 mg / kg); Grupos de quercetina (7,5, 15 y 30 mg / kg) y mercaptotión + quercetina (7,5, 15 y 30 mg / kg). Los tratamientos se administraron por vía intraperitoneal (Mercaptotión) y por vía oral (Quercetina) diariamente durante 65 días. Se evaluaron los parámetros espermáticos, malondialdehído (MDA), capacidad antioxidante total (TAC), nivel de testosterona y altura de la capa germinal. Las expresiones de p53, caspasa-3, Bax y Bcl-2 se midieron mediante PCR cuantitativa en tiempo real. Los valores de todos los factores se redujeron significativamente, excepto el nivel de MDA (que aumentó) en el grupo de mercaptothion en comparación con el grupo de control ($p <0,001$). Los criterios estudiados en los grupos de quercetina y quercetina + mercaptothion en dosis completas aumentaron significativamente, excepto el nivel de MDA (que se redujo) en comparación con el grupo de mercaptothion ($p <0,001$). Además, los niveles regulados a la baja de los genes p53, caspasa-3 y Bax y los niveles no regulados de expresión del gen Bcl-2 se detectaron en los grupos de control y sexto de tratamiento de manera significativa en el grupo de quercetina en comparación con el grupo de Mercaptothion ($p <0,001$). No se detectaron alteraciones significativas en los grupos de quercetina en comparación con el grupo de control ($p> 0,05$). La quercetina redujo los efectos tóxicos del mercaptothion sobre los parámetros de fertilidad masculina.

**Palabras clave:** Quercetin, Fertilidad, Mercaptothion, Rata.

## INTRODUCCION

Human infertility is a highly critical process which is influenced by various factors such as parents' age, maternal related conditions, smoking, alcohol and coffee consumption, socioeconomic status, genetics, hormonal imbalance, and pesticides (Shukala et al., 2010). Swan et al. (2003) showed that pesticides might reduce the potential of male fertility. Occupational exposure to pesticides can induce detrimental effects on male infertility (Guimarães et al., 2019).

Mercaptothion is an unsystematic organic phosphorous compound that exists in a yellow to dark brown oil belonging to the family of organophosphates (Madzorera et al., 2019). This toxin is extensively used in agricultural fields and gardens to kill pests (Choudhary., 2008). Reduced weight of reproductive organs, reduced motility, increased abnormality, and sperms deaths have been reported due to the administration of organophosphates (Selmi et al., 2018; Pathak et al., 2011; Sellandi et al., 2012). Uzun et al. (2009) showed that the Mercaptothion could induce the production of free radicals and oxidative stress and increase the activity of antioxidant enzymes in testis.

In non-physiological conditions, there is imbalance between elimination and generation of free radicals in body of living organisms, which can lead to oxidative stress, and severe cell damages (Kuchewar et al., 2014). Antioxidant enzymes are responsible for detoxification of free radicals. Catalase and superoxide dismutase are key enzymes available in this system. Further, glutathione and thiol are the most frequent non-enzymatic intracellular antioxidants (Hariri et al., 2011). Organophosphates are able to change the antioxidant system of cells, causing membrane lipid peroxidation and induce cell membrane damage via production of free radicals (Valdiglesias et al., 2010). Increased lipid peroxidation and production of free radicals from metabolism of organophosphates have been proposed as the main mechanisms involved in damage to cells and body tissues (Altuntas et al., 2004). Some studied organophosphates can affect the sperm chromatin structure by changing the phosphorylation of protamines of nucleus, especially in the final stages of maturation. Finally, in these situations, adverse side-effects could be revealed on sperm viability, motility, and morphology (Sánchez-Peña et al., 2004). Organophosphates are alkylating agents that can influence spermatogenesis, sperm chromatin structure (by protamine binding), and sperm degeneration (Evenson et al., 2000).

Quercetin ($C_{15}H_{10}O_7$, yellow crystalline powder, Density: 1.799 g/cm3, practically insoluble in water, IUPAC name: 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one, Molar mass: 302.236 g/mol) is a plant flavanol from the flavonoid group of polyphenols that are produced by stilbene synthase enzyme in response to environmental stresses (Khaki et al., 2010; Chen et al., 2010; Tabrizi et al., 2020). Quercetin exists in at least 72 plant species and fruits, especially grape skin (50-100 µg/1 g of wet weight) (Jalili et al., 2017). Quercetin inhibits inflammation through direct inhibition of COX-1 and COX-2 activity (Subbaramaiah et al., 1998). Quercetin can reduce the production of prostaglandin E2 and ROS from lipopolysaccharides of activated microglial cells via suppressing NF-Kappa β and I-Kβ kinase (Udenigwe et al., 2008). This agent with antioxidant properties has inhibitory effects on free radicals and also can increase the number of cellular anti-oxidative enzymes. Antioxidant ability of this polyphenol is dependent on the properties of its polyphenolic hydroxyl groups (Cucciolla et al., 2007).

Based on the antioxidant effects of Quercetin, and according to the previous evidences in literatures, in the present study the Mercaptothion was used. Thus, this study was aimed to determine the effects of Quercetin against Mercaptothion-induced oxidative stress in reproductive parameters of male rats.

## MATERIAL AND METHODS

### *Chemicals and Drug*

In this experimental study, the powder of Quercetin (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one; $C_{15}H_{10}O_7$) and Mercaptothion solution ([(dimethoxyphosphorothioyl)sulfanyl]butanedioate, Diethyl]; $C_{10}H_{19}O_6PS_2$) were purchased (Merk-Germany). They were diluted in normal saline (0.9%) for various doses preparation. Also, formalin, sodium acetate, ferric chloride, Iron sulphate, hematoxylin-eosin, and zinc sulphate powder were purchased from Sigma Co. (USA). For biochemical analysis, the Commercial Pars Azmun colorimetric kits (Pars Azmun, Tehran, Iran) was also prepared. Other buffer additives and solvents were obtained from Merck Co. (Germany).

### *Animals*

This study was applied to 64 male Wistar rats (weighing 220-250 g). All animals were treated in accordance with guidelines of National Institute of Health for the Care and Use of Laboratory Animals approved by Research Deputy at Kermanshah University of Medical Sciences based on WMA Declaration Ethic of Helsinki (Ethic number; IR.KUMS.REC.1397.306). The rats were kept in 12:12 h light/dark cycle at 23°C ± 2°C room temperature in an animal house of medical school of Kermanshah University of Medical Sciences by considering one-week adaptation prior to the experiment (Jalili et al., 2018).

### *Study groups and treatment of animals*

Rats were randomly divided into 8 groups (8 animals in each). Control group received normal saline (orally or injection); Mercaptothion group received a single dose of Mercaptothion (250 mg/kg) orally (Choudhary et al., 2008); Quercetin groups received 7.5, 15, and 30 mg/kg of Quercetin intraperitoneally for 65 consecutive days (Khaki et al., 2010); Mercaptothion + Quercetin groups received a single dose of 250 mg/kg Mercaptothion orally in order to induce reproductive parameters damage followed by 7.5, 15 and 30 mg/kg of Quercetin intraperitoneally for 65 days (Sánchez-Peña et al., 2004; Swan, et al., 2003).

### *Animals' dissection and sampling*

At the end of the treatment period, all rats were deeply anesthetized by intraperitoneal injection of ketamine HCl (100 mg/kg) and Xylazine (10 mg/kg). Blood sample was taken from lateral vein of rat`s tail. The samples were kept in a 37°C incubator for 20 minutes and then centrifuged at 255 g for 15 minutes. Blood serum was isolated and kept at -70 °C for TAC, nitrite oxide and testosterone evaluations. Chest and abdomen of animals were then cut, and epididymis tail was isolated from

the testes and placed in DMEMF12 / FBS5% culture medium. The left testis was removed from the abdominal cavity and fixed in 10% formalin solution for histological and morphometric examinations. Also, the right organ was dissected for other analyses (Salahshoor et al., 2016).

***Sperm cells collection***

Both cauda epididymis 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. After 15 min, the cauda was removed, and suspension was slightly shaken to be normalized. Then it was observed under a light microscope (400x) (Jalili et al., 2018).

***Progressive motility***

Four degrees of sperm motility was studied based on WHO methods. Progressive motility of sperm cells for each sample was examined by an optical microscope with magnification of 40x in 10 fields of view. For this purpose, 50 µl of Semen liquid culture medium was taken and placed on a sterile slide culture (cleaned by 70% alcohol). The slide culture was placed on it and examined under a microscope. Sperm cell counting was performed through a cell count device, and about 100 sperm cells were counted in each sample. In all experimental and control groups, the cell count was repeated (Khaki et al., 2010).

***Survival rate***

In this method, eosin staining was used to distinguish among the living and dead sperms. The basis of this protocol is on stain absorption by cell membrane of dead cells and its disposal by membrane of living cells. At the end of the given time, 20 µl of the medium containing semen fluid was collected from each dish; then it was mixed with equal volume of eosin staining solution (20 µl). 2 to 5 minutes later, part of the mixture was poured onto a neobar slide culture. The prepared slide culture was examined with magnification of 40×. At least 100 sperm cells were calculated from each random sample from 10 fields of imagining, and percentage of live sperm cell was documented (Salahshoor et al., 2016).

***Sperm cells morphology***

Morphology of normal sperm cells was assessed through examination of sperm smears collected from the right cauda epididymis. An aliquot of the sample was used to make the smears to appraise the spermatozoa malformations. Eosin/nigrosine stain was used to assess the normal spermatozoa morphology. One drop of eosin stain was added to the suspension and mixed slightly. The slides were then observed underneath a light microscope (400×). 400 spermatozoa were studied based on respective slides (4000 cells in each group) for irregularities of head and tail (Khaki et al., 2010).

***Sperm calculation***

To analyze the quantity of sperm cells, 400 µL of the sperm suspension was diluted through formaldehyde fixative (Sigma; USA). 15 µL was removed from the diluted solution into a haemocytometer by a Pasteur pipette. The haemocytometer was located into a Petri dish with dampened filter paper and allowed to stand for 10 min. Stable sperms were counted and assessed per 250 small squares of the haemocytometer (using a ×40 objective). Amount of sperm per $mm^3$ equated the number of sperm counted x dilution/number counted in $mm^2$ x depth of chamber (Salahshoor et al., 2016).

***Tissue preparation and staining for evaluation of germinal layer in seminiferous tubules***

Non-parenchymal tissues (fat, fascia and vessels) of removed left testis were dissected. Paraffin-embedded blocks were gotten using Automatic Tissue Processor. The steps of this process were consequently included fixation with 10% formal saline (for 72 hours), washing thoroughly under running water, dehydrating by raised doses of ethanol (50, 60, 70, 80, 90 and 100%, which included 3 min for each step and 100% ethanol step was repeated for three times), clearing by xylene (three times and 10 min in each), and embedding in soft paraffin. At this stage, 5 µm coronal histological thin sections were cut from paraffin-embedded blocks, undertaken by a microtome instrument (Leica RM 2125, Leica Microsystems Nussloch GmbH; Germany), and 5 sections per animal were chosen. For the unification of the selected selection, the first section was considered as 4th, and the last was the 24th (5 sections interval), and finally, the routine protocol for Hematoxylin and Eosin staining was implemented. At the end of tissue processing, the stained sections were mounted by entalan glue and assessed under a microscope Olympus BX-51T-32E01 research microscope connected to a DP12 Camera with 3.34 million-pixel resolution and Olysia Bio software (Olympus Optical Co. LTD, Tokyo, Japan) (Jalili et al., 2018).

***Testosterone measurement***

Collected blood was centrifuged at 23°C for 15 minutes with 5000 g to get the blood serum. The serum samples were kept in a freezer (-18°C). The serum testosterone level was examined through ELISA (Abcam 108666, USA) technique (Khaki et al., 2010).

***Measurement of testis malondialdehyde***

MDA levels in right testis tissues were evaluated as an index of lipid peroxidation. In this regard, homogenizing of the samples were carried out by homogenization buffer containing 1.15% KCl solution. The specimens were centrifuged at 1,500 g for 10 min. Then, the homogenated subjects were added to a reaction mixture containing SDS, acetic acid (pH 3.5), thiobar-bituric acid, and distilled water. Following boiling the mixture (for 1 h at 95°C) and centrifuging (at 3000 g for 10 min), the absorbency of the supernatant was measured by spectrophotometry at 550 nm light length (Badehnoosh et al., 2018).

***Estimation of testicular TAC***

To measure the TAC levels in testis, an acquisition kit (Cat No: TAC-96A, ZellBioGmbH-Germany) was purchased. Function of this kit is based on the oxidation colorimetry resuscitation. The kit contains 1 reagent ready to use, buffer X 100, dye powder, reaction suspension solution, 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 value. The kit's sensitivity was equal to 0.1 mM, and the diagnostic range was mM 2-125 / 0, and final absorbance was read at 490 nm, and unit conversion was performed (Badehnoosh et al., 2018).

***RNA extraction and real-time quantitative PCR***

Total RNA was extracted using a QIAGEN RNA purification mini kit according to the manufacturer's protocol. In this procedure, 30 mg of testicular tissue was placed in RLT buffer, ethanol (96%) was added to the lysate, and resulting mixture was centrifuged. The supernatant was added to the RNeasy mini spin column. Then, the total RNA was bound to the column membrane, the contaminants were efficiently washed away, and high-quality RNA was eluted in RNase-free water. The quality of extracted RNA was checked by a spectrophotometer (UV1240, Shimadzu, Kyoto, Japan) at 260/280 nm wavelength absorbance ratio. DNA was synthesized using a commercial BioFact kit (BioFact RT Series, Korea). According to the kit instructions, 1 µg of total RNA, 10 µl of mastermix, 0.5 µl of oligo-d (T) primer, and 0.5 µl of Random Hexamer primers were added. Then the final volume with RNase-free water was increased to 20 µl. RT reaction was carried out at 70°C (45 min) followed by heat inactivation at 95°C (3 min). The expressions of p53, Bax, and Bcl-2 were evaluated using High ROX BioFact™ 2X Real-Time PCR Smart mix SYBR Green PCR master mix. The function of real-time PCR light cycler device (StepOne™ Real-Time PCR System, USA) was based on the manufacturer's protocol. PCR primers were designed by Oligo software, and the sequences were blasted according to the NCBI database. Sequences of all genes are listed in Table 1. PCR reactions for mRNAs expression consisted of 95°C for 5 min (denaturing cycle) followed by variable amplification cycles (38–42 cycle) at 90°C for 30 sec (annealing cycle) and 72°C for 1 min (extension cycle). All qRT-PCR reactions were carried out in duplicate, and β-actin was used as a housekeeping gene. Gene expression levels were measured using the Ct (2-ΔΔt) method (fold changes) (Dastjerdi et al., 2013).

**Table1**. Primers used in real-time PCR

| Primer ID | Primer sequences |
|---|---|
| β-actin | F: 50-GGCACCACACCTTCTACAATG-30<br>R: 50-GGGGTGTTGAAGGTCTCAAAC-30 |
| Caspase 3 | F: 50-CTGGACTGTGGCATTGAGAC-30<br>R:50-G CAAAGGGACTGGATGAACC-30 |
| Bcl2 | F:50-TGG GATGCCTTTGTGGAACT-30<br>R:50-GAGAC AGCCAGGAGAAATCA-30 |
| p53 | F:50-AGAGACCGCCGTACAGAAGA-30<br>R:50-GCATGGGCATCCTTTAACTC-30 |
| Bax | F: 50-CCGGCGAATTGGAGATGAACT-30<br>R: 50-CCAGCCCATGATGGTTCTGAT-30 |

***Statistical analysis***

Kolmogorov–Smirnov test was conducted to confirm data compliance of normal distribution. The data were analyzed by SPSS software for windows (version 20) using one-way ANOVA postulation followed by Tukey's post hoc test. $P < 0.05$ was considered as significant, and variables were represented as mean ± standard error of the mean.

**RESULTS**

***Progressive motility and viability of sperm cells***

Mercaptothion caused a significant reduction in sperm cell viability and progressive motility compared to the control group ($p<0.001$). No significant variations were detected in Quercetin groups in comparison with control group ($p>0.05$). Also, sperm cell viability and progressive motility in all treated Quercetin and Mercaptothion + Quercetin groups increased significantly compared to the Mercaptothion group ($p<0.001$) (Table 2).

**Table2.** Effect of Mercaptothion, Quercetin and Quercetin + Mercaptothion on sperm parameters (n=6 for each group).

| Groups | Mean of sperm count ($10^6$) | Sperm progressive motility (%) | Sperm viability (%) |
|---|---|---|---|
| Control | 74.22±3.81 | 14.54±2.74 | 91.34±5.73 |
| Mer | 25.96±2.11* | 3.04±1.49* | 47.03±2.74* |
| Que 7.5 mg/kg | 73.75±4.08† | 15.41±2.93† | 92.22±5.34† |
| Que 15 mg/kg | 74.12±3.34† | 14.64±2.11† | 91.43±6.71† |
| Que 30 mg/kg | 72.25±5.17† | 16.74±1.74† | 90.28±4.31† |
| Que +Mer 7.5 mg/kg | 38.44±3.99¶ | 9.98±2.65¶ | 72.73±3.25¶ |
| Que+Mer 15 mg/kg | 42.09±2.84¶ | 6.07±1.01¶ | 69.64±3.64¶ |
| Que+Mer 30 mg/kg | 44.85±3.25¶ | 7.71±1.24¶ | 64.01±2.32¶ |

Data are presented as mean ± SD. * $P < 0.001$ compared to the control group. † $P < 0.001$ compared to Mercaptothion group. ¶ $P < 0.001$ compared to the Mercaptothion group. Que: Quercetin; Mer: Mercaptothion

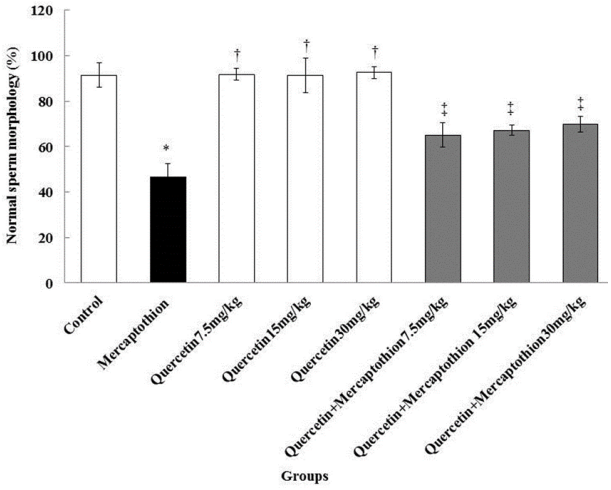


**Figure 1**. Comparison of normal sperm cell morphology in treatment groups. *Significant increase compared to the control group ($p < 0.001$). †Significant increase compared to the Mercaptothion group ($p < 0.001$). ‡Significant increase compared to the Mercaptothion group ($p < 0.001$).

***Sperm cells count and morphology***

The sperm cell count and normal morphology reduced significantly in Mercaptothion group compared to control group ($p<0.001$). No significant deviations were detected in Quercetin groups in contrast with the control group ($p>0.05$). However, the sperm cell count and normal morphology were enhanced significantly in all treated Quercetin and Mercaptothion + Quercetin groups compared to the Mercaptothion group ($p<0.001$) (Figure 1 and Table 2).

***Height of germinal layer in seminiferous tubules***

Mercaptothion caused a signifi¬cant reduction in height of germinal layer in comparison with the control group ($p<0.001$). No significant alterations were observed in comparison with control group ($p>0.05$). This value in entire treated groups of Quercetin and Mercaptothion + Quercetin was improved sig¬nificantly com¬pared to the Mercaptothion group ($p<0.001$) (Figure 2 and Figure 3).

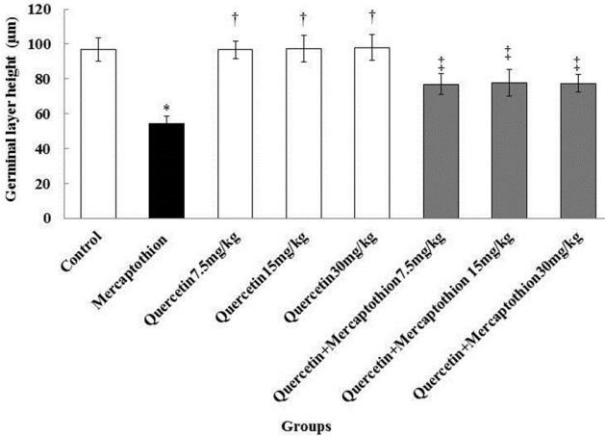


**Figure 2.** Comparison of height of germinal layer seminiferous tubule in treatment groups. *Significant decrease compared to the control group ($p < 0.001$). †Significant increase compared to the Mercaptothion group ($p < 0.001$). ‡Significant increase compared to the Mercaptothion group ($p < 0.001$).

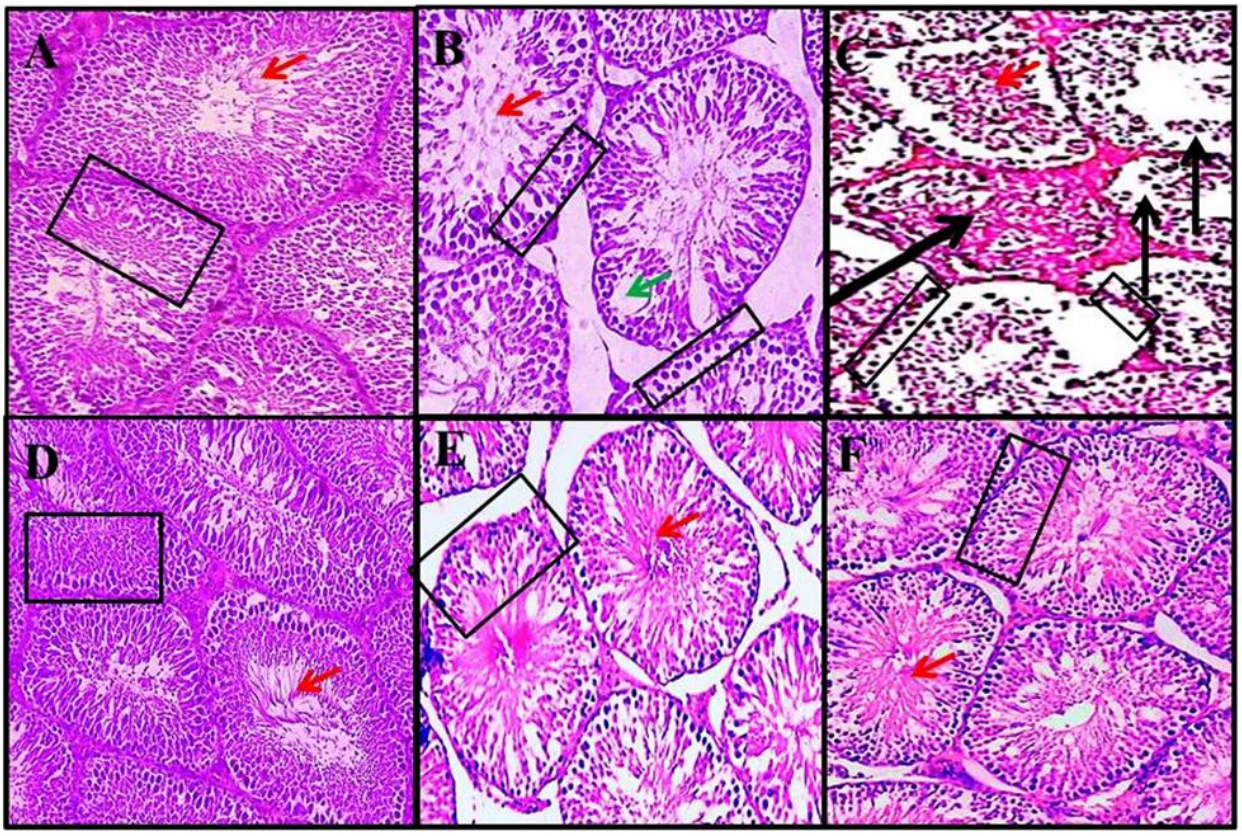


**Figure 3.** Effect of Mercaptothion Quercetin and Quercetin + Mercaptothion on seminiferous tubules (magnification of ×400). Normal seminiferous tubule structure was observed in control group (A), Quercetin group (30 mg/kg) (F), Quercetin + Mercaptothion group (15 mg/kg) (D) and Quercetin + Mercaptothion group (30 mg/kg) (E). A decrease in germinal layer of seminiferous tubules height, destruction of the cells sequence, vacuolization, and reduce sperm cells density were observed in Mercaptothion group (B and C). Rectangles identify germinal layer height, red arrows identify sperm cells density, black arrows identify irregularities in the structure of the margin of tubules (destruction of the membrane seminiferous tubules structure), and green arrow identifies vacuolization.

***Testosterone***

Mercaptothion caused a significant decrease in testosterone hormone levels compared to the control group ($p<0.001$). No significant alterations were detected in Quercetin groups in comparison with control group ($p>0.05$). Furthermore, testosterone hormone levels in all treated Quercetin and Mercaptothion + Quercetin groups improved significantly compared to the Mercaptothion group ($p<0.001$) (Figure 4).

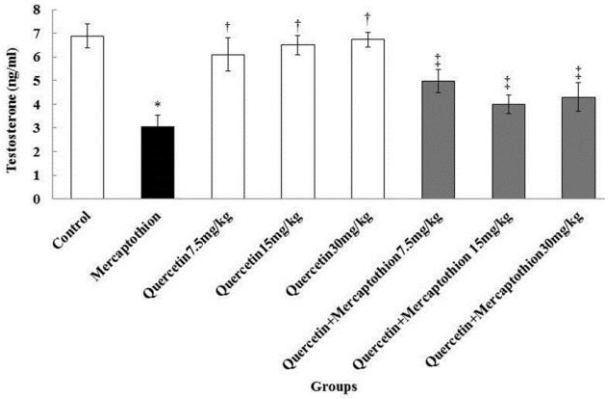


**Figure 4**. Comparison of testosterone hormone levels in treatment groups. * Significant decrease compared to the control group ($p < 0.001$). † Significant increase compared to the Mercaptothion group ($p < 0.001$). ‡ Significant increase compared to the Mercaptothion group ($p < 0.001$).

***MDA levels***

Serum levels of MDA showed a significant increase in Mercaptothion group compared to the control group ($p<0.001$). Also, a significant decrease in MDA levels was showed in all Quercetin and Quercetin + Mercaptothion groups compared to the Mercaptothion group ($p<0.001$). In contrast, no significant effects on the levels of MDA in all Quercetin groups were found compared to the control group ($p>0.05$) (Figure 5).

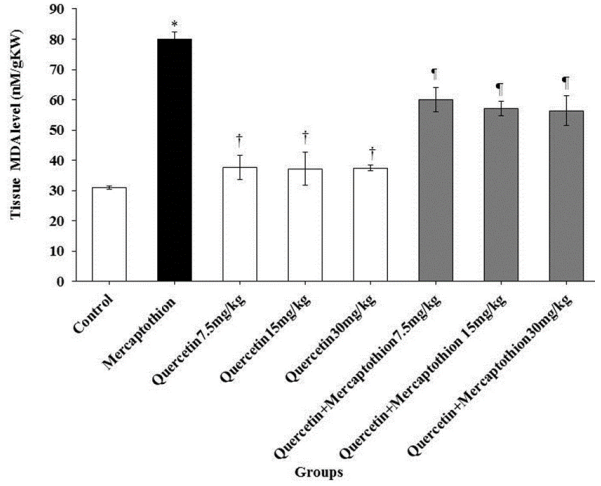


**Figure 5.** Comparison of testis MDA level among treatment and control groups. * $P < 0.001$ compared to the control group. † $P <0.001$ compared to the Mercaptothion group. ¶ $P < 0.001$ compared to the Mercaptothion group. MDA, malondialdehyde.

***TAC levels***

The results of measured TAC levels in study groups showed a significant decrease in Mercaptothion group compared to the control group ($p<0.001$). Also, a significant increase in TAC levels was showed in all Quercetin and Quercetin + Mercaptothion groups compared to the Mercaptothion group ($p<0.001$) while had no significant effects on the levels of TAC in all Quercetin groups compared to the control group ($p>0.05$) (Figure 6).

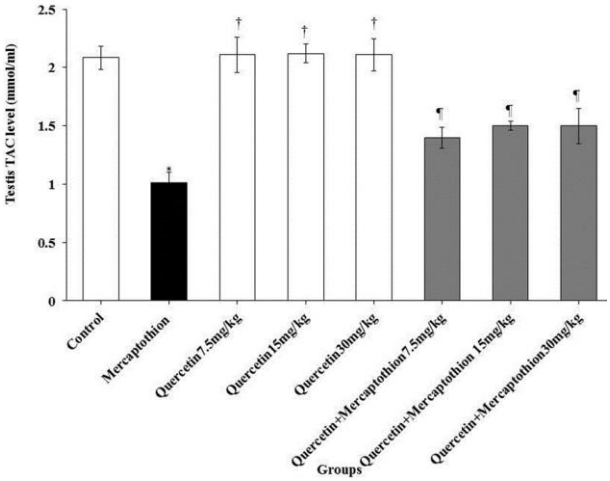


**Figure 6**. TAC level changes in male rats. * $P < 0.001$ compared to the control group. † $P < 0.05$ compared to Mercaptothion group. ¶ $P < 0.05$ compared to the Mercaptothion group. TAC, Total Antioxidant Capacity.

***P53, Bax, Bcl-2 and caspase-3 expression levels***

Mercaptothion significantly up-regulated the apoptosis-regulating genes of caspase-3, p53 and Bax, and also significantly downregulated the Bcl-2 mRNA in the Mercaptothion group compared to the control group. In all Quercetin and Quercetin + Mercaptothion groups, significant down regulatation of apoptosis-regulating genes, including caspase-3, p53, and Bax, were detected. Also, up-regulation of Bcl-2 mRNA in Quercetin and Quercetin + Mercaptothion groups was seen compared to the Mercaptothion group. However, all doses of Quercetin significantly up-regulated the Bcl-2 mRNA in Quercetin and Quercetin + Mercaptothion groups compared to the Mercaptothion group [Figure 7].

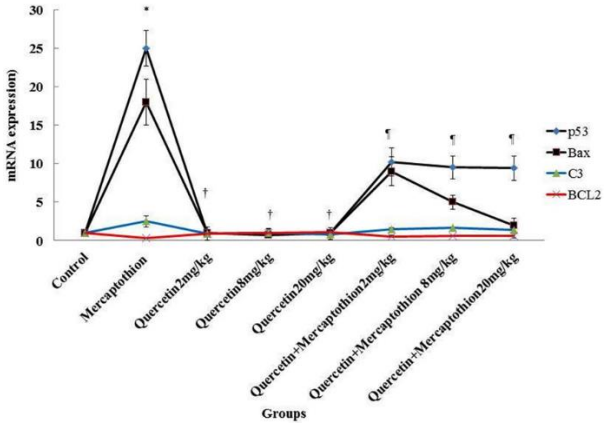


**Figure 7**. The effect of Quercetin and Mercaptothion on C3, p53, Bax and Bcl-2 gene expression of testis ($n = 8$). * $P < 0.001$ compared to the control group. † $p<0.001$ compared to Mercaptothion group. ¶ $p<0.001$ compared to the Mercaptothion group. C3: Caspase 3.

**DISCUSSION**

The World Health Organization (WHO) has reported that about 20,000 people die annually due to toxic effects of pesticides, and 30,00,000 people suffer from nonlethal poisoning of pesticides. This number is increasing that more than 700,000 people experience the chronic effects of contact with pesticides every year (CN et al., 2019). Organophosphates have the highest effects on immune and reproductive systems (Uzun., 2009). These substances can disturb male fertility potential through various ways, such as direct impairment of cell structure and interference with biological processes (Mruk et al., 2011). Quercetin as a natural flavonoid (D'Andrea, 2015) attenuates the diverse effects of Mercaptothion, obviously in male reproductive parameter. It also recovers the cell damage induced by increased level of MDA, altered histological features of tissue and rate of oxidation (by calculating the TAC level). The results of the current study also showed that Quercetin is able to reduce lipid peroxidation (decreased levels of MDA) and increase anti-oxidant capacity (increased TAC levels) of testis tissue, which reduces the oxidative stress. Consistent with these findings, a large body of studies has shown anti-oxidant properties of Quercetin (Khaki et al., 2010; Subbaramaiah et al., 1998). Quercetin is also a lipophilic molecule that is able to inhibit the production of lipid peroxidation via Fenton reaction (Collodel et al., 2011). Thus, it appears that Quercetin, with its anti-oxidant properties, could reduce MDA and increase TAC in treatment groups by inhibiting the production of reactive oxygen species.

The present study also indicated the recovery effects of Quercetin on some male reproductive parameters as well as decreasing the oxidative stress by presentation of decreased levels of MDA. Since sperms lose a large amount of their cytoplasm during spermatogenesis (lack of antioxidant systems), they seem to have a higher sensitivity to elevated ROS than somatic cells (Khaki et al., 2010). The first outcome of ROS attack on membrane structures can be cellular peroxidation in the cell membrane and organelles (Madzorera et al., 2019). The use of antioxidants such as Quercetin for elimination of toxic materials and free radicals can inhibit lipid peroxidation to maintain the biochemical structure of cells (Arabi et al., 2004). According to Nahid et al. (2016), our results showed that Mercaptothion administration reduced catalase and serum total antioxidant levels, increased lipid peroxidation, malondialdehyde and spermatogenesis damage, decreased the height of germinal epithelium, and reduced number of primary spermatocytes in male rats compared to control group (Nahid et al., 2016). The results of the present study showed that sperm parameters (motility, morphology, viability and count) in Mercaptothion group reduced significantly compared to the control group. In Quercetin and Mercaptothion + Quercetin groups, a significant increase was observed in all sperm cells parameter compared to the Mercaptothion group. Spermatogenesis is a highly complex process that is influenced by numerous detrimental factors, leading to infertility and reduced fertility levels (Aitken et al., 2011). One of these factors is oxidative stress, which is induced by accumulation of ROS due to imbalance between oxidant and antioxidant systems (Kuchewar et al., 2014). ROS can affect DNA and RNA synthesis in sperm cells and inhibit their mitochondrial function (Jalili et al., 2018). Mercaptothion-induced oxidative stress seems to disrupt the cell division and differentiation of sperms. Thus a number of spermatogonia are impaired on the basement membrane, and the number of primary and secondary spermatocytes, spermatids, and mature sperms is reduced (Selmi et al., 2018).

The results of Aitken et al. (2011) confirmed the findings of the present study in that the oxidative stress disrupted spermatogenesis and led to defective gametes with remodelled chromatin vulnerable to the attack of free radicals and caused a reduction in the number of spermatogonia, spermatocytes, spermatids, and spermatozoa. Reduced number of sperms in Mercaptothion group might be due to the direct increase of oxidative stress-induced lipid peroxidation, which could have altered natural properties of the membrane and consequently resulted in the sperm count transmitted to the epididymis (Selmi et al., 2018). High levels of ROS cause mitochondrial impairment and consequently release of proapoptotic proteins in intermembrane space, activation of caspases, reduction of ATP synthesis, elevated release of ROS, increased concentration of intracellular calcium, and release of calcium from mitochondria into cytosol, which in turn may lead to activation of apoptosis process (Valko et al., 2005). The findings of Selmi et al. (2018) were in agreement with the results of the current study, indicating that oral administration of Mercaptothion significantly decreased total weights of testis and animal body, sperm count, sperm motility, sperm viability, and normal sperm morphology and also increased sperm DNA damage in comparison with control group. Elevated free radicals levels can lead to impairment of Sertoli cells and destruction of cytoplasmic bridges through loss of epithelial cells. Thereby this event decreases sperm counts and increases sperm cell deformity (Roshankhah et al., 2017).

Quercetin seems to have inhibitory effects on free radicals action and possess antioxidant properties which increase the number of anti-oxidative enzymes. Antioxidant ability of this polyphenol depends on the properties of its polyphenolic hydroxyl groups (Khaki et al., 2010). Quercetin can exert its therapeutic effects through a mechanism involved in the expression of oxidative phosphorylation genes and mitochondrial biogenesis (Lee et al., 2008). Quercetin is also able to stabilize the blood-testis barrier and protect sperm DNA against the oxidative stress induced by free radicals (Khaki et al., 2010). Reval et al. (2001) reported that the Quercetin could inhibit apoptotic induction and DNA damage against benzo[a]pyrene-induced oxidative stress in sperm, which confirms the results of the present study. Also, the present research showed a significant decrease in testosterone levels in serum blood and diameter of seminiferous tubules in Mercaptothion group compared to the control group. Moreover, Quercetin significantly elevated testosterone levels and height of germinal layer in seminiferous tubules in all groups receiving Mercaptothion plus Quercetin in comparison with the Mercaptothion group. Organophosphates can disrupt the expression of steroidogenic acute regulatory protein (StAR). This protein is a determinant agent for biosynthesis of steroids, such as testosterone. Organophosphates directly disturb steroidogenesis in Leydig cells by disruption of StAR expression (Ikeda et al., 2011). The results of Maliji et al. (2014) confirmed the findings of the current research in which the administration of diazinon five days per week for a period of one month significantly elevated interleukin-1 and reduced testosterone in rats. In addition, it seems that organophosphates increase ACTH and cortisol, which can inhibit the activity of hypothalamic-pituitary-gonadal axis, thereby disrupt spermatogenesis

process (Selvage et al., 2003). Considering its potent antioxidant properties, the Quercetin has positive effects on hypothalamic-pituitary-gonadal axis, testosterone level, and sperm production and motility.

Furthermore, Quercetin is able to reduce apoptosis in germinal cells (Mojica-Villegas et al., 2014). Apparently, elevated ROS levels due to administration of Mercaptothion increases lipid peroxidation, which in turn induces atrophy in the germinal layer thickness of seminiferous tubules (Huang et al., 2009). Salahshoor et al. showed a reduction in the epithelial volume of seminiferous tubules due to oxidative stress, which was in line with the findings of the present study (Salahshoor et al., 2016). Quercetin seems to protect lipids against peroxidation, prevent testicular oxidative stress, and play a role in production of testicular steroids (Khaki et al., 2010). The findings of Bitgul et al. were also in agreement with the results of the current study, indicating that oxidative stress impaired the germinal layer of seminiferous tubules compared to control group and also Quercetin improved the height of germinal layer in seminiferous tubules, reduced GSH and MDA, and elevated testosterone in groups exposed to oxidative stress (Erthal et al., 2018). In this study, it was shown that the administration of Mercaptothion increased the expression of preapoptotic factors (such as p-53, Bax and caspase-3), whereas the expression of antiapoptotic (Bcl-2) factors was decreased. P-53 regulates the activities of preapoptotic factors such as Bax, caspases, and endonucleases by releasing cytochrome c from the mitochondria, while Bcl-2 prevents the release of cytochrome c complex (Jiang et al., 1996). Studies showed that Mercaptothion leads to up-regulation of preapoptotic factors in tissues (Ghorbani-Taherdehi et al., 2020). Mercaptothion binds to the related receptor to be translocated into the nucleus and may up- or downregulate the genes with appropriate hormone response elements (Geng et al., 2015). Cells resist environmental damage and oxidative stress attack by cellular enzymes, while Mercaptothion decreases the activity of these enzymes and facilitates the process of apoptosis and cellular damage (Penna-Videau et al., 2012). ROS regulates the apoptosis-associated transcription factors, such as p53, caspase3 and Bax/Bcl-2 ratio. The p53 gene is responsible for cell cycle control at G1/S and G2/M checkpoints and overexpression during cellular damage to induce apoptosis in the cells arrested at these stages (Li et al., 1999). In the process of spermatogenesis, the germ cells are damaged or overexpressed by Sertoli phagocytes through Fas/FasL apoptotic system-dependent manner to maintain equilibrium, which is essential to normal spermatogenesis. Following damage to testicular tissue, the expression of caspase-3, along with the activation of cytochrome c, could increase the rate of the apoptosis process (Kim et al., 2007). In this study, Quercetin reduced the caspase-3, p53, and Bax and increased the Bcl-2 expression and ultimately protected the cells from ROS-induced apoptosis. Studies have shown that the PAC reduced the expression of Bax by reduction in the amount of ROS and increased Bcl-2 gene expression by decreasing the p53 expression (Priyadarsini et al., 2010). The present study showed that Mercaptothion-induced male reproductive damage could be reduced by plant-based antioxidants such as Quercetin. Therefore, Quercetin can improve some male reproductive dysfunctions caused by Mercaptothion-induced toxicity considering its antioxidant properties.

## CONCLUSIONS

The outcomes of this study demonstrated that the Mercaptothion could produce defects in some of male reproductive parameters, and Quercetin with antioxidant features deals with its detrimental effects. In this study, elevated levels of spermatozoa quality, normal morphology, sperm cell viability, and increased levels of height of germinal layer in seminiferous tubules, TAC, motility and count and reduced testis MDA level were found. Quercetin could be valuable for treatment of infertile men to enhancement male fertility. The antioxidant properties of Quercetin could be a main reason for its optimistic outcome on reproductive parameters. Supplementary studies are essential to explain its precise mechanism of action.

## CONFLICT OF INTEREST

The authors have no conflicts of interests to declare.

## AUTHOR CONTRIBUTIONS

MRS and SR carried out the experiments, analyzed and interpreted the data, and drafted the manuscript. MRS and AA designed the study and participated in analysis and interpretation of data. SR coordinated the study, revised the manuscript and approved the final version to be submitted for publication and helped in the analysis and interpretation of data. All authors read and approved the final manuscript.

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