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Original Article

Effects of *Tanacetum parthenium* on Chromatin Quality, Sperm Parameters and Oxidative Stress in Mice

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ABSTRACT

Background: Studies indicate that phytoestrogens and phytosterols have adverse effects on the male reproductive system. To our knowledge, the effects of *Tanacetum parthenium* on testicular tissue, spermatozoa chromatin integrity and free radical damage have not yet been investigated. Therefore, this study aimed to evaluate the effect of *T. parthenium* administration on sperm parameters, testis histology, sperm DNA integrity and oxidative damage in adult male mice.

Methods: Eighteen adult male mice (2-3 months old) were randomly divided into 3 groups: control, TP1 and TP2. TP1 and TP2 groups were separately gavaged with 50 and 100 mg/kg *T. parthenium*. After harvesting the epididymis, sperm analysis was performed according to the guidelines of the World Health Organization (WHO). The testicular tissue also passaged through the tissue routine process after being placed in a formalin fixative solution. To check the quality of sperm chromatin, a sperm smear was prepared and then stained with acridine orange dye and was examined with a fluorescent microscope. Biochemical parameters, including malondialdehyde (MDA), thiol, catalase enzyme, and superoxide dismutase (SOD), were measured in testicular tissue. Finally, data were analyzed by the analysis of variance in SPSS software, version 16.

Results: A significant reduction was seen in sperm count and sperm morphology percentage and the germinal epithelium thickness in the TP1 and TP2 groups versus the control group. The spermatozoa with DNA damage in percentage were higher in the TP1 group (21.22±3.70) and TP2 group (42.60±3.73) compared to the control group (2.40±4.3). There were remarkable differences between the three groups in MDA ($P \leq 0.001$) and thiol ($P \leq 0.001$) levels. Catalase level ($P \leq 0.001$) was lower in the TP1 and TP2 groups than in the control group.

Conclusion: The results of this study showed that *T. parthenium* caused a significant decrease in sperm chromatin quality, MDA level and germinal epithelium thickness at both doses. A reduction was found in the antioxidant enzyme level in the mice administrated with 50 and 100 mg/kg of *T. parthenium*.

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Introduction

Infertility is one of the major problems in the countries of the world. According to the statistics, 15% to 18% of couples suffer from infertility worldwide [1, 2]. Nearly half of these infertility problems are related to male factors. Many factors, such as insecticides, obesity, drugs, toxic substances, and infection, lead to infertility in men [3]. The use of medicinal plants to treat infertility has a long history in different cultures, and it has increased in recent years because of lower side effects compared to chemical drugs [4]. The administration of medicinal herbs such as ginseng, ginger, *Nigella sativa* and saffron increases male fertility and improves semen parameters.

On the other hand, other natural products such as *Ruta graveolens*, *Mentha pulegium* and *Achillea millefolium* significantly reduce fertility and sperm quality [5-7]. *Tanacetum parthenium* is a medicinal plant from the Asteraceae family and the Asterales order found in America, Asia, Europe and Africa. In Iran, it is mostly grown on the river banks and forest areas, as well as in the cities of Kurdistan, Mazandaran, Gillan, Golestan, Khorasan, and Fars provinces. This plant has various compounds, including sesquiterpene lactone, especially parthenolide, germacronolide, guaianolide, flavonoid, phytoestrogen, phytosterol and camphor [8-11]. *T. parthenium* is widely used in the pharmaceutical, cosmetics, hygiene, and agriculture industries. Studies indicate that this plant has anti-rheumatic, anti-flatulent, antiseptic, anti-fungal, anti-parasitic, anti-inflammation and cytotoxic effects. It is used to treat asthma, indigestion, dizziness, migraine, and skin disease [12-15]. Considering that studies report that phytoestrogens and phytosterols of the plants can lead to an adverse effect on the male reproductive system, and based on our search, few documents exist on the administration of *Tanacetum* extract on testicular histology and sperm chromatin quality in the literature. Therefore, the purpose of research was to evaluate the effects of *Tanacetum* extract on sperm parameters, testicular tissue, sperm DNA integrity and free radical damage in adult male mice.

Materials and Methods

This experimental research was conducted on 18 adult BALB/c male mice (2-3 months). Mice were randomly divided into 3 groups: Control, TP1 and TP2. The control group received normal saline. TP term refers to *T. parthenium*. The TP1 group received 50 mg/kg of *Tanacetum* extract, and the TP2 group was gavaged 100 mg/kg of *Tanacetum* using a gavage needle once a day for 2 weeks. These doses were selected based on Soleimani's study [16].

Tanacetum extraction

T. parthenium was collected by a botanist from Zoshk village and confirmed by the Herbarium of the Ferdowsi University of Mashhad (Code: 17895-FUMH). First, 100 g of the powder was mixed with a solvent (50% ethanol+50% distilled water). After shaking, it was passed through a Whatman filter paper (England) and after solvent removal, it was dissolved in normal saline [17].

Sperm analysis

Sperm analysis was performed according to the World Health Organization's (WHO) guidelines for sperm analysis. The crushed epididymis was placed on a plate containing saline in a CO_2 incubator. Then, using a Neubauer hemocytometer, the sperm count and morphology were evaluated [18, 19].

Histology examination

After the testis was isolated, it was placed in 10% formalin fixative (Merck, Germany). Following specimen processing, the tissue was cut into 5 µ slides. After staining, the slides were examined by an expert pathologist [20, 21].

Sperm chromatin quality

Smear sperms were fixed with methanol (Sigman, Germany) and stained with acridine orange solution (Merck, Germany). Then, they were examined with a fluorescent microscope. Red sperm heads were counted as damaged DNA, yellow sperm heads were considered intermediate damage of DNA and green sperm heads indicated healthy DNA [22].

Measurement of malondialdehyde (MDA) level

First, the thiobarbituric acid (TBA) reagent (Merck, Germany) was prepared, and then the testicular tissue was homogenized. The homogenous tissue was heated with TBA reagent, trichloroacetic acid and hydrogen chloride (Merck, Germany) at boiling temperature for 50 minutes. After centrifugation, the absorption was read at 535 nm. The concentration of MDA was expressed in nmol/g [23].

Measurement of thiol level

Tris EDTA buffer was added to homogenous tissue, and the absorbance was read. The absorption of the dithionitrobenzoic acid solution to the sample was measured. Also, the absorption of the dithionitrobenzoic acid solution was considered blank. Thiol concentration was calculated as µmol/g at the wavelength of 412 nm [24].

Measurement of catalase enzyme level

First, hydrogen peroxide (Merck, Germany) was added to the phosphate buffer (solution 2, 3). Homogenous testicular tissue was diluted 1:10 and was added to solution 3. After two minutes, the absorbance changes were read at a wavelength of 240 nm. Enzyme concentration was expressed in U/g [24].

Measurement of superoxide dismutase (SOD) enzyme level

To prepare the tissue homogenate, the testicular tissue was first weighed and then homogenized with 10 mM phosphate buffer saline with pH=4.7. Next, 65 µL of phosphate buffer saline, 30 µL of 25.1 mmol MTT and 75 µL of pyrogallol (Merck, Germany) were mixed with 10 µL of tissue homogenate and incubated for a minute at room temperature. Afterward, 75 µL of dimethyl sulfide oxide was added, and the optical absorption of the obtained solution was read at a wavelength of 570 nm using an ELISA device. It was expressed in U/g [25].

Statistical analysis

Data analysis was done using analysis of variance (ANOVA) and Tukey's post hoc test in SPSS software, version 16.

Results

Sperm analysis

Figures 1 and 2 display the sperm analysis results in different experimental groups. The mean sperm number was 4.8±0.22 million/mL in the control group, while it was remarkably reduced in the TP1 and TP2 groups (4±0.21 vs 3.9±0.20 million/mL). The sperm count in the TP1 group (P=0.006) and TP2 group (P=0.004) indicated a significant decrease versus the control group. The abnormal morphology of sperm was 11% in the control group, 15% in the TP1 group, and 19% in the TP2 group. A significant decrease was observed in the percentage of normal sperm morphology in the TP1 group (P=0.006) and TP2 group (P=0.006) compared to the control group.

Testicular histology

Figure 3 displays the transverse section of the seminiferous tubules in different experimental groups. In the control group, seminiferous tubules were normal. A slight hyperplasia of Leydig cells and a reduction in the germinal epithelium thickness were observed in some tubules of the TP1 group. A remarkable decrease of the germinal epithelium thickness in some tubules and edema, congestion, and apoptotic vacuoles in the TP2 group were found.

Sperm chromatin quality

According to Figure 4, sperm with healthy DNA were observed in green color, and red sperm heads were counted as denatured DNA and yellow sperm heads were considered intermediate DNA damage. The percentage of sperms with damaged DNA in the control group was (2.40±4.3), while after receiving 50 mg/kg of *T. parthenium*, the sperm with damaged DNA remarkably increased (21.22±3.70). The sperm with denatured DNA in the group receiving 100 mg/kg of *T. parthenium* was 42.60±3.73, while it remarkably elevated versus the control group (P≤0.001).

MDA and thiol level in testicular tissue

The level of MDA in the control group was 6.85±1.35 nmol/g of tissue, in TP1, was 8.20±0.91 U/g of tissue, and in the TP2 group was 11.71±2.26 nmol/g of tissue (Figure 3). A significant difference was found in the level of MDA TP groups compared to the control group (P≤0.001). The level of thiol in the control group was 7.00±0.50 µmol/g tissue, in the TP1 group was 5.93±0.70 µmol/g tissue, and in the TP2 group was 4.52±1.01 µmol/g tissue (Figure 4). There was a significant difference in the level of thiol in groups receiving *T. parthenium* compared to the control group (P≤0.001).

Catalase and SOD enzymes in testicular tissue

The catalase enzyme level was 0.37± 0.04 U/g in the control group, 0.27±0.04 U/g in the TP1 group, and 0.19±0.02 U/g in the TP2 group (Figure 5). A significant decrease in the catalase level was observed in the groups receiving *T. parthenium* versus the control group (P≤0.001). The SOD level in the groups receiving 50 mg/kg (P=0.73) and 100 mg/kg (P=0.82) *T. parthenium* decreased compared to the control group (Figure 6), but this reduction was not statistically significant (P>0.05).

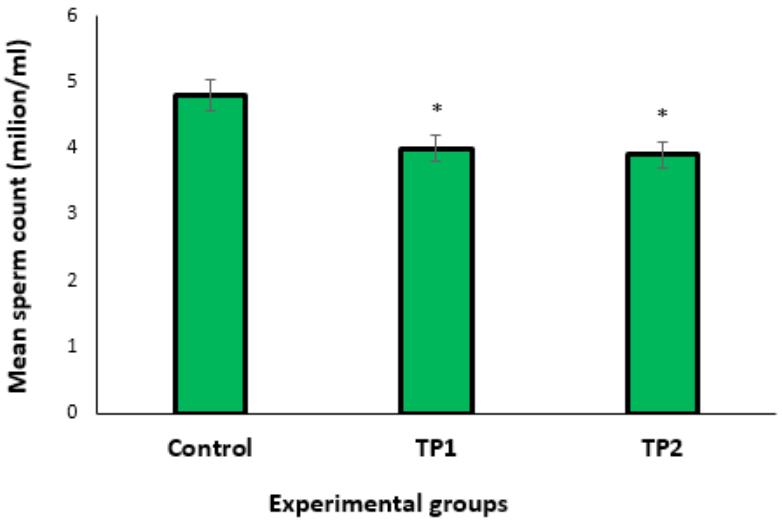

Figure 1. The effect of *T. parthenium* on sperm count in different experimental groups

*P<0.05 statistically difference with the control group.

Notes: The TP1 group received 50 mg/kg of *T. parthenium.* The TP2 group received 100 mg/kg of *T. parthenium*.

Discussion

This study presents data about reduced sperm parameters and DNA integrity after administering *Tanacetum* extract in male mice. In addition, we observed a decrease in the thickness of the germinal epithelium and apoptotic vacuoles, as well as hyperplasia of Leydig cells in seminiferous tubules of the TP1 and TP2 groups. A remarkable increase in MDA level, which indicates oxidative stress, was observed in mice receiving *T. parthenium*. The level of antioxidant enzymes, a useful product for the body and neutralizing free radicals, was reduced compared to the control group. Studies indicate that administering medicinal herbs such as ginseng, ginger, *N. sativa* and saffron increases male fertility and improves semen parameters. On the other hand, other natural products such as *R. graveolens*, *M. pulegium*, *A. millefolium*

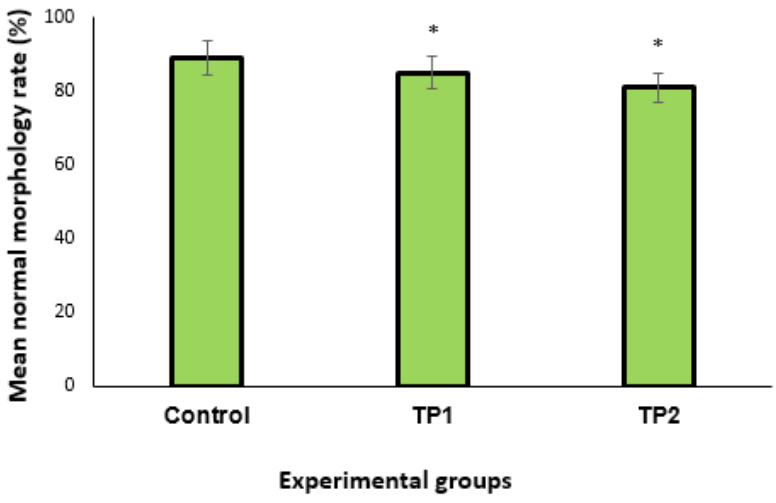

Figure 2. The effect of *T. parthenium* on normal sperm morphology in different experimental groups

*P<0.05 statistically difference with the control group.

Notes: The TP1 group received 50 mg/kg of *T. parthenium*. The TP2 group received 100 mg/kg of *T. parthenium.*

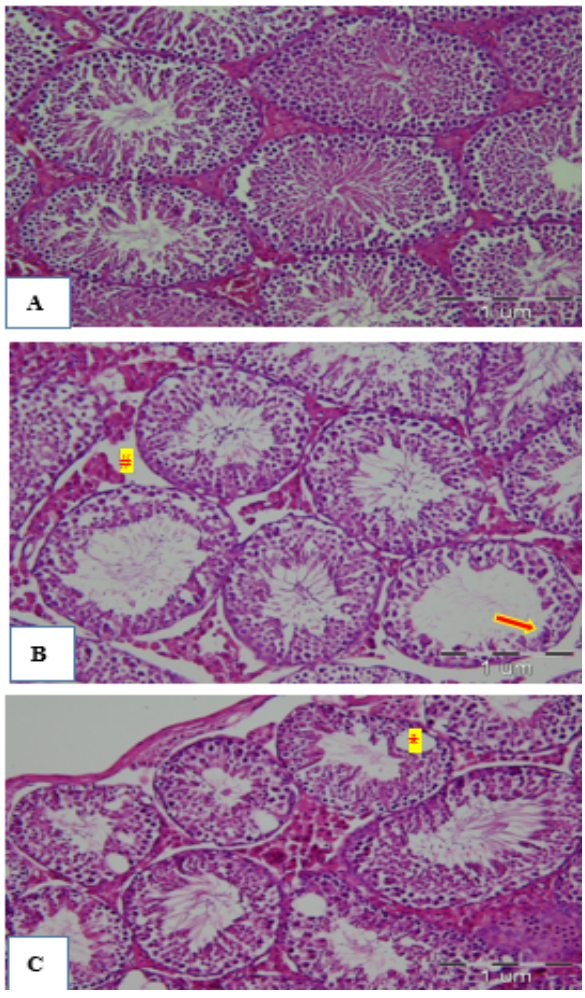

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Figure 3. Testicular histology in different study groups (H&E staining)

*Apoptotic vacuole, #Slight leydig hyperplasia.

Note: Low height in the germinal epithelium.

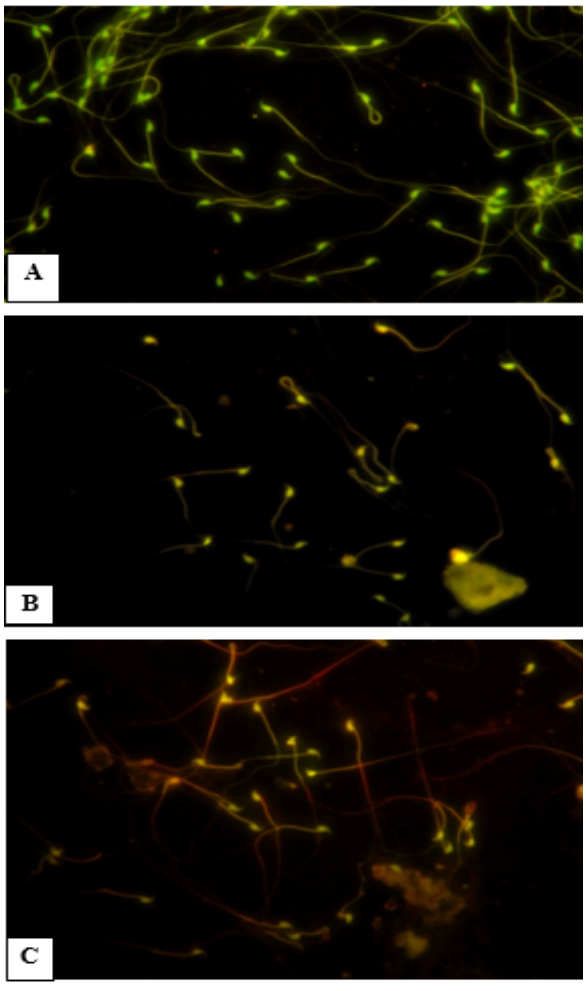

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Figure 4. The Effect of *T. parthenium* on sperm DNA in different study groups (acridine orange staining)

and *Foeniculum vulgare* significantly reduce fertility and sperm quality [5-7].

Pareek et al. in a review article, reported that phytoestrogen compounds, phytosterols, camphor, and apigenin are present in *T. parthenium* [8]. Research indicates that phytoestrogens may damage the male reproductive system [16]. In addition, phytosterols inhibit the activity of the 5-alpha reductase enzyme, thereby reducing the dihydrotestosterone level, an active form of testosterone in the testicular tissue [26]. Phytosterol compounds reduce the sensitivity of tissues to androgens and, through activation of enzymes such as 5-alpha reductase and aromatase, reduce the level of testosterone hormone [27]. These compounds have a similar structure to cholesterol; lowering the cholesterol level reduces the precursor of testosterone hormone synthesis [28, 29]. It was observed that camphor in *T. parthenium* improves bacterial and fungal infections [30]. In addition, the reduction of cytochrome p450 enzyme level decreases some enzymes involved in the synthesis of testosterone hormone. Apigenin also inhibits important enzymes such as phosphatidyl inositol and aromatase, synthesizing testosterone hormone [31]. Consistent with our results, Soleimani et al. injected 50, 100 and 150 mg/kg of *T. parthenium* extract intraperitoneally into mice for 14 days. They reported that the hydroalcoholic extract of *T. parthenium* caused a remarkable reduction in testosterone, dihydrotestosterone, luteinizing hormone, and follicle-stimulating hormone, especially at a dose of 50 mg/kg [16]. Similar to this study, in our research, *T. parthenium* decreased sperm quality and germinal epithelium thickness. The reduction in hormones seems to be caused by the inhibitory effect of phytoestrogens in the *T. parthenium* on the hypothalamus-pituitary-gonadal axis.

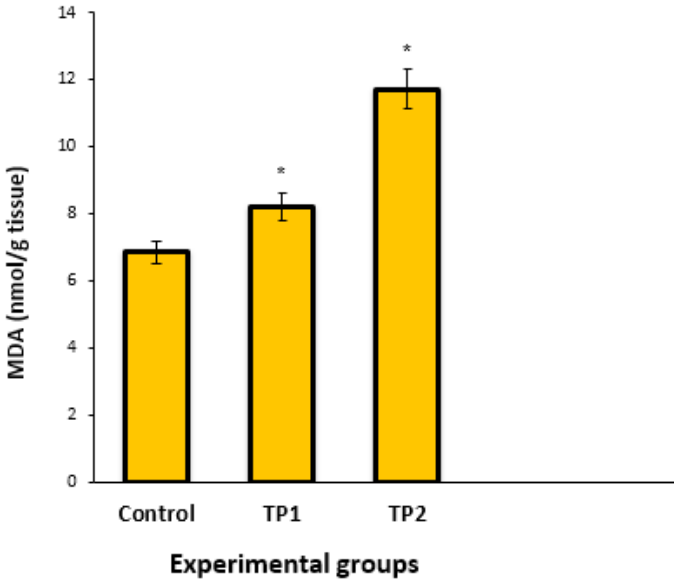

Figure 3. Malondialdehyde level in different study groups

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*P<0.05 statistically difference with the control group.Notes: TP1 group received 50 mg/kg of *T. parthenium*. TP2 group received 100 mg/kg of *T. parthenium*.

On the other hand, Mazani et al. treated rats exposed to carbon tetrachloride with *T. parthenium* [32]. In this study, 7 groups were considered: Group 1 (control) fed pellet+water, group 2 (sham) fed olive oil+pellet, groups 3, 4 and 5 were pretreated with 40, 80 and 120 mg/kg of *T. parthenium* for 14 days, groups 6 and 7 were treated with *T. parthenium* after exposure to carbon tetrachloride. Antioxidant levels increased, and testicular histopathology improved in the groups pretreated with 80 and 120 mg/kg of *Tanacetum*. In Mazzini's study, no group received only *T. parthenium*. Research indicates that phytoestrogens may cause a reduction in fertility. In addition, phytosterols inhibit the activity of the 5-alpha reductase enzyme and thereby reduce the dihydrotestosterone level, an active form of testosterone in the testicular tissue. Phytosterol compounds lessen the sensitivity of tissues to androgens and activating enzymes such as 5-alpha reductase and aromatase reduce the testosterone hormone level. In our study, it would have been better to investigate the effects of parthenolide as an active ingre-

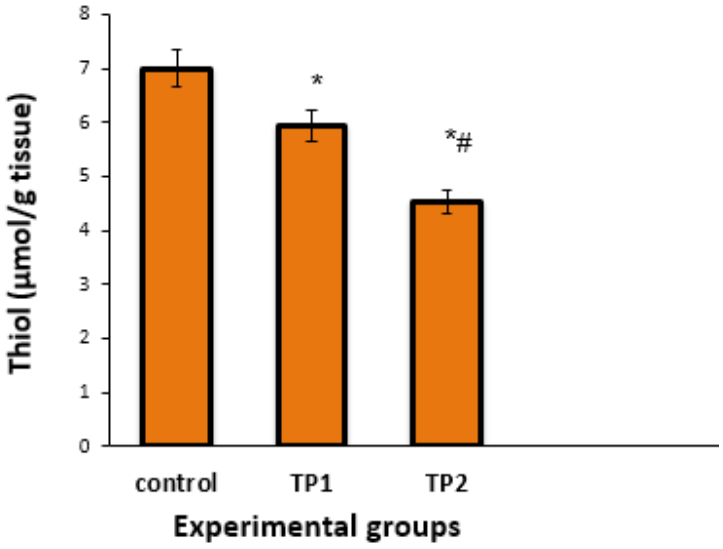

Figure 4. Thiol levels in different study groups

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*P<0.05 statistically difference with the control group, #P<0.05 statistically difference with TP1 group. Notes: TP1 group received 50 mg/kg of *T. parthenium*. TP2 group received 100 mg/kg of *T. parthenium*.

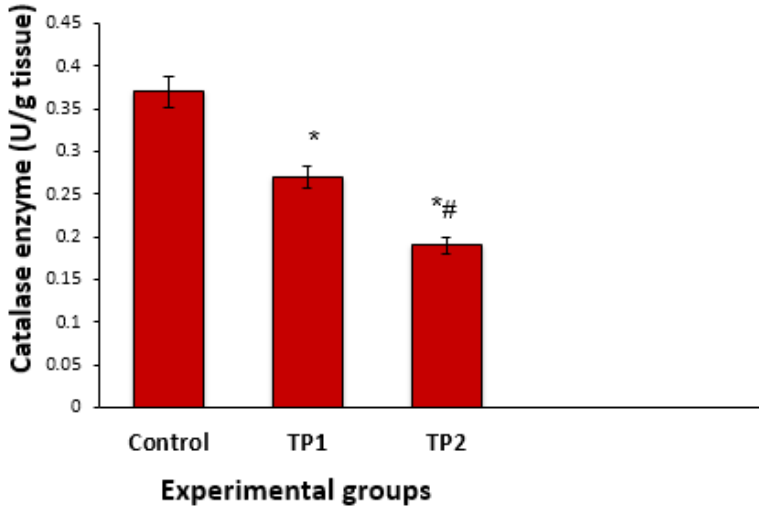

Figure 5. Catalase enzyme level in different study groups

*P<0.05 statistically difference with the control group, #P<0.05 statistically difference with TP1 group.

Notes: TP1 group received 50 mg/kg of *T. parthenium*. TP2 group received 100 mg/kg of *T. parthenium*.

dient of *T. parthenium*, recommended to dear researchers for further research.

Conclusion

The results of this study showed that *T. parthenium* caused a significant decrease in sperm chromatin quality, MDA level, and germinal epithelium thickness at both doses. A reduction was found in the antioxidant enzyme level in the mice administrated with 50 mg/kg and 100 mg/kg of *T. parthenium*.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Ethics Committee of Mashhad University of Medical Sciences (Code: IR.MUMS.MEDICAL.REC.1397.614).

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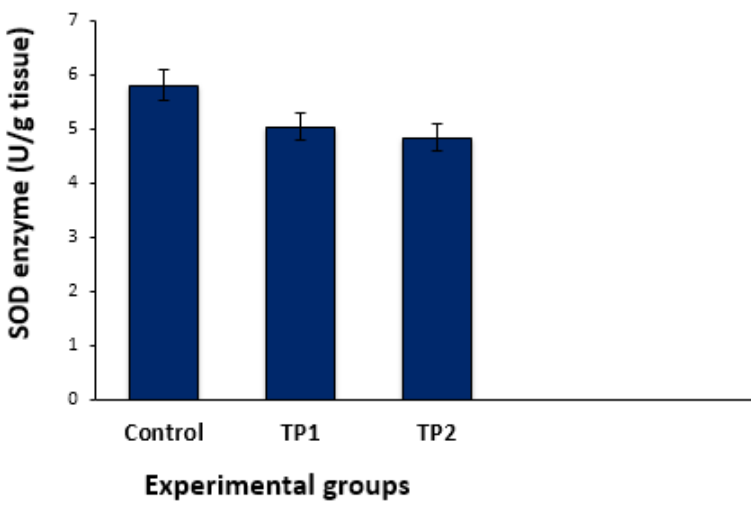

Figure 6. SOD enzyme level in different study groups

Notes: TP1 group received 50 mg/kg of *T. parthenium*. TP2 group received 100 mg/kg of *T. parthenium*.

Authors' contributions

Conceptualization and supervision: Shabnam Mohammadi; Methodology and data collection: Farimah Beheshti; Investigation: Abbas Mohammadipour; Review, editing and final approval: All authors.

Conflict of interest

The authors declared no conflict of interest.

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