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Exploring the main effects of *phoenix dactylifera* on destructive changes caused by cyclophosphamide in male reproductive system in mice

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Abstract

Cyclophosphamide leaves an undesirable effect on testes. This study was conducted to explore the effects of the *Phoenix dactylifera* (DP) on testes following the cyclophosphamide treatment. Thirty-six male mice were divided into six groups, one control, one cyclophosphamide, two groups of cyclophosphamide with a dose of 200 mg kg^{-1} and a dose of 400 mg kg^{-1} DP, and two of only high and low doses of DP. All groups were gavaged daily for 28 days. The animals were euthanized 24 hr after implementing the last treatment. Then, the testes and epididymis samples were removed and weighed. The main sperm characteristics such as the number of sperm and sperm viability, the morphometric changes, biochemical analysis of testes and enzyme activity were investigated. With the cyclophosphamide group, only body weight, testes weight, epididymis weight, sperm viability and the fertilization percentage were decreased significantly compared to the control group. Moreover, the spermatogenesis indices and morphometric parameters in this group indicated a significant decrease. Furthermore, the morphological changes were observed in the testicular tissue, including seminiferous tubule atrophy, vacuolation, hyperemia of blood vessels and increased space in the interstitial tissue. In the biochemical study of cyclophosphamide group, the amount of glutathione peroxidase in serum was decreased, whereas, the amount of malondialdehyde in testicular tissue showed a significant increase. The DP group included the antioxidant and anti-apoptotic properties. It seemed that the compounds in the DP would lead to the inhibition of the production of active metabolites released from the cyclophosphamide.

Introduction

Nowadays, the cancer treatment has become a critical challenge in the world. To this end, several methods can be utilized in cancer treatment. One of these methods is the use of chemotherapy drugs.[1] These drugs cause the production of reactive oxygen species (ROS) in tissues which cause DNA fragmentation as well as the loss of normal sperm function.[2] In this regard, the spermatogenesis is defined as the process by which the immature male germ cells are converted into adult spermatozoa.[3] The use of some drugs to treat diseases would lead to infertility in men.[4] It should be mentioned that one of the most important side-effects of the cyclophosphamide are its toxic effects on the sexual organs which may lead to infertility.[5] Some studies have confirmed that the men receiving such drugs for some time have experienced sperm abnormalities.[6] Several studies have confirmed the beneficial effects of compounds with antioxidant properties such as *Satureja khuzistanica Jamzad*, Spirulina, Ginseng, Hawthorn on the created damage to testicular tissue following the cyclophosphamide treatment.[7-9]

The palm tree is a perennial, two embryose, and a single base plant. In ancient Egypt, the *Phoenix dactylifera* (DP) had been used for female fertility in traditional medicine.[10] It is worth mentioning that the DP contains cholesterol and carotenoids which cause gonadotropin activity in rat.[11] As the testes is a vulnerable organ to

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cyclophosphamide, this combination may have some negative effects such as reducing sperm quality in the testes.[12] In this regard, considerable attention has been paid to coping with the side-effects of anticancer drugs and reducing oxidative stress by the antioxidant elements.[13] On the other hand, the antioxidant properties of DP have been investigated and proven in various conducted studies.[14,15] The aim of this study was to evaluate the effect of palm pollen (*Phoenix dactylifera*) as an antioxidant on sperm parameters and testicular tissue of mice treated with cyclophosphamide.

Materials and Methods

Preparation of cyclophosphamide. Cyclophosphamide (Baxter Oncology GmbH, Weiterstadt, Germany) was prepared as a single dose 100 mg kg^{-1} with insulin syringe and dissolved in 0.20 mL of sterile distilled water. In this experiment, according to the mean weight (33.44 ± 2.00 g) of mice (n = 18) receiving cyclophosphamide, to prepare a dose of 100 mg kg^{-1} cyclophosphamide, 0.034 g of cyclophosphamide powder in 0.2 mL of sterile distilled water was immediately dissolved for each of the mice, then the volume was made-up with water. The mice were intraperitoneally injected with a single dose.[8]

Preparation of *Phoenix dactylifera*. The DP was purchased from Khuzestan province, which was identified (Herbarium number 8026) and confirmed by the botanist of the Herbarium Center of The Faculty of Natural Resources and Desertology of Yazd University (Yazd, Iran). The DP were sliced into small pieces, then grond to fine powder.The obtained DP powder was divided into two groups of low-dose DP alone and low-dose DP with cyclophosphamide (n = 12). Based on the weight of mice (33.67 g), 0.0673 g of DP powder was dissolved in 12.00 mL of sterile distilled water to prepare a dose of 200 mg kg^{-1} of DP and then 1.00 mL of the above solution was administered as oral gavage. Based on the mean weight of mice, the powdered DP was divided in two groups of high-dose (HD) DP alone and high-dose DP with cyclophosphamide (n = 12). Based on the weight of mice (34.83g), to prepare a dose of 400 mg kg^{-1} of DP, 0.0348 g of DP powder was dissolved in 12.00 mL of sterile distilled water, and then 1.00 mL of the above solution was administered as oral gavage to each mice.[6]

The animal models. Thirty-six male mice (NMRI) weighing approximately 30.00 – 36.00 g were prepared from the Laboratory Animal Breeding Center. The animals were kept in propylene cages for 35 days in 12 hr light/dark cycle at a temperature, of 22.00 ± 1.00 ˚C under a relative humidity of 50.00 ± 10.00%. All animals were fed with a standard foot of maize (pellet) and wheat under equal proportions, and were provided free access to water and food. It should be noted that the animals were adapted one week before the start of the 28-day treatment period. The study was approved by the Local, Ethical Committee for Animal Experiments (No. IR.SSU.REC.1398.177).

Sampling. All available mice in the six groups: Control group, CP group received cyclophosphamide (100 mg kg^{-1}). The DP (LD) group received low dose (LD) of DP (200 mg kg^{-1}) and DP (HD) group received high dose of DP (400 mg kg^{-1}). The CP+DP (LD) and CP+DP (HD) groups were weighed 24 hr after the last treatment, then were anesthetized with xylazine (Alfasan, Woerden, The Netherlands) 10.00 mg kg^{-1} and ketamine (Alfasan) 90.00 mg kg^{-1} then sacrificed. First, the blood samples were collected directly from the heart using sterile syringes, and were kept at the laboratory temperature. After taking the animal blood samples, testes and epididymis were weighed after separation. After that, the testes were placed immediately under laminar hood under the sterile conditions. The sperm characteristics evaluation such as the number of sperm and viability, morphometric changes, spermatogenesis indices of testicular tissue, biochemical analysis of testicle, malondialdehyde (MDA) level, and glutathione peroxidase (GPX) activity in serum were also assessed.

Biochemical evaluation. The collected blood samples were centrifuged at 3,000 rpm for 5 min. Then, the serum was separated and transferred to a micro-tube and stored in a freezer at – 70.00 ˚C to measure the levels of GPX and MDA enzymes. The GPx level was measured by the GPx Kit as described in the instructions provided by manufacturer (ZellBio GmbH, Ulm, Germany). The MDA was measured in serum using spectrophotometer (Bio-Rad Laboratories, Hercules, USA) at 535 nm.[8]

Histological parameters. The testes were transferred to a 10.00% formalin stabilization solution for fixation, after confirming tissue fixation and paraffin molding, some serial sections of a 5.00 - 7.00 µm thickness was prepared from the testicular tissue. All slides were stained using Hematoxylin and Eosin and examined using a light microscope (Olympus, Tokyo, Japan). TCapture Software (Photonics Co., Fujian, China) was used for analysis of all photomicrographs. The qualitative changes of testes were recorded and degenerating Leydig cells and abnormal Sertoli cells were considered. Twenty seminiferous tubules in one hundred microscopic field were counted and tubule differentiation index (TDI), the percentage of seminiferous tubules containing at least three differentiated germ cells,[8] spermiation index (SPI) and the percentage of seminiferous tubules with normal spermiation were determined.

Epididymal sperm characteristics. In order to prepare the sperm, the abdominal skin was sterilized with 70.00% ethanol after anesthesia and sacrificing of the animals. An Incision was made in the abdomen and the tail of the epididymis was cut into small pieces and placed in a microtube containing 2.00 mL of PBS culture medium (Sigma, St. Louis, USA), which had already been equilibrated in the incubator at 5.00% CO_2 at 37.00 ˚C.

Next, the tail of the epididymis was dissected with sterile scissors and was placed in the incubator for 15 min to obtain the capacity and remove the sperm from the epididymis.[16] The Neubauer slide was utilized to count the number of sperm. Distilled water (95.00 µL) with a blue sampler head was added to 5.00 µL of sperm-containing suspension in the microtubule. A homogeneous solution of sperm culture and environment was prepared in a dilution of 1 in 20. After pipetting off the resulting suspension, 5.00 µL of the solution was transferred to a Neubauer slide and was then immobilized for 5 min to reduce the sperm motility. The number of sperms per milliliter was measured using the formula: $n \times 50{,}000 \times d$ in the cells related to red blood cell count using the light microscope with 400× magnification. Where, n denotes the number of sperm counted in the five squares of the Neubauer slide, d represents the inverse dilution of the sperm-containing suspension.[17] The number of sperms in epididymis were counted and then averaged. The Eosin- Nigrosin staining was carried out to determine the viability of the live sperm. 7.00 µL drops of culture medium containing sperm were put on a slide and placed in the incubator. Besides, 3.00 µL of Eosin-Nigrosine were added to the slide. The pipetting was done for 10 sec to mix the dye and the solution. A smear was prepared using another slide and observed under a microscope. The live sperm had a light white head and trunk, whereas, the dead sperms were pink in which all parts, especially the sperm head, were much darker than the background. The number of unstained sperms for all available sperms was calculated for the survival rate and then the mean of the obtained results was calculated. In this way, 200 sperms were evaluated for each sample with a 400× magnification, and then the achieved results were expressed as a percentage.

Statistical analysis. Numerical data obtained in this study were evaluated by SPSS software (version 24.0; IBM Corp., Armonk, USA). For the purpose of comparison between the experimental groups, the One-way variance method (One Way ANOVA) and Tukey's range test were used. Finally, the results were expressed as average ± standard deviation and the difference was considered significant at the level of $p < 0.05$.

Results

Clinical observations. The results of the mean comparison in the current research indicated that the body and testicle weight were significantly decreased in mice in the cyclophosphamide group compared to the control group ($p < 0.05$). In groups that received the DP at high and low doses simultaneously with cyclophosphamide, a significant increase was observed compared to the cyclophosphamide group ($p < 0.05$; Table 1).

Biochemical results. The serum level of GPX in the cyclophosphamide group was significantly decreased compared to control group ($p < 0.05$). Nevertheless, the treatment of DP in high and low doses following cyclophosphamide treatment exhibited a significant increase in the serum level of GPX ($p < 0.05$). In the group receiving low dose of DP following cyclophosphamide treatment, a significant decrease was observed compared to the control group ($p < 0.05$). Also, the serum levels of MDA were significantly increased in the cyclophosphamide group ($p < 0.05$). However, in the DP treatment in high and low doses, the serum level was significantly decreased compared to the cyclophosphamide group ($p < 0.05$; Fig. 1).

Histomorphometrical results. In the morphometric evaluation, the mean comparison showed that the thickness of the testicular capsule as well as the thickness of the interstitial tissue in the mice receiving the cyclophosphamide was significantly increased compared to the control group ($p < 0.05$). In contrast, in the groups which received the DP at high and low doses simultaneously with the cyclophosphamide a significant decrease was observed compared to the cyclophosphamide group ($p < 0.05$). The thickness of the epithelium of seminiferous tubules as well as the diameter of tubules in the mice receiving the cyclophosphamide group was significantly decreased compared to the control group ($p < 0.05$). In contrast, in the groups which received DP at high and low doses concomitantly with cyclophosphamide, the thickness of the epithelium of the seminiferous tubules as well as the diameter of the seminiferous tubules a significant increase was observed compared to the cyclophosphamide group ($p < 0.05$; Table 1).

Sperm characterization results. It was revealed that the number of sperms and sperm survival percentage in the cyclophosphamide group were significantly reduced compared to the control group ($p < 0.05$). In contrast, in the groups co-administered with cyclophosphamide which received DP in high and low doses there was a significant increase in terms of the number of sperm as well as the sperm viability compared to the cyclophosphamide group ($p < 0.05$; Table 2).

Spermatogenesis results. The TDI, SPI, regeneration index (RI; percentage of seminiferous tubules that were differentiated from type A spermatogonia to higher grades), Sertoli cell index (SCI; the ratio of the number of germ cells to the number of Sertoli cells), meiotic index (MI; the ratio of the number of round spermatozoa to primary spermatocytes)[9] were significantly decreased in the group that received the cyclophosphamide ($p < 0.05$). In the group that received the DP at high and low doses of the cyclophosphamide treatment, it was significantly increased compared to cyclophosphamide group ($p < 0.05$). Besides, in these groups, TDI showed a significant increase ($p < 0.05$) compared to the control group. In those groups receiving DP at high and low doses, there was no significant difference in the TDI (Table 1).

Table 1. Comparison of body and testis weights (g), and spermatogenesis index in the studied groups as mean ± SD (n = 6).

Parameters	Control	CP	DP (HD)	DP (LD)	CP+DP (HD)	CP+DP (LD)
Testicle weight (g)	0.10 ± 0.00	0.06 ± 0.00[a]	0.11 ± 0.00[b]	0.10 ± 0.00[b]	0.08 ± 0.00[abcd]	0.08 ± 0.00[abcd]
Primary body weight (g)	35.52 ± 1.54	30.10 ± 1.47	34.33 ± 1.36	33.17 ± 1.16	35.33 ± 1.03	34.17 ± 2.13
Final body weight (g)	37.33 ± 0.51	25.50 ± 1.76[a]	40.67 ± 2.42[ab]	36.50 ± 1.37[ab]	36.33 ± 0.51[abcd]	32.83 ± 1.47[abcd]
Diameter of STs (µm)	146.32 ± 13.75	96.77 ± 9.94[a]	148.32 ± 13.71[b]	146.92 ± 13.36[b]	133.56 ± 16.92[abcd]	130.88 ± 11.14[abcd]
Epithelium thickness of STs (µm)	63.12 ± 1.96	22.52 ± 4.92[a]	65.36 ± 3.55[b]	64.12 ± 2.90[b]	54.24 ± 1.98[abcd]	47.56 ± 1.82[abcd]
Meiotic index	3.15 ± 0.21	2.53 ±0.19[a]	3.20 ± 0.16[b]	3.15 ± 0.16[b]	2.89 ± 0.20[b]	2.88 ± 0.33[b]
Sertoli cell index	4.16 ± 0.20	3.33 ± 0.44[a]	4.27 ± 0.37[b]	4.22 ± 0.18[b]	4.16 ± 0.39[b]	3.97 ± 0.20[b]
Regeneration index	95.33 ± 1.52	33.67 ± 2.08[a]	96.67 ± 2.51[b]	95.67 ± 2.88[b]	75.67 ± 1.52[abcd]	72.67 ± 2.08[abcd]
Spermiogenesis index	95.04 ± 1.54	31.92 ± 2.43[a]	95.64 ± 1.55[b]	94.88 ± 1.96[b]	75.44 ± 1.68[abcd]	72.04 ± 1.67[abcd]
Tubule differentiation index	93.88 ± 2.60	36.04 ± 1.06[a]	95.36 ± 1.65[b]	94.20 ± 1.89[b]	75.56 ± 1.98[abcd]	72.20 ± 2.23[abcd]

CP: Cyclophosphamide, DP (HD): High dose of *Phoenix dactylifera*, DP (LD): Low dose of *Phoenix dactylifera*, STs: seminiferous tubules.
[abcd] The dissimilar letters indicate a significant difference among groups ($p < 0.05$).

Table 2. Comparison of cells count and viability between the groups in the testicular tissue as mean ± SD (n = 6).

Parameters	Control	CP	DP (HD)	DP (LD)	CP+DP (HD)	CP+DP (LD)
Viability (%)	91.00 ± 1.00	35.67 ± 1.15[a]	96.33 ± 1.52[ab]	92.67 ± 1.52[b]	85.67 ± 3.05[abcd]	62.67 ± 2.51[abcd]
Sperm count (10^6 mL^{-1})	46.21 ± 2.14	18.17 ± 1.10[a]	54.16 ± 1.90[ab]	52.29 ± 2.43[ab]	37.40 ± 0.54[abcd]	34.20 ± 0.83[abcd]
Leydig cells per 0.18 mm^2	23.30 ± 8.54	10.10 ± 9.91[a]	24.34 ± 3.47[b]	23.38 ± 3.40[b]	19.65 ± 2.12[abcd]	18.28 ± 1.66[abcd]
Spermatids per 20 ST	122.30 ± 6.38	60.40 ± 4.87[a]	128.27 ± 4.98[b]	122.27 ± 4.22[b]	100.04 ± 6.88[abcd]	90.32 ± 1.58[abcd]
Sertoli cells per 20 ST	13.39 ± 0.48	11.71 ± 1.01[a]	13.48 ± 1.07[b]	13.22 ± 0.34[b]	12.35 ± 1.08[abcd]	12.04 ± 0.58[abcd]
Primary spermatocytes per 20 ST	39.08 ± 2.05	23.88 ± 1.48[a]	40.10 ± 1.12[b]	38.92 ± 1.48[b]	34.16 ± 3.20[abcd]	31.37 ± 1.28[abcd]

CP: Cyclophosphamide, DP (HD): High dose of *Phoenix dactylifera*, DP (LD): Low dose of *Phoenix dactylifera*. ST: seminiferous tubule.
[abcd] The dissimilar letters indicate a significant difference among groups ($p < 0.05$).

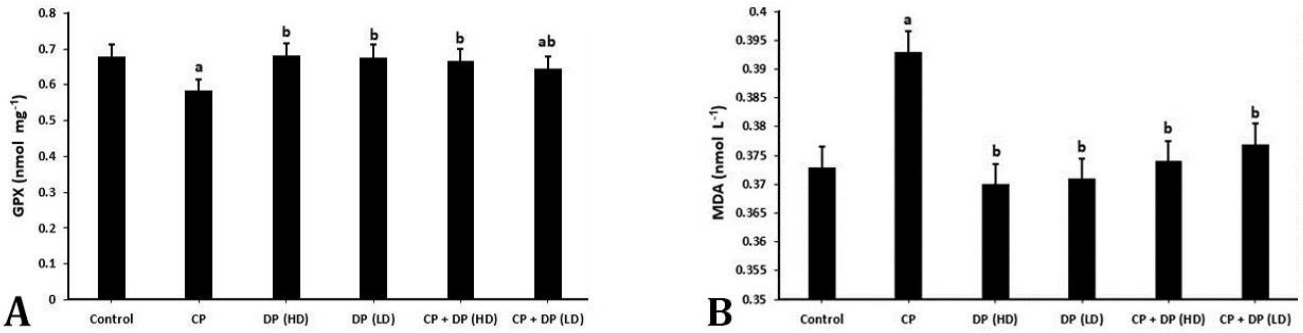

Fig. 1. A) Level of glutathione peroxidase and **B)** Level of malondialdehyde in the study groups (Mean ± SD). CP: Cyclophosphamide, DP (HD): High dose of *Phoenix dactylifera*, DP (LD): Low dose of *Phoenix dactylifera*.
[ab] The dissimilar letters indicate a significant difference between groups ($p < 0.05$).

The results of counting germ cell, Sertoli cell, and Leydig cell. The results of the mean comparison of counting the germ cell (spermatogonia B), Sertoli, and Leydig (all parameters were examined in 20 seminiferous tubules in 100 microscopic fields) indicated a significant decrease in the mean of spermatogonia, primary spermatocytes, spermatids, Sertoli and Leydig cells in the group receiving the cyclophosphamide ($p < 0.05$). In the groups which received the DP at high and low doses simultaneously with the cyclophosphamide, the number of spermatogenic, Sertoli,[9] and Leydig cells revealed a significant increase compared to the cyclophosphamide group ($p < 0.05$; Table 2).

The results of testicular tissue morphology. The testicular tissue in the control group: The spermatogenic cells along with Sertoli cells could be observed in the seminiferous tubules of different classes. The Leydig cells could be observed in clusters with other connective cells. The morphological structure of testicular tissue in the cyclophosphamide-treated group showed severe atrophy in the seminiferous tubules, disintegration and vacuolation of the germinal epithelium, hyperemia of blood vessels, overlapping of the seminiferous tubules, tissue edema, rupture of the germinal epithelium isolated from the basement membrane, decrease in the number of germinal epithelial layers , decrease in the number of different germ cell lines and spermatogenesis, decrease in the number, shrinkage, and irregularity of Sertoli cell nuclei, degeneration of Leydig cells and wrinkled or pyknotic nuclei. The testicle tissue could be observed in the high and low doses DP treatment group, similar to the control group. Regarding the testicular tissue in the treatment group with high dose and low DP, following the cyclophosphamide treatment in these two groups, the coherence and uniformity of the seminiferous tubules without any signs of the tissue edema could be seen (Fig. 2).

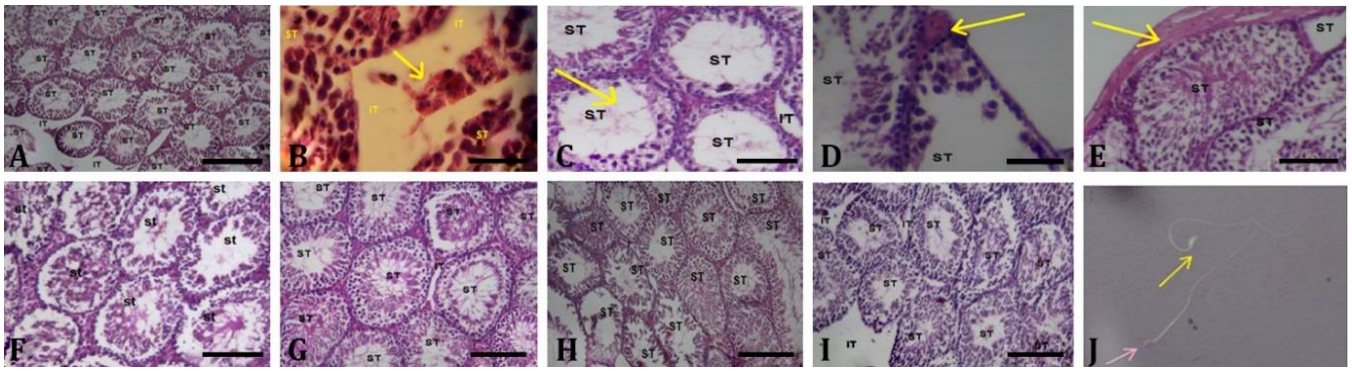

Fig. 2. The image of testicular tissue sections in the studied groups by Hematoxylin and Eosin staining. **A)** Testicular tissue in the control group (Scale bar = 210 µm). **B)** Yellow arrow indicates Leydig cells in clusters (Scale bar = 21 µm). **C)** Testicular tissue in the cyclophosphamide-treated group. Yellow arrow indicates decrease in the number of germinal epithelial layers (Scale bar = 84 µm). **D)** Testicular tissue in the cyclophosphamide-treated group. Yellow arrow indicates degeneration of Leydig cells, the existence of wrinkled or pycnotic nuclei (Scale bar = 21 µm). **E)** Testicular tissue in the cyclophosphamide-treated group. Yellow arrow indicates rupture of the germinal epithelium isolated from the basement membrane (Scale bar = 84 µm). **F)** Testicular tissue in low dose *Phoenix dactylifera* treatment group (Scale bar = 210 µm). **G)** Testicular tissue in high dose *Phoenix dactylifera* treatment group (Scale bar = 210 µm). **H)** Testicular tissue in the treatment group with high dose *phoenix dactylifera* following cyclophosphamide treatment (Scale bar = 210 µm). **I)** Testicular tissue in the treatment group with low dose *phoenix dactylifera* following the cyclophosphamide treatment (Scale bar = 210 µm). **J)** Yellow arrow refers to the live sperm (no colored) compared to dead sperm (pink arrow), (Eosin- Nigrosin staining, 200×). ST: seminiferous tubule, IT: interstitial space.

Discussion

In this study, the treatment of DP powder in mice treated with the cyclophosphamide caused an increase in the testicle damages compared to mice receiving the cyclophosphamide alone. Based on the obtained results of mean comparison in the groups receiving the cyclophosphamide, a significant decrease was observed in the testicle weight, body weight, and epididymis, which was consistent with the results of previous studies.[8] Moreover, in another study by Kaya *et al.*,[18] the weight loss due to a single dose of cyclophosphamide was reported. Based on the conducted studies, lower dose of the cyclophosphamide could affect the male reproductive system locally.[19] The results of this study confirmed that the cyclophosphamide could significantly reduce the number of sperm, which could significantly increase the dead sperm and sperm with abnormal morphology,[8] which was consistent with previous studies. Moreover, Jalali *et al.*[7] explained that the cyclophosphamide had some toxic effects on the male reproductive system via creating the oxidative stress, which could decrease the number of sperm and its motility, increase the abnormal sperm and reduce the testosterone and antioxidant capacity of testicle tissue. It was confirmed that cyclophosphamide resulted in a lack of DNA repair mechanism and apoptosis in cells, which could ultimately lead to accumulation of damage in the sperm.[20] Here, it should be mentioned that more than half of the people undergoing chemotherapy suffered from severe and irreversible damages to the epithelium of the seminiferous tubules.[1] Bakhtiary *et al.*[21] stated that the cyclophosphamide could reduce the Sertoli cells which had some negative effects on the germ cells and, as a result, reduced the spermatozoa production. Mehraban *et al*,[22] confirmed that cyclophosphamide could decrease the number of spermatids in adult male rats. In this regard, Wetzels *et al.*[23] described that the toxic effects of the cyclophosphamide on the reproductive system of males and females could disrupt the process of the spermatogenesis and oogenesis which was consistent with the present study. Besides, it was found that compared to control rats, CP treatment also induced marked histological alterations in testis including reduction in the numbers of germ cells, vacuolization of the seminiferous epithelium, spermatogenesis arrest and degeneration of Leydig cells confirming previous reports.[9] The germ cells reduction, seminiferous epithelial vacuolization and spermatogenesis arrest were in agreement with decreased sperm count observed in the current study and could be the results of oxidative stress induced by CP. The testicular tissue sections for histomorphometric examination obtained from the studied groups showed that the previous results were in accordance with our results. Furthermore, Jalali *et al.*[7] showed that cyclophosphamide had some toxic effects on the male reproductive system by generating the oxidative stress, which could decrease the number of sperms, increase the abnormal sperm and decrease the testosterone. Some studies showed that the cyclophosphamide treatment was associated with a decrease in the number of spermatogenic cells as well as the disruption of the germ cell process which confirmed the obtained results of the evaluation of spermatogenesis in this paper**.** In addition, the results of Ghobadi *et al.*[24] were in accordance with the results of the present study. The current study revealed that oxidative stress was confirmed by decreased antioxidant enzyme GPx as well as

increased MDA level. Administration of DP significantly attenuated the severity of CP-induced histological changes and effects on spermatogenesis which could be attributed to its antioxidant activities. Some studies have shown that the glutathione peroxidase, an antioxidant in the protection of sperm, could play a critical role to decrease the oxygen free radicals in the body.[25] Here, it is worthwhile to mention that one of the indices of lipid peroxidation of red blood cell membrane is MDA.[26] This chemical product is exploited in several biochemical evaluations to estimate the amount of the peroxidative damage based on which in the work conducted by Asleiranifam *et al.,*[27] the effects of hydro-alcoholic extract of *Achillea millefolium* on testicular tissue affected by single dose of the cyclophosphamide could increase the level of the malondialdehyde of the testicular tissue. In another study performed by Ilbey *et al.*[28] it was reported that cyclophosphamide could increase the level of the malondialdehyde and cause a change in the glutathione peroxidase in testicular tissue which was consistent with the results of the present study. The results of this study confirmed that the DP could play a significant protective role in the reproductive system as an antioxidant against the toxic effects of cyclophosphamide. In this way, based on the Jashni and Jahromi,[6] the effect of DP on the folliculogenesis process after treatment was investigated using cyclophosphamide in rats. The results revealed that the aqueous extract of DP could improve the harmful effects of cyclophosphamide on the folliculogenesis process.[6] Consistent with another study, it has been shown that the DP was significantly effective to protect and enhance the testicular structure in diabetic rats due to its potent antioxidant activity in which it had an adjusting effect on changing testosterone levels caused by diabetes.[29] In addition, the DP included the gonadotropins and several types of natural antioxidants such as flavonoid and glycoside.[15] Several studies implied that the plants with antioxidant compounds such as flavonoids could decrease the toxicity of cyclophosphamide treatment. The DP improved testicular function after its toxicity induced by cyclophosphamide in rats. The protective role of DP could improve the biochemical oxidative enzyme restoring which might occur moderately by reduction of testicular damaged cell. Therefore, DP might potentially be a protective agent for cyclophosphamide-induced toxicity. Further studies are necessary to investigate the effect of DP on the metabolism of cyclophosphamide.

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Conflict of interest

The authors declare no potential conflict of interest concerning the research, authorship and publication of this article.

References

1. Koff JL, Ramachandiran S, Bernal-Mizrachi L. A time to kill: targeting apoptosis in cancer. Int J Mol Sci 2015; 16(2): 2942-2955.
2. Mohammadi F, Nikzad H, Taghizadeh M, et al. Effect of pumpkin extract regimen on testicular structure and serum biochemical parameters in cyclophosphamide-treated adult rats. KAUMS Journal (FEYZ) 2013; 17(5): 438-446.
3. Zhou R, Wu J, Liu B, et al. The roles and mechanisms of Leydig cells and myoid cells in regulating spermatogenesis. Cell Mol Life Sci 2019; 76(4): 2681-2695.
4. Panner Selvam MK, Agarwal A, Pushparaj PN, et al. Sperm proteome analysis and identification of fertility-associated biomarkers in unexplained male infertility. Genes (Basel) 2019; 10(7): 522. doi:10.3390/genes10070522.
5. Hamzeh M, Hosseinimehr SJ, Karimpour A, et al. Cerium oxide nanoparticles protect cyclophosphamide-induced testicular toxicity in mice. Int J Prev Med 2019; 10: 5. doi: 10.4103/ijpvm.IJPVM_184_18.
6. Jashni HK, Jahromi HK. Effects of palm pollen on folliculogenesis process after treatment with cyclophosphamide among rats. J Fundam Appl Sci 2016; 8(4): 1998-2007.
7. 7Jalali AS, Hasanzadeh S, Malekinejad H. Chemoprotective effect of *Crataegus monogyna* aqueous extract against cyclophosphamide-induced reproductive toxicity. Vet Res Forum 2011; 2(4): 266-273.
8. Afkhami-Ardakani M, Hasanzadeh S, Shahrooz R, et al. Antioxidant effects of Spirulina platensis (Arthrospira platensis) on cyclophosphamide-induced testicular injury in rats. Vet Res Forum 2018; 9(1): 35-41.
9. Abd El Tawab AM, Shahin NN, AbdelMohsen MM. Protective effect of Satureja Montana extract on cyclophosphamide-induced testicular injury in rats. Chem Biol Interact 2014; 224: 196-205.
10. Moshfegh F, Baharara J, Namvar F, et al. Effects of date palm pollen on fertility and development of reproductive system in female Balb/C mice. J Herbmed Pharmacol 2016; 5: 23-28.
11. Abedi A, Karimian SM, Parviz M, et al. Effect of aqueous extract of *Phoenix dactylifera* pollen on dopamine system of nucleus accumbens in male rats. Neurosci Med 2014; 5(1): 49-59.
12. Selvakumar E, Prahalathan C, Mythili Y, et al. Protective effect of dl-alpha-lipoic acid in cyclophosphamide induced oxidative injury in rat testis. Reprod Toxicol

2004; 19(2): 163-167.
13. Patra K, Bose S, Sarkar S, et al. Amelioration of cyclophosphamide induced myelosuppression and oxidative stress by cinnamic acid. Chem Biol Interact 2012; 195(3): 231-239.
14. Abedi A, Parviz M, Karamian SM, et al. Aphrodisiac activity of aqueous extract of *Phoenix dactylifera* pollen in male rats. Adv Sex Med 2013;3: 28-34.
15. Biglari F, AlKarkhi AFM, Easa AM. Antioxidant activity and phenolic content of various date palm (*Phoenix dactylifera*) fruits from Iran. Food Chem 2008; 107(4): 1636-1641.
16. Pedersen HS, Liu Y, Foldager L, et al. Calibration of sperm concentration for *in vitro* fertilization in a mouse reprotoxicity model. Toxicol In Vitro 2019; 55: 58-61.
17. Aponte PM, van Bragt MP, de Rooij DG, et al. Spermatogonial stem cells: characteristics and experimental possibilities. APMIS 2005; 113(11-12): 727-742.
18. Kaya C, Barbaros Baseskioglu A, Yigitaslan S, et al. The therapeutic potential of amifostine on cyclophosphamide-induced testicular dysfunction in rats: An experimental study. Int J Reprod Biomed 2019; 17(4): 245-252.
19. Arbabian M, Amirzadegan M, Tavalaee M, et al. Oxidative stress and its effects on male infertility: a review study. J Rafsanjan Univ Med Sci 2018; 17(3): 253-274.
20. Aladaileh SH, Abukhalil MH, Saghir SAM, et al. Galangin activates Nrf2 signaling and attenuates oxidative damage, inflammation, and apoptosis in a rat model of cyclophosphamide-induced hepatotoxicity. Biomolecules 2019; 9(8):346. doi: 10.3390/biom9080346.
21. Bakhtiary Z, Shahrooz R, Ahmadi A, et al. Protective effect of ethyl pyruvate on testicular histology and fertilization potential in cyclophosphamide treated mice. Vet Res Forum 2020; 11(1): 7-13.
22. Mehraban Z, Ghaffari Novin M, Golmohammadi MG, et al. Protective effect of gallic acid on apoptosis of sperm and *in vitro* fertilization in adult male mice treated with cyclophosphamide. J Cell Biochem 2019; 120(10): 17250-17257.
23. Wetzels GE, Nelemans P, Schouten JS, et al. Facts and fiction of poor compliance as a cause of inadequate blood pressure control: a systematic review. J Hypertens 2004; 22(10): 1849-1855.
24. Ghobadi E, Moloudizargari M, Asghari MH, et al. The mechanisms of cyclophosphamide-induced testicular toxicity and the protective agents. Expert Opin Drug Metab Toxicol 2017; 13(5): 525-536.
25. Abarikwu SO, Otuechere CA, Ekor M, et al. Rutin ameliorates cyclophosphamide-induced reproductive toxicity in male rats. Toxicol Int 2012; 19(2): 207-214.
26. Samie A, Sedaghat R, Baluchnejadmojarad T, et al. Hesperetin, a citrus flavonoid, attenuates testicular damage in diabetic rats via inhibition of oxidative stress, inflammation, and apoptosis. Life Sci 2018; 210: 132-139.
27. Asleiranifam N, Hasanzadeh S, Sam MR, et al. The effects of hydro-alcoholic extract of *Achillea millefolium* on sperm parameters and apoptotic changes in cyclophosphamide treated mice [Persian]. J Mazandaran Univ Med Sci 2016; 25(133): 77-90.
28. Ilbey YO, Ozbek E, Simsek A, et al. Potential chemoprotective effect of melatonin in cyclophosphamide- and cisplatin-induced testicular damage in rats. Fertil Steril 2009; 92(3): 1124-1132.
29. Kazeminia SM, Kalaee SEV, Nasri S. Effect of dietary intake alcoholic extract of palm pollen (*Phoenix dactylifera* L.) on pituitary-testicular axis in male diabetic rats [Persian]. J Mazandaran Univ Med Sci 2014; 24(Supple 1): 167-175.