Original Article

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The Restorative Effect of Prosopis Farcta on Fertility Parameters and Antioxidant Status in Diabetic Rats

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ABSTRACT

BACKGROUND AND OBJECTIVE: *Prosopis farcta* has antidiabetic and antioxidant properties and may be effective in preventing destructive effects of diabetes on the testes. The present study aims to analyze the effect of *Prosopis farcta* on fertility parameters, antioxidant status and the structure of testicular tissue in diabetic rats.

METHODS: In this experimental study, 32 male adult Wistar rats (200-220 g) were divided into 4 groups including control, *Prosopis farcta*, diabetic and diabetic plus *Prosopis farcta*. Streptozotocin (55 mg/kg) was administered intraperitoneally to induce diabetes in rats. *Prosopis farcta* group and *Prosopis farcta* extract treated diabetic group received 300 mg/ml hydroalcoholic extract of *Prosopis farcta* intraperitoneally for 30 days. At the end of the study, the animals were weighed and dissected. Then, fertility, antioxidant and histopathology parameters of testis were analyzed.

FINDINGS: The level of total antioxidant capacity (12.4±0.5) and superoxide dismutase (1.9±0.5) in testicular tissue of diabetic rats treated with *Prosopis Farcta* increased significantly compared with diabetic group (7.9±0.7 and 0.6±0.18, respectively) (p=0.001), while *Prosopis Farcta* extract significantly decreased the level of Malondialdehyde in the diabetic group (373.9±16.6) (p=0.000). Furthermore, a significant increase in serum testosterone levels, count and motility of sperm was observed in diabetic rats treated with *Prosopis Farcta* (p=0.003). Moreover, *Prosopis Farcta* decreased testicular tissue damage caused by diabetes.

CONCLUSION: Results of the study demonstrated that hydroalcoholic extract of *Prosopis farcta* improves fertility parameters and testicular tissue structure in diabetic rats through increasing antioxidant activity and decreasing oxidative stress.

KEY WORDS: *Prosopis Farcta, Testis, Rat, Malondialdehyde, Antioxidant, Fertility*.

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Introduction

Infertility is one of the problems of industrial societies, and approximately 8 – 12% of the populations are infertile (1). Oxidative stress caused by increased free radicals as well as lipid peroxidation are the most important causes of functional damage and decrease the quality of sperm and have a close relationship with infertility (2). Diabetes has many side effects on the organs, including the genital tract. One of its complications is fertility disorder, which leads to reduced erection up to three times (3), testicular atrophy and decrease in the number and motility of the sperm in men (4).

On the other hand, diabetes induces oxidative stress, increases lipid peroxidation and reduces the activity of antioxidant enzymes and total antioxidant capacity of testicular tissue and degrades it, decreases testosterone levels, prevents spermatogenesis, and can lead to apoptosis in testicular cells of rats (5).

Medicinal herbs are rich in natural antioxidants and are used in traditional medicine to control and cure many diseases. In recent years, much attention has been paid to the study of the effect of different plants on fertility in laboratory animals (6). Researchers consider finding an effective drug for improving diabetes and reducing its complications, and many plant species are used in different countries to treat diabetes, and it has been estimated that more than 800 types of herbs are used to treat diabetes (7). The leaves and seeds of *Prosopis Farcta* plant are used as a traditional drug in different parts of Asia, including India and Iran.

Prosopis farcta plant is a member of Leguminosea family with anti-microbial, anti-microbial and antioxidants properties, increased high-density lipoprotein and decreased low-density lipoprotein in ostriches, and has a protective effect on liver function in diabetic rats (8–10). It also has the properties for treating gastric ulcer, diarrhea, rheumatism, joint swelling, laryngeal inflammation, heart disease, dyspnea and abortion (11).

Some of the active compounds in this plant are 5-hydroxy-tryptamine, lectin, L-arabinose, crocin and epinephrine (12). In addition, a number of phenolic compounds with strong antioxidant activity, such as vicenin-2, epinephrine, glycoside, isoorientin, rutin, caffeic acid derivatives, and luteolin have been identified in this plant (13). In Iran, the leaves of this plant are used to treat diabetes and a wide range of diseases. Some applications of this plant have been proven in new researches. Nevertheless, most of its uses lack scientific support. Recently, the neuronal protective effect of *Prosopis farcta* leaves has been reported in rats (9). The leaf of this plant is used to treat wounds caused by diabetes, but the exact mechanism of its effect has not been properly understood yet. In a study, *Prosopis farcta* administration decreased glucose levels in diabetic rats (10).

Medicinal plants are used to increase fertility and to eliminate such issues as hormonal disorders, impotence and reduced sperm count, and their antioxidant compounds inhibit the production of free radicals and lipid peroxidation and sperm membrane damage and improve fertility parameters. Since *Prosopis farcta* has antioxidant activity, it is useful for diabetic patients and there is no report on the effect of this plant on the damaging effects of diabetes in fertility, the present study was conducted to investigate the effect of hydroalcoholic extract of *Prosopis farcta* on fertility parameters, testicular structure and its antioxidant status in diabetic rats.

Methods

Animals: In this experimental study, 32 male Wistar rats (200-220 g) were prepared from the Pasteur Institute and were kept at a temperature of 22 ºC and under 12/12 h day/night photoperiod with free access to standard food and water.

The protocol of this study was conducted according to the International Committee on Laboratory Animals as well as the Ethics Committee for Labor Animals in Kermanshah University of Medical Sciences with the code KUMS.REC.1395.168. The rats were divided into 4 groups of 8.

Diabetes induction: Diabetes was induced by intraperitoneal injection of Streptozotocin (STZ) (Sigma, USA) at a dose of 55 mg / kg dissolved in fresh phosphate buffer of 0.05 M with pH = 4.5 (14). Diabetes was confirmed by polydipsia, polyuria, body weight loss and glucose greater than 300 mg / dL, 72 hours after STZ injection (15). *Prosopis farcta* injection was considered as the first day of the study.

Control group and diabetic group: Healthy and diabetic rats received 0.5 cc distilled water daily (extract solvent), the *Prosopis farcta* group and the diabetic group were treated with the *Prosopis farcta*, while healthy rats and diabetic who received the extract of the *Prosopis farcta* and received intraperitoneal injection of 300 mg/kg/day for 30 days.

At the end of the study (30 days after the administration of *Prosopis farcta*), the rats were first weighed and then anesthetized with chloroform. Blood

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collection was performed in the fasting state from the left ventricle. Blood serum was isolated by centrifugation and testosterone was measured by ELISA using specific kits (Pishtaz Teb Co., Iran). After taking blood samples, the epididymis tail was isolated and incubated in $HamsF_{10}$ culture medium at 37 ° C and 5% Co_2.

After creating an excision in the epididymis tail, the sperms were placed into the free medium and then, their number and mobility were determined (5). The left testis of each animal was then removed and weighed and was immediately placed in formalin 10%. After fixing and performing tissue passage, 5 – micrometer sections were prepared and stained with Hematoxylin-Eosin staining, and the status of seminiferous tubule including the order and arrangement of spermatogenic cells was investigated (16).

The right testis of the rats was used to measure the concentration of malondialdehyde, superoxide dismutase, catalase and total antioxidant capacity. Testis to body weight ratio of rats was also calculated.

Preparation of hydroalcoholic extract of *Prosopis farcta* fruit: After collecting the plant, it was identified and confirmed by Herbarium experts at Zabul University. Since the plant's fruits were wet and fresh, they were placed in a shade and air for a few days to dry completely. Then the fruit of the plant was powdered with electric mill. 100 ml ethanol 70% was added to 50 g of the powder and it was completely mixed for 24 hours with a magnetic stirrer in the laboratory. The solution was then passed through a filter paper and placed in an oven at 45°C for 24 hours to dry. The dried powder was weighed and stored at 4°C (17).

Measuring the malondialdehyde level of testicular tissue: 2 mg of testicular tissue was stored at 70 °C in 2 cc phosphate buffer solution and was homogenized by homogenizer. The suspension was then centrifuged for 10 minutes at 10,000 rpm (5). 500 µl of homogenized tissue solution was removed and added to 2.5 ml of trichloroacetic acid solution 10% and centrifuged for 10 minutes. 1.5 ml of supernatant was added to 2 ml of thiobarbituric acid 0.67% and placed in bain-marie for 30 minutes at a temperature of 90˚C. After cooling, samples were re-centrifuged for 10 minutes at 4000rpm, and the optical absorption of the supernatant was measured by a spectrophotometer at 593 wavelengths. The concentration of malondialdehyde was calculated according to the standard diagram (18).

Superoxide dismutase and catalase assay: Suroxide dismutase levels were measured as antioxidant enzymes (tissue protection against free radicals) using the Kit colorimetric assay (19). The catalase tissue levels were measured by the method proposed by Ghanbari et al. (5).

Measuring the total antioxidant capacity using Ferric Reducing Ability of Plasma (FRAP): The total antioxidant capacity was measured using FRAP method. This method is based on the strength of the sample antioxidants (serum) in regeneration Fe^{+3} ions to Fe^{+2} in the presence of a substance called tripyridyltriazine. The regeneration ability of each sample was measured by increasing the concentration of Fe^{+2} complexes with tripyridyltriazine at 593 nm wavelengths using the spectrophotometer (5).

Statistical analysis: Data were analyzed using unilateral ANOVA and Tukey post hoc test. $P<0.05$ was considered significant.

Results

At the end of experiment, the mean body weight of rats decreased significantly in diabetic group (163.9±6.9) compared with control group (235.5±5.2) and *Prosopis farcta* group (238±2.4) (p=0.000). However, there was no significant difference between the diabetic group treated with *Prosopis farcta* (210±11.7) and the control and *Prosopis farcta* groups (Fig 1A). Comparison of testicular weight in the groups of control, *Prosopis farcta*, diabetic and diabetic treated with *Prosopis farcta* did not show any significant difference (Fig 1B).

The results of sperm analysis showed that the diabetic group had the lowest sperm count (p=0.003), and there was no significant relationship in terms of reduction in the number of sperm in the diabetic group treated with *Prosopis farcta* and the control group (Fig2A).

The percentage of moving sperm in diabetic groups was significantly lower and the percentage of motionless sperm was higher than in other groups (p=0.000) (Fig 2B). The serum testosterone levels in the diabetic group (0.34±0.03) were significantly lower than control (0.66±0.04) and *Prosopis farcta* (0.64±0.03) (p=0.000).

Meanwhile, the use of *Prosopis farcta* extract significantly increased serum testosterone levels in diabetic rats (0.57±0.025). There was no significant difference between serum testosterone levels in diabetic group treated with *Prosopis farcta* and the control group (Fig 3).

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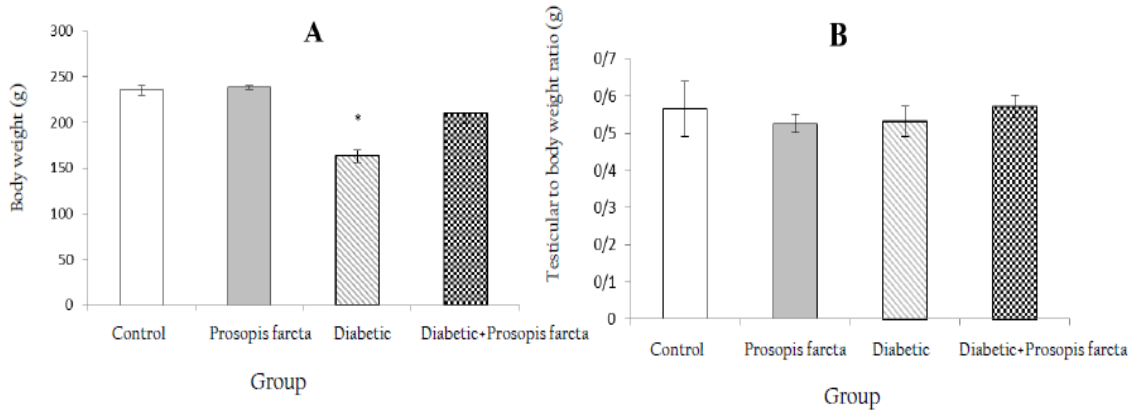

Figure 1. The mean body weight at the end of the study (A) and testicular to body weight ratio (B) in the studied groups. *: Indicating significant difference with control group at the level of p <0.05.

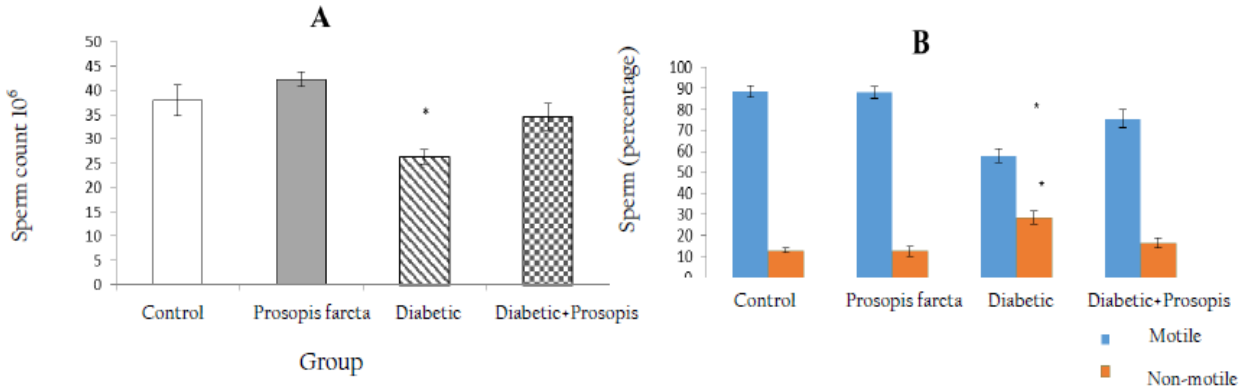

Figure 2. Effect of *Prosopis farcta* on sperm count (A), percentage of sperm motility and percentage of inactive sperm (B) in different experimental groups after 30 days of using hydroalcoholic extract of *Prosopis farcta*. The values are expressed based on mean ± standard deviation, *: indicates a significant difference with the control group at the level of p <0.05.

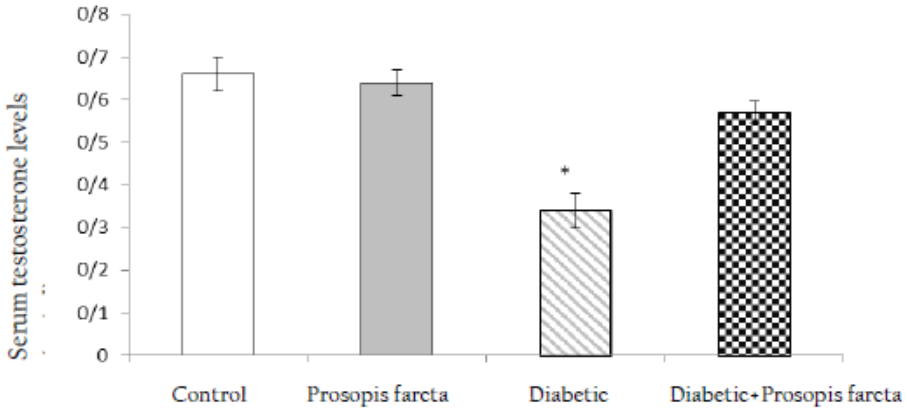

Figure 3. The effect of hydroalcoholic extract of *Prosopis farcta* on serum testosterone concentrations in the studied groups. *: Significant difference was observed with the control group at the level of p <0.05.

The mean levels of malondialdehyde in diabetic group (489.26±8.9) increased compared to control (308.17±4.8) and *Prosopis farcta* (279.12±1.4) groups, while the diabetic group treated with *Prosopis farcta* (373.16±9.4) showed a significant decrease compared with the diabetic group (p=0.000) (Fig 4A). Comparison of total antioxidant capacity of testicular tissue in different groups showed that its level in control group (7.0±9.7) was significantly less than the diabetic group (12.0±7.6) (p=0.001). The hydroalcoholic extract of *Prosopis farcta* increased the total antioxidant capacity in the diabetic group treated with *Prosopis farcta* (12.0±4.5), and in other groups there was no significant difference (Fig 4B). The level of superoxide

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dismutase in the testicular tissue was 2.3±0.3 in the control group, 2±0.2% in *Prosopis farcta* group, 0.6±0.2 in diabetic group, and 9.0±1.5 in diabetic group treated *Prosopis farcta*, indicating a significant difference (p=0.004), and there was no significant difference in other groups. There was no significant correlation in mean catalase levels of testicular tissue between control and other groups (p=0.7) (Fig 5).

Investigating the histological sections of the control groups, the seminiferous tubule were completely normal in terms of cellular ordering and cellular spermatogenesis (Fig 6A). In the *Prosopis farcta* group, the natural appearance of the seminiferous tubules and cells was observed (Fig 6B). However, the testicular tissues of diabetic rats (Fig 6) showed tissue damage including destruction of tubes and spermatogenic cell lines (g), and decreased bonding between cells (f) with increasing sperm density between seminiferous tubules. In the diabetic group treated with *Prosopis farcta*, all spermatogenic cells were observed to be natural. The space between the seminiferous tubules was also normal (Fig 6D).

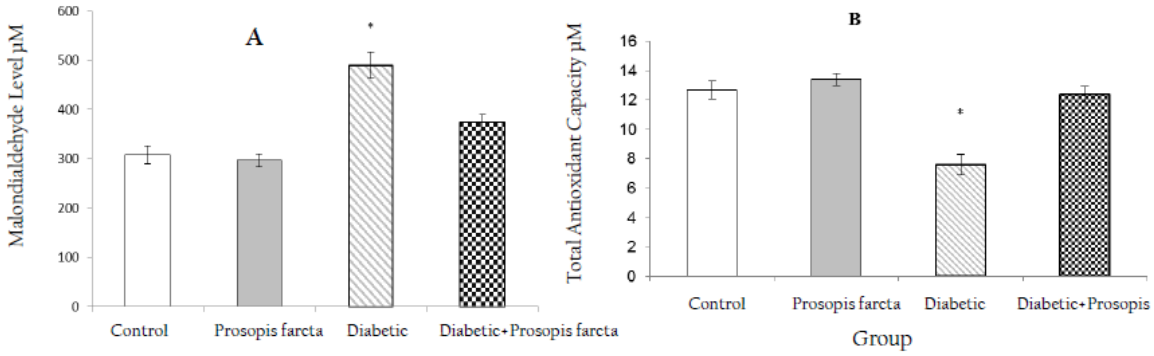

Figure 4. The comparison of Mean malondialdehyde Level (A) and Total Antioxidant Capacity (B) between the studied groups. All values are shown as mean ± Mean standard error, *: indicates a significant difference with the control group at the level of p<0.05.

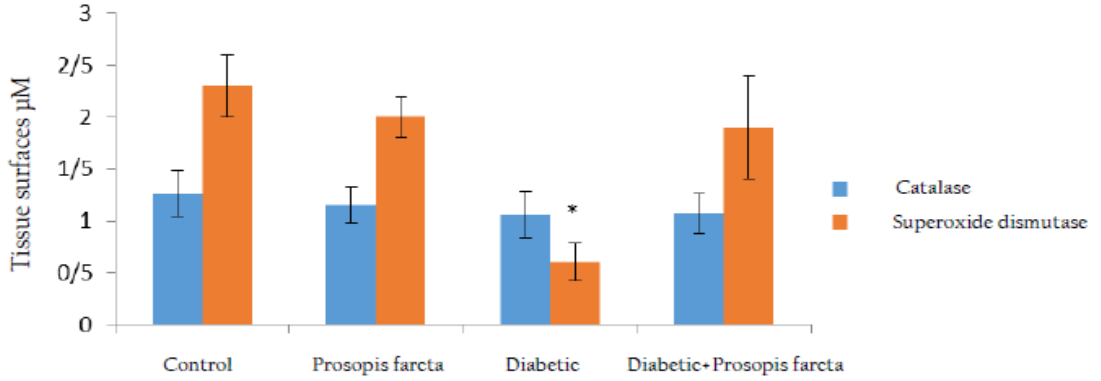

Figure 5. The effect of *Prosopis farcta* on the levels of testicular tissue of superoxide dismutase and catalase in different groups. Data are presented as mean ± standard error of the mean, *: indicates a significant difference with the control group at the level of p <0.05.

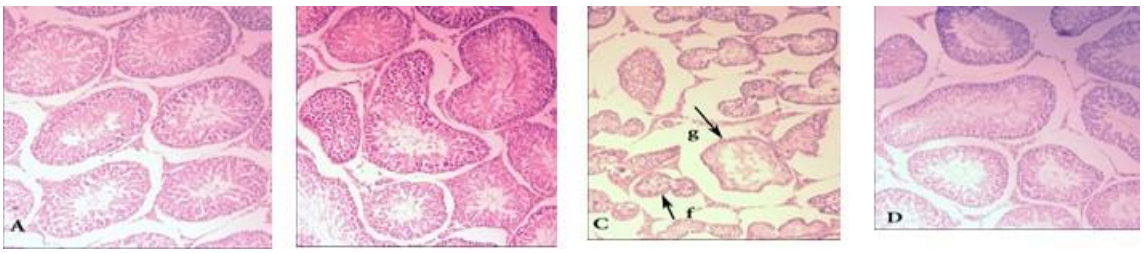

Figure 6. Testicular tissue sections (hematoxylin-eosin staining, 10-x magnification). A: Control group: seminiferous tubules have structural order and tissue integrity, and interstitial cells have normal appearance. B: The *Prosopis farcta* group has a cellular structure in normal seminiferous tubules and is similar to the normal group. C: The Diabetic group, (g) disrupted cellular order in seminiferous tubules, and (f) degeneration of cells and a significant reduction in the number of spermatocytes. D: The diabetic group treated with *Prosopis farcta* (300 mg / kg), the structure of the seminiferous tubules and cellular order is improved.

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Discussion

The present study showed that treatment with *Prosopis farcta* extract (300 mg / kg for 30 days) increased total antioxidant capacity and superoxide dismutase levels of testicular tissue and increased serum testosterone levels, motility and sperm count. On the other hand, the *Prosopis farcta* reduced the damage caused by diabetes in testicular tissue. Previous studies have shown that STZ causes a significant reduction in insulin secretion and diabetes induction by damaging beta cells of islets of langerhans (20, 21). In STZ-induced diabetes models, damage to the male reproductive system is associated with increased oxidative stress in testicular tissue, both in the initial phase (first week) and in the advanced phase (sixth week) of diabetes. The induction of diabetes in animals causes body weight loss, sexual organs weight loss, and the number and mobility of sperm (22). In addition, lowering serum testosterone concentrations in diabetic rats has also been reported (4). In a study by Lotfi et al. on the effect of STZ-induced diabetes, serum testosterone levels, testicular weight, seminiferous tubules diameter, and total sperm count decreased (16) and our results were consistent with these results in terms of reduction in testosterone, mobility, and total sperm count.

The study of Molan et al. showed that the *Prosopis farcta* extract has the ability to clear free oxygen radicals and this extract can reduce the activity of α-glucosidase and α-amylase enzymes, which confirms the traditional use of this plant for the treatment of diabetes. Diabetes increases the level of lipid peroxidation and decreases the activity of the superoxide dismutase enzyme in the testicular tissue of diabetic rats (23, 24). In the present study, the administration of hydroalcoholic extract of *Prosopis farcta* fruit could reduce lipid peroxidation in testicular tissue. Therefore, it is likely that the protective effects of the testosterone extract on testicular tissue are due to the presence of antioxidants (quercine and apigenin) in the plant, which probably prevents the oxidative stress caused by diabetes by increasing the total antioxidant capacity of the testicle and prevents tissue changes. Previous studies have shown that quercine has a positive effect on serum testosterone levels in men (25). The present study showed that extract of *Prosopis farcta* in diabetic rats increased testosterone levels and may thereby protect testicular tissue and reduce the complications of diabetes. Previous researches have shown that *Prosopis farcta* extract in rats increase the expression of pyruvate kinase gene, which may decrease glucose and lipid peroxidation (26).

In the present study, administration of the extract of *Prosopis farcta* could reduce the amount of testicular lipid peroxidation in diabetic rats. The extract also increased total antioxidant capacity and superoxide dismutase level in diabetic rats. The beneficial effects of *Prosopis farcta* in this study can be attributed to the antioxidant compounds in it. Diabetes causes the testicular tissue changes by reducing the diameter of the seminiferous tubules and reducing the spermatogenic cell population (5), which was also observed in the present study. On the other hand, the treatment of diabetic rats with *Prosopis farcta* extract reduces the damage to testicular tissue and cell-based cells. These results indicate the protective effects of *Prosopis farcta* on testicular tissue in diabetic rats.

As a result, the hydroalcoholic extract of the *Prosopis farcta* fruit improves the sperm parameters, the antioxidant status of the testicular tissue and reduces oxidative stress. *Prosopis farcta* probably inhibits the damage of free radicals caused by diabetes and reduces the destruction of testicular tissue with its antioxidant properties.

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