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Evaluation of the Effect of Ferula Rigidula Extract on Sperm Parameters, Antioxidant Parameters and Testicular Structure in Male Rats in Experimental Diabetic Condition

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ABSTRACT The study was conducted to investigate how Ferula rigidula extract affected sperm profile, antioxidant parameters, and stereological profile in experimental diabetic rats. Performed on forty-nine male rats. The rats were randomly assigned to control group, diabetic group, diabetic + Ferula rigidula group 1, diabetic + Ferula rigidula group 2, diabetic + glibenclamide group, Ferula rigidula group 1, and Ferula rigidula group 2. While sperm count, motility, antioxidant parameters, testosterone hormone, germinal epithelial volume, and germinal epithelial height decreased in the diabetic group, abnormal sperm count, malondialdehyde level, and lumen volume increased. When Ferula rigidula (extract) was given to diabetic rats, it brought the stereological findings to the same level as the control group. In addition, it was determined that there were improvements in biochemical parameters, approaching the values of the control group. Specifically, when Ferula rigidula extract was administered alone, testosterone levels and stereological findings improved in group 1. In addition, it was determined that there were significant improvements in sperm parameters. However, it was determined that the positive effect of Ferula rigidula extract was very significant at low doses (250 mg/kg) and decreased at high doses (500 mg/kg). As a result, Ferula rigidula extract has an antioxidant role and can be used to alleviate the problems caused by diabetes in the male reproductive system.

Keywords: *Diabetes, Ferula, Rat, Sperm.*

ÖZ

Deneysel Diyabetik Durumda Erkek Sıçanlarda Ferula Rigidula Ekstraktının Sperm Parametreleri, Antioksidan Parametreler ve Testis Yapısı Üzerine Etkisinin Değerlendirilmesi

Çalışma, deneysel diyabetik sıçanlarda Ferula rigidula ekstraktının sperm profilini, antioksidan parametreleri ve stereolojik profili nasıl etkilediğini araştırmak için yapıldı. Kırk dokuz erkek sıçan üzerinde gerçekleştirildi. Sıçanlar kontrol grubu, diyabetik grup, diyabetik + Ferula rigidula grup 1, diyabetik + Ferula rigidula grup 2, Diyabetik + glibenklamid grubu, Ferula rigidula grup 1 ve Ferula rigidula grup 2 olarak rastgele ayrıldı. Diyabetik grupta sperm sayısı, motilite, antioksidan parametreler, testosteron hormonu, germinal epitel hacmi ve germinal epitel yüksekliği azalırken, anormal sperm sayısı, malondialdehit düzeyi ve lümen hacmi arttı. Diyabetik ratlara Ferula rigidula (ekstrakt) verildiğinde stereolojik bulguları kontrol grubu ile aynı düzeye getirdi. Ayrıca biyokimyasal parametrelerde kontrol grubu değerlerine yaklaşacak şekilde iyileşmelerin olduğu tespit edildi. Özellikle, Ferula rigidula ekstresi tek başına uygulandığında, 1. grupta testosteron düzeylerinde ve stereolojik bulgularda iyileşme oldu. Bunun yanı sıra sperm parametrelerinde anlamlı olarak düzelmelerin olduğu belirlendi. Ancak, Ferula rigidula ekstraktının olumlu etkisinin düşük dozda (250 mg/kg) çok belirgin olduğu, yüksek dozlarda (500 mg/kg) ise azaldığı tespit edildi. Sonuç olarak, Ferula rigidula ekstresi antioksidan role sahiptir ve erkek üreme sisteminde diyabetin neden olduğu sorunları hafifletmek için kullanılabilir.

Anahtar Kelimeler: *Diyabet, Ferula, Sıçan, Sperm.*

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INTRODUCTION

Diabetes mellitus is a metabolic disease that is characterized by hyperglycemia in particular and affects the male reproductive system at various levels (Vlad and Popa 2016). It is known that prolonged hyperglycemia causes deterioration in libido and spermatogenesis (Mulholland et al. 2011; Ghasemi et al. 2016). Diabetic condition causes excessive oxygen radical production in many tissues such as testis and epididymis (Jain and Jangir 2014). The resulting oxidative stress leads to spermatogenic dysfunction, testicular dysfunction and hypogonadism (Feyli et al. 2017; Shi et al. 2017).

Research results have indicated that diabetes reduces sperm count, motility and viability and causes significant increases in abnormal sperm count and malondialdehyde (MDA) level (Jiao et al. 2020; Sahu et al. 2020). In addition, diabetic people suffer from decreases in follicle stimulating hormone, luteinizing hormone and testosterone levels as well as body and testicular weight (Jiao et al. 2020).

Although many chemicals have been synthesized for therapeutic purposes, herbal medicines have always maintained their importance. Turkey has a great tradition of folk medicine due to its diverse flora (Tufan et al. 2018). Some Ferula species (Ferula caspica Bieb: Stomach pain, gynecological diseases and diabetes; Ferula elaeochytris Korovin: Aphrodisiac; Ferula rigidula DC: Diabetes, hypercholesterolemia) are used in the traditional medicine especially in Eastern Anatolia (Altundağ 2011). Bagheri et al. (2015) found that administration of Ferula assa foetida increased sperm count, motility, morphology and viability in rats.

Glibenclamide, which works by stimulating insulin secretion, is an antidiabetic agent (Serrano-Martín et al. 2006). Although it is preferred by diabetics, there is no study reporting that glibenclamide has antioxidant properties. It is known that the formation of reactive oxygen species in diabetes induced by streptozotocin administration exceeds physiological levels (Bolzan and Bianchi 2002). Therefore, the combined use of antioxidant agents and antihyperglycemic agents may be a correct approach to minimize the possible problems of free oxygen radicals that occur at pathological levels in diabetes.

Ferula rigidula, which is preferred in traditional medicine, is consumed by diabetics, especially in the eastern Turkey. However, there is no scientific report in the literature to support this traditional use. The effect of Ferula rigidula on sperm profile, antioxidant parameters, testosterone level and stereological profile in diabetic rats was evaluated. This study will help close the gap in this field.

MATERIAL AND METHODS

Collection of Plant material and Preparation of the extract

Ferula rigidula plant was collected from districts of Van Province between May-June of the year as a result of taxonomic analysis. Its stem and leaf parts were washed with distilled water. They were then dried in a shade and dry environment. After they were dried and pulverized with a grinder, the extract was prepared by following the steps in the method described by (Farkhad et al. 2012).

Animal Material

Forty nine rats weighing 200-300 g were supplied from Van Yuzuncu Yıl University Experimental Research Center. They were weighed and clinically examined. Appropriate conditions for temperature, humidity and light were provided and daily observations were made on them. There were given feed and drinking water ad libitum. Experimental studies were carried out in the same center. The study procedures were approved by the ethics committee (Van Yuzuncu Yıl University Animal Experiments Local Ethics Committee, Van, Turkey, Approval number: 2020/08-05), and the principles of Care and Use of Laboratory Animals were followed in all applications.

Diabetes Induction

Basal glucose levels were determined before administration of streptozotocin. Later, 45 mg/kg streptozotocin was administered intraperitoneally (i.p) to the rats which were fasted the night before. Blood glucose level was measured 72 hours after administration of streptozotocin. Those who showed a glucose level above 200 mg/dL in the measurement were considered as diabetic (Naghibi et al. 2022).

Experimental Planning

Experimental groups in this study were 7 groups including an equal number of rats in each group.

Control group (n=7): No specific application was made except for giving feed and drinking water.

Diabetic group (n=7): Streptozotocin 45 mg/kg i.p. administered and diabetes was induced.

Diabetic+Ferula rigidula group 1 (n=7): Ferula rigidula was administered to rats with diabetes induction at a dose of 250 mg/kg via gastric gavage.

Diabetic+Ferula rigidula group 2 (n=7): Ferula rigidula was administered at a dose of 500 mg/kg by gastric tube to diabetes-induced rats.

Diabetic+glibenclamide group (n=7): 5 mg/kg glibenclamide was administered by gastric tube to diabetes-induced rats (Andrade-Cetto 2011).

Ferula rigidula group 1 (n=7): Ferula rigidula was administered via gastric gavage at a dose of 250 mg/kg.

Ferula rigidula group 2 (n=7): Ferula rigidula was administered at a dose of 500 mg/kg via gastric gavage.

All rats were anesthetized 24 hours after the last application, and after blood collection and testicular tissue collection, the experimental application was terminated upon sacrifice of the rats.

Spermatological Evaluation

While the rats were anesthetized, motility examination was performed from the cauda epididymis of the testis removed from the scrotal sac (before the body and testicles were cooled). Spermatological evaluation was performed as in our previous study (Belhan et al. 2020).

Biochemical Evaluation

While testosterone was measured in serum, antioxidant parameters were examined in testicular homogenate. First, the mass of the removed testicular tissue was determined and phosphate buffer (pH: 7.4) was added. It was then homogenized using a homogenizer (Samarghandian et al. 2015). Glutathione (GSH), MDA, Catalase (CAT) and Superoxide dismutase (SOD) were measured using spectrometric method according to the methods given, respectively (Beutler et al. 1963; Aebi 1984; Sun et al. 1988; Dubovskiy et al. 2008). A commercial kit from Abbott was used to measure testosterone. The measurement was made through the ARCHITECT c1616200 autoanalyzer.

Stereological Evaluation

Randomly isotropic identical sections were taken from testicular tissue. The orientation method was used to take the sections. 8-10 tissue samples were taken from each rat and embedded in paraffin blocks. 10-15 consecutive sections were obtained from these blocks. The sections were then stained by using Masson's trichrome dye and evaluated in a microscopic environment (light microscope).

Estimation of Volume density and Germinal epithelial height

The volume density of the germinal epithelium, lumen and interstitial tissue was calculated using the dot grid (Figure 1A) and the following formula (Mayhew and Gundersen 1996).

$$\text{The volume density} = \frac{V_{(\text{Structure})}}{V_{(\text{Testis})}} \times 100$$

Germinal epithelium height was estimated according to previously described methods (Gundersen et al. 1988; Noorafshan 2014). Attention was paid to associate each test line with a point (Figure 1B).

Histopathological Evaluation

Samples followed for tissue were embedded in paraffin blocks. Later, sections (4 μm) were stained through Masson trichrome dye and evaluated microscopically (Light microscope-Nikon Y-IM 7551012, Japan). The findings was assessed semi-quantitatively.

Statistical Analysis

Statistical analysis was carried out by using the package program (SPSS 21.0). ANOVA (One Way Analysis of Variance) and Post-Hoc Tukey test were applied for sperm profile and histopathological parameters. For biochemical parameters, ANOVA and Duncan's test were used. Data were expressed as mean±standard deviation (SD). Data with $p \leq 0.05$ were considered significant.

RESULTS

Sperm Findings

Table 1 shows findings related to sperm profile. While the diabetic group had lower sperm motility and density, the abnormal sperm count was higher ($p<0.05$). Sperm motility and density increased significantly in rats treated with Ferula rigidula (250 mg/kg), while the abnormal sperm count decreased significantly. However, motility and sperm density were significantly lower in rats treated with Ferula rigidula of 500 mg/kg than the controls ($p<0.05$). No significant difference was detected between diabetic, diabetic + Ferula rigidula and diabetic + glibenclamide groups in terms of sperm motility, sperm density and abnormal sperm count ($p>0.05$).

Table 1: Effects of glibenclamide and Ferula rigidula on sperm profile in diabetic rats.

Groups	Motility (%)	Density (x10⁶)	Abnormal sperm rate (%)		
			Head	Tail	Total
Control	73.57±2.43^{b}	111.14±1.21^{b}	5.71±0.75^{c}	5.00±0.57^{c}	10.71±0.75^{c}
Diabetic	27.85±2.67^{d}	33.14±0.69^{d}	25.00±1.29^{a}	23.42±0.78^{a}	48.42±1.27^{a}
Diabetic+Ferula rigidula (250)	28.57±2.43^{d}	33.57±0.53^{d}	23.85±1.06^{a}	23.85±1.34^{a}	47.71±1.25^{a}
Diabetic+Ferula rigidula (500)	26.42±2.43^{d}	34.00±0.81^{d}	24.57±0.97^{a}	23.71±0.75^{a}	48.28±1.11^{a}
Diabetic+Glibenclamide	25.71±1.88^{d}	32.42±0.53^{d}	24.71±0.95^{a}	24.42±0.97^{a}	49.14±0.69^{a}
Ferula rigidula (250)	81.42±3.77^{a}	130.00±1.52^{a}	4.00±0.57^{d}	4.42±0.78^{c}	8.42±0.53^{d}
Ferula rigidula (500)	58.57±2.43^{c}	91.14±0.89^{c}	8.00±0.57^{b}	8.00±0.81^{b}	16.00±1.00^{b}

The letters a, b, c, d denote the statistical difference between different groups in the same column ($p<0.05$).

Biochemical Findings

Table 2 shows findings on biochemical parameters. In the present study, the diabetic group had significantly lower level of testosterone ($p<0.05$). Testosterone value of Ferula rigidula group 1 (250 mg/kg) was significantly higher when compared to the value of the control group ($p<0.05$). The diabetic + Ferula rigidula group had higher testosterone value when compared to the diabetic + glibenclamide group ($p>0.05$) however it was not statistically significant. GSH, CAT, SOD values increased and MDA levels decreased in diabetic + Ferula rigidula group compared to the diabetic group.

Stereological Findings

As seen in Table 3, germinal epithelial thickness significantly decreased in the diabetic group and the diabetic + glibenclamide group ($p<0.05$). On the other hand, these parameters significantly increased in in the diabetic + Ferula rigidula (250 mg/kg) and diabetic + Ferula rigidula (500 mg/kg) groups than the diabetic group ($p<0.05$). No significant difference was detected among the groups.in terms of the interstitial space volume ratio. The volume density of the germinal epithelium, lumen, and interstitial tissue and the height of the germinal epithelium are presented in Figure 1.

Histopathological Findings

Control group had a normal histological structure. All spermatogenic cells and Sertoli cells were observed in the germinal epithelium. Normal connective tissue cells were observed along with Leydig cells in the interstitial area (Figure 2NC). Upon comparison made between the diabetic and diabetic + glibenclamide groups and the control group, it was found that the number of germinal epithelial layers decreased, seminiferous tubules had degeneration and atrophy, and the basal lamina got thickened and the area between sertoli and spermatogenic cells opened up (Figure 2DC, DG). Diabetic + Ferula rigidula (250 mg/kg) (Figure 2DFR1), diabetic + Ferula rigidula (500mg/kg) (Figure 2DFR2), Ferula rigidula (250 mg/kg) (Figure 2FR1) and Ferula rigidula (500 mg/kg) (Figure 2FR2) groups were histopathologically similar to the control group.

Table 2: Effects of glibenclamide and Ferula rigidula on biochemical profile in diabetic rats.

Groups	GSH (µmol/g)	CAT (U/g)	MDA (nmol/g)	SOD (U/g protein)	Testosterone (nmol/L)
Control	6.82±0.05^{a}	271.88±3.55^{a}	23.77±1.18^{c}	605.87±10.79^{a}	2.14±0.39^{b}
Diabetic	4.66±0.27^{e}	161.55±5.28^{d}	42.83±1.75^{a}	426.24±10.09^{f}	0.31±0.02^{d}
Diabetic+Ferula rigidula (250)	5.51±0.19^{b,c,d}	187.47±4.09^{b,c}	33.29±1.33^{b}	471.68±8.07^{d,e}	0.43±0.02^{d}
Diabetic+Ferula rigidula (500)	5.13±0.15^{d,e}	169.63±5.54^{c,d}	40.74±0.98^{a}	451.52±7.27^{e,f}	0.32±0.01^{d}
Diabetic+Glibenclamide	5.68±0.23^{b,c}	191.86±6.23^{b}	31.25±0.89^{b}	511.09±3.18^{c,d}	0.24±0.02^{d}
Ferula rigidula (250)	6.03±0.13^{b}	271.75±10.20^{a}	24.63±0.60^{c}	557.65±23.52^{b}	3.01±0.20^{a}
Ferula rigidula (500)	5.43±0.09^{c,d}	206.88±7.73^{b}	26.11±0.64^{c}	537.17±22.64^{b,c}	1.14±0.18^{c}

The letters a, b, c, d, e denote the statistical difference between different groups in the same column ($p<0.05$). **GSH:** Glutathione, **CAT:** Catalase, **MDA:** Malondialdehyde, **SOD:** Superoxide dismutase.

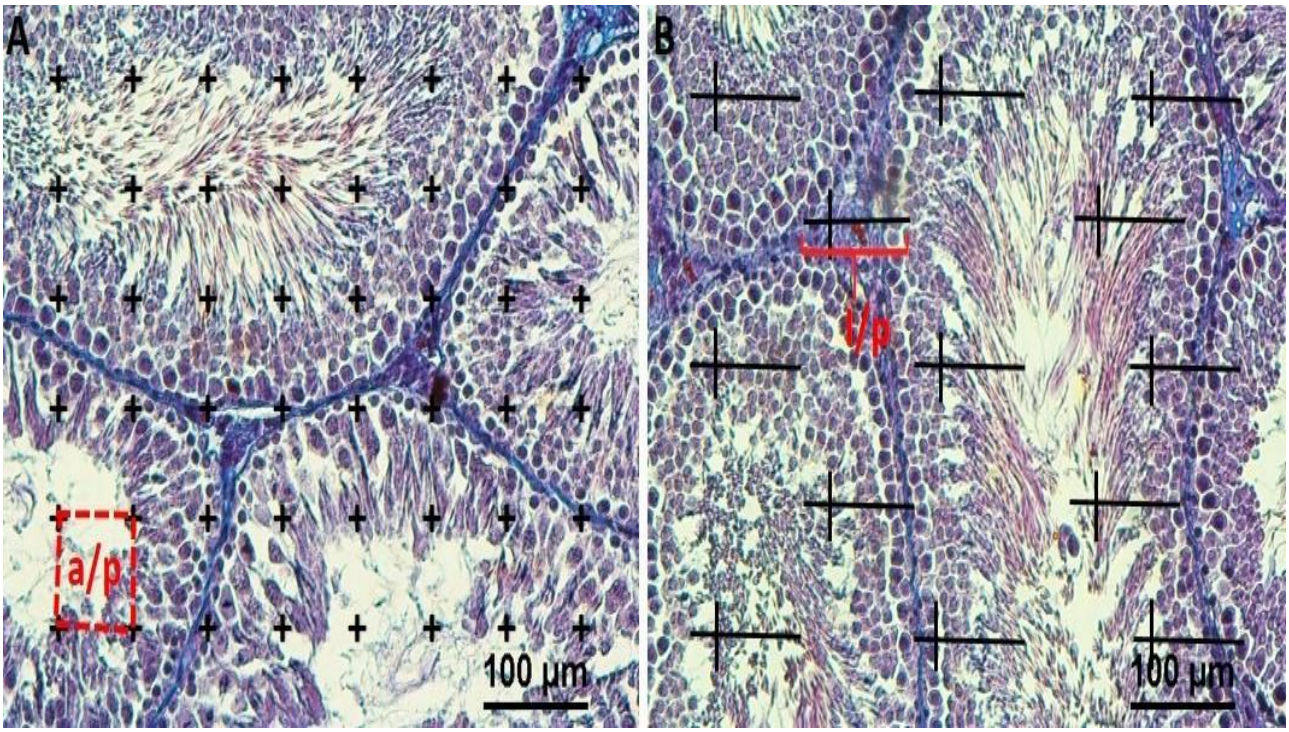

Figure 1: A. The superimposed of a point grid on the image of section. **B**. The superimposed of a grid of test lines on the image of section.

Table 3: Effects of glibenclamide and Ferula rigidula on stereological profile in diabetic rats.

Groups	Germinal epithelial volume (mm3)	Interstitial tissue volume (mm^3)	Lumen volume (mm^3)	Germinal epithelial height (µm)
Control	58.23±2.00^b	16.43±3.24	25.33±2.45^b	61.19±2.77^b
Diabetic	52.83±7.33^a	15,36±5.31	31.79±3.52^a	51.04±2.08^a
Diabetic+Ferula rigidula (250)	59.71±7.87^b	14.58±3.97	25.72±4.67^b	58.89±3.05^b
Diabetic+Ferula rigidula (500)	59.67±7.81^b	14.47±3.82	27.18±1.14^b	59.67±2.89^b
Diabetic+Glibenclamide	51.76±1.87^a	14.79±1.30	32.44±1.71^a	53.01±3.59^a
Ferula rigidula (250)	60.05±5.41^b	15.14±6.25	24.80±1.08^b	62.05±3.64^b
Ferula rigidula (500)	59.69±5.59^b	14.32±4.82	26.14±1.40^b	63.46±3.76^b

The letters a, b denote the statistical difference between different groups in the same column ($p<0.05$).

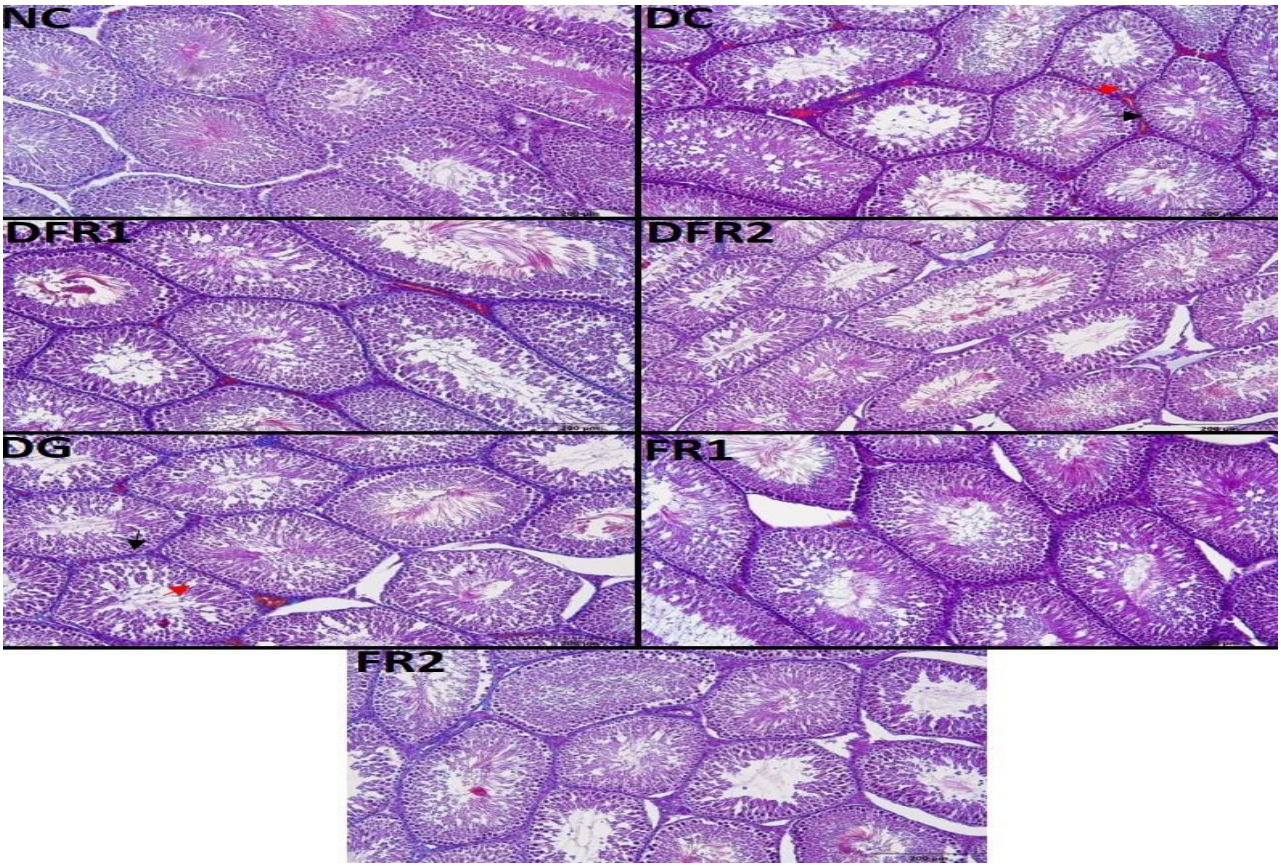

Figure 2: Photomicrographs of testicular sections. NC; in normal histological appearance. DC; there was a decrease in the number of epithelial layer, opening in the lumen, degeneration and atrophy in the tubules. D+FR1, D+FR2 and D+G; there was a decrease in the number of epithelial layer, opening in the lumen, degeneration and atrophy in the tubules. FR1; a slight increase in the number of epithelial layer compared to the control group. FR2; moderate increase in the number of epithelial layer compared to the control group. (**NC;** normal control, **DC;** Diabetic control, **D+FR1;** Diabetic + Ferula Rigidula 250 mg / kg , **D+FR2;** Diabetic + Ferula Rigidula 500 mg / kg , **D+G;** Diabetic + Glibenclamide, **FR1;** Ferula Rigidula 250 mg / kg, **FR2;** Ferula Rigidula 500 mg / kg).

DISCUSSION AND CONCLUSION

Many natural resources have a biological activity (Mohammed et al. 2020; Sevindik 2020). Today, many people increasingly tend to use phytotherapy due to a number of difficulties they experience in modern medicine (Dahl 2001). However, scientific research needs to show how people here in Turkey - just as in other parts of the world - use herbal products for therapeutic purposes and how that impacts their health (Sucaklı et al. 2014). Although testicular dysfunction caused by diabetes is not a life-threatening factor, associated psychological and emotional problems are of great importance (Zhang et al. 2020). This is because sexual dysfunction seriously affects the quality of life of men.

When the sperm profile was evaluated, it was found that the diabetic group had lower sperm motility and density compared to the controls, and the abnormal sperm count was higher. This result is compatible with the diabetes studies (Jiang et al. 2020; Jiao et al. 2020; Sahu et al. 2020). The undesirable result obtained regarding sperm parameters in the diabetic group can be explained by the disrupted activities of the hypothalamic -pituitary-gonadal axis (Brüning et al. 2020). It was determined that the motility obtained by administrating the glibenclamide in the diabetic group was lower than the motility value of the diabetic group, although it was not significant. The decrease in sperm motility in rats treated with glibenclamide may be a result of oxidative stress because streptozotocin damages the DNA of pancreatic beta cells and causes the formation of reactive oxygen species (Bolzan and Bianchi 2002). It is an expected result that glibenclamide, which does not have anti-oxidant properties, cannot protect diabetic rats from oxidative stress (Odo et al. 2018). Sperm motility value was found to be significantly higher in ferula rigidula group 1 (250 mg/kg) than the control group. This situation can be associated with the antioxidant properties of Ferula rigidula (Köse and Ocak 2018). However, Ferula rigidula decreased the motility significantly in ferula rigidula group 2 (500 mg/kg) than the control group. This result reveals the importance of the dose. When examining sperm density, it was determined that while 250 mg/kg dose of Ferula rigidula significantly increased sperm count compared to the control group, 500 mg/kg dose significantly decreased sperm count. This indicated the importance of administration dose of Ferula rigidula. Bagheri et al. (2015) found that Asafoetida derived from some Ferula species (F. assa - foetida and Ferula foetida, Ferula rubricaulis, Ferula rigidula, Ferula alliacea) increased sperm count at all doses (25, 50, 100, 200). In the present study, the effect of Ferula rigidula at the dose of 250 mg/kg on sperm morphology was remarkable. However, the same dose did not have any positive effect on diabetic rats. Sperm morphology was highly impaired in the diabetic group, which is compatible with previous studies (Jiang et al. 2020; Sahu et al. 2020).

In the present study, the testosterone levels of the diabetic group were low, which is compatible with previous studies (Jiao et al. 2020; Sahu et al. 2020). Low testosterone level is likely to be associated with the change in proliferation and differentiation of Leydig cells due to diabetes. It was found in the present study that the Ferula rigidula (250 mg/kg) group 1 had significantly higher testosterone level compared to the control group. The decrease in testosterone level when Ferula rigidula was administered at a dose of 500 mg/kg is consistent with the testosterone result that Ayoubi et al. (2013) received when they administrated asafoetida at high doses (300 mg/kg). Both studies reported lower testosterone levels at high doses. This situation may be associated with vacuuming of Leydig cells (Bagheri et al. 2015). The testosterone value of the diabetic + Ferula rigidula group was higher than the value of the diabetic + glibenclamide group but it was not significant. This may be due to the fact that while Ferula rigidula has antioxidant properties, glibenclamide does not have antioxidant properties (Köse and Ocak 2018; Odo et al. 2018).

In the present study, it was found that while both doses of Ferula rigidula administrated to diabetic rats increased GSH, CAT, SOD values compared to the diabetic group, it decreased the MDA level. It is possible to see the expected effect of ferula rigidula in diabetic rats particularly at a dose of 250 mg/kg. As a matter of fact, the GSH, CAT, SOD values in the diabetic + Ferula rigidula (250 mg/kg) group 1 were significantly higher than the diabetic group, while the MDA value was significantly lower. This result is compatible with the previous study (Yusufoglu et al. 2015). In the ferula rigidula group 1 (250 mg/kg), the CAT, MDA, GSH and SOD values were similar to values of the control group, which show us the antioxidant power of the relevant dose. As a matter of fact, it has been reported that Ferula rigidula has antioxidant properties (Köse and Ocak 2018).

When examining stereological findings in diabetic and control groups, it was found that the germinal epithelial height and germinal epithelial volume decreased in the diabetic group, which is compatible with other studies (Kianifard et al. 2011; Keyhanmanesh et al. 2018; Gholizadeh et al. 2019; Hosseinipour et al. 2019). It has been reported that apoptosis occurring during the course of spermatogenesis is effective in this decrease (Hosseinipour et al. 2019), resulting in suppression of the activity of spermatogenic cells (Kianifard et al. 2011). Interstitial tissue volume did not change in the present study, which is compatible with other studies (Gholizadeh et al. 2019; Hosseinipour et al. 2019). It was found that the lumen volume increased in the diabetic group than the control group, and this result supports a previous study (Keyhanmanesh et al. 2018).

The results of this study showed that negative changes on sperm parameters in streptozotocin induced diabetic rats were significantly restored by administration of Ferula rigidula extract (250 mg/kg). It is especially important that its effect on sperm parameters has been studied for the first time. Positive changes in antioxidant enzyme levels suggested that the extract may have antioxidant properties. As a result of the findings of the present study, it is of great importance in terms of revealing the scientific validity of the use of Ferula rigidula extract among the public and shedding light on the studies to be conducted. However, it can be asserted that the therapeutic effect of Ferula rigidula extract depends on dose.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

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AUTHOR CONTRIBUTIONS

Idea / Concept: SB, UO
Supervision / Consultancy: SB
Data Collection and / or Processing: SB
Analysis and / or Interpretation: SB, UO, AUK, FA
Writing the Article: SB
Critical Review: SB, UO, AUK, FA, YD

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