Original Article

Ameliorating Effects of *SaturejaedmondiBriq* Essential Oil on Reproductive Parameters in Pentylenetetrazol Induced Epilepsy Model in Adult Male Rats

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Abstract

Background& Objective: Epilepsy is a common neurological disorder that causes unpredictable, recurrent seizures. Oxidative stress and epilepsy have negative effects on the reproductive system and cause infertility. Antioxidants are effective in reducing infertility disorders. *Satureja edmondi Briq* is known as a powerful antioxidant that can reduce the effects of oxidative stress. The aim of this study was to evaluate the effects of *Saturejaedmondi* on reproductive parameters in pentylenetetrazol (PTZ)-induced epileptic and normal male rats.

Materials & Methods: A total of 48 Wistar adult male rats were used in this experimental study. Animals were randomized into normal and kindled groups that were treated with 150 and 300 *ppmS. edmondi* essential oil. At the end of the study, the rats' testes were removed following anesthesia. The testes were weighed, and sperm parameters were measured. Data were analyzed by one-way ANOVA test, and $P<0.05$ was considered statistically significant.

Results: This study showed that sperm parameters, including sperm count, sperm viability, and progressive sperm motility significantly decreased in epileptic rats compared to normal rats ($p\leq.001$). These parameters significantly increased at 150 and 300 ppm doses of S. *edmondi* essential oil in the Kindled groups) $p\leq.001$ (. Sperm parameters significantly increased at 300 ppm dose of S. *edmondi* essential oil in normal groups ($p\leq.001$). Moreover, changes in testis weight were not significant in the treatment groups.

Conclusions: *Satureja edmondi Briq,* with abundant antioxidant compounds, improves the pathological changes induced by testicular oxidative stress in tic rats. S. *edmondi* as a good source of antioxidants can improve sperm parameters and reproductive potential in PTZ-induced epileptic male rats.

Keywords: Epilepsy, Satureja edmondiBriq, infertility, reproductive parameters, rat

Introduction

Epilepsy is one of the most common central nervous system disorders after stroke that affects the people's lives. It is caused by unusual electrical activities in the brain. Epilepsy is determined by uncontrolled and recurrent seizures that disrupt the brain activity and mental and physical functions (1). Continuous seizures for at least 30 minutes and discontinuous seizures shorter than 30 minutes (even 5 min) are defined as status epilepticus (2). Seizure symptoms are variable from person to person. Epilepsy can cause periods of unusual behavior, uncontrollable movement of arms and legs,

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and sometimes loss of awareness.

Pentylenetetrazol (PTZ) is a chemical that stimulates seizure in the central nervous system and is used as a convulsing drug in experimental studies. It is the most preferred and common drug for kindling (3).The results of the studies indicate that sexual disorder occurs in both genders (men and women) in patients with epilepsy. Sexual dysfunction is known as hyposexuality, hyposexual activity, or decreased sexual interest and awareness (4). Reproductive disorders, sexual dysfunction, and infertility have been noted in men with epilepsy and have been attributed to epilepsy and antiepileptic drugs (5). Epilepsy and antiepileptic drugs may lead to sexual dysfunction, dysregulation of sex hormones, and alterations in semen analysis in male patients with epilepsy, especially in young male patients. Infertility has been confirmed to be associated with epilepsy and antiepileptic drugs (6). Epilepsy has negative effects on the male reproductive parameters in epileptic human and epilepsy models in animals (7, 8).

Experimental studies suggest that oxidative stress is a possible mechanism and an important factor that contributes to the development of epilepsy. Oxidative stress is basically involved in seizures and epilepsy (9). Antioxidant compounds can delay or inhibit oxidative damage to a target molecule and scavenge free radicals. Experimental evidence shows that antioxidant therapy may reduce the negative effects of oxidative free radicals in chemical kindling and seizure models in animals, especially in rats (10, 11). Some plants are known as powerful antioxidants that have been used for the treatment of wounds and illnesses since ancient time. Many herbs are known to have antioxidant effects on the growth and immunological parameters in someanimals (12, 13).

Satureja edmondi Briq is belongs to the Lamiaceae family and usually grows on rocks in the west of Iran, Kermanshah. S. edmondi is often used to treat digestive and respiratory disorders such as enteritis, chronic ulcer, and asthma. It can also help to treat nervous dysfunctions, neuralgia. *S. edmondi* is also known to have antimicrobial, antifungal, anti-inflammatory, and specially antioxidant effects. *Satureja edmondiBriq* contains many antioxidant compounds such as p-Cymene, gamma terpinene, thymol, and alpha-Terpineol, which act as a natural antioxidant (14). The antimicrobial effects of *Saturejaedmondi* essential oil on *Staphylococcus aureus* in food products have been studied. This study showed that *S. edmondi* essential oil could be used to inhibit the growth of *S. aureus* in food products (15). *S. edmondi* has been observed to have a positive effect on memory impairment induced by chemical kindling in adult male rats (16). The effect of *Satureja edmondi* on sexual hormones in epileptic and normal male rats has also been researched, indicating its positive effect on testosterone and lutein hormone (LH) in epileptic rats compared to normal rats (17). Due to the effect of epilepsy and antiepileptic drugs on infertility in epileptic patients and the use of medicinal plants to treat infertility and increase fertility and the weakness of semen in antioxidant defense in epileptic patient, finally the positive effect of the *Satureja edmondi* on reducing seizures, this study aimed to evaluate the effect f *Satureja edmondi Briq essential* oil on reproductive and sperm parameters in normal and pentylenetetrazole-induced epileptic male rats.

Materials & Methods

Preparation of plant and essential oil

SaturejaedmondiBriq was obtained from the mountains of Kermanshah, Iran. It was

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identified and authenticated by the herbarium of Islamic Azad University of Kermanshah. The plant was dried in the shade at a proper temperature and crushed. Essential oil extraction was performed by steam distillation and a Clevenger-type apparatus. The obtained essential oil was stored in a closed container at 4 °C away from sunlight. The chemical composition and concentrations of the Saturejaedmondiessential oil were evaluated and identified by gas chromatography/mass spectrometry (GC/MS) (15).

Animals and experimental design

A total of 48 Wistar adult male rats (240-260g) were purchased from Razi Institute (Tehran, Iran) and housed under controlled environmental conditions,12/12 h light/dark cycle and 22±2ºC temperature. Food and water were freely available throughout the study. The rats were kept in the laboratory one week before the start of the experiment so that they could adapt to the environment. The experiment protocol was approved by the ethics committee of animal research (IR.KUMS.REC.1398.977). The animals were randomly divided into normal and kindling groups. Rats were randomly divided into 6 groups, with 8 rats in each group, and treated for 28 days with different doses of Satureja edmondi Briq essential oil:

Group 1: Normal control group (without any essential oil)

Group 2: Normal group received 150ppm/kg essential oil daily

Group 3: Normal group received 300ppm/kg essential oil daily

Group 4: Kindled control group (without any essential oil)

Group 5: Kindled group received 150ppm/kg essential oil daily

Group 6: Kindled group received 300ppm /kg essential oil daily

Treatment groups were kindled by repetitive intraperitoneal injection of PTZ (25 mg/kg bodyweight) (Sigma, St. Louis, MO, USA),1ml/kg body-weight every 15 minutes until seizure occurred (two or three injections) (16).

The stages of epilepsy model by PTZ in rats in clued no answer in stage 0, ear and facial shrinking in stage 1, myoclonic convulsion with no rearing in stage 2, myoclonic convulsion and rearing in stage 3, turn over into side status and clonic-tonic seizures in stage 4, turn over into back status and generalized tonic-clonic seizures (GTCS) in stage 5. The rats reaching phase 4 or 5 were considered kindled rats. The kindled and normal treatment groups received different doses (150 and 300 ppm) of essential oil daily for 28 days. The normal and kindled control groups received normal saline intraperitoneally during the treatment. All experiments were carried out in the morning. At the end of the treatments, the animals were anesthetized with ketamine (2.5 mg/kg) and xylazine (30 mg/kg).

Semen analysis

After anesthesia with ketamine and xylazine, the rats' testes were removed and washed in normal saline solution (0.9%). Cauda epididymis was separated, and testis was weighed. After the cauda epididymis was separated, it was cut in Hams/f10, including 10% FBS, and was kept in an incubator (37˚C and 5% CO2) for thirty minutes. The prepared suspension was used for the analysis of sperm parameters, including sperm viability, motility, and count. To count the sperm cells, the suspension was pipette to both counting chambers of a hemocytometer. To determine the motility, one drop of the sperm cell suspension was placed on the chamber, and the motile and immotile sperm cells were analyzed by microscope at 40x magnification (18). To determine the

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sperm viability, cells on the four corners of the central square of Neubauer slide were counted, and data were expressed as the number of sperms per ml. To assess viability, a drop of semen was placed on a slide and stained with Eosin-Nigrosindye. The non-stained sperms were expressed as viable sperms (19).

Statistical analysis

The results were statistically analyzed by one-way analysis of variance (ANOVA) and were presented as Mean±SEM. Additional analysis for multiple comparisons was carried out by post-hoc Tukey test. In all evaluations, $p<0.05$ was considered statistically significant.

Results

The chemical composition of the *Satureja edmondi Briq* essential oil was identified by gas chromatography/mass spectrometry (GC/MS). The predominant compounds of *S. edmondi* include ethymol, gamma terpinene, p-Cymene, alpha-terpinene, and beta-myrcene. The results of testis weight (g) in the treatment groups are shown in Chart 1. Testis weight was decreased in the kindled group compared to the normal group. The kindled and normal groups receiving different doses of essential oil showed an increase in testis weight, but it was not significant. According to the results of this study, sperm count, sperm viability, and progressive sperm motility parameters increased in the normal group receiving 150 ppm *Satureja edmondi Briq* essential oil compared to the normal control group, but this increase was not significant. The group that received 300 ppm *S. edmondi* essential oil showed a statistically significant increase in these parameters compared to the control group ($p\leq0.001$). The results of these parameters in normal groups are presented in Table 1. The sperm parameters including sperm viability, motility, and count, significantly decreased in the kindled groups compared to the normal control group ($p\leq0.001$). The results of sperm count in the normal and kindled groups are shown in Chart 2.

Sperm count increased in kindled groups receiving 150 ppm of essential oil compared to the kindled control group ($p\leq0.01$). However, there was a significant increase in the kindled group receiving 300 ppm *Satureja edmondi Briq* essential oil compared to the kindled control group ($p\leq0.001$). The results of these parameters in kindled groups are shown in Table 2. Sperm viability and motility increased in the kindled groups that received 150 ppm and 300 ppm doses of *Satureja edmondi Briq* essential oil compared to the kindled control group ($p\leq0.001$). The results of these parameters in the kindled groups are shown in Table 2. The results of sperm viability and motility in the normal and kindled groups are shown in Chart 3and 4.

Table1. The effect of *Satureja edmondi Briq* essential oil on sperm parameters in normal groups. Data are expressed as Mean± SE. In comparison with control group, significant differences are shown by ***$p\leq0.001$

Sperm parameter	Control	150ppm	300ppm
Sperm count ($\times10^6$)	64.5± 1.1	67.3 ± 0.76	71.83 ± 0.79***
Viability (%)	72.3 ± 3.8	75 ± 0.57	79.3 ± 0.61***
High motility (%)	61.5 ± 0.96	64 ± 0.84	69.6 ± 0.84***

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Table 2. The effect of *Satureja edmondi Briq* essential oil on sperm parameters in the kindled groups. Data are expressed as Mean± SE. In comparison with control group, significant differences are shown by ***p≤0.001

Sperm parameter	Control	150ppm	300ppm
Sperm count ($\times 10^6$)	41.6 ± 1.05	46 ± 0.57**	52.6± 0.88***
Viability (%)	41.2 ± 1.2	47.4 ± 0.54***	58.6 ± 0.85***
High motility (%)	42 ± 0.84	47.08± 0.53***	59.6 ± 0.67***

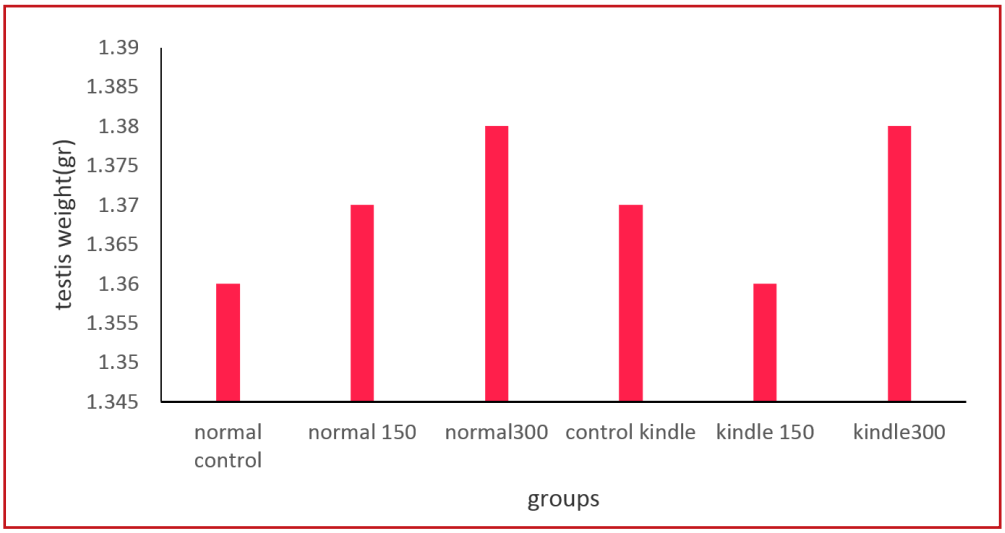


Chart 1. The effect of *Satureja edmondi Briq* essential oil on testis weight in kindled and normal groups. (Data are indicated as Mean± SE). In comparison with control group, significant differences are shown by *P<0.05.

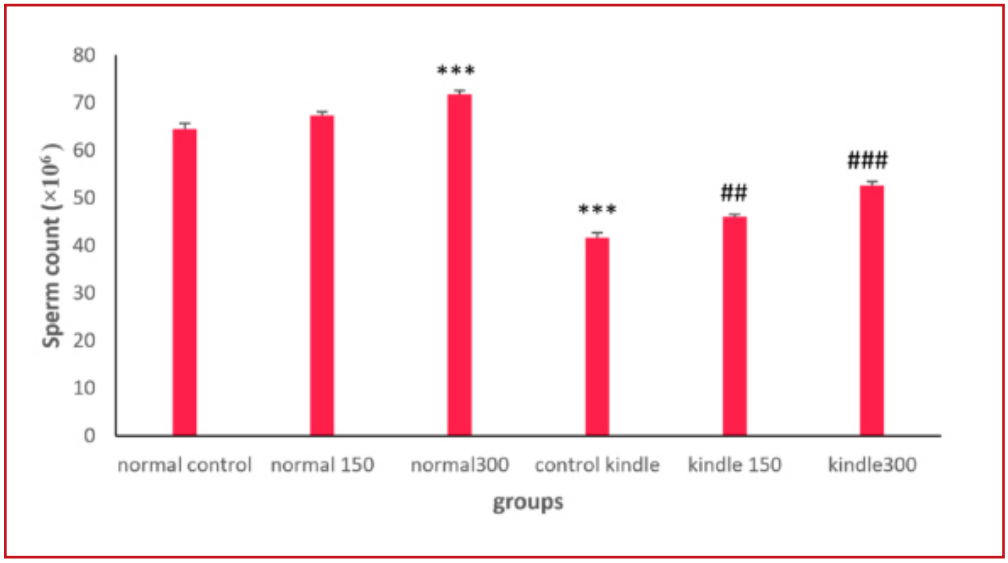


Chart 2. The effect of *Satureja edmondi Briq* essential oil on sperm count in treatment groups, including normal control, normal groups with 150 and 300 ppm of *S. edmondi* essential oil, kindled control group, and kindled groups with 150 and 300 ppm *S. edmondi* essential oil.

*** p≤0.001: Statistically significant difference with control normal group
p≤0.001: Statistically significant difference with kindled control group
p≤.01: Statistically significant difference with kindled control group

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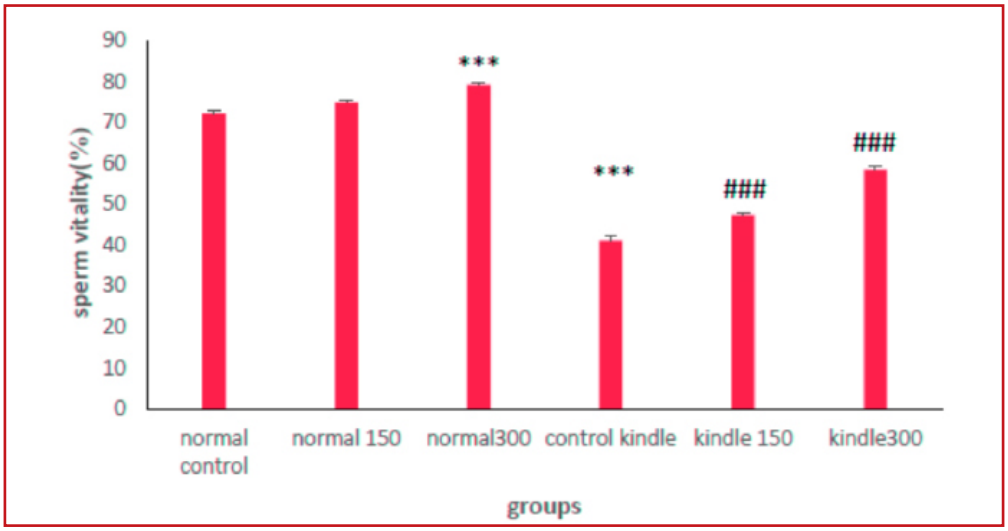


Chart 3. The effect of *Satureja edmondi Briq* essential oil on sperm viability in treatment groups, including normal control, normal groups with 150 and 300 ppm *S. edmondi* essential oil, kindled control group, and kindled groups with 150 and 300 ppm of *S. edmondi* essential oil

*** p≤0.001: Statistically significant difference with control normal group
p≤0.001: Statistically significant difference with kindled control group

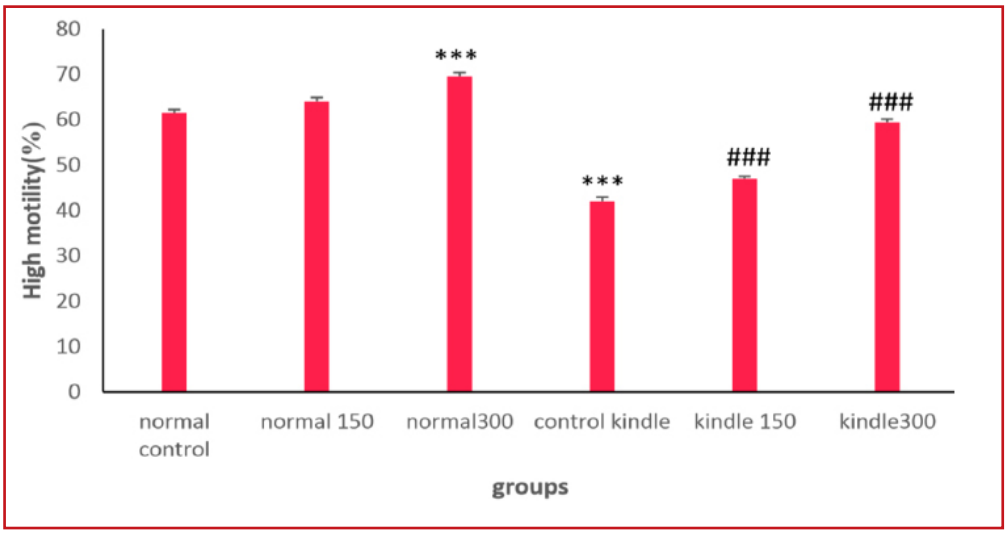


Chart 4. The effect of *SaturejaedmondiBriq* essential oil on sperm motility in treatment groups, including normal control, normal groups with 150 and 300 ppm *S. edmondi* essential oil, kindled control group, kindled groups with 150 and 300 ppm *S. edmondi* essential oil

*** p≤0.001: Statistically significant difference with control normal group
p≤0.001: Statistically significant difference with kindled control group

Discussion

Epilepsy is one of the most important clinical problems and ranks third among all organic brain diseases. Epileptic patients have frequently sexual and reproduction dysfunctions, on the other hand, these problems also occur in patients that use anti-epileptic drugs (AEDs). There are potential reproductive dysfunctions, endocrine disorders, loss of libido, low sperm count, decreased sperm viability, unhappiness,

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disinterest, and low fertility in epileptic men. These dysfunctions also occur in patients that use antiepileptic drugs (AEDs). The most common causes of infertility in men are inability to produce healthy and active sperm and reduced sperm motility (20). Ineffectiveness of drug in some cases and serious side effects of the AEDs lead to the use of herbal medicine as a form of complementary and alternative medicine. Oxidative stress is a main factor involved in epilepsy and epileptogenesis process. Antioxidant therapy can reduce reproductive dysfunctions and oxidative stress-induced infertility in patients with epilepsy. Herbal drugs containing antioxidant compounds are used for the therapy of disorders induced by oxidative stress. Therefore, the present study was designed to investigate the role of *Satureja edmondi Briq* essential oil on sperm parameters, including sperm count, sperm viability, progressive sperm motility, and testis weight, in normal and PTZ-induced epilepsy in male rats. Our data revealed that all sperm parameters, including sperm count, sperm viability, progressive sperm motility, and testis weight changed in the epileptic and normal groups by increasing the dose of *Satureja edmondi Briq* essential oil. The increase in these variables was significant in some instances. The total sperm count, sperm viability, and progressive sperm motility in both normal and epileptic groups raised with an increase in *Satureja edmondi Briq* essential oil dose. The highest dose of *S. edmondi* essential oil significantly increased sperm parameters in normal groups, and different doses of *S. edmondi* essential oil caused a significant increase in the kindled groups. Sperm parameters such as sperm viability, sperm motility, and sperm count was decreased in epileptic rat. Previous studies have shown that most men with epilepsy have abnormal sperm parameters, including decreased sperm count, abnormal morphology, and reduced motility or immobility (21). In patients with epilepsy, sperm motility decreased and caused sperm abnormalities (22). Focal epilepsy may affect sperm morphology, sperm concentration, and sperm motility (23). In experimental epilepsy in rats, sperm count was reduced significantly. Epilepsy can also reduce sperm count and motility and decrease sperm viability in the kindled Wistar male rats (8).

In this study, significant differences in sperm count, viability, and motility were observed in the epileptic rats treated with *S. edmondi* essential oil, which is due to the high antioxidant properties of this plant. In our previous study, we evaluated the effect of *Satureja edmondi* on sexual hormones in epileptic and normal rats. Testosterone and lutein hormone (LH) decreased significantly in epileptic rats compared to normal rats. Testosterone and LH were increased significantly at higher doses of *Satureja edmondi* essential oil in treatment groups (17). Antioxidant compounds such as thymol in this herb caused an increase in testosterone and LH in treatment groups. Testosterone levels play an important role in maintaining the process of spermatogenesis in males. Increased oxidative stress decreases the level of important enzymatic and non-enzymatic antioxidants in Leydig cells, so reduction of testosterone synthesis is an effective factor in disrupting spermatogenesis and reducing the number of epididymal sperms (24).

Excessive production of reactive oxygen species (ROS) stops the cell cycle and increases the apoptosis process, thus reducing daily sperm production as well as the total number of sperms (25). Epilepsy causes proteolysis and reduces antioxidant defense in testis due to increased ROS level,

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thereby reducing sperm count and decreasing motility (26).

Different doses of *Ferollago angulata*, a traditional herb in the west of Iran with high antioxidant power, significantly increase sperm parameters such as sperm count, motility, and viability, thus improving sperm parameters and enhancing spermatogenesis (27). The protective effect of reproductive system degeneration on cyclosporine in male moths was also investigated by Saturejakhuzestanica (28). Antioxidants can affect the testicular tissue in experimental animals (29). In a study, the antioxidant effects as well as the stimulating effects of *Saturejakhuzestanicaessential* oil on fertility in male rats were investigated. The results showed the positive effects of *Saturejakhuzestanicaon* fertility in male rats (30).

In this study, the effect of *Satureja\edmondi* on testis weight changes was evaluated in the epileptic and normal rats. Testis weight was decreased in the kindled rats. Different doses of *Satureja edmondi* essential oil increased testis weight. Testis weight is associated with its function. Increased testicular weight increases spermatogenesis and testosterone level. Epilepsy impairs spermatogenesis and reduces total antioxidant capacity, so there are disorders in testicular weight and sperm parameters such as sperm count, viability, and motility. Different doses of *Satureja edmondi Briq* increase the antioxidant defense of testicular tissue and improve sperm quality in disorders caused by stress, including epilepsy.

Conclusion

Satureja edmondi Briq contains many antioxidant compounds such as p-Cymene, gamma terpinene, thymol, and alpha-Terpineol, which act as a natural antioxidant. This herb can remove free radicals in the cell membrane. The antioxidant compounds of *Satureja edmondi Briq* reduce the destructive effects of epilepsy on the sperm parameters and reproductive system. To complete the results of this article, the hydroalcoholic and aqueous extracts of *Satureja edmondi* are suggested to be examined in future research. The effect of *Satureja edmondi* on oxidative stress index and total antioxidant capacity in reproductive system is also advised to be studied.

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Conflicts of Interests

The authors declare that they have no competing interests.

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