Effects of Satureja Edmondi Briq on Ameliorate Reproductive Parameters in Pentylenetetrazol-Induced Epilepsy Models in Rats

somayeh bohlouli (✉ sbohloli@yahoo.com)

kermansh brunch, islamic azad university, kermanshah, iran. <https://orcid.org/0000-0003-0854-1875>

gelavij mahmoodi

kermanshah brunch, islamic azad university, kermanshah, iran

Research

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Abstract

Background

Epilepsy is one of the common neurological brain defects that causes unpredictable, recurrent seizures. It has adverse effects on the reproductive functions. Oxidative stress contributes to the evolution of epilepsy, including reduction of sperm count and motility, increase of abnormal sperm morphology, and low testosterone. Antioxidants are beneficial to spermatogenesis and sperm parameters. *Satureja edmondii* 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 *Satureja edmondii* reproductive potential in pentylenetetrazol (PTZ)-induced epileptic male rats.

Methods

In this experimental study, 48 Wistar male rats with an average age of 10 weeks and weight of 240- 260g were used. Animals were randomized into normal and kindled groups that were treated with different doses of *S. edmondii* essential oil. Treatment of animals lasted 4 weeks. In the end, testosterone, follicle-stimulating hormone, luteinizing hormone, and sperm parameters were measured according to the World Health Organization (WHO) standards.

Results

This study showed that all sperm parameters, including sperm count, sperm viability, and progressive sperm motility were increased and testosterone, follicle-stimulating hormone and luteinizing hormone (FSH, LH) changes in the epileptic and normal groups were changed with an increase in the dose of *S. edmondii* essential oil.

Conclusions

The changes were significant in some parameters. *S. edmondii* as a good source of antioxidants can improve sperm parameters and reproductive potential in PTZ-induced epileptic male rats.

Introduction

Epilepsy is one of the most common central nervous system disorders after stroke that affects the people's lives. It is caused by the unusual electrical activities in the brain. Epilepsy is determined by uncontrolled and recurrent seizures that disrupt 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, and sometimes loss of awareness. Epilepsy has an impact on the reproductive system [3]. Epilepsy also influences the pituitary-gonadal hormone and reproductive parameters. Sexual dysfunction and fertility

problems have been noted in men with epilepsy [4]. Reproductive impairment is attributed both to epilepsy itself and antiepileptic drugs (AEDs) [5]. Epilepsy, antiepileptic drugs (AEDs), and the reproductive system have intricate interactions [6]. Reproductive **endocrine dysfunction** is unusually common among women and men with **epilepsy**. Epilepsy has negative effects on the male reproductive system in epileptic patients and animals.

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 [7]. Oxidative stress is considered a possible mechanism involved in epileptogenesis. Experimental studies have pointed out that oxidative stress is a contributing factor to the onset and evolution of epilepsy [8].

some plants are regarded as powerful antifungal, antimicrobial and antioxidant since ancient times.

An antioxidant can be broadly defined as any substance that delays or inhibits oxidative damage to a target molecule. Antioxidant compounds scavenge free radicals [9, 10]. Many herbs are known to have antioxidant effects on the growth and immunological parameters in some animals [11, 12].

The essential oil of *S. edmondi* contains predominant compounds like thymol, gamma terpinene, and p-cymene. These compounds are known to have antimicrobial, antifungal, anti-inflammatory, and specially antioxidant properties [13]. In our previous study, we observed the positive effect of *S. edmondi* on memory impairment induced by chemical kindling in male rats [14]. This study was conducted to investigate the link between chemical kindling and positive effect of *S. edmondi* on the reproductive parameters and pituitary-gonadal hormone in epileptic male rats.

Materials And Methods

Animals

A total of 48 Wistar male rats (240-260g) were purchased from Razi Institute (Tehran, Iran) and housed under controlled environmental conditions, 12/12 h light/dark cycle and $22 \pm 2^\circ\text{C}$ 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).

Preparation of plant

Satureja edmondi Briq was obtained from the mountains of Kermanshah, Iran. It was identified and authenticated by the herbarium of Islamic Azad University of Kermanshah. Essential oil extraction was performed by steam distillation and a Clevenger-type apparatus [13].

Experimental design

The rats were randomly divided into control, kindling, and treatment groups (6 groups of 8 rats) and treated for 28 days:

Group 1: Normal control group

Group 2: Normal control group received 150ppm essential oil

Group 3: Normal control group received 300ppm essential oil

Group 4: Kindled control group

Group 5: Kindled group receiving 150ppm essential oil

Group 6: Kindled group receiving 300ppm essential oil

All efforts were made to minimize the number of animals and their suffering. Treatment groups were kindled by repetitive intraperitoneal injection of PTZ (25 mg/kg bodyweight) (Sigma, St. Louis, MO, USA) every 15 minutes until seizure occurred (two or three injections) [15]. Seizure activity was observed for 45. The rats reaching phase 4 or 5 were considered kindled rats. PTZ was administered intraperitoneally in a single dose every other day to the kindled and normal groups receiving different doses of essential oil. Control groups were recipient of normal saline. 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). Then, blood samples were taken from the heart and serum was separated by centrifuge (3000 RPM, 20 min). To measure the hormones level, the serum samples were kept in the freezer at -20°C.

Semen analysis

After anesthesia was conducted and blood was taken from heart, cauda epididymis was separated and 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 such as sperm viability, motility, and count. The suspension was pipetted into the counting chamber and immediately assessed for sperm count and motility. The sperm heads on the four corners of the central square of Neubauer slide were counted, and data were expressed as the number of sperm per ml. To assess viability, a drop of semen was placed on a slide and stained with Eosin-Nigrosin's dye. The non-stained sperms were expressed as viable sperms. Non-viable sperms had red or dark-pink heads and viable sperms had white or faintly-pink heads. Testosterone and pituitary-gonadal hormone (FSH, LH) levels were measured by the ELISA kit and plasma-optical emission spectroscopy (EUROIMMUN Analyser I-2P) [16].

Statistical analysis

The results were statistically estimated 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 results of changes in sperm count, sperm viability, and progressive sperm motility in normal groups are presented in Table 1 and those of kindled groups are shown in Table 2. According to the results of this study, these parameters increased in the group that

received 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 ($p \leq 0.001$).

Moreover, these variables indicated a significant decrease in the kindled group compared to the control group ($p \leq 0.001$). Further, these variables increased significantly in the kindled groups receiving the 150 ppm and 300 ppm doses compared to the kindled control group ($p \leq 0.001$). The results of testosterone measurement (ng / ml) in the normal group showed that this variable increased in the group that receiving *S.edmondi* essential oil with 150 (2.28 ± 0.08) and 300 (2.51 ± 0.07) doses compared to the control group (2.08 ± 0.08), but this increase was not significant.

On the other hand, this variable indicated a significant decrease in the kindled group (0.99 ± 0.03) compared to the normal control group ($p \leq 0.001$). Also, the comparison between the kindled groups receiving the 300 ppm and 150 ppm doses of *S.edmondi* essential oil and kindled control group showed an increase in testosterone levels. This increase was significant with 300 ppm dose ($p \leq 0.001$) (Figure 1). The LH hormone (mIU / ml) increased in normal groups that received 150 ppm *S.edmondi* essential oil (1.36 ± 0.88) compared to the control group (1.06 ± 0.88); however, this increase was not significant.

Further, this variable significantly increased in the group that received 300 ppm *S.edmondi* essential oil (1.65 ± 0.77) compared to the control group (1.06 ± 0.08) ($P \leq 0.001$). On the other hand, this variable indicated a significant decrease in the PTZ receptor group (0.05 ± 0.03) compared to the normal control group (0.6 ± 0.08) ($p \leq 0.001$). Moreover, the comparison between kindled groups receiving 300 and 150 doses of *S.edmondi* essential oil and kindled control group showed an increase in the LH levels. This increase was significant with 300 ppm dose ($p \leq 0.001$) (Figure 2).

Changes in FSH levels (mIU / ml) in the groups are shown in Figure 3. This variable increased in normal groups that received different doses of *S.edmondi* essential oil compared to the normal control group, but this increase was not statistically significant. This variable decreased significantly in the kindled group compared to the normal control group ($p \leq 0.01$). Moreover, the comparison of this variable between the kindled groups receiving different doses of *S.edmondi* essential oil and kindled control group did not show a significant difference.

Table 1. The effect of *Satureja edmondi* Briq essential oil on sperm parameters in normal groups.

the data are expressed as Mean $\pm$ SE. In comparison with control group significant differences are shown by *** $p \leq 0.001$

Difference of <i>Satureja edmondi</i> Briq essential oil in normal rats			
	Control	150ppm	300ppm
Sperm count ($\times 10^6$)	64.5$\pm$ 1.1	67.3 $\pm$ 0.76	71.83 $\pm$ 0.79***
Viability (%)	72.3 $\pm$ 3.8	75 $\pm$ 0.57	79.3 $\pm$ 0.61***
High motility (%)	61.5 $\pm$ 0.96	64 $\pm$ 0.84	69.6 $\pm$ 0.84***

Table 2. The effect of *Satureja edmondi* Briq essential oil on sperm parameters in kindle groups.

the data are expressed as Mean $\pm$ SE. In comparison with control group significant differences are shown by ***p $\leq$ 0.001

Difference of <i>Satureja edmondi</i> Briq essential oil in kindle rats			
Sperm parametr	Control	150ppm	300ppm
Sperm count ($\times 10^6$)	41.6 $\pm$ 1.05	46 $\pm$ 0.57***	52.6 $\pm$ 0.88***
Viability (%)	41.2 $\pm$ 1.2	47.4 $\pm$ 0.54***	58.6 $\pm$ 0.85***
High motility (%)	42 $\pm$ 0.84	47.08 $\pm$ 0.53***	59.6 $\pm$ 0.67***

Discussion

Fertility is lower in both men and women with epilepsy than in the general population. Moreover, reproductive endocrine disorders are more common among patients with epilepsy than the general population. In this study, all sperm parameters such as sperm count, sperm viability, progressive sperm motility, and testosterone and pituitary-gonadal hormone changes in the epileptic and normal groups were changed by increasing the dose of *Satureja edmondi* Briq essential oil. The increase in these variables was significant in some instances. The total number of sperms, sperm viability, and progressive motility in both normal and epileptic groups were raised with an increase in the dose of *Satureja edmondi* Briq essential oil. Thus, the highest dose of *S. edmondi* essential oil increased significantly in normal groups compared to normal control group, and different doses of *S. edmondi* essential oil caused a significant increase in epileptic groups compared to the epileptic control group.

Previous studies have shown that most men with epilepsy have abnormal sperm parameters, including decreased sperm count, abnormal morphology, and impaired motility [17]. In a study, the antioxidant effects as well as the stimulating effects of *Satureja Khuzestanica essential oil* on fertility in male rats

were investigated. The results showed the positive effects of *Satureja Khuzestanica* on fertility in male rats [18]. In patients with epilepsy, sperm motility decreased and caused sperm abnormalities [19]. Focal epilepsy may affect the sperm morphology, concentration, and motility [20]. Induction of experimental epilepsy significantly reduced the number of natural sperms and total sperm count in experimental rats compared to the normal rats. It can also reduce sperm count and motility and decrease sperm viability in the kindled Wistar male rats [21].

On the other hand, a significant difference was observed in the group treated with *S. edmondi* essential oil compared to the epileptic control group in terms of sperm count, viability, and motility, which is due to the high antioxidant properties of this plant. The most common causes of infertility in men are their inability to produce enough healthy and active sperms and their reduced mobility [22]. 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 [23].

There is a steady decrease in sperm viability and progressive motility and a decrease in motility, which is associated with increased ROS level [24]. Epilepsy produces a variety of biochemical processes, including proteolysis and ROS emission, which can damage the fats and proteins and reduce antioxidant properties [25]. 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 survival, thus improving sperm parameters and enhancing spermatogenesis [26].

The protective effect of reproductive system degeneration on cyclosporine in male moths was also investigated by *Satureja Khuzestanica* [27]. The results of Cao et al. showed that, the levels of important enzymatic and non-enzymatic antioxidants in Leydig cells decreased with an increase in oxidative stress, which reduced the synthesis and secretion of testosterone and was an effective factor for spermatogenesis and dysfunction. As a result, there is a significant reduction in the number of epididymal sperm [28]. Testosterone levels in the body play an important role in maintaining spermatogenesis, and lower levels of testosterone reduce sperm count [29].

The physiological effects of oxidative stress on male infertility have been investigated. It has been reported that oxidative stress causes endocrine disorders in the testicles and damages the testicular morphology [30]. The antioxidant properties and role of *Satureja Khuzestanica* in infertility treatment have also been investigated [31]. The LH, FSH, and testosterone levels in the experimental epileptic group increased due to PTZ. A significant decrease was observed compared to the control group. However, there was a significant increase compared to the normal control group and the epileptic control group only in the highest dose (300 ppm).

The changes in follicle-stimulating hormone (FSH) in the study groups, whether in the normal rats or epileptic rats, increased slightly, which was not significant. Due to the production of free radicals by PTZ and the induction of experimental epilepsy in the studied rats, a significant decrease was observed in the testosterone and lutein hormones compared to the control group. Due to the presence of antioxidant compounds, *Satureja edmondi* Briq significantly increased serum testosterone and lutein hormone

compared to normal and epileptic control groups. The results are consistent with the results of a study that found that the kindling of Wistar male rats reduced serum testosterone and luteinizing hormone levels, but did not change the serum levels of follicle stimulating hormone between groups [21].

An important factor in reducing serum testosterone is increased free radicals and oxidative stress. As a chronic neurological disorder, epilepsy can increase the active species of oxygen and the production of superoxide in the brain [32]. Studies have shown that epilepsy reduces serum LH concentrations and lowers LH, which consequently decreases the testosterone secretion [33]. In the present study, serum testosterone levels decreased significantly in the epileptic group without treatment compared to the normal group. Different doses of plant extract with abundant antioxidant properties cause a significant increase in testosterone in Wistar male rats with oxidative diseases, thus improving and enhancing the fertility potential in these patients [16].

Conclusion

Epilepsy causes disorders in sperm parameters such as count, viability, and motility. It also impairs the testicular tissue and lowers the testosterone, LH, and FSH levels by damaging spermatogenesis and reducing total antioxidant capacity. The use of different doses of *Satureja edmondiBriq* increases the antioxidant power of testicular tissue and improves sperm quality and pathological changes caused by epilepsy-induced stress. Based on the results of this study, *Satureja edmondiBriq*, which contains many antioxidant compounds such as thymol, gamma terpinene, p-Cymene, alpha-Terpinene, acts as a natural antioxidant, removes the free radicals in the cell membrane, and protects lipid membrane against peroxidation. The antioxidant compounds of *Satureja edmondiBriq* reduce the destructive effects of epilepsy on the reproductive process and potential.

Abbreviations

PTZ, pentylenetetrazol; WHO, World Health Organization; LH, luteinizing hormone; FSH, follicle-stimulating hormone; ppm, Parts per million

Declarations

Ethics approval and informed consent

The experiment protocol was approved by the ethics committee of animal research (IR.KUMS.REC.1398.977).

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Consent for publication

Not applicable.

Availability of data and materials

Data are available upon request from the corresponding author.

Competing Interests

The authors declare that they have no competing interests.

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Authors'contributions

Bohlouli designed the experiments and performed the experiments, mahmoodi analyzed the data and bohlouli wrote the manuscript with assistance from mahmoodi. They approved the final manuscript.

Author details

¹Department of veterinary medicine ,College of Agriculture, Kermanshah Branch, Islamic Azad University, Kermanshah, Iran.²Department of Biology, College of Basic Science, Kermanshah Branch, Islamic Azad University, Kermanshah, Iran.

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Figures

Figure 1

The effect of Saturejaedmondi Briq essential oil on testosterone (ng/ml) treatment groups included: normal control, normal groups with 150 and 300 ppm of S. edmondi essential oil, kindle control, kindle groups with 150 and 300 ppm of S. edmondi essential oil. *** $p \leq 0.001$: Statistical difference with control normal group ### $p \leq 0.001$: Statistical difference with kindle control group

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Figure 2

The effect of Satureja edmondi Briq essential oil on LH(mlu/ml) in treatment groups included: normal control, normal groups with 150 and 300 ppm of Satureja edmondi Briq essential oil, kindle control, kindle groups with 150 and 300 ppm of Satureja edmondi Briq essential oil. *** $p \leq 0.001$: Statistical difference with control normal group ### $p \leq 0.001$: Statistical difference with kindle control group

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The effect of Satureja edmondi Briq essential oil on LH (mlu/ml) in treatment groups included: normal control, normal groups with 150 and 300 ppm of Satureja edmondi Briq essential oil, kindle control, kindle groups with 150 and 300 ppm of Satureja edmondi Briq essential oil. *** $p \leq 0.001$: Statistical difference with control normal group ### $p \leq 0.001$: Statistical difference with kindle control group

Figure 3

The effect of Satureja edmondi Briq essential oil on FSH (mIU/ml) in treatment groups included: normal control, normal groups with 150 and 300 ppm of S. edmondi Briq essential oil, kindle control, kindle groups with 150 and 300 ppm of S. edmondi Briq essential oil. ** $p \leq 0.01$: Statistical difference with control normal group

Figure 3

The effect of *Satureja edmondi* Briq essential oil on FSH(mIU/ml) in treatment groups included: normal control, normal groups with 150 and 300 ppm of *S. edmondi* Briq essential oil, kindle control, kindle groups with 150 and 300 ppm of *S. edmondi* Briq essential oil. ** $p \leq 0.01$: Statistical difference with control normal group