Title: Effects of Pretreatment with Satureja Bachtiarica Essential Oil in Preventing Seizure in Pentylenetetrazol-Kindled Mice

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Highlights:

- The administration of *S. bachtiarica* essential oil at 50 and 100 mg/kg to PTZ-treated mice caused significant increase in latency to first seizure.
- The administration of *S. bachtiarica* essential oil at 50 and 100 mg/kg to PTZ-treated mice caused significant decrease in the frequency of the head and upper limbs seizure, total body seizures, tonic seizures, and spin and jump.
- The administration of flumazenil significantly inhibited *S. bachtiarica* essential oil-induced effects and increased the head and upper limbs seizures, tonic seizures, jumping.

Plain Language Summary

Epilepsy is one of the most common disorders of the central nervous system from which one in every 100 people is suffering globally. Despite the developments of antiepileptic drugs, novel strategies are being sought out due to drug resistance and the side effects resulting from these drugs at high concentrations. For certain reasons such as availability, the history of long-term use, being nature-based, and relative safety, researchers have focused on plants. In the current study, the effect of the pretreatment with *S. bachtiarica* essential oil in preventing seizure was studied in the PTZ-kindled mice. The injection of 50 and 100 mg/kg *S. bachtiarica* essential oil caused significant increase in latency to first seizure and survival duration, and significant decrease in the frequency of the head and upper limbs seizures, tonic seizures, and spin and jump in the PTZ-receiving mice.
Abstract

Introduction: Epilepsy is a group of chronic neurological disorders characterized by seizures. The aim of the present study was to investigate the effects of pretreatment with *Satureja bachtiarica* essential oil in preventing epilepsy.

Methods: In this experimental study, 50 mice were randomly assigned to five groups of 10 each. The control group received normal saline plus tween80 and, 30 min later, PTZ. Groups 2 and 3 were treated with *S. bachtiarica* essential oil at 50 and 100 mg/kg and 30 min later received PTZ, respectively. Group 4 received diazepam and 30 min later received PTZ. Group 5 received flumazenil and 30 min later received PTZ. After the last injection of PTZ, the time of seizure onset, seizure severity and score, the completion time of each seizure (attack episode), and mortality rate in different groups were recorded and compared.

Results: The administration of *S. bachtiarica* essential oil at 50 and 100 mg/kg to PTZ-treated mice caused significant increase in latency to first seizure and survival, and significant decrease in the frequency of the head and upper limbs seizure, total body seizures, tonic seizures, and jumping. *S. bachtiarica* essential oil at 100 mg/kg caused a significant decrease in the head ticks frequency. The administration of flumazenil significantly inhibited *S. bachtiarica* essential oil induced effects and increased the head and upper limbs seizures, tonic seizures, and jumping.

Conclusion: The present study demonstrated that *S. bachtiarica* essential oil can prevent PTZ-induced seizure and these findings authenticate the traditional claims about use of *Satureja bachtiarica* in treatment of epilepsy.

Key words: *Satureja bachtiarica*, Epilepsy, GABAergic activity, PTZ – kindled
Introduction

Epilepsy is the second leading neurological disorder after stroke. Nearly 1% of the global population (65 million) have epilepsy and approximately 80% of the people with epilepsy live in developing countries (Karimi, Naseri et al. 2014).

GABA is an inhibitory neurotransmitter in the brain that effectively blocks the transmission of neural signals in the brain. Excessive reduction in GABA causes certain conditions in which a stimulatory neurotransmitter seems to be continuously firing and functioning. In such conditions, epilepsy may occur. The drugs that cause an increase in synaptic GABA levels through inhibiting GABA catabolism or increasing GABA reabsorption, including antiepileptic drugs, are considered to be effective. Benzodiazepines (e.g. diazepam) are a group of these drugs that cause increase in binding the GABA to its receptors and therefore the frequency of chloride channels opening. A number of the inhibitors of the GABA synthesis such as thiosemicarbazide-4, isoniazid, deoxypyridoxine, and L-allylglycine can cause seizure (Treiman 2001). As the most important inhibitory neurotransmitter, GABA interacts with two types of receptors, GABA_A and GABA_B. GABA_A receptors are located postsynaptically and GABA_B ones are located presynaptically, and cause modulation of synaptic release. In the adult brain, the GABA_A receptors are permeable to chloride ions. Following activation, the permeation of the chloride ion causes membrane hyperpolarization and action potential inhibition. Therefore, the compounds that serve as the GABA_A receptors, such as barbiturates and benzodiazepines can effectively suppress seizure. The GABA_B receptors are associated with the subsequent signaling and cause weakening of the release of neurotransmitters according to their presynaptic positions (Gey, Gernert et al. 2016).

Mitochondrial dysfunction due to ROS toxicity often occurs after seizure. Epileptic seizures cause calcium to flow into the cell through NMDA-dependent ion channels. Increased intracellular ions cause a cascade of biochemical events. High levels of intracellular calcium are associated with increased production of ROS. ROS are inactivated by enzymatic antioxidants such as superoxide dismutase (SOD), catalase, glutathione peroxidase, glutathione reductase, and peroxiredoxins as well as none-enzymatic antioxidants such as vitamin C, vitamin E, and glutathione (Kim 2004). Studies have indicated that plant-based antioxidants are useful in decreasing the severity of and damage due to epileptic seizures (Gupta, Kumar et al. 2003).

_Satureja bachtiarica_ species grows widely in Iran and have been identified western, central, and southwestern provinces of this country (Ahmadi, Sefidkon et al. 2009).

In traditional medicine, _S. bachtiarica_ is considered to have a relatively warm and dry nature. It is anti-flatulent and appetizing, and contributes to improving sexual prowess. _S. bachtiarica_ is used for relieving pain, and if consumed with fig juice, is useful for treating cough, dyspnea, and brilliant facial paint. _S. bachtiarica_ is highly useful for treating diarrhea. Its compress with olive oil is suitable for different types of pains due to abdominal torsion. _S. bachtiarica_, as with thyme, can be used to remove weakness and stomach cramp. Besides that, this plant can be used for treating indigestion, intestinal fermentation, and bloating (Memarzadeh, Pirbalouti et al. 2015).
In a study, the essential oils of *S. bachtiarica* aerial parts were extracted by steam distillation, and their active compounds were identified by gas chromatography-mass spectrometry (GC/MS). Out of the 26 identified compounds, thymol (44.5%), gamma-terpinene (23.9%), p-cymene (7.3%), beta-caryophyllene (5.3%), and borneol (4.2%) were found to be the main compounds (Sefidkon and Jamzad 2000). Sefidkon et al. reported that the essential oil of pre-flowering *S. bachtiarica* contained 20% carvacrol and 19% thymol, and that of flowering *S. bachtiarica* contained approximately 26% carvacrol and 5% thymol. They also reported that *S. bachtiarica* essential oil with 32 μg/ml IC50 had potential antioxidant effects in inhibiting DPPH radicals (Sefidkon, Sadeghzadeh et al. 2007).

Nowadays, despite many advances in the treatment of epileptic disorders, millions of people are still suffering from uncontrolled epilepsy that is resistant to common antiepileptic drugs such that one of every three patients do not respond appropriately to these drugs. In addition, about one-third of the patients who have recovered from epilepsy experience recurrent epilepsy after discontinuation of the drug. The complications due to antiepileptic drug toxicity lead to restricted use and not achieving the desired therapeutic effect. It is therefore essential to consider epilepsy treatment and seek out appropriate drugs for epilepsy with comparatively fewer side effects. As a result, it is obviously necessary to take into account traditional medicine and medicinal plants with the aim of achieving safe drugs with minimal side effects. *S. bachtiarica* has many therapeutic applications in traditional medicine. There was no scientific report presented on the antiepileptic activity of *S. bachtiarica* essential oil. Hence, the present study is designed to validate the uses of *S. bachtiarica* essential oil in epilepsy.

1. Methods

2.1. Preparation of *S. bachtiarica* essential oil

*S. bachtiarica* was purchased from the groceries across Shahrekord city and authenticated by Dr. Gooini, Botanical Survey of IRAN. The essential oil was extracted by distillation using Clevenger apparatus. For this purpose, 50 g pulverized *S. bachtiarica* and 500 ml water were poured into the distilling flask and then heated to reach a distillation rate of 2-3 ml/min. After 4 hours, the essential oil was collected and mixed with anhydrous sodium sulfate for 24 hours to remove water content. The essential oil was stored in -20 °C.

1.2. Determining antioxidant capacity using DPPH radicals

First, different concentrations of *S. bachtiarica* essential oil were prepared. Also, DPPH in 0.1 mM concentration (in methanol) was prepared. 2 ml DPPH was added to 2 ml of *S. bachtiarica* essential oil prepared in different concentrations and left in the dark for 15 min. The tube containing 2 ml methanol and 2 ml DPPH was prepared near the samples. After 15 minutes, spectrophotometer wavelength was set at zero using blank and then absorbance was read at 517 nm wavelength. The
inhibition percentage of the DPPH radicals was calculated by the formula below:

\[ I = 100 \times \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \]

The concentration of the essential oil in which 50% of the DPPH radicals were neutralized (IC50) was derived by plotting the graph. In this graph, Y axis represents inhibition percentage and X axis represents extract concentration (Choi 1988).

1.3. Animals

Albino NMRI male mice weighing 20–25 g were used. The animals were maintained under standard laboratory conditions at temperature 24 ± 2 °C and relative humidity (30–70%) with a 12:12 h light:dark cycle throughout all the experiment. The animals were fed with standard pellet diet and free access of water. The animals were shifted to the laboratory one hour prior to the experiment.

1.4. Drugs or Chemicals

All solvents, chemicals and materials for extraction were purchased from Sigma Aldrich. Diazepam and flumazenil were product of Daru Pahsh; all other compounds for animal experiments were obtained from Sigma–Aldrich Chemical Company.

1.5. Experimental design and animal grouping

Group 1 (PTZ group): i.p. received vehicle (normal saline+ tween80 and 30 minutes after received PTZ (30 mg/kg).

Groups 2 and 3 (SB-EO groups); i.p. received 50 and 100 mg/kg SB essential oil approximately 30 minutes before receiving PTZ, respectively;

Group 4 (diazepam group): i.p. received 20 mg/kg diazepam (a GABA receptor antagonist) approximately 30 minutes before receiving PTZ (Bakhtiarpoor and Setorki 2018).

Group 5 (flumazenil group): Received flumazenil(Daru Pahsh, Iran, a selective antagonist of benzodiazepine GABA\(_A\) receptors) (10 mg/kg) 10 minutes before i.p. injected of 100 mg/kg S. bacthiarica essential oil, and PTZ 30 minutes after injection of S. bacthiarica essential oil.

In this study, 35 mg/kg PTZ was i.p. injected every other day (once every 48 hours). The mice were injected with 50 and 100 mg/kg S. bacthiarica essential oil every day for 10 days, and on the 10th day, 30 minutes after injection of S. bacthiarica essential oil, normal saline, and diazepam, the mice were administered with 60 mg/kg body weight (BW) PTZ, and the severity and rate of seizure were recorded and analyzed for 30 minutes by filming. Diazepam and flumazenil were injected only once and on the last day.
2.6. Induction of seizures by PTZ

After the last injection of PTZ (Sigma Aldrich), latency to first seizure, seizure severity (score), the completion time of each seizure (attack duration), and mortality rate in different groups were measured and compared. Scoring was as follows:

0: Normal behavior: normal behavior in adult rat involves initial orientation lasting 2 to 3 minutes.
1: Head tick
2: Repeated seizures of the head and jerk of the anterior limb
3: The seizure of the entire body and standing up
4: Tonic seizures: while the animal was on its belly (without the loss of balance)
5: Repeated spin and jump
6: Death (Solati, Rabiei et al. 2019)

2.7. Data analysis

Data analysis was conducted by SPSS 16. One-way analysis of variance and Tukey's test were used to investigate the significance of difference among different treatments and respective mean values, respectively. Data were expressed as mean ± standard error, and p < 0.05 was considered significance level.

2. Results

3.1. S. bachtiarica essential oil inhibitory effect on DPPH radicals

According to the results, 50% of the DPPH radicals were inhibited by 0.095 mg/ml S. bachtiarica essential oil (IC50=0.095 mg/ml).
2.2. Latency to first seizure

Figure 2 illustrates the effect of *S. bachtiarica* essential oil on latency to first seizure in the mice receiving PTZ, 50 and 100 mg/kg *S. bachtiarica* essential oil, and flumazenil + 100 mg/kg *S. bachtiarica* essential oil. As illustrated, 50 and 100 mg/kg *S. bachtiarica* essential oil caused significant increase in latency to first seizure in the PTZ-receiving mice (*p* < 0.05). First latency to first seizure was significantly longer in the diazepam-receiving group than the groups receiving *S. bachtiarica* essential oil + PTZ (*p* < 0.001). First latency to first seizure was not significantly different between the flumazenil (10mg/kg i.p.) + 100mg/kg *S. bachtiarica* essential oil-receiving group and the groups receiving *S. bachtiarica* essential oil + PTZ (*p* > 0.05).
Figure 2. The effect of *Satureja bachtiarica* essential oil on latency to first seizure in mice; *** significant difference compared to pentylenetetrazol group (*p*<0.001); * significant difference compared to the pentylenetetrazol group (*p*<0.05); SB: *Satureja bachtiarica*

Figure 3 illustrates the effect of *S. bachtiarica* essential oil on survival duration in the mice receiving PTZ, 50 and 100 mg/kg *S. bachtiarica* essential oil, and flumazenil + 100 mg/kg *S. bachtiarica* essential oil. As illustrated, 50 and 100 mg/kg *S. bachtiarica* essential oil caused significant increase in survival duration in the PTZ-receiving mice (*p*<0.01 and 0.001, respectively). Survival duration decreased in the flumazenil + 100 mg/kg *S. bachtiarica* essential oil-receiving group compared to the groups receiving 100 mg/kg *S. bachtiarica* essential oil yet insignificantly. In the diazepam group, no mouse died and therefore survival duration was not measured.
Figure 3. The effect of SB essential oil on survival duration in mice; ** significant difference compared to PTZ group (p<0.01); *** significant difference compared to the pentylenetetrazol group (p<0.01). SB: Satureja bachtiarica; PTZ: pentylentetrazol; Flumaz: Flumazenil

2.3. Head ticks frequency

Figure 4 illustrates the effect of S. bachtiarica essential oil on the head ticks frequency in the mice receiving PTZ, 50 and 100 mg/kg S. bachtiarica essential oil, and flumazenil + 100 mg/kg S. bachtiarica essential oil. As illustrated, 100 mg/kg, unlike 50 mg/kg, S. bachtiarica essential oil caused significant decrease in the head ticks frequency in the PTZ-receiving mice (p<0.01). The head ticks frequency was significantly lower in the diazepam-receiving group than the groups receiving S. bachtiarica essential oil and PTZ (p<0.001). The head ticks frequency was significantly higher in flumazenil + 100 mg/kg S. bachtiarica essential oil than the group receiving 100 mg/kg S. bachtiarica essential oil (p<0.05).
2.4. Total frequency of the head and upper limbs seizures

Fifty and 100 mg/kg *S. bachtiarica* essential oil caused significant decrease in the total frequency of the head and upper limbs seizures in the PTZ-receiving mice (*p*<0.05). The total frequency of the head and upper limbs seizures was significantly lower in the diazepam-receiving group than *S. bachtiarica* essential oil + PTZ-receiving group (*p*<0.001). The total frequency of the head and upper limbs seizures was higher in the flumazenil + 100 mg/kg *S. bachtiarica* essential oil than the groups receiving 50 and 100 mg/kg *S. bachtiarica* essential oil (*p*<0.05) (Figure 5).
Figure 5. The effect of SB essential oil on the total frequency of the head and upper limbs seizures in mice; *** significant difference compared to PTZ group ($p<0.001$); * significant difference compared to the PTZ group ($p<0.05$). SB: *Satureja bachtariaca*

2.5. The frequency of tonic seizures

Figure 6 illustrates the effect of *S. bachtariaca* essential oil on the frequency of tonic seizures in the mice receiving PTZ, 50 and 100 mg/kg *S. bachtariaca* essential oil, and flumazenil + 100 mg/kg *S. bachtariaca* essential oil. As illustrated, 50 and 100 mg/kg *S. bachtariaca* essential oil caused significant decrease in the frequency of tonic seizures in the mice receiving PTZ ($p<0.001$). The frequency of tonic seizures was significantly lower in the diazepam-receiving group than the groups receiving 50 and 100 mg/kg *S. bachtariaca* essential oil + PTZ ($p<0.001$). The frequency of tonic seizures was significantly higher in the flumazenil + 100 mg/kg *S. bachtariaca* essential oil than the groups receiving 50 and 100 mg/kg *S. bachtariaca* essential oil ($p<0.001$).
Figure 6. The effect of SB essential oil on the total frequency of tonic seizures in mice; *** significant difference compared to PTZ group (p<0.001). SB: Satureja bachtiarica

2.6. The frequency of repeated spin and jump

Figure 7 illustrates the effect of *S. bachtiarica* essential oil on the frequency of repeated spin and jump in the mice. As illustrated, 50 and 100 mg/kg *S. bachtiarica* essential oil caused significant decrease in the frequency of repeated spin and jump in the PTZ-receiving mice (p<0.001). The frequency of repeated spin and jump was significantly lower in the diazepam-receiving group than the groups receiving 50 and 100 mg/kg *S. bachtiarica* essential oil + PTZ (p<0.001). (p<0.001).
2.7. Survival rate

Figure 8 illustrates the effect of *S. bachtiarica* essential oil on the survival rate of the mice. As illustrated, the survival rate was 50% in the PTZ-receiving mice. In the group receiving PTZ + 50 mg/kg *S. bachtiarica* essential oil, the survival rate was 75%. In the group receiving PTZ + 100 mg/kg *S. bachtiarica* essential oil, the survival rate was 91%. In the group receiving flumazenil + 100 mg/kg *S. bachtiarica* essential oil, the survival rate was 50%; and in the diazepam-receiving group, the survival rate was 100%.
Figure 8. The effect of *Satureja bacthiarica* essential oil on the survival rate of mice
Discussion

In the current study, the effect of the pretreatment with *S. bachtiarica* essential oil in preventing seizure was studied in the PTZ-kindled mice. The injection of 50 and 100 mg/kg *S. bachtiarica* essential oil caused significant increase in latency to first seizure and survival duration, and significant decrease in the frequency of the head and upper limbs seizures, tonic seizures, and spin and jump in the PTZ-receiving mice.

However, *S. bacterica* essential oil at 50 mg/kg had no significant effect on the frequency of the head ticks. Flumazenil significantly inhibited the effects of this essential oil, and caused significant increase in the frequency of the head and upper limbs seizures, tonic seizures, and spin and jump. Besides that, flumazenil decreased latency to first seizure and survival duration yet insignificantly.

Assaei et al. study reported that *S. khuzestanica* essential oil, at 40, 80, and 120 mg/kg, caused increase in latency to first seizure and decrease in seizure severity and durability in mice (Assaei R 2012). Different studies to identify the chemical compounds of *S. bachtiarica* essential oil, demonstrated that thymol and carvacrol are two main compounds of this essential oil (Sefidkon and Jamzad 2000, Memarzadeh, Pirbalouti et al. 2015). Aliabadi et al. reported that i.p. injection of thymol caused the severity and latency to first seizure in mice to decrease (Aliabadi, Izadi et al. 2016). Quintans-Júnior et al. study showed that carvacrol caused PTZ-induced seizure to relieve (Quintans-Júnior, Guimarães et al. 2010).

As already mentioned, thymol and carvacrol are two main compounds in *S. bachtiarica* essential oil. García et al. study on thymol effect on GABA<sub>A</sub> receptors in cortical neurons culture showed that thymol caused increase in chloride ion transport from the GABA<sub>A</sub> receptors, which significantly decreased in the presence of the GABAA receptors. Thymol caused increase in the combined appetite of GABA agonists for the GABA<sub>A</sub> receptors including muscimol and flunitrazepam (García, Bujons et al. 2006). Melo et al. study showed that carvacrol exerted anti-anxiety effects in mouse model. In Melo et al. study, flumazenil significantly inhibited carvacrol and diazepam-induced anti-anxiety effects. They argued that carvacrol anti-anxiety effects were due to the GABAA receptors (Kalueff, Lehtimaki et al. 2004).

In the present study, flumazenil significantly inhibited the effects of *S. bachtiarica* essential oil, and it can therefore be argued that the anticonvulsant effects of this plant are exerted via the GABAergic system.

It is argued that acetylcholine and its nicotinic receptors may play role in stimulating and maintaining seizure. Inducing epilepsy through injecting lithium, pilocarpine, and kinate causes significant increase in the acetylcholine levels in the brain and hippocampal membrane. In addition, treatment with the NMDA receptor antagonists causes significant decrease in the brain acetylcholine levels (Steinlein 2002). Recently, the role of certain mutations in the subunits of the nicotinic acetylcholine receptors has been confirmed in the incidence of certain types of epilepsy. Steinlein et al. study showed that the point mutations in the α<sub>4</sub> subunits of the acetylcholine...
receptors were associated with autosomal dominant nocturnal frontal lobe epilepsy (Steinlein, Mulley et al. 1995). Potier et al. study indicated that adding acetylcholinesterase inhibitor caused a significant increase in the incidence rate of spontaneous seizure. All these effects were inhibited by the muscarinic antagonists. Potier et al. argued that inhibiting cholinergic activity caused termination of seizure, while stimulating this activity induced seizure (Potier and Psarropoulou 2001).

The anticholinergic effects of thymol and carvacrol have already been demonstrated. Jukic et al. reported that carvacrol-induced inhibition of acetylcholinesterase was 10 fold more potent than thymol-induced inhibition of this enzyme while these two compounds have similar chemical structures (Jukic, Politeo et al. 2007). Hejazian et al. observed that $S. bacthiarica$ essential oil relieved acetylcholine-induced contractions in guinea pig ileum(Hejazian, Bameri et al. 2014). It seems that $S. bacthiarica$ essential oil inhibits the PTZ-induced seizures through decreasing the brain acetylcholine levels; however, this argument deserves further investigation.

The mechanism of action of certain anticonvulsants occurs through blocking calcium channels. Suzuki et al. reported that zonisamide decreased calcium ion flow into T-type calcium channels but did not cause any significant change in the L-type calcium channels(Suzuki, Kawakami et al. 1992). Fletcher et al. reported that $\alpha1A$ calcium channels in the brain of the mouse with epilepsy were dysfunctional (Fletcher, Lutz et al. 1996). Jouvenceau et al. argued that absence epilepsy was associated with dysfunction of P/Q-type voltage-gated calcium channel (Jouvenceau, Eunson et al. 2001). Because thymol is able to decrease the ion calcium flow into the skeletal muscle calcium channels (Szentandrássy, Szentesi et al. 2003), the anticonvulsant effects of $S. bacthiarica$ essential oil can be attributed to the effects of its active compounds on the ions flow into the calcium channels; however, this should be studied more vigorously.

Oxidative stress causes cell dysfunction and several cell damages. It has been reported that seizure is associated with the hemostatic imbalance between antioxidants and oxidants(Kim 2004). Oxidative stress contributes significantly to brain injury and neuronal death due to seizure(Gupta, Kumar et al. 2003). Animal studies have indicated that epileptic attacks cause production of free radicals and oxidative damage to proteins, lipids, and cell DNA. Excessive production of mitochondrial superoxide, inactivation of iron and sulfur-dependent enzymes such as aconitase, and iron-induced toxicity may contribute to the oxidative stress of the neurons after epileptic attacks(Kim 2004). In a study on 50 patients with epilepsy, lipids peroxidation and the percentage of erythrocytes hemodialysis were significantly higher in these patients than healthy people. Besides that, plasma ceruloplasmin concentration was significantly higher in patients with epilepsy.

The levels of glutathione reductase and vitamins A, C, and E in the erythrocytes were significantly lower in patients with epilepsy than healthy people(Sudha, Rao et al. 2001). Because the antioxidant effects of $S. bacthiarica$ essential oil (Hashemi, Niakousari et al. 2011) and its active compounds such as thymol (Youdim and Deans 2000) and carvacrol (Aeschbach, Löliger et al.
have been confirmed, it can be argued that the protective effects of this essential oil against PTZ-induced seizure are due to its antioxidant effects.

In vitro studies have indicated that in rodents, inducing epilepsy causes significant increase in the inflammatory mediators in regions involved in epilepsy, and anti-inflammatory drugs decrease the severity of certain types of epilepsy in animal models (Kalueff, Lehtimaki et al. 2004). High expression of inflammatory cytokines such as TNF-alpha and interleukin-6 in the astrocytes caused decrease in seizure threshold and spontaneous seizure frequency (Virta, Hurme et al. 2002). Deng et al. reported that thymol significantly relieved depression due to irreversible chronic stress model(Deng, Li et al. 2015). The anti-inflammatory effects of carvacrol and thymol on carrageena-induced ear edema and inflammation in rats have been also demonstrated(Fachini-Queiroz, Kummer et al. 2012).

Aliabadi et al. studied the effect of pretreatment with thymol at different concentrations in PTZ-induced epilepsy model. PTZ caused generalized seizure and was associated with increased lipids peroxidation and decreased SOD activity. In addition, consecutive PTZ injections caused significant increase in the levels of the inflammatory markers in mouse hippocampus. In that study, intraperitoneal injection of thymol caused decrease in the severity of seizure and latency to first seizure. Thymol also decreased the levels of malondialdehyde in the brain and inflammatory markers and increased SOD activity (Aliabadi, Izadi et al. 2016).

The present study demonstrated that *S. bacthiarica* essential oil significantly decreased seizure severity, latency to first seizure, and survival duration. The anticonvulsant effects of this essential oil are due to its effect on the GABA<sub>A</sub> receptors such that flumazenil inhibited these effects. The anticonvulsant effects of *S. bacthiarica* essential oil may be related to its active compounds including thymol and carvacrol. Besides that, other mechanisms such as preventing oxidative stress and decreasing the levels of acetylcholine in the brain and inflammatory mediators can contribute to the anticonvulsant activity of this essential oil. It is recommended to investigate such possibilities.

**Conflict of interest statement**
The authors have no conflict of interest.

**Ethical Considerations**
Shahrekord university of Medical Science Research Ethics Committee reviewed all procedures and experiments as the local referral Biomedical Committee for Research Ethics. Protocols and guidelines were carried on in accordance with the National Institutes of Health (NIH) for the care and use of experimental animals. Supervision of animal protocols was carried out according to the research design.

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Authors' contributions

Study concept, design, student mentorship: Zahra Rabiei; Acquisition of animal data: Manochehr Shirchi Data analysis, interpretation of the findings: Zahra Rabiei and Reviewing the manuscript and approving the final version for publication: All authors.

Conflict of interest

The authors declare no conflict of interest regarding this study and manuscript preparation after reading the Journal’s position on issues concerning the ethical publication and confirm that this report is consistent with those guidelines.

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