

# Research Paper: Effect of Hydroalcoholic Extract of *Stachys lavandulifolia* on Pentylentetrazole-induced Seizures in Male Mice: The Role of GABAergic and Opioidergic Systems



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## ABSTRACT

**Introduction:** Epilepsy is one of the most common neurological disorders. Though there are several effective drugs for treating epilepsy, most drugs are associated with side effects and drug interactions. *Stachys lavandulifolia* used in Iranian traditional medicine has proven anti-anxiety and sedative properties. The current study aimed to evaluate the anticonvulsant effect of hydroalcoholic extract of *S. lavandulifolia* on the Pentylentetrazole (PTZ)-induced seizure in male mice and the role of benzodiazepine and opioid receptors.

**Methods:** This study was conducted on 100 male mice, randomly categorized into 10 groups: Normal Saline (NS), two diazepam groups (0.025 and 0.1 mg/kg), three *S. lavandulifolia* extract groups (50, 100, and 200 mg/kg), diazepam 0.025 mg/kg+*S. lavandulifolia* extract 50 mg/kg, and three groups that pretreated with NS, flumazenil, or naloxone, 5 min before injection of 200 mg/kg *S. lavandulifolia* extract. After 30 min, PTZ (80 mg/kg) was injected into animals, and seizure indices were evaluated.

**Results:** The *S. lavandulifolia* extract attenuated the PTZ-induced seizures in a dose-dependent manner, and pretreatment with flumazenil reversed this effect. However, pretreatment with naloxone could not reverse this effect because seizure indices in the naloxone pretreated group were lower than that in the normal saline group. The combination of an ineffective dose of diazepam and *S. lavandulifolia* extract decreased PTZ-induced seizures.

**Conclusion:** The results of our study showed the anticonvulsant properties of hydroalcoholic extract of *S. lavandulifolia*. These effects might be due to the impact of the components of this extract on the central benzodiazepine system.

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## Highlights

- Hydroalcoholic extract of *S. lavandulifolia* attenuated the PTZ-induced seizures in a dose dependent manner.
- Pretreatment with flumazenil (blocker of benzodiazepines receptor) reversed anti-seizure effect of *S. lavandulifolia* extract.
- Combination of an ineffective dose of diazepam and *S. lavandulifolia* extract decreased PTZ-induced seizures.

## Plain Language Summary

Epilepsy is one of the most common neurological disorders after stroke and is characterized by recurrent seizures due to abnormal excessive neural activity in the brain. Although there are many anticonvulsant drugs on the market, not all patients with epilepsy can be treated and one-third of patients suffer from recurring epilepsy despite using different antiepileptic drugs and more than 50% of them show side effects during treatment. So, it is necessary to conduct further studies to develop more effective anti-epilepsy drugs with the minimum side effects. In recent years, plenty of studies have been conducted on medical plants, and *S. lavandulifolia* reported among the Iranian traditional medicine with anti-anxiety and sedative features. Some studies have mentioned the sedative and anti-inflammatory function of *S. lavandulifolia*, and its significant effects on anxiety have been approved comparable to diazepam. Overall, considering the anti-anxiety, analgesic, and sedative effects of the hydroalcoholic extract of *S. lavandulifolia*, it might possess anti-convulsive effects, too. The purpose of the current study was designed to investigate whether the effect of intra peritoneal injection of hydroalcoholic extract of *S. lavandulifolia* on the PTZ-induced convulsion in male mice and assessed the role of benzodiazepine and opioid receptors. Results of this study demonstrated that *S. lavandulifolia* extract attenuated the PTZ-induced seizures in a dose dependent manner, and pretreatment with flumazenil (blocker of benzodiazepines receptor) reversed this effect. However, pretreatment with naloxone (Non-selective blocker of opioids receptor) could not reverse this effect but the combination of an ineffective dose of diazepam and *S. lavandulifolia* extract decreased PTZ-induced seizures, thus anti-epileptic effect of *S. lavandulifolia* mediated by benzodiazepine receptors.

## 1. Introduction

**E**pilepsy is one of the most common neurological disorders after stroke and is characterized by recurrent seizures due to abnormal excessive or synchronous neural activity in the brain (Katzung, Masters, & Trevor, 2012). Seizure arises due to excessive excitation or loss of inhibition in the brain (Stafstrom, 2010). Although there are many anticonvulsant drugs on the market, not all patients with epilepsy can be treated, and one-third of patients suffer from recurring epilepsy despite using different antiepileptic drugs and multidrug regimens (Mohanraj & Brodie, 2006). Moreover, more than 50% of epileptic patients show side effects of anti-epilepsy drugs during treatment (Krug, Koch, Grecksch, & Schulzeck, 1997). So, it is necessary to conduct further studies to develop more effective anti-epilepsy drugs with the minimum side effects. Ethnopharmacology and medicinal plants are considered new fields of interest in this area.

Using animal models is a proper way to establish epileptic models and identify the mechanisms involved in such diseases, evaluate novel antiepileptic medications,

and finally reach efficient therapeutic approaches in epileptic patients (Löscher, 2002; Trojnar, Wojtal, Trojnar, & Czuczwar, 2005). Pentylentetrazole (PTZ) is one of the derivatives of tetrazole and acts as an antagonist of Gamma-Aminobutyric Acid (GABA), which can lead to epilepsy in animal models through the inhibition of the GABA<sub>A</sub> receptors (Löscher, 2002; Mohammadi-Khanaposhtani et al., 2016) and blockade of chloride inflow (Naseer, Shupeng, & Kim, 2009).

In recent years, plenty of studies have been conducted on medical plants, and *Stachys lavandulifolia* is among the Iranian traditional medicine with approved anti-anxiety and sedative features (Kumar & Bhat, 2014; Rabbani, Sajjadi, & Jalali, 2005). This plant has shown many therapeutic features such as anti-oxidative (Saeedi, Morteza-Semnani, Mahdavi, & Rahimi, 2008), sedative, anti-inflammatory (Delfan, Bahmani, Rafieian-Kopaei, Delfan, & Saki, 2014), anti-anxiety (Rabbani et al., 2005), anti-diarrhea (Bahmani et al., 2014) and analgesic (Hajhashemi, Ghannadi, & Sedighifar, 2007). Some studies have mentioned the sedative and anti-inflammatory function of *S. lavandulifolia*, and its significant effects on anxiety have been approved comparable to diazepam.

It is proposed that the mentioned properties may be attributed to the presence of flavonoid, propanoid, and terpenoid components (Monji, HOSSEIN, Halvaei, & ARBABI, 2011; Neshat, Pour, & Balanejad, 2017; Rabbani et al., 2005). Nasri et al. showed that the hydroalcoholic extract of *S. lavandulifolia* aerial parts has analgesic and anti-inflammatory effects in male mice (Nasri, Ramezanghorbani, & Kamalinejad, 2011). In addition, Nasri et al. reported that the chloroformic fraction of *S. lavandulifolia* extract had a spasmolytic effect on ileum contractility of mice and this effect is mediated mainly via disturbing the calcium mobilization and partly by opioid receptors' activation (Nasri, Adibpour, Namjooyan, Rezaee, & Shahbazi, 2011). Overall, considering the anti-anxiety, analgesic, and sedative effects of the hydroalcoholic extract of *S. lavandulifolia*, it might possess anti-convulsive effects, too. So, in this study, we explored the effect of intraperitoneal injection of hydroalcoholic extract of *S. lavandulifolia* on the PTZ-induced convulsion in male mice and the role of benzodiazepine and opioid receptors in this reaction.

## 2. Methods

### Study animals

This study was conducted on 100 mature male mice weighing 25-30 g. The mice were purchased from Pasteur Institute (Karaj, Iran) and were kept at 12 h day-night circumstances, at 23°C temperature and fed ad libitum in the animal room of Guilan University of Medical Sciences. After one week accommodation time, the animals were randomly divided into 10 groups (n=10 mice in each group) as following: Normal Saline (NS) group, two diazepam positive control groups (0.025 and 1 mg/kg) (Rashidian et al., 2017), three groups of hydroalcoholic extract of *S. lavandulifolia* (50, 100, and 200 mg/kg) (Nasri et al., 2011; Rabbani et al., 2005), 0.025 mg/kg diazepam+50 mg/kg *S. lavandulifolia* extract (the simultaneous injection of extract ineffective dose and diazepam), NS+200 mg/kg *S. lavandulifolia* extract, flumazenil 2 mg/kg+S. *lavandulifolia* extract 200 mg/kg, and naloxone 5 mg/kg+S. *lavandulifolia* extract 200 mg/kg. In the last 3 groups, normal saline, flumazenil, and naloxone were injected 5 minutes before (Rashidian et al., 2017) the injection of *S. lavandulifolia* extract (Figure 1). In all study groups, the convulsion dose of PTZ (80 mg/kg) was injected 30 minutes after the administration of mentioned interventions (Keshavarz, Fotouhi, & Rašti, 2016). Drugs and saline injections were performed intraperitoneally.

### Drugs

PTZ was purchased from Sigma-Aldrich, and flumazenil ampoule from Hmlen, Germany. Naloxone and diazepam were purchased from Tolid Daru and Daru Pakhsh companies (Iran). The medications were injected intraperitoneally at 5 mL/kg. All drugs and extract were freshly prepared to the desired concentration before being used.

### Plant extraction

The aerial parts of *S. lavandulifolia* were collected from the Deilaman-Pirkooch area in summer. After the taxonomical confirmation of the plant herbarium by Department of Pharmacology, Tehran University of Medical Sciences, they were left to be air-dried and then thoroughly pulverized. The plant extract was prepared by two times percolation method using hydroalcoholic solvent (80% methanol). The concentration of the yielded extract was performed by the rotary at almost 50°C. This extract was weighed precisely and 50, 100, and 200 mg concentrations were prepared in the volume of 5 mL/kg for the animal injection.

### PTZ-induced seizure

The anticonvulsant activity of the hydroalcoholic extract of *S. lavandulifolia* was determined with a PTZ-induced seizure test. In this model of induced seizure, the ability of the novel compounds to protect mice against convulsion doses of PTZ (80 mg/kg) was evaluated. Vehicle group mice received an equal volume of normal saline. After 30 min, PTZ was injected intraperitoneally, and then the animals were left in a fiberglass chamber with the dimensions of 70×70×50 cm, and convulsive behaviors were observed at least 30 min after the administration of PTZ by video recording. These convulsive parameters included the latency period for initiation of clonic seizure, delay time to start tonic-clonic seizure, and finally, the mortality rate in 24 h (Aghaei et al., 2015; Keshavarz et al., 2016; Rostampour, Ghaffari, Salehi, & Saadat, 2014). The latency period for the initiation of clonic seizure shows the time interval between PTZ injection and the start of clonic seizure, which was calculated as the needed time for the initiation of the clonic seizure (latency period). In case of not observing tonic-clonic seizure in a 30-minute follow-up, 1800 s was considered in the calculations. The seizure occurrence was assessed after video recording by two observers blinded to the treatments. Observer reliability was assured via assessment using the kappa coefficient, where >80% reflect a satisfactory level of agreement between observers.

### Statistical analysis

The results of this study were presented as mean  $\pm$  Standard Error of the Mean (SEM). Statistical analysis was performed using SPSS (version 23). Data comparison among groups was performed using 1-way ANOVA followed by Tukey's post hoc test. The Kruskal-Wallis test and binary comparison of results were used for non-parametric data and assessing 24 hours mortality rate. The significance level was considered  $P < 0.05$ . This study was approved by the Regional Research Ethics Committee of Guilan University of Medical Sciences (approval code: IR.GUMS.REC.1397.4.12).

### 3. Results

#### Dose-dependent anticonvulsant effect of *S. lavandulifolia* extract compared to positive control group

Administration of *S. lavandulifolia* extract (50, 100, and 200 mg/kg) increased the latency period of clonic seizure initiation so that this time in the two groups which received 100 and 200 mg/kg of *S. lavandulifolia* extract were significantly more than that in the NS group ( $74.3 \pm 15.51$  and  $85.3 \pm 36.6$  versus  $45.8 \pm 6.28$  s, respectively,  $P < 0.01$ , Figure 2 A). Also, hydroalcoholic extract in concentrations of 100 and 200 mg/kg significantly increased the delay time of tonic-clonic seizure initiation ( $442.5 \pm 159.32$  and  $669 \pm 394.5$  s, respectively), compared to the NS group ( $66.1 \pm 31.5$  sec,  $P < 0.01$  and  $P < 0.001$  respectively, Figure 2 B). However, only 200 mg/kg of plant extract could significantly decrease the mortality rate compared to that in the NS group ( $P < 0.05$ , Figure 2 C).

In the positive control groups (0.025 and 1 mg/kg diazepam), the latency periods of clonic seizures initiation were  $48.6 \pm 5.66$  and  $113.4 \pm 46.66$  s, respectively, and the delay time periods of tonic-clonic initiation were  $126.6 \pm 94.81$  and  $1095 \pm 332.39$  s, respectively. However, 0.025 mg/kg dose of diazepam did not have any notable effects on convulsive indices compared to that in the NS group, but in the group that received 1 mg/kg of diazepam, the latency period of clonic and delay time of tonic-clonic initiation were significantly longer than those in the NS group ( $P < 0.001$ ). Moreover, the PTZ-induced mortality rate of this group was significantly lower than that in the NS group.

#### Exploration of the roles of benzodiazepine and opioid receptors on the anticonvulsant effect of hydroalcoholic extract of *S. lavandulifolia*

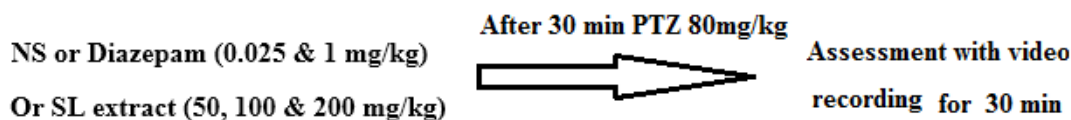
To assess the roles of benzodiazepine and opioid receptors on the anticonvulsant effect of hydroalcoholic extract of *S. lavandulifolia*, flumazenil (2 mg/kg) (GABA<sub>A</sub> benzodiazepine receptor blocker) and naloxone (5 mg/kg) (non-selective antagonist of opioid receptors) were separately injected 5 minutes before the injection of *S. lavandulifolia* extract (200 mg/kg). The latency periods of clonic seizure initiation was  $53 \pm 10.07$  and  $75.3 \pm 30.75$  s after pretreatment by flumazenil and naloxone, respectively, so the administration of flumazenil significantly decreased this latency period compared to the NS+200 mg/kg *S. lavandulifolia* extract group ( $85.88 \pm 38.76$  s,  $P < 0.01$ ), but this reduction was not significant compared to the NS group. In other words, pretreatment with flumazenil decreased the anticonvulsant effect of *S. lavandulifolia* extract. However, pretreatment with naloxone could not significantly affect the anticonvulsant effect of *S. lavandulifolia* extract, and the latency period was significantly higher than that in the NS group ( $P < 0.01$ , Figure 3 A). Pretreatment with flumazenil and naloxone decreased the delay time of tonic-clonic seizure initiation compared to NS+200 mg/kg *S. lavandulifolia* extract group ( $83 \pm 36$  and  $369.3 \pm 441.56$  vs  $656.67 \pm 416.68$  s, respectively). Pretreatment by flumazenil decreased the anticonvulsant effect of *S. lavandulifolia* extract compared to the NS+S. *lavandulifolia* extract group ( $P < 0.001$ ), but pretreatment with naloxone did not significantly reduce the anti-convulsive effect of 200 mg/kg *S. lavandulifolia* extract compared to the NS+S. *lavandulifolia* extract group and the delay time was still higher than that in NS group ( $P < 0.05$ , Figure 3 B).

Furthermore, pretreatment with flumazenil significantly inhibited the anticonvulsant effect of *S. lavandulifolia* extract so that the 24 h mortality rate was not significantly different between this group and the NS group. Nevertheless, naloxone pretreatment could not inhibit the anticonvulsant effects of *S. lavandulifolia* extract, and the mortality rate of the animals significantly decreased compared to that in the NS group ( $P < 0.05$ , Figure 3 C).

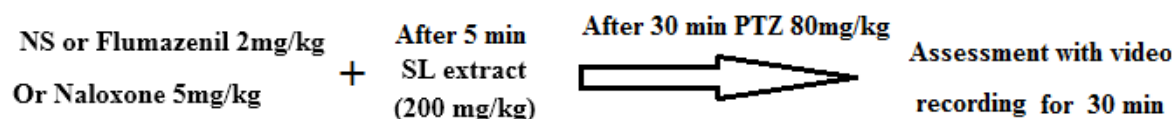
#### Anticonvulsant effect of simultaneous administration of non-effective dose of *S. lavandulifolia* extract and diazepam represented of GABAergic pathway

The separate administration of diazepam (0.025 mg/kg) and *S. lavandulifolia* extract (50 mg/kg) decreased seizure indices significantly compared to those indices in the NS group. However, simultaneous injection of these ineffective doses could significantly increase the latency period of clonic and delay time of tonic-clonic seizure initiation ( $76.7 \pm 22.19$  and  $538.2 \pm 677.52$  s, re-

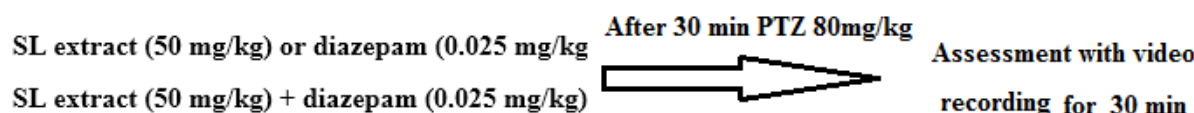
**Phase 1:**



**Phase 2:**



**Phase 3:**



**Figure 1.** Flowchart for different groups and drug administration

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Phase 1, dose-response of hydroalcoholic extract of *S. lavandulifolia* (SL) compared with Normal Saline (NS) and diazepam (positive control groups) on Pentylentetrazole (PTZ)-induced seizure;

Phase 2, exploring benzodiazepine and opioids receptor roles on the antiepileptic effect of SL extract by pretreatment with flumazenil and naloxone;

Phase 3, comparing the effects of alone and simultaneous administration of low doses of SL extract and diazepam on PTZ-induced seizure.

Drugs and saline were injected intraperitoneally.

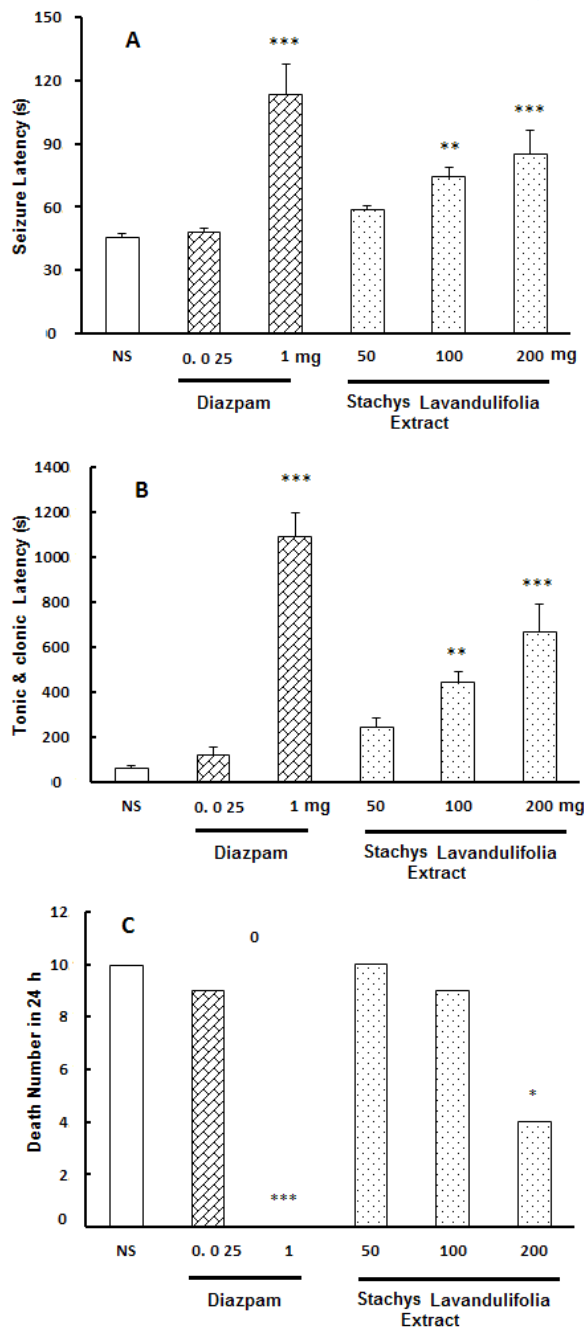
spectively), which were significantly longer than those in the NS group (48.6±5.66 and 66.10±31.53 s, respectively, P<0.001 and P<0.05, [Figure 4 A and B](#)). Also, the mortality rate significantly decreased after simultaneous administration of ineffective doses of diazepam and hydroalcoholic extract of *S. lavandulifolia* compared to that in the NS group (P<0.01, [Figure 4 C](#)).

#### 4. Discussion

The current study results revealed that hydroalcoholic extract of *S. lavandulifolia* has an anticonvulsant effect that attenuated the PTZ-induced seizures in a dose-dependent manner. Administration of hydroalcoholic extract of *S. lavandulifolia* increased the latency period of clonic seizure initiation and delay time of tonic-clonic initiation and decreased mortality rate compared to the NS group. In addition, the simultaneous administration of ineffective doses of diazepam and hydroalcoholic extract of *S. lavandulifolia* had an anti-convulsive effect. The blockade of benzodiazepine receptors by pretreatment with flumazenil decreased the anti-convulsive effects of *S. lavandulifolia* extract; however, the blockage of opioid receptors by pretreatment with naloxone could not inhibit the anti-convulsive effect of the *S. lavandulifolia* extract.

In the current study, the administration of 200 mg/kg hydroalcoholic extract of *S. lavandulifolia* increased the latency period of clonic seizure initiation and the delay time of tonic-clonic initiation and decreased mortality rate just like 1 mg/kg dose of diazepam as a positive control treatment group. Similar to our study, Bahramnejad et al. reported that peritoneal injection of diazepam could increase the delay time of clonic and tonic-clonic seizure initiation in male mice ([Bahramnejad et al., 2018](#)). Also, the mortality rate reached following the injection of the effective dose of diazepam, which was following the results of Rezvani Nejad et al. ([Nejad et al., 2017](#)).

*Stachys lavandulifolia*, a plant in Iranian traditional medicine, has anti-anxiety, sedative ([Kumar & Bhat, 2014](#); [Rabbani et al., 2005](#)), and spasmolytic effects ([Duke, 2002](#); [Narayan & Kumar, 2003](#)). Some studies reported that *S. lavandulifolia* extract has components such as flavonoid, propanoid, and terpenoid, which contribute to the sedative function of *S. lavandulifolia*. The significant effects of this plant on anxiety have been approved comparable to diazepam ([Monji et al., 2011](#); [Neshat et al., 2017](#); [Rabbani et al., 2005](#)). Because flavonoids have a similar structure to GABA<sub>A</sub> receptor ligands ([Wasowski & Marder, 2012](#)), thus the plant may have a modulatory

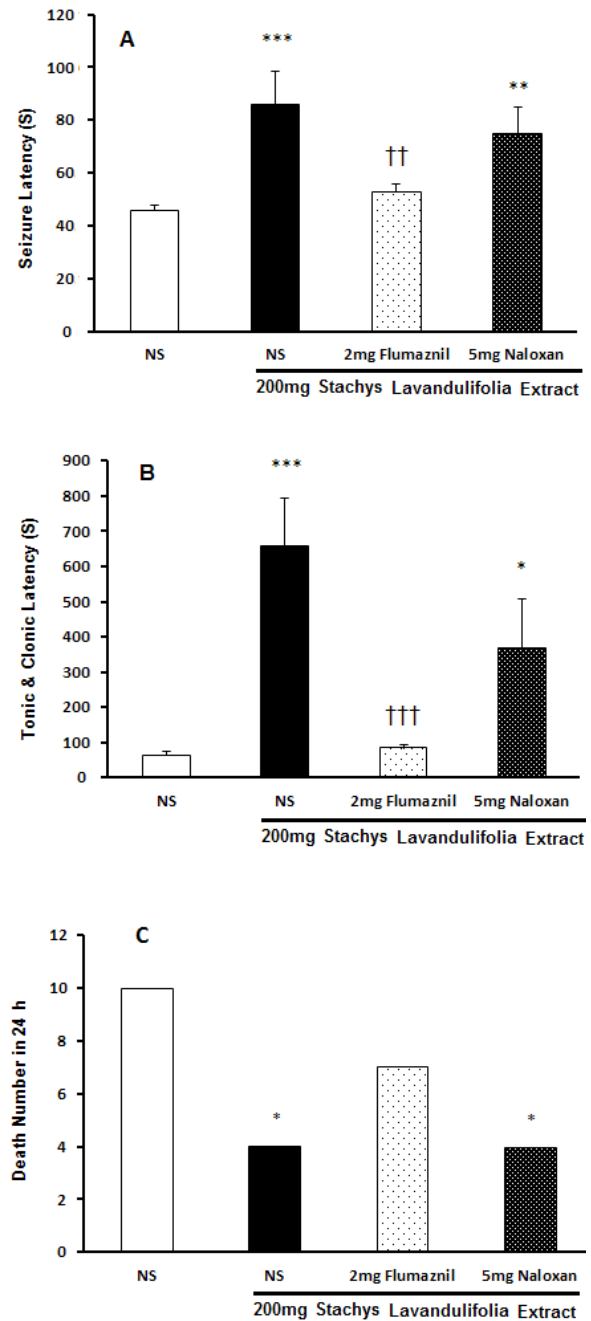


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**Figure 2.** The effects of hydroalcoholic extract of *S. Lavandulifolia* on Pentylene tetrazole (PTZ)-induced seizure parameters and comparing them with the normal saline and diazepam groups

A) The latency period of clonic seizure initiation, B) the delay time of tonic-clonic seizure initiation, and C) the mortality rate after 24 hours.

\*P<0.05, \*\*P<0.01 and \*\*\*P<0.001 compared to the normal saline group.

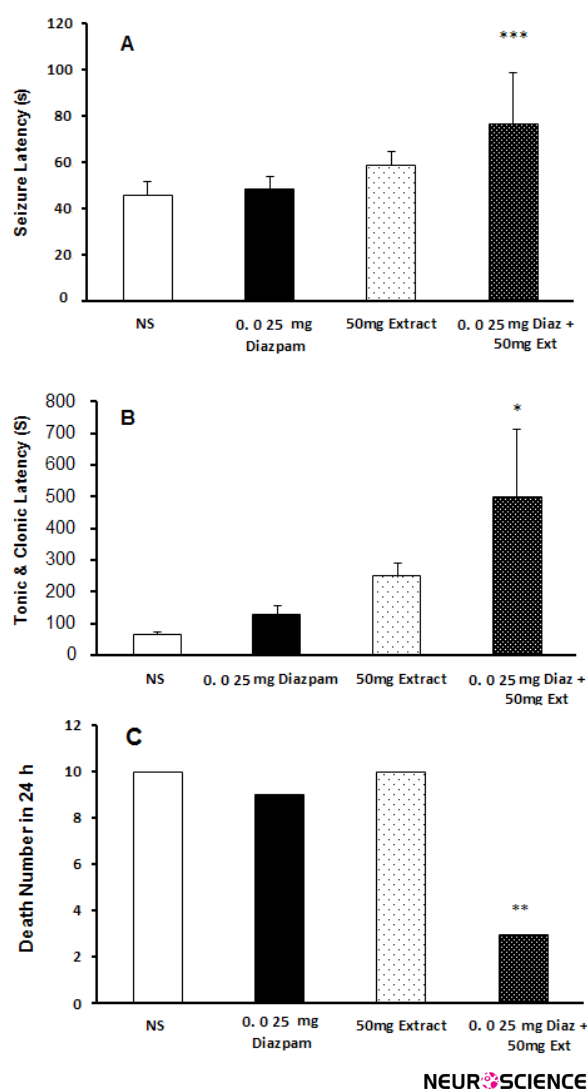


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**Figure 3.** The effects of flumazenil and naloxone pretreatment on anticonvulsant properties of hydroalcoholic extract of *S. lavandulifolia* (SL)

A) The latency period of clonic seizure initiation, B) the delay time of tonic-clonic seizure initiation, and C) the mortality rate after 24 hours.

\*P<0.05, \*\*P<0.01 and \*\*\*P<0.001 compared to the normal saline group, and ††P<0.01 and †††P<0.001 compared to 200 mg/kg SL extract group.



**Figure 4.** The effect of simultaneous administration of hydroalcoholic extract of *S. lavandulifolia* and diazepam on Pentylentetrazole (PTZ)-induced seizure parameters

A) The latency period of clonic seizure initiation, B) the delay time of tonic-clonic seizure initiation, and C) the mortality rate after 24 hours.

\* $P < 0.05$ , \*\* $P < 0.01$ , and \*\*\* $P < 0.001$  compared to the normal saline group.

ry effect on seizure. The current study was conducted to explore anti-convulsive effect of hydroalcoholic extract of *S. lavandulifolia* on PTZ-induced seizure.

In the current study, the hydroalcoholic extract of *S. lavandulifolia* (100 and 200 mg/kg) had anti-convulsive effects, and the combination of ineffective doses of diazepam and extract showed anti-convulsive effects, too. This effect of *S. lavandulifolia* extract can be mediated by benzodiazepines receptors via hyperpolarization of neural resting membrane potential. In agreement with our study, Rabbani et al. argued that *S. lavandulifolia* ex-

tract has sedative and anti-anxiety effects. They proved the significant effects of this extract in sedation compared to diazepam and suggested that these effects could be due to the presence of components such as flavonoids, phenylpropanoids, and terpenoids (Rabbani et al., 2005). However, they did not evaluate the role of benzodiazepine receptors.

Furthermore, Nasri et al. showed that hydroalcoholic extract of *S. lavandulifolia* had analgesic and anti-inflammatory effects (Nasri et al., 2011). In another study, Naseri et al. evaluated the spasmolytic effects of chloroformic fraction of *S. lavandulifolia* plant on mouse ileum. They claimed that *S. lavandulifolia* had inhibitory effects on the movements and contractions of the intestine, which originated from disturbing calcium mobilization and the activation of opioid receptors, and antispasmodic effect was reduced by naloxone (Naseri et al., 2011). It seems that the anti-anxiety, analgesic, and spasmolytic effects of *S. lavandulifolia* extract may be mediated by benzodiazepines and or opioid receptors that lead to hyperpolarization of pain receptors and visceral smooth muscle. In the current study, the role of benzodiazepine receptors (by pretreatment with flumazenil) was evaluated alongside the role of opioid receptors (by pretreatment with naloxone).

Based on our study results, the blockade of benzodiazepine receptors before the injection of 200 mg/kg of *S. lavandulifolia* extract reversed the anti-convulsive effects of this plant extract. However, the blockade of opioid receptors could not significantly diminish the anti-convulsive effects of this extract. Based on these observations, the anti-convulsive effects of *S. lavandulifolia* extract mainly act through the impact on the GABA<sub>A</sub> receptor because the benzodiazepines have stimulatory effects on the GABA<sub>A</sub> receptor. In this study, pretreatment with flumazenil significantly decreased the anti-convulsive effect of hydroalcoholic extract of *S. lavandulifolia*. Also, co-injection of ineffective doses of diazepam and *S. lavandulifolia* extract caused notable anti-convulsive activities, while neither could show such effects when injected alone. This simultaneous effect might be partially described by the signal amplification of the GABA<sub>A</sub> receptor.

Moreover, *S. lavandulifolia* extract has active components such as phenylethanoid, terpenoid, and flavonoid with biological functions (Mohammadhosseini, Akbarzadeh, & Hashemi-Moghaddam, 2016; Monji et al., 2011; Neshat et al., 2017). Flavonoids are an important category of natural antioxidant components (Hajhashemi et al., 2007) with several neuro-pharmacologic features.

Some of these features are linked to GABA<sub>A</sub> receptors in the Central Nervous System (CNS) (Rabbani et al., 2005; Wasowski & Marder, 2012). PTZ induces convulsion mainly by antagonizing the GABA<sub>A</sub> receptor in the chloride channel complex, and the brain effects of flavonoids are associated with this receptor, too (Dirscherl et al., 2010). Hence, based on this information and according to previous studies, such as the study of Pages et al., which showed positive responses to flavonoid components in PTZ-induced convulsion (Abbasi, Nassiri-Asl, Shafeei, & Sheikhi, 2012), a part of anti-convulsive effects of hydroalcoholic extract of *S. lavandulifolia* can be attributed to its flavonoid components.

Also, the alleviating effect of the central opioid system on convulsion is known (Naseer et al., 2009). Many studies have shown that low doses of morphine ( $\mu$ -opioid receptor agonist) have anti-convulsive effects, while higher doses would make the model animals vulnerable to the convulsion induced by epileptogenic agents such as PTZ (Pages et al., 2010). Naloxone, which is considered a nonspecific opioid receptor antagonist (claimed by Lauretti et al. in Hong, 1992) and can mimic these effects of morphine. Similarly, Kazemi Roodsari et al. showed that pretreatment with different doses of methadone before the injection of PTZ significantly decreased the convulsion threshold. In contrast, the injection of various doses of naltrexone, as an opioid receptor antagonist, decreased the pre-convulsive activity of methadone in the acute phase (Kazemi Roodsari, Bahramnejad, Rahimi, Aghaei, & Dehpour, 2019). Similar to this observation, we showed that naloxone pretreatment before *S. lavandulifolia* extract injection decreases the delay time of PTZ-induced seizure; however, this reduction was not statistically significant because of the utilization of single doses of naloxone and extract. Therefore, the effects of hydroalcoholic extract of *S. lavandulifolia* can be related to the central opioid system, too, which needs to be explored in detail.

In the current study, LD<sub>50</sub> was not assessed, but the toxicity profile of hydroalcoholic extract of *S. lavandulifolia* has been evaluated in some studies (Monji et al., 2011; Taghikhani, Afrough, Ansari Samani, Shahinfard, & Rafeian-Kopaei, 2014). Monji et al. reported that acute (24 h), sub-acute (14 days), and sub-chronic (45 days) administration of *S. lavandulifolia* extract (140 mg/kg oral gavages) causes hepatic and renal toxicity in female mice. So that after 45 days administration of *S. lavandulifolia* extract, abnormal changes in kidney and liver weight as well as biochemical parameters were significantly increased in treatment groups suggesting the possible role of this extract with doses higher than 140 mg/

kg. They proposed that a dose up to 70 mg/kg could be considered with no observable adverse effect and used it in further study (Monji et al., 2011). Therefore, a low dose of *S. lavandulifolia* extract can be used with another antiepileptic drug for treating seizures in future studies.

## 5. Conclusion

Our study showed the anti-convulsive properties of hydroalcoholic extract of *Stachys lavandulifolia*. These effects might be due to the impact of the components of this extract on the central benzodiazepine system. It seems that hydroalcoholic extract of *S. lavandulifolia* could be used as a proper approach to control convulsion seizures if more detailed mechanistic studies take place in this field.

## Ethical Considerations

### Compliance with ethical guidelines

This study was approved by the Regional Research Ethics Committee of Guilan University of Medical Sciences (Code: IR.GUMS.REC.1397.4.12).

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### Authors' contributions

All authors equally contributed to preparing this article.

### Conflict of interest

The authors declared no conflict of interest.



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