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Original Article

In Vivo and *In Silico* Study of Lavender Extract in Pentylenetetrazol-induced Seizures Compared to Diazepam and Phenytoin in Male Mice

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Abstract

Objective: Lavender contains essential oils that can cross the blood-brain barrier and affect the activity of brain neurons. Therefore, its antiepileptic effects were investigated in this study.

Methods: Forty two male mice were randomly assigned to 7 groups of 6 mice each. Included: (1) control group receiving distilled water; (2) the kindled group receiving only pentylenetetrazol; (3) experimental groups that received extract at different doses of lavender; and (4) two experimental groups receiving diazepam and phenytoin. Epileptogenesis was assessed by monitoring chemical seizure scores. Memory was assessed using a novel object recognition (NOR) test. Histological changes were assessed by hematoxylin and eosin staining. The antioxidant effect of lavender extract was assessed by measuring malondialdehyde (MDA) levels. Binding affinities of phytochemical compounds were assessed by molecular docking.

Results: Data analysis indicated that treatment with *Lavadula* extracts had a pronounced effect on chemical kindling. The result of the NOR test demonstrated that lavender extract ameliorated memory impairment. Histological examination showed that lavender extract had neuroprotective effects in the CA3 region of the hippocampus. The data showed that pretreatment with lavender extract decreased brain MDA levels. *In silico* studies showed that lavender phytochemicals have a high affinity for $GABA_A$ receptors and voltage-gated sodium channels.

Conclusion: The findings suggest that lavender extract has considerable anticonvulsant, neuroprotective, and antioxidant effects on pentylenetetrazol-induced kindling in hippocampal tissue. These medicinal herbs may be beneficial for the treatment of seizures in humans; however, further investigation is warranted.

Keywords: Epilepsy, pentylenetetrazol, kindling, lavender, diazepam, phenytoin, hippocampus, *in silico* study

INTRODUCTION

Epilepsy is one of the most common disorders of the central nervous system (CNS); it is characterized by spontaneous seizures and affects about 1% of the world's population. Epilepsy cannot be cured, but seizures can be effectively controlled in about 70% of cases with medication alone. However, low availability, and side effects of antiepileptic drugs and drug-resistant epilepsy negatively affect the quality of life of patients in countries with weak health systems.[1,2] Diazepam and phenytoin, like other drugs used to treat this condition, have long been considered anticonvulsants. Since the 1960s, benzodiazepines have continued to play an important role in the control of epilepsy and are among the first-line drugs in convulsive states. The main clinical advantages of diazepam are its high efficacy, rapid onset of action, and low toxicity. Diazepam is a GABA receptor agonist (about 20-50% of all synapses use GABA as a neurotransmitter), which increases the inhibitory state of the brain and suppresses seizures.[3] GABA via $GABA_A$ receptors has an inhibitory effect on synaptic communication in the CNS. Phenytoin is commonly recognized as a broad-spectrum sodium-channel blocker and affects nearly all voltage-gated sodium channels. Its anticonvulsant effect is mainly achieved by disrupting the positive feedback mechanism responsible for the spread of rapid, repetitive neuronal action potentials, thereby reducing seizure activity and selectively blocks high-frequency neuronal activity through the voltage-dependent blockade of membrane sodium channels.[4,5] In addition, phenytoin has been shown to that phenytoin inhibits calcium influx into presynaptic terminals, suggesting a mechanism for its membrane stabilizing properties.[6]

Species of the genus *Lavadula* are widely recognized as important medicinal and aromatic plants and have considerable economic significance for the pharmaceutical sector. Lavender essential oil has been used as a therapeutic agent in traditional medicine for centuries because of its sedative and antidepressant properties. Lavender essential oil contains monoterpenes (linalool, terpinen-4-ol, α-terpineol, camphor, 1,8-cineol, and borneol).[7,8] Lavender oil has been suggested to be anticonvulsant, antianxiety, analgesic.[9,10]

Research has indicated that certain brain regions are more prone to triggering and developing seizure activity compared to other areas, the most important and best-known areas are the temporal lobe and hippocampus.[11] The hippocampus is the primary tissue in which epileptic attacks occur, and hippocampal sclerosis is the most common tissue damage observed in the temporal lobe.[12] One method of studying epilepsy is to create animal models using kindling. The kindling model is a well-known method for studying the mechanisms of epilepsy and investigating new anticonvulsant drugs. Pentylenetetrazol (PTZ)-induced kindling is one of the animal models used to study epilepsy.[13,14] Epilepsy is characterized by recurrent seizures that are frequently accompanied by cognitive impairments and psychosocial complications, although the underlying mechanisms have not yet been fully clarified. Stimulation with PTZ has been reported to markedly influence the hippocampus, a brain region that plays a central role in the formation of new declarative memories.[15] Evidence also suggests that oxidative stress contributes substantially to both the onset and progression of epileptic seizures. The results of several studies indicate that, under certain procedural conditions, temporary or permanent lesions of the hippocampus affect object memory processes as measured by the spontaneous object recognition task. Oxidative stress has been shown to play a significant role in the initiation and progression of epileptic seizures.[16] Malondialdehyde (MDA), a final product of the peroxidation of polyunsaturated fatty acids, is recognized as a major metabolite of arachidonic acid and a reliable marker of oxidative stress. Therefore, measuring MDA concentrations in biological systems is widely considered a useful approach for assessing lipid peroxidation under both *in vitro* and *in vivo* conditions associated with different pathological states.[17,18]

Plants represent an important reservoir of undiscovered compounds for early drug discovery. Many plant extracts and chemical compounds from around the world have demonstrated anticonvulsant properties in experimental animal models exhibiting the phenotypic characteristics of epilepsy. In addition, cognitive-enhancing, neuroprotective, and anti-inflammatory activities of many plant extracts and compounds may help in the treatment of epilepsy. Several studies have been conducted to investigate the effects of different types of lavender on neurodegenerative diseases. For example, it has been shown that in Alzheimer's disease, treatment with lavender extract improves spatial learning deficits.[19] It has also been shown in a PTZ-induced epilepsy model that in brain tissue homogenates, lavender treatment can modulate inflammatory factors such as MDA, nitric oxide, and superoxide dismutase.[20] Studies have also shown that the species lavender dentata can reduce pro-oxidant markers in hippocampal homogenates from a pilocarpine-induced kindling model. This study also showed that levels of inflammatory markers were reduced. Also, the extract of this plant can reduce the level of brain excitability by membrane repolarization.[21,22] However, due to limitations in research on the extract and essential oils of this plant, further studies are needed to draw conclusions about its effectiveness in treating epilepsy. In this study, we therefore used the PTZ kindling model of epilepsy in mice to assess the antiepileptic effects of lavender extract and to compare its activity with diazepam and phenytoin. In addition, cognitive, antioxidant, histological, behavioral, and computer modeling studies were simultaneously conducted, which allowed for more comprehensive information on the effectiveness of the extract in an animal model of epilepsy.

MAIN POINTS

- Lavender extract shows efficasy in pentylenetetrazol-induced seizures.
- Lavender antiepileptic effects were demonstrated in histological studies, antioxidants assessment and behavioral studying.
- *In silico* studies were also used to confirm the *in vivo* findings.

METHODS

Chemical Substances

PTZ (Sigma Chemical Co., United States) was used in this study. In this experiment, mice were intraperitoneally (i.p.) injected with PTZ dissolved in 0.9% normal saline. Phenytoin (Atipharmed Pharmaceuticals Co., Iran). was freshly dissolved in distilled water using dilute NaOH (final pH of the solution was approximately 11) and administered orally via gavage in a volume of 0.5 mL[23] and diazepam (Atipharmed Pharmaceuticals Co., Iran) dissolved in distilled water and administered orally via gavage in a volume of 0.5 mL.[24]

Preparation of the Aqueous Extract

In this experiment, an aqueous extract was prepared from lavender using the soaking method. 2 g of lavender powder was mixed with 50 mL of water, and the mixture was heated at 35 °C using a magnetic stirrer for 30 minutes. The mixture was then subjected to an ultrasonic bath for 30 min. One of the main advantages of ultrasonication is that it disrupts plant cell walls, facilitating the release of intracellular compounds and metabolites from lavender. After sonication, the extract was evaporated to separate the solvent from other components. For evaporation, sample was then dried in an oven (UF30; MEMMERT Co.) at 35 °C to prevent degradation of secondary metabolites.[25]

Experimental Animals

Swiss male albino mice (26-32 g) were obtained from Tabriz University and acclimatized in animal housing. They were kept under controlled conditions: temperature 23±2 °C, relative humidity 51±10%, and a 12:12 h light/dark cycle. Animals had free access to standard rodent chow and water.

Evaluation of Behavior-induced Induced Epileptic Animals

PTZ (45 mg/kg, i.p.) was injected 45 min after gavage, at a subconvulsive dose every 48 h, to induce seizures in the mice. This procedure was repeated 15 times at 48 h intervals. Animals were placed in a glass box to monitor their behavior after gavage with the extract and injection of the drug; their activity was recorded using a camera. Seizure behavior was observed for 30 min after each PTZ injection. The intensity of seizure responses was scored according to the chemical kindling scale: phase 0 (no response), phase 1 (myoclonic jerk), phase 2 (straub tail), phase 3 (clonic jerkwithout loss of righting reflex), phase 4 (clonic seizure with loss of righting reflex) phase 5 (tonic seizure) and phase 6 (death).[26,27]

Kindling is considered to occur when an animal reaches Stage 4 or Stage 5 for 3 consecutive days after PTZ administration. The experimental groups were as follows: Group 1: normal control (vehicle only); Group 2: kindling group (PTZ 45 mg/kg; i.p.);

Groups 3-5: treatment groups receiving different doses of lavender extracts. Group 3 received PTZ+LAV 200 (45 mg/kg PTZ+200 mg/kg lavender extract). Group 4 received PTZ+LAV 400 (45 mg/kg PTZ+400 mg/kg lavender extract). Group 5 received PTZ+LAV 800 (45 mg/kg PTZ+800 mg/kg lavender extract). Each doses of lavender extract was dissolved in distilled water and administered orally (0.5 mL) 30 min before PTZ injection.[22]

Phenytoin and Diazepam

Phenytoin and diazepam were used at doses of 40 mg/kg and 2 mg/kg, respectively.[27,28]

Histopathological Examination

The brains of the dissected mice were fixed in 10% formalin and embedded in paraffin. Formaldehyde infiltration into tissues depends on factors such as tissue type, fixation time, tissue-to-fixative volume ratio, and temperature. In this study, mice were first anesthetized, and their brains were removed within a golden period of 2 min. The tissues were then fixed at room temperature in formalin with a fixative-to-tissue ratio of 5:1 for 3 days. Laboratory experience has shown us that, because the softness and thickness of mouse brain tissue differ from those of rats, obtaining good-quality slices from mouse brains does not require transcardial perfusion fixation, unlike rat brains. Coronal 6µm thick sections were cut at the hippocampal level using a microtome and fixed on standard glass slides (75×25×1 mm).[29] Each section was deparaffinized in xylene, rehydrated through graded ethanol, and stained with hematoxylin and eosin (H&E). The slides were then stained and examined under a light microscope for histological examination.[30,31]

On the other hand, in the H&E-stained coronal sections of the CA3 across the different lavender extract and drug groups, pyramidal neurons were similar to those in the control group, and no obvious pathological changes were observed.

Novel Object Recognition Test

The novel object recognition test (NORT) is a behavioral test currently used to assess learning and memory in rodents. NORT is conducted over 4 days: one day for habituation, two days for training, and a final day for testing. The stages of NORT are shown in Figure 1. On the first day, each mouse was placed in a box with a length of 65 cm, a width of 45 cm, and a height of 45 cm for 5 min. During training, 24 h later, two identical objects (matched for color, shape, and texture) were placed in different corners of the box, each at a distance of 10 cm from the wall. On the third day, the same procedure was repeated. Animals were allowed to explore similar objects. On the test day, one of the familiar objects was replaced by a new object. The time spent exploring the familiar and novel objects was recorded. The difference in exploration time was considered an index of recognition memory. The test was performed every 48 h after PTZ administration and after extract and drug pretreatments.[32]

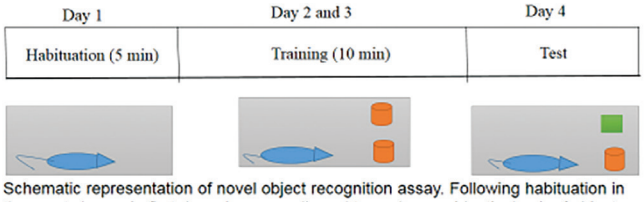


Figure 1. Schematic representation of NOR
NOR: Novel object recognition

Assessment of Antioxidant Index

Brain tissues from the experimental groups were rapidly removed after anesthesia of mice with ketamine and xylazine at doses of 100 mg/kg and 10 mg/kg, respectively,[33] frozen at -24 °C, and were homogenized in phosphate buffer (pH 7.4). Samples were then centrifuged at 10,000 rpm for 10 min, and the supernatant was collected. For derivatization of the MDA-thiobarbituric acid (TBA) complex, 50 µL of each supernatant was transferred to a 2 mL plastic tube, to which 50 microliters of 0.05% butylated hydroxytoluene solution, 100 microliters of 0.44 M phosphoric acid solution, and 100 microliters of 42 mM TBA solution were added, and the mixture was vortexed for 5 min. Samples were placed in a 100 °C water bath for one hour and then transferred to an ice bath for 5 min. After cooling the samples for MDA-TBA derivatization, 25 microliters of butanol were added to each sample. After 5 min, the samples were centrifuged at 10,000 rpm for 10 min to obtain a biphasic system. Finally, 200 µL of the supernatant solution containing MDA-TBA was collected, and fluorescence was measured at an excitation wavelength of 532 nm using a spectrophotometer.[34]

Molecular Docking

Molecular docking simulations were performed to identify target proteins and their ligands for epilepsy. The selected targets for this study were GABA and voltage-dependent sodium channel (VDSC). To perform molecular docking, we first prepared our files for the docking process; our ligands' chemical-induced dimerization identifiers are 6549, 11230, 3016, and 1775 for linalool, terpineol, diazepam, and phenytoin, respectively. For protein preparation, we retrieved our protein data bank (PDB) files from research collaboratory for structural bioinformatics PDB database. PDB ID of our proteins are: 8S9C, 6X3X respectively named after VDSC and $GABA_A$. After obtaining the required files, several modifications were performed: the ligand file formats were converted from structure data format to PDB format using Open Babel software. Subsequently, molecular docking was performed using PyRx software. Among the results achieved, the most suitable ones were chosen for further investigation. To achieve ligand-receptor interaction plot we used Discovery Studio software.[35,36]

Statistical Analysis

In this study, data are presented as mean ± standard deviations. Statistical analyses were performed using SPSS software. Comparisons between experimental groups were conducted using the Kruskal-Wallis test, followed by Dunn's post-hoc test to compare the maximum seizure stage among groups. To compare seizure onset latency and seizure duration, a one-way analysis of variance was performed, followed by Tukey's post-hoc test for multiple comparisons between groups. A p-value <0.05 was considered statistically significant.

Ethics Committee Approval

The study was approved by the Azarbaijan Shahid Madani University Research Ethics Committee (approval no: IR.AZARUNIV.REC.1404.013, date: 02.06.2025).

RESULTS

Effect of Different Doses of Lavender Extract on Seizure Onset Latency

Figure 2 compares seizure onset latency among the treatment groups (different doses of lavender extract), the drug-treated groups (diazepam and phenytoin), and the kindled group. Data are presented as mean ± standard deviation and a p-value <0.05 was considered statistically significant. Different doses of lavender extract, as well as diazepam and phenytoin, significantly increased latency to seizure onset compared with the kindled group (as shown in the graph). Statistical analysis demonstrated that the treatment and drug-treated groups differed significantly from the kindled group.

The Effect of Different Doses of Lavender Extract on the Duration of Seizure

Figure 3 compares seizure duration between the treatment groups, drug treated groups, and the kindled group. Data are presented as mean ± standard deviation and a p-value <0.05 was considered statistically significant. As shown in the graph, pretreatment with different doses of lavender extract reduced seizure duration compared with that in the kindled group. However, the reduction in seizure duration was not statistically significant compared with the kindled group. In contrast, pretreatment with diazepam and phenytoin significantly reduced seizure duration. Statistical analysis showed significant difference between the diazepam and phenytoin treated groups and the kindle group.

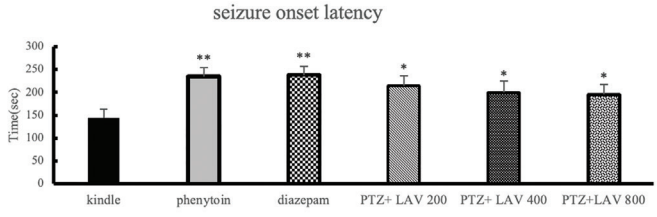


Figure 2. Pretreatment with diazepam, phenytoin and different dose of lavender extract compare to PTZ group significantly increased latency of seizure onset
*: p-value ≤0.05, **: p-value ≤0.01
PTZ: Pentylenetetrazol

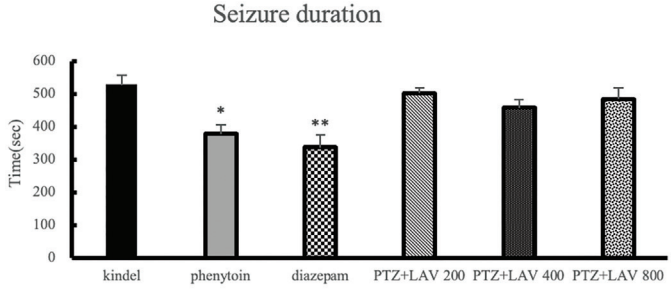


Figure 3. Pretreatment with diazepam and phenytoin significantly decreased seizure duration. Different dose of lavender extract in compare to PTZ group didn't decreased significantly. *: p-value ≤0.05, **: p-value ≤0.01
PTZ: Pentylenetetrazol

Effect of Different Doses of Lavender Extracts on the Progression of Seizure Stages

Figure 4 shows the progression of seizure stages over 15 days in groups treated with different doses of lavender extract and in drug-treated groups, compared with the kindled (PTZ) group. Data is presented as mean ± standard deviation and a p-value <0.05 was considered statistically significant. As shown in the graph, lavender extracts had an inhibitory effect on seizure progression compared with that in the kindled group. All doses of lavender extract kept seizure stages below Stage 5. Statistical analysis revealed a significant difference in seizure stage progression in the lavender extract-treated and drug-treated groups, compared with the kindled group.

Effect of Different Doses of Lavender Extract on the Discrimination Ratio

The novelty object recognition test, as shown in Figure 5, demonstrated that the discrimination index (DI) was significantly decreased in the kindled group compared with the control group. The discrimination ratio represents the proportion of time an animal spends exploring the novel object (T new) relative to the familiar object (Told), calculated with respect to the total exploration time (T total; Eq. 1). Findings from lesion-based and neurophysiological studies using the NORT paradigm indicate that the DI mainly reflects memory performance and sensitivity.

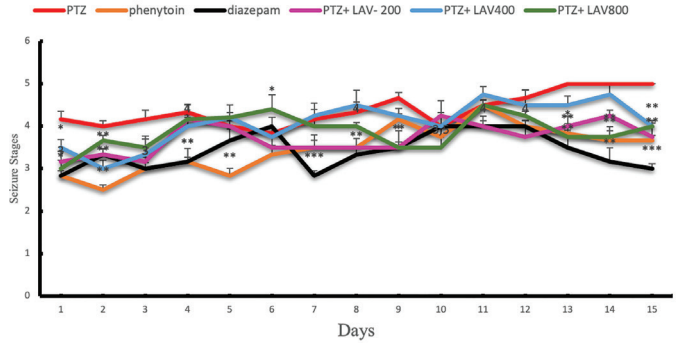


Figure 4. Pretreatment with diazepam and phenytoin and different doses of lavender extract significantly decreased seizure stages in compare to PTZ group. *: p-value ≤0.05, **: p-value ≤0.01 and ***: p-value ≤0.001
PTZ: Pentylenetetrazol

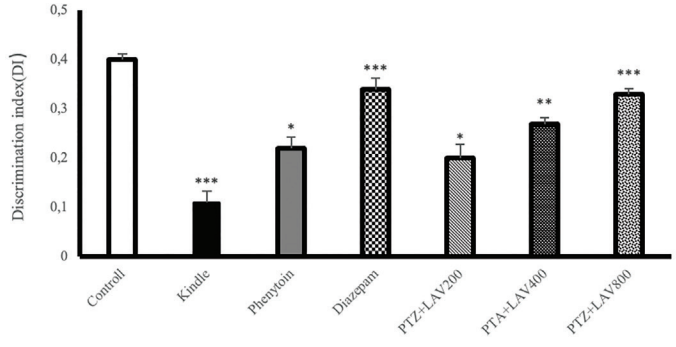


Figure 5. Pretreatment with different dose of lavender extract and diazepam and phenytoin improved of memory. In PTZ group memory decrease significantly in comparison to control group. Administration of diazepam, phenytoin and different doses of lavender extract improve memory significantly in comparison to kindle group. *: p-value ≤0.05, **: p-value ≤0.01 and ***: p-value ≤0.001
PTZ: Pentylenetetrazol

Pretreatment of animals with different doses of lavender extract predominantly increased the DI, an effect comparable to that of diazepam and phenytoin. Data is presented as mean ± standard deviation.

Equation 1: DI = T (new) – T (old) / T (total)

Fluorescence was measured using a spectrophotometer at an excitation wavelength of 532 nm. Data are presented as mean ± standard deviation.

Effect of Different Doses of Lavender Extract on the MDA Levels

As shown in Figure 6, MDA levels in hippocampal tissue, measured as an index of oxidative stress, were significantly increased in the kindled group compared with the control group. Absorbance measurements showed that the highest MDA levels were observed in the kindled group. Pretreatment with 200 mg/kg of lavender did not significantly change the MDA level. In contrast, pretreatment with dose 400 mg/kg decreased MDA levels, while 800 mg/kg produced a reduction comparable to the effects of diazepam and phenytoin. Fluorescence was measured using a spectrophotometer at an excitation wavelength of 532 nm. Data is presented as mean ± standard deviation.

Effect of Different Doses of Lavender Extract on the CA3 Region of the Hippocampus

Figure 7 shows the H&E staining of hippocampal tissue. Figure 7-A shows the general structure of hippocampal tissue in the control group. The coronal sections of the CA3 region of the hippocampus from the control group showed regular cellular architecture. The pyramidal cell layer(P)contained neurons that were uniform in size and distribution. Each neuron had a round, central nucleus with a prominent nucleolus (Figure 7-B). In contrast, H&E-stained sections of the CA3 region of the hippocampus in the kindled group did not show uniformity in neuronal size or distribution. The kindled group exhibited hyperchromatic nuclei and vacuolated cytoplasm (Figure 7-B). Pretreatment with diazepam and phenytoin (Figure 7-C), as well as with different doses of lavender extract (Figure 7-D), reduced neuronal degeneration in the hippocampus. Dark-stained neurons with severe degeneration are indicated by a thin arrow, whereas normal neurons are indicated by a thick arrow. Scale bar: 500 µm.

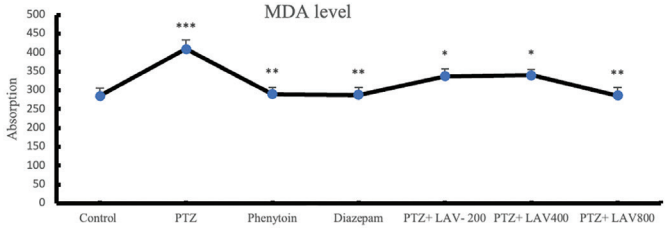


Figure 6. Administration of diazepam, phenytoin and different doses of lavender extract reduced MDA compared to the PTZ group. Administration of diazepam, phenytoin and different doses of lavender extract significantly, improved memory compared to the PTZ group. *: p-value ≤0.05, **: p-value ≤0.01 and ***: p-value ≤0.001
PTZ: Pentylenetetrazol, MDA: Malondialdehyde

Molecular Docking Studies of Linalool, Terpineol, Phenytoin and Diazepam with $GABA_A$ Receptor and VDSC Channels

In silico study of linalool showed this compound has high affinity for $GABA_A$ receptors and sodium channels. Recent studies have demonstrated that several monoterpenoids, including the acyclic compound linalool, potentiate $GABA_{ergic}$ currents through an allosteric mechanism *in vitro*, particularly after overexpression of inhibitory α1β2 $GABA_A$ receptors in different expression systems. In $GABA_A$ receptor: diazepam makes interactions with amino acids: tyrosine D: 160, glutamine D: 205, serine D: 204 and phenylalanine E: 77, tyrosine E: 58, histidine D: 102, phenylalanine D: 100. They also interact with these amino acids, exhibiting different interaction energies (ΔG). ΔG values for the compounds diazepam, phenytoin, linalool, and terpineol are -10.3 kJ, -7.7 kJ, -6.6 kJ, and -7.3 kJ, respectively.

In VDSC channels, diazepam interacts with the amino acids phenylalanine A: 1748, isoleucine A: 1744, tyrosine A: 1404, and alanine A: 1403. However, in this case, linalool binds to phenylalanine A: 1748, phenylalanine A: 1452, and other residues. But terpineol does not bind to the same amino acids as linalool and diazepam. The ΔG values for these compounds, diazepam, phenytoin, linalool, and terpineol, are -9.1 kJ, -8.6 kJ, -7.4 kJ, and -5.4 kJ, respectively.

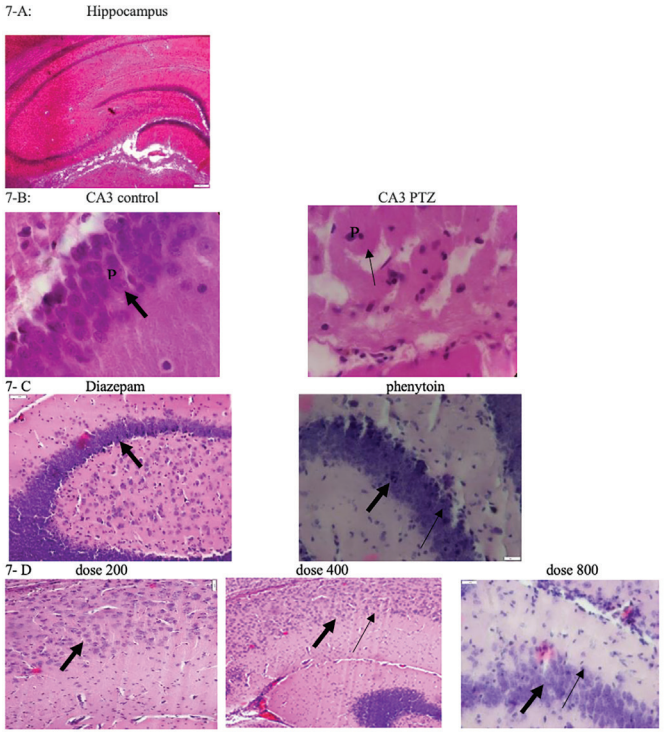


Figure 7. H&E staining of coronal sections of the hippocampal region in the PTZ kindling model. Figure of 7-A shows general shape of hippocampus in control group. 7-B: CA3 region of control and PTZ (×40). 7-C: CA3 region of diazepam and phenytoin group (×10) and 7-D: CA3 region of dose of 200 and 400 mg/kg of lavender extract (×10) and dose of 800 mg/kg 800 (40). Photomicrographs showing changes in staining of damaged neurons (dark-stained neurons with severe degeneration indicated by a thin arrow as compared to normal neurons indicated by a thick arrow. Scale bar: 500 µm
PTZ: Pentylenetetrazol, H&E: Hematoxylin and eosin

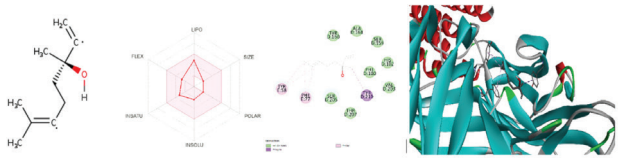
These compounds, linalool and terpineol, have shown acceptable binding affinity to $GABA_A$ receptors at the same binding site and with similar interacting amino acids as a diazepam and phenytoin. On the other hand, regarding VDSC, only linalool showed a binding pattern similar to that of phenytoin and diazepam, although with lower binding affinity. According to these results, with some modifications, linalool and terpineol may become suitable candidates as alternatives to diazepam and phenytoin. However, further investigation is required to fully evaluate their potential. The molecular docking results are shown in Figure 8. The binding affinities and ADME properties of compounds with target proteins are shown in Table 1.

DISCUSSION

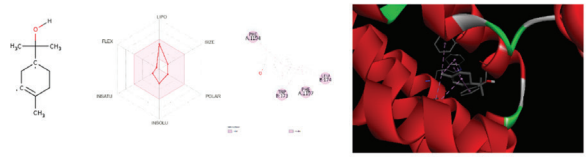
Different doses of lavender extract showed anti-seizure effects in PTZ-induced epilepsy. Administration of lavender extract 30 minutes prior to intraperitoneal injection of PTZ may allow its metabolites to reach the brain via the bloodstream, thereby reducing neuronal excitability. Consequently, the ability of PTZ to interact with the picrotoxin-binding site on GABA receptors is likely diminished; this reduction may contribute to the observed anticonvulsant effects.

Given that linalool and terpineol are the most abundant metabolites in lavender, the anticonvulsant effects are likely mediated by these compounds. Several studies have shown that linalool and terpineol can cross the blood-brain barrier and affect brain function. However, since lavender also contains other secondary metabolites, a more accurate conclusion requires comparing the effects of lavender extract with isolated of linalool and terpineol.[37,38]

Linalool is a secondary metabolite that can be isolated from a variety of aromatic plant species, such as lavender, *Ocimum,* and *Eucalyptus*. Among secondary metabolites, linalool is a major constituent (35-51%) of essential oils derived from various types of lavender. Several animal studies, have demonstrated that inhalation of linalool induces sedative-like behavior.[37] Recent studies have demonstrated that linalool, because of its low molecular weight and high lipophilicity, can cross the blood-brain barrier. This characteristic makes it a potential candidate for pharmaceutical applications aimed at influencing behavioral, cognitive, and sleep patterns. Linalool and its related compounds, by modulating different brain circuits, may have therapeutic potential in the management of several neurological and psychiatric conditions.[38] Evidence has demonstrated that linalool probably through anti-neuroinflammatory, antioxidant and neuroprotective effects, can be used for improvement of neurodegenerative diseases including Alzheimer's and Parkinson's.[38,39] In addition, some investigations suggest that linalool reduces glutamatergic hyper-stimulation and inhibits seizure and apoptotic processes.[39-41] Other studies have shown that linalool possesses anti-inflammatory properties and can decrease amounts of inflammatory cytokines, nitric oxide, and reactive oxygen species through inhibition of the NF-kB pathway.[42] Clinical studies have shown that inhaling lavender oil and petitgrain oil, both of which contain linalool, reduces anxiety-like behavior in humans. Another study in mice demonstrated that inhalation of linalool reduces anxiety-like behavior and improves sleep. Furthermore, the measurement of linalool in mouse brain tissue using a gas chromatography system equipped with a tandem quadrupole mass spectrometer revealed significant levels in different brain regions, indicating that it crosses the blood-brain

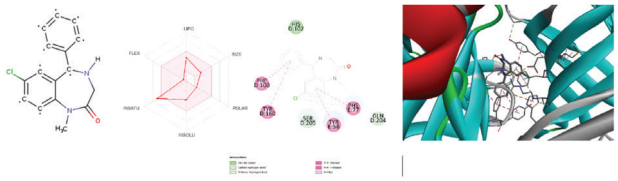


Linalool. (a) Structure (b) ADME radar graph (c) 2D interaction with $GABA_A$ graph (d) 3D interaction with $GABA_A$ graph



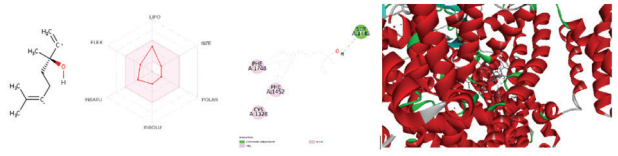
Terpineol. (a) structure (b) ADME radar graph (c) 2D interaction with $GABA_A$ graph (d) 3D ineraction with $GABA_A$ graph

Phenytoin. (a) Structure (b) ADME radar graph (c) 2D interaction with VDSC graph (d) 3D interaction with VDSC graph



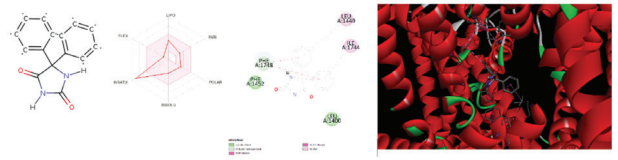
Diazepam. (a) Structure (b) ADME radar graph (c) 2D interaction with $GABA_A$ graph (d) 3D interaction with $GABA_A$ graph

Diazepam. (a) Structure (b) ADME radar graph (c) 2D interaction with VDSC graph (d) 3D interaction with VDSC graph



Linalool. (a) Structure (b) ADME radar graph (c) 2D interaction with VDSC graph (d) 3D interaction with VDSC graph

Terpineol. (a) Structure (b) ADME radar graph (c) 2D interaction with $GABA_A$ graph (d) 3D interaction with $GABA_A$ graph



Phenytoin. (a) structure (b) ADME radar graph (c) 2D interaction with VDSC graph (d) 3D interaction with VDSC graph

Figure 8. Molecular docking studies demonstrated intraction of linalool, terpineol, diazepam and phenytoin with $GABA_A$ receptors and VDSC channels VDSC: Voltage-dependent sodium channel

Table 1. Binding affinity and ADME properties of compounds with target proteins

	Binding affinity GABA (Kcal/mol)	Binding affinity VDSC (Kcal/mol)	Hydrogen bound GABA	Bioavailability score	Synthetic accessibility	GI absorption	BBB permeant
Linalool	-6.6	-7.4	0	0.55	2.67	High	Yes
Terpineol	-7.3	-5.4	1	0.55	3.17	High	Yes
Diazepam	-10.3	-9.1	0	0.55	2.68	High	Yes
Phenytoin	-7.7	-8.6	0	0.55	1.51	High	Yes

VDSC: Voltage-dependent sodium channel, GI: Gastrointestinal, BBB: Blood-brain barrier

barrier.[43] Results of some studies have shown that terpinen-4-ol, another phytochemical component found abundantly in lavender, efficiently reduces the severity of PTZ induced seizures and delays the onset of seizures.[44] Results of another study showed that terpinen-4-ol probably induces a depressant effect on the CNS through interaction with GABA receptors and suppresses convulsion activity of the brain significantly.[45] On the other hand, α-terpineol, an abundant compound in lavender, has neuroprotective effects in the hippocampus. It can inhibit neural damage and improve synaptic plasticity, which may in turn enhance hippocampal function and improve spatial memory following transient cerebral ischemia in rats.[46] In addition, investigations have demonstrated that α-terpineol, a phytochemical component of lavender, has antioxidant properties. Terpineol reduces nitric oxide synthase induces anti-inflammatory effects.[47] Recently, a study demonstrated the antioxidant, anti-inflammatory, and anti-fibrillatory properties of alpha-terpineol in an animal model of Alzheimer's, suggesting that this compound can cross the blood-brain barrier and affect brain tissue.[48] Another study showed that, in zebrafish with Parkinson's disease induced by the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), terpineol improved motor function and acetylcholinesterase activity in MPTP larvae. Terpineol also showed potent neuroprotective effects by reducing intracellular reactive oxygen species, lipid accumulation, and apoptosis markers.[49]

Lavender has antioxidant, anti-inflammatory, neuroprotective, and anticonvulsant properties, which are attributed to compounds such as linalool, terpinen-4-ol, and α-terpineol. The antioxidant effect of lavender is demonstrated by a reduction in MDA levels in hippocampal tissue. Evidence suggests that cellular death and tissue damage mediated by free radicals may contribute to neurological disorders.[50] MDA, an end-product of lipid peroxidation, was present at significantly higher levels in the kindling group than in the control group, and pretreatment with lavender extract significantly reduced MDA levels. As a result, lavender extract may act as a neuroprotective agent through a reduction in MDA levels, as seen in H and E-stained hippocampal slices and an improvement in memory in the NORT.

An *in silico* study of linalool showed that this compound has a high affinity for GABA receptors and sodium channels. Recent research has indicated that several monoterpenoid compounds, including the acyclic linalool, can increase $GABA_{ergic}$ currents through allosteric modulation *in vitro*, particularly when inhibitory α1β2 $GABA_A$ receptors are overexpressed in heterologous expression systems.[50-52]

In silico studies showed that linalool, terpineol, diazepam and phenytoin bind to $GABA_A$ receptors with high affinity. Interactions with affinity higher than -5 Kcal/mol are considered indicative of good binding. At these values, molecules bind tightly, forming a stable complex even at low concentrations.

Among these substances, diazepam has the highest and linalool the lowest affinity for $GABA_A$ receptors. Our results show that linalool, terpineol, diazepam, and phenytoin bind to VDSC with high affinity. Diazepam binds to this channel with the highest affinity, whereas terpineol binds with the lowest affinity.

Study Limitations

To confirm the effects of lavender extract, it would have been preferable to inject pure linalool and terpineol into an animal model; this approach could have validated *in silico* findings and would have yielded more accurate results.

CONCLUSION

These results suggest that phytochemicals in lavender extract, due to their low molecular weight and high lipophilicity, can cross the blood-brain barrier, enter the brain, and exert antioxidant, anti-inflammatory, and neuroprotective effects. *In silico* studies conducted as part of this research showed that lavender phytochemicals bind with high affinity to GABA receptors and to VSCD. Therefore, this plant may be considered a potential candidate for the development and synthesis of novel, effective drugs for patients with epilepsy and other neurological disorders.

Ethics

Ethics Committee Approval: The study was approved by the Azarbaijan Shahid Madani University Research Ethics Committee (approval no: IR.AZARUNIV.REC.1404.013, date: 02.06.2025).

Informed Consent: It was not deemed necessary, as it was an animal experiment.

Footnotes

Authorship Contributions

Surgical and Medical Practices: S.A.P., Concept: S.N.A., Design: S.N.A., Data Collection or Processing: S.A.P., A.P., Analysis or Interpretation: S.N.A., S.A.P., Literature Search: S.A.P., A.P., Writing: S.N.A., A.P.

Conflict of Interest: No conflict of interest was declared by the authors.

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REFERENCES

1. Abdollahi Fard M, Shojaii A. Efficacy of Iranian traditional medicine in the treatment of epilepsy. *Biomed Res Int.* 2013;2013:692751. [Crossref]

2. Browne TR, Holmes GL. Handbook of epilepsy. 4th ed. Philadelphia: Lippincott Williams & Wilkins; 2008. [Crossref]
3. Riss J, Cloyd J, Gates J, Collins S. Benzodiazepines in epilepsy: pharmacology and pharmacokinetics. *Acta Neurol Scand.* 2008;118(2):69-86. [Crossref]
4. Yaari Y, Selzer ME, Pincus JH. Phenytoin: mechanisms of its anticonvulsant action. *Ann Neurol.* 1986;20(2):171-184. [Crossref]
5. Sohn RS, Ferrendelli JA. Anticonvulsant drug mechanisms. Phenytoin, phenobarbital, and ethosuximide and calcium flux in isolated presynaptic endings. *Arch Neurol.* 1976;33(9):626-629. [Crossref]
6. van Vliet EA, van Schaik R, Edelbroek PM, et al. Inhibition of the multidrug transporter P-glycoprotein improves seizure control in phenytoin-treated chronic epileptic rats. *Epilepsia.* 2006;47(4):672-680. [Crossref]
7. Radu D, Alexe P, Stănciuc N. Overview on the potential role of phytochemicals from lavender as functional ingredients. *Ann Univ Dunarea de Jos Galati Fascicle VI Food Technol.* 2020;44(2):173-188. [Crossref]
8. Białoń M, Krzyśko-Łupicka T, Nowakowska-Bogdan E, Wieczorek PP. Chemical composition of two different lavender essential oils and their effect on facial skin microbiota. *Molecules.* 2019;24(18):3270. [Crossref]
9. Aprotosoaie AC, Gille E, Trifan A, Luca VS, Miron A. Essential oils of Lavadula genus: a systematic review of their chemistry. *Phytochem Rev.* 2017;16(4):761-799. [Crossref]
10. Bahr TA, Rodriguez D, Beaumont C, Allred K. The effects of various essential oils on epilepsy and acute seizure: a systematic review. *Evid Based Complement Alternat Med.* 2019;2019:6216745. [Crossref]
11. Battaglia FP, Benchenane K, Sirota A, Pennartz CM, Wiener SI. The hippocampus: hub of brain network communication for memory. *Trends Cogn Sci.* 2011;15(7):310-318. [Crossref]
12. Marangoz C. Deneysel epilepsi modelleri. J Exp Clin Med. 2009;14(3):147-186. [Crossref]
13. McNamara JO. Kindling model of epilepsy. *Adv Neurol.* 1986;44:303-318. [Crossref]
14. Ahmadian SR, Ghasemi-Kasman M, Pouramir M, Sadeghi F. Arbutin attenuates cognitive impairment and inflammatory response in pentylenetetrazol-induced kindling model of epilepsy. *Neuropharmacology.* 2019;146:117-127. [Crossref]
15. Ahmadi M, Dufour JP, Seifritz E, Mirnajafi-Zadeh J, Saab BJ. The PTZ kindling mouse model of epilepsy exhibits exploratory drive deficits and aberrant activity amongst VTA dopamine neurons in both familiar and novel space. *Behav Brain Res.* 2017;330:1-7. [Crossref]
16. Shehta N, Kamel AE, Sobhy E, Ismail MH. Malondialdehyde and superoxide dismutase levels in patients with epilepsy: a case-control study. *Egypt J Neurol Psychiatry Neurosurg.* 2022;58(1):51. [Crossref]
17. Gaweł S, Wardas M, Niedworok E, Wardas P. Dialdehyd malonowy (MDA) jako wskaźnik procesów peroksydacji lipidów w organizmie [Malondialdehyde (MDA) as a lipid peroxidation marker]. *Wiad Lek.* 2004;57(9-10):453-455. Polish. [Crossref]
18. Khoubnasab Jafari M, Ansarin K, Jouyban A. Comments on "use of malondialdehyde as a biomarker for assesing oxidative stress in different disease pathologies: a review". *Iran J Public Health.* 2015;44(5):714-715. [Crossref]
19. Ahghari P, Afshar S, Dastan D, Ahmadieh Yazdi A. Protective effect of Lavadula officinalis on pentylenetetrazol-induced epilepsy in mice: expression of NOS genes and caspase-3. *Int J Ep.* 2024;10(01/02):11-19. [Crossref]
20. Kashani MS, Tavirani MR, Talaei SA, Salami M. Aqueous extract of lavender (Lavadula angustifolia) improves the spatial performance of a rat model of Alzheimer's disease. *Neurosci Bull.* 2011;27(2):99-106. [Crossref]
21. Antar A, Abdel-Rehiem ES, Al-Khalaf AA, et al. Therapeutic efficacy of Lavadula dentata's oil and ethanol extract in regulation of the neuroinflammation, histopathological alterations, oxidative stress, and restoring balance treg cells expressing FoxP3+ in a rat model of epilepsy. *Pharmaceuticals (Basel).* 2024;18(1):35. [Crossref]
22. Rahmati B, Khalili M, Roghani M, Ahghari P. Anti-epileptogenic and antioxidant effect of Lavadula officinalis aerial part extract against pentylenetetrazol-induced kindling in male mice. *J Ethnopharmacol.* 2013;148(1):152-157. [Crossref]
23. Löscher W, Reissmüller E, Ebert U. Kindling alters the anticonvulsant efficacy of phenytoin in Wistar rats. *Epilepsy Res.* 2000;39(3):211-220. [Crossref]
24. Khalid S, Rasool MF, Imran I, et al. A physiologically based pharmacokinetic model for predicting diazepam pharmacokinetics after intravenous, oral, intranasal, and rectal applications. *Pharmaceutics.* 2021;13(9):1480. [Crossref]
25. Tăbărașu AM, Anghelache DN, Găgeanu I, Biriș SȘ, Vlăduț NV. Considerations on the use of active compounds obtained from lavender. *Sustainability.* 2023;15(11):8879. [Crossref]
26. Akyüz E. A new view of the racine scoring system in the pentylenetetrazol epilepsy model. Harran Univ Tip Fak Derg. 2020;17(2):306-310. [Crossref]
27. Zivanovic D, Stanojlovic O, Susic V, Stojanovic J. The effects of phenytoin and phenobarbital on seizures induced by imipenem/cilastatin in rats. *Acta Neurol Belg.* 2004;104(1):20-26. [Crossref]
28. Naderi N, Aziz Ahari F, Shafaghi B, Najarkolaei AH, Motamedi F. Evaluation of interactions between cannabinoid compounds and diazepam in electroshock-induced seizure model in mice. *J Neural Transm (Vienna).* 2008;115(11):1501-1511. [Crossref]
29. Grizzle WE. Special symposium: fixation and tissue processing models. *Biotech Histochem.* 2009;84(5):185-193. [Crossref]
30. Tucker DK, Foley JF, Hayes-Bouknight SA, Fenton SE. Preparation of high-quality hematoxylin and eosin-stained sections from rodent mammary gland whole mounts for histopathologic review. *Toxicol Pathol.* 2016;44(7):1059-1064. [Crossref]
31. Fischer AH, Jacobson KA, Rose J, Zeller R. Hematoxylin and eosin staining of tissue and cell sections. *CSH Protoc.* 2008;2008:pdb.prot4986. [Crossref]
32. Lueptow LM. Novel object recognition test for the investigation of learning and memory in mice. *J Vis Exp.* 2017;(126):55718. [Crossref]
33. Levin-Arama M, Abraham L, Waner T, et al. Subcutaneous compared with intraperitoneal ketaminexylazine for anesthesia of mice. *J Am Assoc Lab Anim Sci.* 2016;55(6):794-800. [Crossref]
34. Agarwal R, Chase SD. Rapid, fluorimetric-liquid chromatographic determination of malondialdehyde in biological samples. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2002;775(1):121-126. [Crossref]
35. Miller PS, Aricescu AR. Crystal structure of a human GABAA receptor. *Nature.* 2014;512(7514):270-275. [Crossref]
36. Shaheen U, Akka J, Hinore JS, et al. Computer aided identification of sodium channel blockers in the clinical treatment of epilepsy using molecular docking tools. *Bioinformation.* 2015;11(3):131-137. [Crossref]
37. Milanos S, Elsharif SA, Janzen D, Buettner A, Villmann C. Metabolic products of linalool and modulation of GABAA receptors. *Front Chem.* 2017;5:46. [Crossref]
38. da Silva PR, de Andrade JC, de Sousa NF, et al. Computational studies applied to linalool and citronellal derivatives against Alzheimer's and Parkinson's disorders: a review with experimental approach. *Curr Neuropharmacol.* 2023;21(4):842-866. [Crossref]
39. Kaur J, Famta P, Famta M, et al. Potential anti-epileptic phytoconstituents: an updated review. *J Ethnopharmacol.* 2021;268:113565. [Crossref]
40. Elisabetsky E, Brum LF, Souza DO. Anticonvulsant properties of linalool in glutamate-related seizure models. *Phytomedicine.* 1999;6(2):107-113. [Crossref]
41. Dos Santos ÉRQ, Maia JGS, Fontes-Júnior EA, do Socorro Ferraz Maia C. Linalool as a therapeutic and medicinal tool in depression treatment: a review. *Curr Neuropharmacol.* 2022;20(6):1073-1092. [Crossref]
42. Younis NS, Abdelnaby RM, Mohamed ME. Hepatoprotective effects of linalool against liver ischemia-reperfusion: the role of Nrf2/HO-1/NQO1 and TLR4/RAGE/NFκB pathways. *Eur Rev Med Pharmacol Sci.* 2023;27(20):10094-10111. [Crossref]
43. Oguro A, Fujiyama T, Sato H, Yoshida T, Miyara M, Kotake Y. Linalool inhalation in mice leads to brain accumulation and metabolite-driven neuronal activation. *Biochem Biophys Res Commun.* 2025;786:152716. [Crossref]

44. de Sousa DP, Nóbrega FF, de Morais LC, de Almeida RN. Evaluation of the anticonvulsant activity of terpinen-4-ol. *Z Naturforsch C J Biosci.* 2009;64(1-2):1-5. **[Crossref]**
45. Vieira G, Cavalli J, Gonçalves ECD, et al. Antidepressant-like effect of terpineol in an inflammatory model of depression: involvement of the cannabinoid system and D2 dopamine receptor. *Biomolecules.* 2020;10(5):792. **[Crossref]**
46. Moghimi M, Parvardeh S, Zanjani TM, Ghafghazi S. Protective effect of α-terpineol against impairment of hippocampal synaptic plasticity and spatial memory following transient cerebral ischemia in rats. *Iran J Basic Med Sci.* 2016;19(9):960-969. **[Crossref]**
47. Gouveia DN, Costa JS, Oliveira MA, et al. α-terpineol reduces cancer pain via modulation of oxidative stress and inhibition of iNOS. *Biomed Pharmacother.* 2018;105:652-661. **[Crossref]**
48. Chinnappan D, Kuppamuthu A, Magudeeswaran S, Kandasamy S, Krishnan M, Elangovan N. A dual-target inhibitor of α-terpineol in Alzheimer's and Parkinson's diseases: a comprehensive active site analysis. *ChemistrySelect.* 2025;10(24):e05631. **[Crossref]**
49. Chinnappan D, Krishnan M, Kuppamuthu A, Elangovan N. Developmental and neuroprotective effects of α-terpineol in MPTP-induced parkinsonism in zebrafish larvae. *Neurochem Res.* 2025;50(3):158. **[Crossref]**
50. Hassan W, Noreen H, Rehman S, Kamal MA, da Rocha JBT. Association of oxidative stress with neurological disorders. *Curr Neuropharmacol.* 2022;20(6):1046-1072. **[Crossref]**
51. Xie HB, Sha Y, Wang J, Cheng MS. Some insights into the binding mechanism of the GABAA receptor: a combined docking and MM-GBSA study. *J Mol Model.* 2013;19(12):5489-5500. **[Crossref]**
52. Chowdhury R, Bhuia MS, Al Hasan MS, et al. Anticonvulsant effect of (±) citronellal possibly through the GABAergic and voltage-gated sodium channel receptor interaction pathways: In vivo and in silico studies. *Neurochem Int.* 2024;175:105704. **[Crossref]**