Anti-Anxiety Effect of *Salvia hypoleuca*

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**Abstract**

**Background and objectives:** Anxiety is a chronic and common disorder worldwide and impairs the quality of life of affected people. Herbal medicines have long been used to treat CNS related disorders. *Salvia* species are important medicinal plants that have shown various pharmacologic activities including CNS effects. *Salvia hypoleuca* is an annual and herbaceous plant which is endemic to Iran. The aim of this study was to evaluate the anxiolytic activity of the hydro-alcoholic, n-hexane, ethyl acetate and methanol extract of *S. hypoleuca* in mice. **Methods:** n-hexane, ethyl acetate, methanol and hydroalcoholic extracts were prepared from aerial parts of *Salvia hypoleuca*. Anxiolytic activity of the extracts was evaluated using open field and hole-board tests. The mice were randomly divided into different groups and were treated with normal saline, diazepam, and the extracts of *S. hypoleuca* (500 mg/kg, ip). **Results:** The results of open field and hole-board tests showed that treatment of mice with the hydro-alcoholic and methanol extracts of *S. hypoleuca* (500 mg/kg, ip) produced a significant anxiolytic effect as compared to the normal control group. **Conclusion:** This study demonstrated that the hydro-alcoholic and methanol extracts of aerial parts of *S. hypoleuca* have anti-anxiety activity. The isolation and identification of the active compounds of the effective extracts are suggested.

**Keywords:** anti-anxiety; behavior tests; *Salvia hypoleuca*


**Introduction**

Anxiety, an emotional state and unpleasant in nature, is characterized by sympathetic hyperactivity, motor tension and vigilance syndromes. Anxiety disorders affect around one eighth of the total population worldwide. It is the most common group of mental disorders and seriously impairs the quality of life of affected people. Many drugs including benzodiazepines are used for the treatment of anxiety; however, these drugs have serious side effects. Herbal medicines have long been used to treat CNS related disorders such as anxiety. Today herbal medicines are preferred by many people due to lower adverse effects and costs [1-4]. *Salvia* is an important genus in the Lamiaceae family. These plants have different pharmacologic
activities especially on the CNS including sedative and hypnotic, hallucinogenic, analgesic, memory enhancing, anticonvulsant, neuroprotective and anti-Parkinson properties [5].

*Salvia hypoleuca* is endemic to Iran that grows in Tehran and Mazandaran provinces. The aerial parts of *S. hypoleuca* has been used as tonic, carminative, digestive, antispasmodic and as an anti-inflammatory agent [6]. Pharmacologic activities of *S. hypoleuca* such as spermatogenesis [7] and antioxidant, antimicrobial [8] and anti-nociceptive properties [9] have been previously studied; however, there is no report about the possible anxiolytic effects of *S. hypoleuca*. This study was designed to evaluate the anxiolytic actions of the *n*-hexane, ethyl acetate, methanol, and hydro-alcoholic extracts of *S. hypoleuca* in mice.

**Materials and Methods**

**Ethical considerations**

Ethical Committee of Tehran University of Medical Sciences approved this study with the code of 135634 on 2015-03-07. All animal procedures were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and with the approval of Tehran University of Medical Sciences Ethics Committee.

**Plant material**

*Salvia hypoleuca* aerial parts were collected from the northern hilly areas of Tehran, Iran in 2015. The taxonomical identification of *S. hypoleuca* was confirmed by an expert botanist (Dr. Ali Sonboli, Shahid Beheshti University, Tehran, Iran). A voucher specimen ((MPH-1320) has been deposited at the Herbarium of the Medicinal Plant and Drug Research Institute (MPH) of Shahid Beheshti University, Tehran, Iran.

**Extraction**

The air-dried aerial parts of *S. hypoleuca* (150g) were powdered and extracted with 500 mL of various solvents including *n*-hexane, ethyl acetate, methanol and 80% ethanol, respectively by maceration method at room temperature. Finally, the extracts were concentrated and dried in rotary evaporator [10]. To evaluate anti-anxiety activity of *S. hypoleuca*, 500 mg of each extract was dissolved in 10 mL of normal saline and DMSO (20 µL) was used to increase the solubility of the extracts. The extracts were injected to mice intraperitonially (i.p.).

**Animals**

Experiments were performed on adult and healthy NMRI mice weighing 20 to 25 g. Animals were maintained under standard environmental conditions (22± 2 °C, 12h light/dark cycle) and were allowed free access to water and standard rodent feed.

**Experimental design**

Mice were divided into five groups (n= 5): 1. Normal control group: mice received saline; 2. Standard group: mice received diazepam (1.5 mg/kg, i.p.); 3. Hydroalcoholic group: mice received hydro-alcoholic extract of *S. hypoleuca* (500 mg/kg, i.p.); 4. Hexane group: mice received hexane extract of *S. hypoleuca* (500 mg/kg, i.p.); 5. Ethyl acetate group: mice received ethyl acetate extract of *S. hypoleuca* (500 mg/kg, i.p.); 6. Methanol group: mice received methanol extract of *S. hypoleuca* (500 mg/kg, i.p.). The dose of *S. hypoleuca* extract used in this study was selected based on our preliminary experiment.

**Behavioral tests**

**Open-field test**

One hour after treatment of the normal control group with saline, standard group with diazepam (1.5 mg/kg), and test groups with extract (500 mg/kg), each mouse was in turn gently placed at the center of the apparatus. The open field is made of plexiglass with 40 × 40 × 60 cm dimension with the floor divided into 16 squares. The duration of time that mice spent in the four central squares was recorded within the observation period of 5 min.

**Hole-board test**

The hole-board apparatus is a box made of plexiglass with approximately 40 × 40 cm dimension. The floor of the box is covered with 16 holes each 2 cm in diameter. One hour after treatment of the normal control group with saline, standard group with diazepam (1.5 mg/kg), and test groups with extract (500 mg/kg), each mouse was placed at one corner of the apparatus and was observed for 5 min. The numbers of head-dipping into the holes within the observation period were recorded for individual mouse.
Statistical analysis
The results were expressed as mean ± SD. Values were analyzed statistically using One-way ANOVA followed by Tukey’s multiple comparison tests. Differences between groups were considered to be significant when p< 0.05.

Result and Discussion
The open-field and hole-board tests are the experimental method used in behavioral pharmacology to measure anxiety, stress and emotionality in rodents. It has been shown that the administration of an anxiolytic agent increases exploration time of mice in the center of the open field whereas stressful stimuli decreases the time spent in the center [11]. In the hole-board test the administration of an anxiolytic agent increases the number of head dipping behavior of mice in the apparatus while stressful stimuli decreases the number of head-dips [12].

The mice were treated with n-hexane, ethyl acetate, methanol and hydroalcoholic extracts from the aerial parts of *S. hypoleuca* at the dose of 500 mg/kg. After one hour, they were tested for the anxiety response by the open field and hole-board tests. As shown in figures 1 and 2, the results revealed that treatment of mice with methanol and hydro-alcoholic extracts of *S. hypoleuca* (500 mg/kg, ip) significantly increased the central time and the frequency of head-dipping as compared to the normal control group.

In conclusion, the results of this study demonstrated that the hydro-alcoholic and methanol extracts of *S. hypoleuca* aerial parts showed significant anti-anxiety activity in the rodent behavioral tests. Further studies are needed for isolation of the active compounds from the extracts *S. hypoleuca*.

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Abbreviations

CNS: Central Nervous System; DMSO: Dimethyl Sulfoxide.