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Short communication

# Evaluation of Sedative and Hypnotic Effects and Acute Toxicity of *Paeonia daurica* subsp. *macrophylla* (Albov) D.Y.Hong Root Extracts in Mice

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## Abstract

**Background and objectives:** *Paeonia daurica* ssp. *macrophylla* (PD) (Paeoniaceae) is a perennial plant, growing in Iran. In Persian medicine, another species, *Paeonia officinalis* "Oode-saleeb" has been used especially for treating epilepsy and brain disorders. This study aimed to evaluate the sedative and hypnotic activity and the acute toxicity of *P. daurica* root extracts in mice. **Methods:** Sedative and hypnotic effects of the aqueous extract, total hydro alcoholic 80% extract, and its fractions, hexane, chloroform, and methanol were examined by the righting reflex method. Plant samples (50, 100, and 200 mg/kg) and vehicle (10 mL/kg) were injected intraperitoneally (i.p.) 30 minutes before sodium thiopental injection (40 mg/kg, i.p.) in groups of seven mice. The time taken before losing the righting reflex and the time taken to regain the righting reflex were recorded as the onset of sleep and sleep duration, respectively. **Results:** The findings of the study indicated that, with the exception of the chloroform fraction and the total hydroalcoholic extract administered at the dose of 50 mg/kg, all the samples demonstrated a reduction in sleep onset time produced by sodium thiopental. Furthermore, chloroform (200 mg/kg) was the most effective sample on sleep duration compared to diazepam (3 mg/kg) ($p<0.001$). **Conclusion:** Results of this study demonstrated that *Paeonia daurica* root can be used as a sedative and hypnotic complementary drug.

**Keywords:** diazepam; hypnotic effects; *Paeonia*; Persian medicine; sedative effects

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## Introduction

Insomnia is a common disorder defined by subjective complaints of falling asleep or staying awake that occur in distress or significant impairment of functioning [1].

*Paeonia daurica* subsp. *macrophylla* (Paeoniaceae), with the common name of peony, is a perennial and rhizomatous plant growing in northern parts of Iran [2]. Roots of *Paeonia officinalis*, known as ''Oode-Saleeb'' in traditional Persian medicine, have been used for some brain diseases, especially epilepsy [3]. The peony roots have several phytochemicals

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including terpenes, phenols, flavonoids, essential oils, and tannins. Among these phytochemicals, paeoniflorin and paeonol glucoside showed sedative, antispasmodic activity on smooth uterine muscle, and anti-inflammatory effects [4, 5].

In this study, the sedative, hypnotic, effects of the aqueous extract, hydroalcoholic extract, and its partitioned fractions hexane, chloroform, and methanol, from *Paeonia daurica* subsp. *macrophylla* root was investigated on the duration of sleep, induced in mice. Also, acute toxicity of hydroalcoholic extract was evaluated intraperitoneally (i.p) in mice.

## Material and Methods

### Ethical considerations

The Ethics and Animal Care Committee of Tehran University of Medical Sciences approved the study with the code IR.TUMS.VCR.REC.1395.687.This study was in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals [6].

### Chemicals

Ethanol, *n*-hexane, chloroform, and DMSO were purchased from Merck (Germany), Diazepam (Chemidarou, Iran), and Sodium thiopental (Loghman Company, Iran) were used in the study.

### Plant material

The roots of *P. daurica* ssp. *macrophylla* (Albov) D.Y.Hong were collected from the southern heights of Alborz, Mazandaran province, in July 2015 after the end of their flowering time. Prof. Gholamreza Amin identified the plant sample (voucher number 6620-TEH) at the herbarium of the Faculty of Pharmacy at Tehran University of Medical Sciences. The air-dried roots (500 g) were powdered and exhaustively extracted by the maceration method (aqueous ethanol, EtOH 80%) (three times for 48 h), and hydroalcoholic extracts were concentrated by a rotary instrument at 40 ºC. The hydroalcoholic extract was consecutively partitioned into *n*-hexane, chloroform, and methanol. To prepare *P. daurica* aqueous extract, the dried roots were suspended in distilled water (190 g dried roots per 2 L water), and the mixture was boiled for 60 min at 90 °C with occasional stirring. The aqueous extract was lyophilized and stored in the refrigerator until pharmacologic tests.

### Animal study

Female Naval Medical Research Institute (NMRI) albino mice (n=161) (25±5 g) were used for animal studies. Mice were stored in a room with a temperature of 25 °C and a dark-light cycle of 12 h with free access to water and food. The animals had free access to food and water in individual cages. On the day of the experiment, groups of seven mice were randomly separated 30 minutes before the start of the experiment.

### Acute toxicity test

Five consecutive doses (1200, 1000, 800, 600, and 400 mg/kg) of hydroalcoholic extract were used intraperitoneally (i.p) to determine acute toxicity. The following formula calculated the acute toxicity, $LD_{50}$; Lethal dose resulting in 50% death. A group of seven mice received normal saline i.p as the control group. Mortality was measured during the first 24 h and then up to 14 days later [7,8].

$$LD_{50} = LD_{100} - \Sigma\, a \times b/n$$

$LD_{100}$ = a lethal dose that kills 100 % of the animals; a = average of deaths between two consecutive doses; b = difference between two consecutive doses; n = number of animals in each group.

### Hypnotic and sedative effects

In order to perform hypnotic tests, different doses of the extracts were dissolved in an appropriate volume of normal saline+DMSO (<20 µL/10 g). The mice were divided into 17 groups of 7: negative control group (normal saline), positive control group (diazepam 3 mg/kg), and groups 3-17: different extracts and fractions including aqueous extract (AE), hydroalcoholic extract (HE), hexane (F-Hex), chloroform (F-$CHCL_3$), and methanol fractions (F-MeOH) with three doses of 50, 100, and 200 mg/kg). The samples were injected intraperitoneally, 30 minutes before sodium thiopental (40 mg/kg i.p.). The time of losing the righting reflex in the mice was measured as the onset of sleep. In the next step, the mice were monitored, and when they regained the ability to return to their right (righting reflex), it was considered the end time of sleep, and the sleeping time was calculated [9].

### Statistical analysis

One-way analysis of variance (ANOVA)

followed by Tukey-Kramer, and multiple comparison tests, were used to compare the differences between various treatment groups using GraphPad Prism 5.01. The statistical probability of $p<0.05$ was considered significant. Time measurements are reported as Mean ± SEM.

## Results and Discussion

The results of acute toxicity showed that the lethal dose of hydroalcoholic extract (i.p.) that caused 100 % death of animals was 1200 mg/kg. The difference between two consecutive doses of the prescribed extract was equal to 200, and due to the death of three mice in 100 mg/kg, the average number of dead animals in two consecutive doses was equal to 5, which according to the formula, acute toxicity was calculated as follows: $LD_{50}= 1200 - \Sigma (200 \times 5)/7= 1057$ mg/kg. According to the calculation, the acute toxicity dose obtained for HE was 1057 mg/kg.

The result of the injection of different extracts and fractions is shown in Figure 1. According to the results, diazepam (3 mg/kg), the F-Hex (50, 100 mg/kg), and the groups of HE, F-$CHCl_3$, F-MeOH, AE (100, 200 mg/kg) significantly reduced the onset of sleep by thiopental sodium compared to the negative control group ($p<0.001$), Figure 1(A).

According to Figure1 (B), diazepam (3 mg/kg), HE (50, 100, 200 mg/kg), F-$CHCl_3$ (100, 200 mg/kg), F-Hex (100, 200 mg/kg), and AE (200 mg/kg), significantly increased the duration of sleep induced by sodium thiopental compared to the normal saline control group ($p<0.001$). A comparison of the effect of extracts on increasing the induced sleep time showed that F-$CHCl_3$ (200 mg/kg) and HE (200 mg/kg) ($p <0.001$ and $p<0.05$, respectively) were more effective than diazepam 3 mg/kg in increasing sleep time.

Based on the results of this study, it seems that the F-Hex, F-$CHCl_3$, and F-MeOH groups could reduce the sleep induction time, dose-independently. However, the effects of plant samples on induced sleep duration showed that F-$CHCl_3$ at a dose of 200 mg/kg was more effective than diazepam.

Recently, antinociceptive and anticonvulsant evaluation of *Paeonia daurica* as well as oleanolic acid, a pentacyclic triterpenoid, isolated from *P. daurica* verified the efficacy of this plant in the treatment of brain disorders [10-13]. On the other hand, current studies reported that total glycosides and paeoniflorin, a monoterpene water-soluble glycoside, isolated from *Paeonia lactiflora,* could improve sleep parameters [14].

## Conclusions

Based on the results, it can be concluded that *Paeonia daurica* subsp. *macrophylla* root can be suggested as a complementary sedative and hypnotic agent in the control of neurological diseases with symptoms of insomnia. Obviously, there is a need for more research to clarify the mechanisms involved and identification of active compounds of this plant.

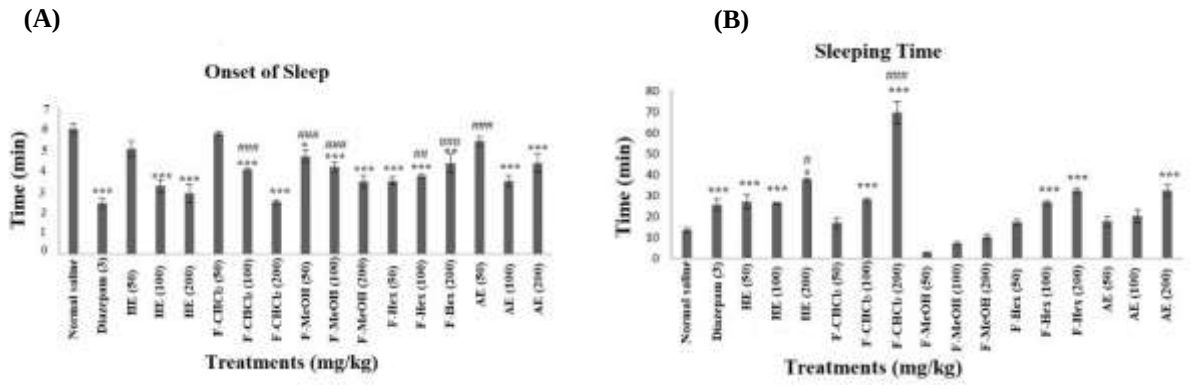


**Figure 1.** Effects of different extracts of *Paeonia daurica* ssp. *macrophylla* root on (**A)** onset of sleep and (**B)** sleep induction time by sodium thiopental; * $p<0.05$, ** $p<0.01$, *** $p<0.001$ compared to the negative control, # $p <0.05$, ## $p <0.01$, ### $p <0.001$ compared to diazepam; number of samples in each group (n = 7); HE: hydroalcoholic extract; F-$CHCL_3$: chloroform fraction; F-MeOH: methanol fraction; F-Hex: *n*-hexane fraction; AE: aqueous fraction

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## Author contributions

Hamid Reza Monsef-Esfahani was involved in the conception and design of the study; Armin Rafizadeh acquired and analyzed data; Paria Sharafi-Badr contributed into drafting the article; Mohammad Sharifzadeh and Mahdi Vazirian contributed to designing of study; Seyede Nargess Sadati Lamardi designed the study and was involved in the analysis of data and critical revision.

## Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper's content.

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## Abbreviations

DMSO: Dimethyl sulfoxide; HE: Hydroalcoholic extract; F-$CHCL_3$: Chloroform fraction; F-MeOH: Methanol fraction; F-Hex: *n*-hexane fraction; AE: Aqueous fraction