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Effect of Hydroalcoholic Extract of *Dactylorhiza Hatagirea* Roots & *Lavandula Stoechas* Flower on Thiopental Sodium Induced Hypnosis in Mice

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ABSTRACT

Dactylorhiza hatagirea (D.Don) Soo belongs to the family orchidaceae. The plant is native and near endemic to Indian Himalayan region. Its distribution extends to Pakistan, Afghanistan, Nepal, Tibet and Bhutan. In India, it is reported from Jammu and Kashmir, Sikkim, Arunachal Pradesh, Uttarakhand and Himachal Pradesh. The Juice extracted from tuber is used as tonic and also used for the treatment of pyorrhea (inflammation of the gum & teeth). Root paste is externally applied as poultice on cuts and wounds and extract is given in intestinal disorders. *Lavandula stoechas*, the Spanish lavender or topped lavender or French lavender is a species of flowering plant in the family lamiaceae, ccurring naturally in several Mediterranean countries, including France, Spain, Portugal, Italy and Greece. It is used commercially in air fresheners and insecticides. Flower spikes have been used internally for headaches, irritability, feverish colds and nausea and externally for wounds, rheumatic pain, antiseptic, digestive, antispasmodic, healing, insect repellent and antibacterial. The neuropharmacological activities were examined by thiopental sodium induced sleeping time in mice at the doses of 100, 200 and 300 mg/kg p.o body weight. All the extracts exhibited significant reduction of onset and duration of sleep in thiopental sodium induced sleeping time test. Altogether, these results suggest that experimental hydroalcoholic extracts of *D. hatagirea* roots & *L. stoechas* flower possesses potent hypnotic properties, which support its use in traditional medicine.

Keywords: *Dactylorhiza hata*, *Lavandula stoechas*, Sleeping time test, Thiopental sodium

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INTRODUCTION

Modern stressful life is linked with variety of psychiatric disorders. Among these disorders, depression is a pervasive psychiatric problem. It occurs usually in the early adult life of patients with decrease in monoamine neurotransmitters and about 10–30% of general population is suffering from these throughout the world[1]. Typically, sedative and hypnotics are used to reduce anxiety as its produce a calming effect by inducing the onset of sleep as well as maintaining sleeping duration[2]. Tricyclic antidepressants (TCAs), selective reversible inhibitors of monoamine oxidase A (RIMAs), selective serotonin reuptake inhibitors (SSRIs), and specific serotonin–noradrenaline reuptake inhibitors (SNRIs) are clinically recommended for drug therapy in psychiatric disorders. Nowadays, these drugs are extensively used in treatment of different psychiatric disorders. However, serious side effects ranging from respiratory, digestive, and immune system dysfunctions to decline of cognitive function, physical dependence, and tolerance have tend for incessant use of these currently available synthetic drugs[3]. Thus, development of new sedative-hypnotic drugs with fewer side effects has been suggested to be a promising approach to counter different psychiatric disorders. That's why the search for new anxiolytic agents with reduced adverse effects is still an area great interest for the researchers. Generally, natural products, specifically medicinal plants are considered to be a fundamental arsenal of chemical substances with therapeutic potentiality. *D. hatagirea* (D.Don, Orchidaceae) Soo is native and near endemic to Indian Himalayan region. Its distribution extends to Pakistan, Afghanistan, Nepal, Tibet and Bhutan. In India, it is reported from Arunachal Pradesh, Uttarakhand, Jammu and Kashmir, Sikkim and Himachal Pradesh[4]. Generally, it is widely and narrowly distributed at an altitudinal ranges between 2500 to 5000 m amsl in open grassy slopes and alpine meadows. It is commonly known as panja, salam-panja, hath-panja or hatajari in Uttarakhand; salem panja in Kashmir and wanglak or angulagpa in various parts of Ladakh. Generally, the plant is a perennial herb, up to 60 to 70 cm in height, having palmately lobed, divided root tubers with broadly lanceolate leaves arranged more or less

along the stem and purple flowers, but some time white[5,6]. The plant tubers of *D. hatagirea* contain a glucoside, a bitter substance, starch, mucilage, albumen, a trace of volatile oil and ash. Chemically, dactylorhins A to E, dactyloses A and B and lipids, etc is found as major constituents. Young leaves and shoots are eaten as a vegetable. The root is expectorant, astringent, demulcent and highly nutritious. Powdered root is spread over wounds to control bleeding. A decoction of the root is given in cases of stomach trouble. It is also used as aphrodisiac and sexual stimulant[7]. *L. stoechas* (Lamiaceae), the Spanish lavender or topped lavender or French lavender is a species of flowering plant in the family , occurring naturally in several Mediterranean countries, including France, Spain, Portugal, Italy and Greece[8]. An evergreen shrub, it usually grows to 30–100 cm (12–39 in) tall and occasionally up to 2 m (7 ft) high in the subspecies *luisieri*. The flowers, which appear in late spring and early summer, are pink to purple, produced on spikes 2 cm long at the top of slender, leafless stems 10-30 cm (4-12 in) long; each flower is subtended by a bract 4-8 mm long. At the top of the spike are a number of much larger, sterile bracts (no flowers between them), 10–50 mm long and bright lavender purple (rarely white). It blooms in spring and early summer, from the month of March, depending on the climate where it inhabits[9]. *L. stoechas* is used commercially in air fresheners and insecticides. Flower spikes have been used internally for headaches, irritability, feverish colds and nausea, antiseptic, digestive, antispasmodic, healing and externally for wounds, rheumatic pain and as an insect repellent. The flowers are used in aromatherapy, to prepare infusions and essential oils that contain ketones (d-camphor and d-fenchone) and alcohols (borneol and terpineol)[10]. To the best of our knowledge, very few pharmacological studies have been reported on these two plants. Present study was design to estimate the role of this plant on neuropharmacological effect in mice.

MATERIALS AND METHODS

Plant material

The roots of plant *D. hatagirea* and flowers of *L. stoechas* were collected from local area of Bhopal (M.P), India in the month of Jan 2017. The taxonomical identification and authentication of the plant material was done by Dr. Zia Ul Hasan, Department of Botany, SAFIA College Bhopal (M.P). The specimens of voucher have been submitted and preserved in the herbarium of SAFIA College Bhopal (M.P).

Chemical reagents

Diazepam and thiopental sodium were purchased from Sigma Aldrich Chemical Co. (Milwaukee, WI, USA), methanol was purchased from HiMedia Laboratories Pvt. Ltd. (Mumbai, India), India. All other chemical used in this study purchased from SD Fine-Chem Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals used in this study were of analytical grade.

Preparation of extract

Defatting of plant material

Powdered material of *L. stoechas* and *D. hatagirea* were shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether (60-80°C) in a soxhlet apparatus. The extraction was continued till the defatting of the material had taken place.

Extraction by hot continuous percolation process

100 gm of *L. stoechas* and *D. hatagirea* dried material were exhaustively extracted with 80% ethanol (Hydroalcoholic) using hot continuous percolation for 24 hrs. Appearance of colorless solvent in the siphon tube was taken as the end point of extraction. The extracts were concentrated to ¾ of its original volume by distillation. The concentrated extracts were taken in a china dish and evaporated on a thermostat controlled water bath till it forms a thick paste and stored in an air tight container free from any contamination until it was used. Finally the percentage yields were calculated of the dried extracts[11].

Experimental animals

Swiss albino male mice (20-25 gm) we're group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2°C, 55–65%). Mice received standard rodent chow and water *ad libitum*. Animal were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate group (n=6) of mice was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

Acute oral toxicity study

Acute toxicity studies were conducted on mice as per the Organization for Economic Co-operation and Development (OECD) 423 guidelines[12]. The hydroalcoholic extract of both plants at doses of 5, 50, 300, and 2000 mg/ kg body weight were administered to four groups of rats (n = 6) after overnight fasting. The animals were observed twice on the day of the dosing and once daily thereafter for 14 days. Animals were observed daily for mortality and for gross changes in activity and behavioral pattern. They were also observed for the presence of tremors, convulsions, salivation, diarrhea and lethargy. The maximum non lethal dose was found to be 2000 mg/kg body weight; hence 1/10th dose was taken as effective dose for hydroalcoholic extracts of *L. stoechas* and *D. hatagirea* to evaluate hypnotic activity.

Thiopental sodium induced hypnosis

Thiopental sodium-induced sleeping time was evaluated according to the previously described method[13]. Thirty min after vehicle or HELS (100, 200 and 300 mg/kg, p.o.), HEDH (100, 200 and 300 mg/kg, p.o.) and 15 min after diazepam treatment, thiopental sodium (TS) was administered to each animal intraperitoneally at the dose of 25 mg/kg. Then the animals were observed for the time to lose their righting reflex, immediately after thiopental sodium injection (latent period) and the duration of sleep (time between the loss and recovery of reflex) induced by TS. The followings are group distribution,

Group 1: Normal received Thiopental
Group 2: received Diazepam & Thiopental as a standard control
Group 3: received HELS-100 mg/kg, p.o.
Group 4: received HELS-200 mg/kg, p.o.
Group 5: received HELS-300 mg/kg, p.o.
Group 6: received HEDH-100 mg/kg, p.o.
Group 7: received HEDH-200 mg/kg, p.o.
Group 8: received HEDH-300 mg/kg, p.o.

Statistical analysis

The values were expressed as mean ± SEM (n=6). The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Tukey's test and P<0.05 were considered to be statistically significant.

RESULTS

The roots of *D. hatagirea* and flowers of *L. stoechas* were collected from the local area of Bhopal, MP, India. Air-dried and extracted by continuous hot extraction process using soxhlet apparatus. The average percentage yield of hydroalcoholic extract of *D. hatagirea* and *L. stoechas* was found to be 6.2 and 8.5%w/w respectively. Acute toxicity studies revealed that *D. hatagirea* and *L. stoechas* extract was safe at all doses when administered orally to mices, up to a dose of 2000 mg/ kg. No mortality was observed during the 14 days of the observation period. Hence three doses, 100, 200 and 300 mg/kg were selected in the present study for both the extracts. In the thiopental sodium induced sleeping time test, the test group treated with the extract of HELS at dose 100, 200, 300 mg/kg p.o. and HEDH at dose 100, 200, 300 mg/kg p.o. body weight showed significant ($p < 0.05$) decrease in onset of action and increased the duration of sleep as shown in Table 1 and Figure 1. In addition, the dose dependently prolonged the duration of sleeping time in test animals compared to normal.

Table 1 Effect of HELS and HEDH extract on thiopental sodium induced hypnosis

Groups	Dose	No. of animals	Duration of sleep (mean ± SD)	% Change in sleeping time
Thiopental	25 mg/kg, i.p.	06	20.51±2.12	-
Diazepam + Thiopental	3 + 25 mg/kg, i.p.	06	80.67±11.23***	372.81
HELS-100	100 mg/kg, p.o.	06	35.28±8.27	72.01
HELS-200	200 mg/kg, p.o.	06	48.83±10.24*	138.14
HELS-300	300 mg/kg, p.o.	06	57.50±12.12***	259.84
HEDH-100	100 mg/kg, p.o.	06	35.30±7.26	151.60
HEDH-200	200 mg/kg, p.o.	06	49.37±15.32*	220.20
HEDH-300	300 mg/kg, p.o.	06	57.00±13.24***	257.40

Values are expressed as mean±S.E.M. (n = 6).Values are statistically significant at***P<0.001, **P<0.01, *P<0.05 vs. control group respectively (One-way ANOVA followed by Tukey's post hoc test).

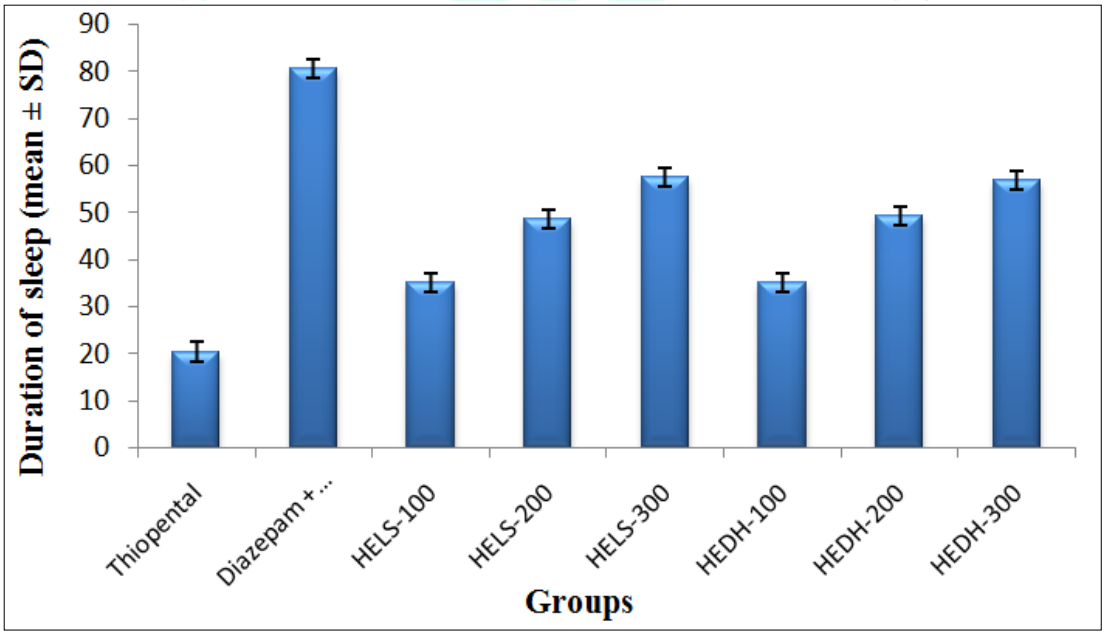

Fig. 1 Effect of HELS and HEDH extract on thiopental sodium induced sleeping time in mice. Data are shown as Mean±SD of six animals in each group

DISCUSSION

Several investigators have proposed that these extract possesses central nervous system activity particularly the ability to inhibit mono amine oxidase-A, an enzyme involved in the degradation of NE and 5HT(serotonin). However, there were studies which have reported the potentiating of thiopental sodium -induced sleeping at lower doses (4 mg/kg) [14]. The doses of the drug seem to be crucial to the type of effects obtained by different researchers in various studies. The present study was designed to find out the CNS depressant action of the extract in graded doses of 100, 200 & 300 mg/kg p.o. In agreement with previous reports, the results of our study show that the lower doses (100 mg/kg) of the HELS and HEDH extract reduced the locomotor activity and thiopentone induced sleeping time where as the dose of 200 mg/kg HELS and HEDH extract, significantly reduced the motility and locomotor activity. There was significant motor in coordination and hypnosis was prolonged to 48.83±10.24 and 49.37±15.32 min as compared to diazepam 80.67±11.23 min. The dose of 300 mg/kg HELS and HEDH extract reduced more significantly the motility and locomotor activity. There was significant motor in coordination and hypnosis was prolonged to 57.50±12.12 and 57.00±13.24 min as compared to diazepam. Furthermore, there are several reports which demonstrated that the alkaloids, glycosides, and flavonoids rich plant and

plant extracts possess sedative, anxiolytic, and antiepileptic properties mediated through their affinity (in vitro) with benzodiazepine site of GABAergic complex system or are direct or indirect modulators of this receptor. Besides, nonspecific CNS depression can also be attributed by tannin. Therefore it appears that the abovementioned phytochemicals present in the HELS and HEDH extract may contribute at least in part to the sedative and hypnotic effects on the CNS. These findings are indicative of a remarkable sedative effect which was further strengthened by potentiation of thiopentone induced hypnosis after oral administration of HELS and HEDH extract. Further exploration of the effect of the extract on activities responsible for increase in GABA concentration, such as potentiation of thiopental sodium-induced hypnosis, motor coordination, anticonvulsant activity. The fact that extract activity was potentiated by the in induced hypnosis suggest a GABA-mediated effect on the CNS since CNS depressants extend sleeping time. It is known that sedative-hypnotic drugs induce their effect on the Gabaergic system in the brain and inhibition of neuronal output could be facilitated by GABA (an inhibitory neurotransmitter) release. Loss of righting reflex induced by phenobarbital is potentiated by GABA agonist (muscimol and 4, 5, 6, 7-tetrahydroisoxazolo [5,4-c]pyridin-3-ol (THIP) and inhibited by GABA antagonist; the activation of GABA receptor partially mediates the sleep response. It is thus plausible to assert that the sedative effect of the extract is due to the facilitation of GABAergic transmission.

CONCLUSION

From the above experiments, it could be concluded that hydroalcoholic extracts of *D. hatagirea* roots & *L. stoechas* flower contains significant neuropharmacological activities. To elucidate the exact mechanism action and bioactive compounds responsible for the neuropharmacological activites of this plant extract, further studies are needed to isolate the pharmacological active compounds responsible for this activity.

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