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Pharmacological evaluation of Sedative and Hypnotic activities of methanolic extract of *Lycopus europaeus* in mice

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Abstract

The methanolic extract of *Lycopus europaeus* was evaluated on central nervous system. The methanolic extract produced significant sedative effect at the doses of 200, 400 and 600 mg/kg (by oral route), compared to reference substance diazepam in hole board and thiopental -induced sleeping time methods. While the hypnotic effect was calculated at the doses of 800 and 1000 mg/kg via oral pathway significantly reduced in both the reestablishment time and number of head dips during the traction and hole-board tests. In conclusion, these results suggest that the methanolic extract of *Lycopus europaeus* possess potent sedative and hypnotic activities, which supported its therapeutic use for insomnia.

Keywords: *Lycopus europaeus*, Sedative, Hypnotic, Methanolic extract.

Introduction

The use of medicinal plants singly or in combination in treating different ailments has been practiced by traditional medical healers for a very long time. People of Mesoamerica have utilized plants and products from plants in curing and relieving ailments for centuries.¹ These medicinal plants are often regarded by traditional healers as safe and effective in curing a variety of disease conditions. However, scientific evaluation of these claims is needed to provide evidence of their safety and efficacy.

Lycopus europaeus is also known as Bugleweed, wolfstrappkraut, bitter bugle, water horehound. *L. virginicus*: Paul's betony and water bugle. *Lycopus europaeus* is an herbaceous perennial mint that grows in wet habitats. The leaves are toothed, and the small white flowers surround the square stem at the leaf axils in dense clusters. The plant has little odour; the European species has a bitter taste, while the American species is not bitter. The whole herb is used medicinally.

Scientists have played their important for the evaluation of traditional uses of *Lycopus europaeus* on different animals. For example extracts of *L. europaeus* administered to healthy rats reduced the weight of the thyroid, decreased thyroid hormone activity, and increased absorption and storage of iodine. The extract retarded goiter formation in propylthiouracil-treated rats. All animals treated with the extract demonstrated reduced metabolism.² Other studies in rats have shown inhibition of serum thyrotropic hormone and thyroxin after oral administration.³

Cardiac signs of hyperthyroidism were reduced in an experiment in rats treated with *L. europaeus* extract.⁴ The plant was also reported for its antitussive activity.⁵ Traditionally *Lycopus europaeus* is being used as an astringent and sedative purposes.^{6, 7} So the following study is being done to evaluate the sedative and hypnotic activities of *Lycopus europaeus* in different cough induced models in mice.

Material and Methods

Collection of plant and preparation of crude extract

The plant was collected from the tropical regions of Pakistan and was identified by a taxonomist. The plant material was made free from soil and other adulterants and vegetative debris. The dried plant material was grinded to coarse powder with the help of a special herbal grinder. The powdered plant material (1 kg) was subjected to maceration in 70% aqueous-methano in amber coloured bottle at room temperature for 7 days with occasional vigorous shaking at room temperature and keeping the extract in the dark room. The filtrate was obtained by passing the mixture through a muslin cloth and then through a Whatman qualitative grade 1 filter paper. The filtrate was evaporated on a rotary evaporator attached to a vacuum pump at 37°C under reduced pressure to thick paste like consistency and then the extract obtained was stored at -4°C in air tight jars.



Figure1: Aerial parts of *Lycopus europaeus*

Animals

Male Swiss mice (20–25 g) were used in pharmacological tests. The animals were fed ad libitum with standard food

and water except when fasting was required in the course of the study.

Drugs

All drugs and extracts were freshly prepared on the day of the experiments. A control group received distilled water (10 mL/kg, p.o.) as vehicle. Diazepam (3mg/kg, i.p., a conventional sedative) and thiopental (60 mg/kg, i.p., a conventional hypnosis) were used as positive control.

Pharmacological Evaluations

The activity of methanolic and aqueous extract from *Lycopus europaeus* on the central nervous system was then studied, using a battery of behavioural tests used in psychopharmacology. We analyzed the effect of different doses of the methanolic extracts (100, 200, 400, and 600 mg/kg, p.o.) for their sedative and hypnotic activities. Following tests were performed for the sedative activity.

Traction Test

Mice were individually suspended by anterior limbs to a wire stretched horizontally. Abnormal mice that fail to make a reestablishment at least one of its posterior limbs to reach the wire are considered as subject under a sedative action. When the animals perform normal reestablishment immediately, the reaction is known as positive; otherwise, the reaction is called negative; also the behaviours of animals were recorded during the period of the experiment.⁸

Hole-Board Test

Mice were individually placed in the centre of a perforated board, and the number of head dips was registered during a 5 min. The perforated board test was made by using a wood floor board, 40 cm × 40 cm × 25 cm, in which evenly spaced holes were made. The number of explored holes provides a measure of the number of head dips.⁹

Thiopental-Induced Sleep in Mice

Thiopental (a sub hypnotic dose) 60mg/kg was injected i.p.30min after administration of methanolic extract. The mice were treated with different doses of methanolic extract (100, 200, 400, and 600 mg/kg p.o., n = 5), the control group (n = 5) was treated with distilled water (10mL/kg, p.o.), and positive control group (n = 5) was administrated with diazepam (3mg/kg, i.p.), respectively.

The effect was recorded for disappearance (latency) and reappearance (duration) of the righting reflex. Hypnotic sleeping time was considered to be the time interval between disappearance and reappearance of the righting reflex.¹⁰

Statistical Analysis

The statistical analysis was done using ANOVA. The results with $P < 0.05$ were considered significant. The data are expressed as mean \pm SD.

Results

Sedative Activity of the Methanolic Extract on the Central Nervous System (CNS)

The result of psychotropic effect of methanolic extract was expressed by comparison with control groups. Pharmacological tests were then performed at nontoxic doses (i.e., 100, 200, 400, and 600 mg/kg, p.o.), for the methanolic extract.

Traction Test

The methanolic extract of *Lycopus europaeus* given by oral route at 100 mg/kg did not significantly alter the reestablishment time; all animals performed normal reestablishment immediately ($P > 0.05$). However, the extract at the dose of 200 mg/kg produced significant sedative effect on the central nervous system (CNS) as indicated by the relatively high time for the reestablishment of the mice (Table 1).

Table 1: Sedative action of *Lycopus europaeus* methanolic extract. Values were expressed as mean \pm SD; $P < 0.001$ versus the control group.

Treatment	Dose (mg/kg)	Traction test (Re-establishment Time)	Hole board test (Explored holes during 5 minutes)	Fireplace test (Time to go back the tube in seconds)
Control		0.10 sec \pm 0.04	11 \pm 1	6 sec
Diazepam	3	12 sec \pm 0.5	0.0 \pm 0.0	>2min
Le.cr	100	0.09 sec \pm 0.12	8 \pm 0.1	1 sec \pm 0.5
Le.cr	200	6 sec \pm 0.04*	3 \pm 0.0*	35 sec \pm 0.5*
Le.cr	400	17 sec \pm 0.2*	1 \pm 0.0*	57 sec \pm 0.1*
Le.cr	600	26 sec \pm 0.1*	0 \pm 0.0*	>2min*

By increasing the doses to 400 and 600 mg/kg, the average reestablishment time was increased. The reestablishment time was notably higher than control group ($P < 0.001$) (Table 1).

So, methanolic extract of *Lycopus europaeus* produced significant sedative effect at the doses of 200 and 400 mg/kg p.o. (Table 1). In addition, the dose of 100 mg/kg did not decreased the reestablishment time effectively.

Hole-Board Test

In the hole-board test, a significant reduction in the number of head dips at the doses of 200, 400, and 600 mg/kg by oral route administration; with the exception at the dose of 100 mg/kg, the methanolic extract did not reduce the number of head dips significantly. The data lead to conclude that the methanolic extract of *Lycopus europaeus* possess potential sedative effects of the central nervous system at the doses of 200, 400, and 600 mg/kg via oral route administration (Table:1).

Thiopental-Induced Sleep in Mice

We observed that the methanolic extract of *Lycopus europaeus* had significant hypnotic effect ($P < 0.001$). Hypnosis induced by methanolic extract (800 and 1000 mg/kg, p.o.) was evaluated by observation of the duration of thiopental-induced sleeping time. The extract showed a reduction in the time of onset of sleep induced by thiopental. The effects of the extract on onset of sleep at 800 and 1000 mg/kg were comparable to that of diazepam at 30 mg/kg. (Table. 2)

Table 2: Effect of the methanolic extract of *Lycopus europaeus* on the onset and duration of sleep in thiopental-treated mice. Data are expressed as mean \pm SD; P <0.001 versus the control group.

Group	Dose (mg/kg)	Sleep latency (min)	Sleeping time (min)
Control	60	9 \pm 0.5	35 \pm 2
Diazepam	30	5 \pm 0.5	78 \pm 3.5
Le.cr	800	13 \pm 1	42 \pm 2
Le.cr	1000	5 \pm 1.5	97 \pm 3

Discussion

During five thousand years of recorded history, we know that from the ancient times people have used different methods and procedures in treatment of various psychiatric disorders and very often these were medicinal preparations of plants. Numerous scientific discoveries in the industrial age media big contribution to medicine development and significantly improved quality of life for psychiatric patients during the last century. However, evidence-based medicine after big bliss faced a lot of disappointments, and an attitude that some natural drugs were unnecessarily thrown out of use step by step came along. On the other hand, there are a huge number of patients that use natural medicinal plants for self-treatment of different psychiatric disorders.¹¹

Diazepam which belongs to the benzodiazepine group is a central nervous system depressant used in the management of sleeps disorders such as insomnia. Benzodiazepines have a binding site on GABA receptor type-ionophore complex.¹² They decrease activity, moderate excitement and calm the recipient. Substances like diazepam (the reference drug used in this study) reduce onset of and increase duration of barbiturate-induced sleep and reduce exploratory activity possessing potentials as sedative.¹³

Plant compounds such as flavonoids, terpenes and saponins have been found to have hypnotic effect. Flavonoids with anxiolytic activities have been described in numerous plant species used in folk medicine to depress the CNS. This effect has been ascribed to their affinity for the central benzodiazepine receptors.¹⁴ It could be suggested that flavonoids of the *Lycopus europaeus* contribute to the hypnotic effect of this plant through central benzodiazepine receptors.

Conclusion

The pharmacological profiles of the present investigation of the methanol extract of *Lycopus europaeus* indicate that the extract possess strong CNS depressant and analgesic activities as it significantly reduced locomotion, onset of sleep, increased duration of sleep and inhibition of central and peripheral mechanisms of mice in different experimental model.

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