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Evaluation of antitussive activity of *Lycopus europaeus* on cough reflex induced by different cough induced models in mice

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ABSTRACT

The present study was carried out to elucidate the potential of, methanol extract of *Lycopus europaeus* plant on antitussive activity in albino mice. The methanol extract (yield 12.5% w/w with respected to dry powdered plant material) was selected for all experimental procedure. Antitussive activity of the methanolic extract of *Lycopus europaeus (Le.cr)* was investigated for its effect on a cough model induced by sulphur dioxide gas and Ammonium liquor induced cough in mice. Results were revealed that the *Le.cr* was found to produce significant antitussive activity ($P<0.001$) when compared with control, codeine phosphate and Dextramethorphane in a dose dependent manner. High dose of *Le.cr* of (500 mg/kg) showed maximum inhibition of cough by 61.21% and 56.63% induced by both inducers. It concludes that *Le.cr* possessed remarkable antitussive effect, which provides pharmacological evidence in support of folklore-claim of *Lycopus europaeus* as an antitussive agent.

Keywords: *Lycopus europaeus*, methanolic extract; Antitussive activity, codeine phosphate and Dextramethorphane.

INTRODUCTION

Lycopus europaeus is also known as Bugleweed, bitter bugle, water horehound. *L. virginicus*: Paul's betony and water bugle. *Lycopus europaeus* is a 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 odor; 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 evaluationof 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 [1]. Other studies in rats have shown inhibition of serum thyrotropic hormone and thyroxine after oral administration [2]. Cardiac signs of hyperthyroidism were reduced in an experiment in rats treated with L. europaeus extract [3].

Freeze-dried extracts of bugleweed and other related plants showed a dose-dependent inhibition of bovine thyroid-stimulating hormone (TSH) binding to human thyroid membranes, with simultaneous inhibition of TSH-stimulated adenyl cyclase activity [4; 5]. Formation of covalent adducts with TSH amino acid residues was postulated; however, the evidence for this is not conclusive [6]. Traditionally *Lycopus europaeus* is being used as astringent, antitussive and sedative purposes, [7-9]. So the following study is being done to evaluate the antitussive activities of *Lycopus europaeus* in different cough induced models in mice.

MATERIALS 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 in Al-Manara college of Pharmacy. 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-methanol 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 37ºC under reduced pressure to thick paste like consistency. And then the extract obtained was stored at -4°C in air tight jars.

Figure 1. Aerial parts of *Lycopus europaeus*

Chemicals

Methanol, ammonium hydroxide, sodium hydrogen sulfate, sulfuric acid, ammonium chloride, sodium bicarbonate, Dextramethorphane and codeine phosphate were used in the antitussive study.

Evaluation of Antitussive Activity

Experimental Animals used

The experiments were carried out in Albino mice of either sex weighing between 20–30 g obtained from animal house of Al-Manara college of Pharmacy Multan, Pakistan were kept in the animal house at 26±2°C in polyacrylic cages with not more than six animals per cage and maintained under standard laboratory conditions with Standard food and water *ad libitum*. The animals were used for the experiment after an acclimatization period of one week before experimental sessions. Animals were divided into six groups, containing 6 mice each. The animal experiment was performed according to the college's ethical committee approval.

The control group was treated with saline solution orally, and the positive control was treated with codeine phosphate and dextramethorphan. The remaining groups were treated with the methanolic extract of *Lycopus europeaus (Le.cr)* extracts at doses of 250 and 500 mg/Kg body weight.

Sulfur dioxide gas induced cough reflex in mice

The experimental model is shown in Figure 1 where A is a 500 ml three-necked flask which contains aqueous saturated sodium hydrogen sulphite solution. By opening the stop-cock of a burette (B), the concentrated sulphuric acid was introduced to generate sulphur-dioxide gas.

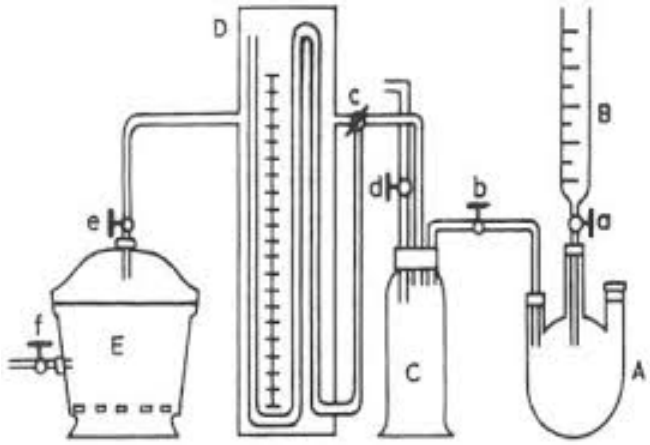

Figure 2. Apparatus used in sulfur dioxide gas induced cough model

A: Saturated NaHSO3 solution in 500ml flask, **B**: Conc. H_2SO_4 in burette, **C**: Gas Cylinder, **D**: Water manometer, **E**: Dessicator and **a, b, c, d, e, f** are stop cocks.

The chemical reaction which occurred in flask A is as follows:

$$2\ NaHSO_3 + H2SO_4 \rightarrow 2\ SO_2 + Na2SO_4 + 2\ H_2O$$

Flask A and gas cylinder C were filled with sulphur dioxide (SO_2) gas. Cocks c and b were opened to elevate pressure in gas cylinder C, which was recorded by water manometer D. Stop-cock b was then closed and stop-cock d was opened slightly until pressure in D (11 mm, i.d.) reached 75 mm H_2O, when stop-cock d was closed. The procedures were conducted in a draught. Cough response of all the groups are observed (0 minute) by placing the animals in desiccators E. The cocks c, f and e are opened in order and when the pressure in D became 0 mm of H_2O, all the cocks are closed immediately.

A certain amount, 5ml sulfur dioxide gas is induced into the desiccator and this way. After a minute of introducing the gas, the animal is taken out of the dedicator and frequency of cough is observed for five minutes in an un-ended filter funnel with a stethoscope at the tip in which mice is confined. In the same fashion

the frequency of cough are observed for all the animal groups after every 30 minutes. [10].

Ammonium liquor induced cough

Healthy mice were divided into six groups: Control, *Le.Cr* (250 and 500 mg/kg) and standard. Briefly, 1 h after oral administration of the test drug, each mice was placed in a glass chamber and exposed to 0.3 ml 25% NH_4OH produced by a nebulizer for 45 s. Animal was monitored during ammonia exposure and cough frequency was recorded.

Statistical analysis

The results of pharmacological studies were reported as Mean ± S.E.M. The total variations present in data were analyzed by using one way analysis of variance (ANOVA) followed by Dunnett's Test.

RESULTS AND DISCUSSION

Lycopus europaeus extract in multiple doses showed good dose‑dependent antitussive activity (Table 1 and Table 2). Methanolic extract (500 mg/kg) exhibited significant activity i.e 56.63%, but lower dose of it showed less activity i.e 23.14% inhibition in Ammonium liquor induced cough**.** While on the other hand*, Lycopus europaeus* Methanolic extract (500 mg/kg) exhibited significant activity i.e 61.21%, but lower dose of it showed less activity i.e 32.73% inhibition in Sulfur dioxide gas induced cough.

Antitussive activity of formulations was evaluated by using method of [11; 12]. A vial containing 2 ml of 500mg/ml solution of sodium hydrogen sulfite in double distilled water was placed at the base of a dessicator and covered with wire gauze to serve as a platform for placement of mice. To the $NaHSO_3$ solution, 0.2 ml of sulphuric acid was added using a pipette. After 15 seconds, the mice were placed on the platform in the dessicator and exposed to SO_2 for 45sec.

The mice were then removed from the dessicator and placed in an observation chamber for counting of bouts of cough for five minutes thereafter. But in laboratory condition, when the mice were placed on the platform, in the dessicator and exposed to SO_2 for 45 s and then removed from the dessicator and placed in an observation chamber for counting of bouts of cough for five minutes thereafter, it produced too much cough, even on exposing for 50 sec to SO2 gas caused death. So, there was a need to standardize the method according to the laboratory condition. Concentration of H_2SO_4 and $NaHSO_3$ was 0.2 ml and 2 ml respectively used through all the experiment. For the standardization of cough induction model according to the laboratory condition, the mice were exposed to SO_2 in different time durations like 5 second to 50 second and cough was counted respectively. The effect exhibited by the entire treated group on sulphur-dioxide induced cough in experimental animals has been presented in Tables 1 and 2.

Table 1. Effect of methanolic extract of Le.cr *on* cough frequency in sulfur dioxide gas induced cough mice

Treatment	Dose (mg/kg)	Number of cough	Percentage of inhibition
Control	10	58.54±	--
Le.Cr	250	39.38±1.90*	32.73
Le.Cr	500	22.71±4.12***	61.21
Codeine phosphate	10	26.30±1.62**	55.18
Codeine phosphate	20	13.80±4.10***	76.43

Values expressed as mean±SEM (n=6). *P<0.05, **P<0.01, and ***P<0.001 for comparison of treated groups with control

Table 2. Effect of methanolic extract of Le.cr on cough frequency in Ammonium liquor induced cough mice

Treatment	Dose (mg/kg)	Number of cough	Percentage of inhibition
Control	10	48.54±3.40	--
Le.Cr	250	37.31±2.30*	23.14
Le.Cr	500	21.04±4.10***	56.63
Dextramethorphane	10	26.52±2.04**	45.34
Dextramethorphane	20	14.31±3.61***	71.52

Values expressed as mean±SEM (n=6). *P<0.05, **P<0.01, and ***P<0.001 for comparison of treated groups with control

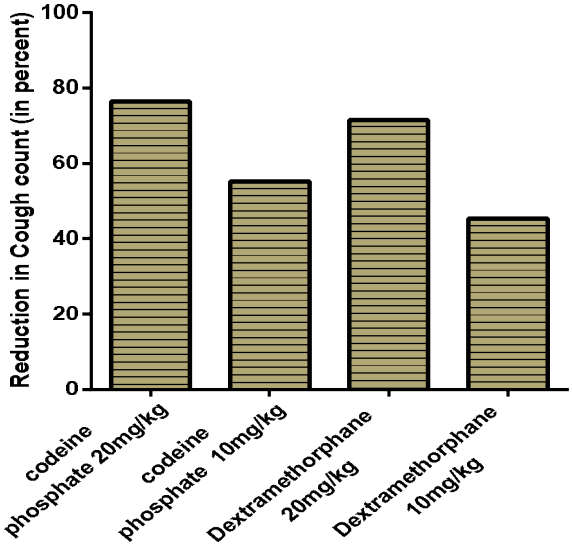

Figure 3. Comparative study of percent inhibition on cough on treatment with both standards

Antitussive animal models could be designed by mechanical stimulus, electrical stimulus, and chemical stimulus. In this experiment, chemicals like ammonium liquor and sulfur dioxide were used to induce cough. These models are widely used animal models for evaluating antitussive activity of a traditionally used drug. Cough is a normal physiological response to an irritation of the laryngo-tracheo-bronchial system caused by mechanical or chemical stimulation. It may be painful and require suppression by antitussive drugs.

In animals, coughing has been elicited by mechanical [13] or chemical irritation [14] and by electrical stimulation [15] of tracheal mucosa or by nerve stimulation [16]. Of all these methods, chemical or mechanical stimulation are more similar to the

physiological event and also the experimental models generally used in man.

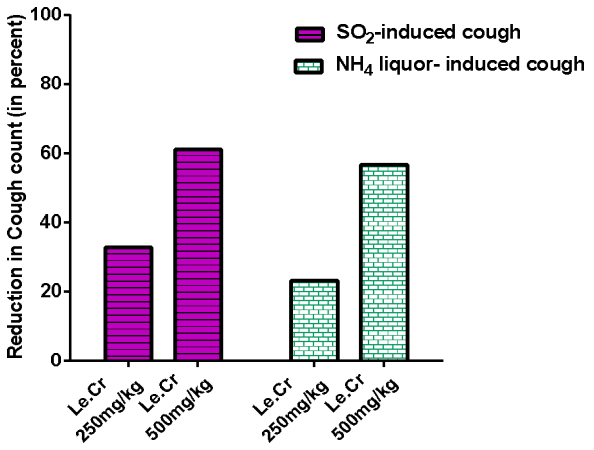

Figure 4. Percentage of reduction in cough count by methanolic extract of Le.cr *on* cough frequency in both cough-induced models in mice

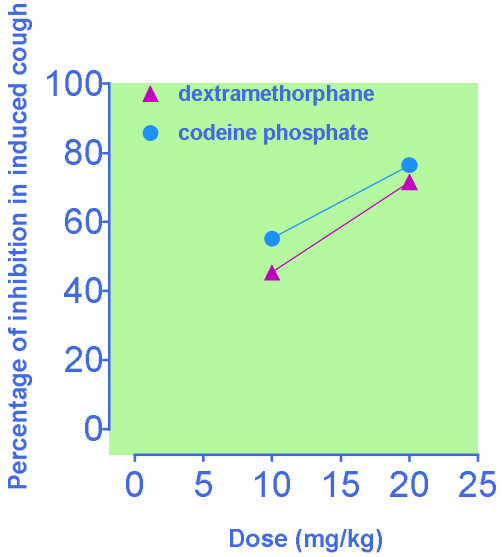

Figure 5. Comparison of the standard drugs for inhibition of induced cough.

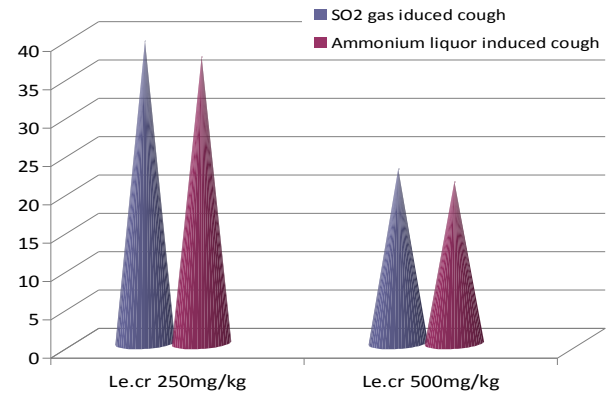

Figure 6. Reduction in cough by *Le.cr* plant extracts on the cough induced by both cough inducers in mice. On Y-axis No. of cough is mentioned while on X-axis, doses of Le.cr is mentioned

Anti-tussive agents are used mainly to suppress dry and painful cough. Cough suppressants act to reduce the urge to cough. Nonmyelinated C-fibres and rapidly adapting receptors, which have myelinated Aδ-fibres, appear to be involved in cough. These putative cough receptors have myelinated afferents and are found mainly in the larynx and the extrapulmonary airways [17]. Vagal afferent nerve provide inputs to brainstem nuclei, primarily the nucleus of the solitary tract (nTS) that receive inputs from airway cough evoking afferents and generate cough reflex in body. Centrally acting antitussives such as codeine phsophate and dextromethorphane act within the central nervous system (CNS) at the level of the brain stem by depolarization or a dulling of the vagus nerve, the nerves leading from the brain stem and serving the chest area. Peripheral antitussive drugs act outside the CNS to inhibit cough by suppressing the responsiveness of one or more vagal sensory receptors that produce cough [18].

CONCLUSION

To conclude, our study indicated that the methanol extract of *Le.cr* demonstrated significant antitussive activity and obtained percentage inhibition of cough reflex is approximately comparable as standard drug. These effects are the important evidence for the traditional use of *Le.cr* in the treatment of cough and respiratory disorders. While other we are further evaluating other traditionally used activities of *Le.cr*

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