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Formulation and evaluation of herbal Antitussive Syrup of Methanolic Extract of *Lycopus europaeus* in Mice

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ABSTRACT

Methanolic extract of *Lycopus europaeus* were investigated for their antitussive effect on citric acid induced cough model in mice. The results showed that the formulated cough syrup exhibited significant antitussive activity in a dose dependent manner the activity was compared with the prototype antitussive agent diphenhydramine HCl. It has been observed that the extract has produced 54%, 70%, 75% reduction in cough bouts at the dose level of 1, 2, 3 ml respectively after 1hr of drug administration. It is evident from the data the highest dose of 3 ml was found to be more effective. It is found that antitussive activity produced by the herbal formulation in the minimum dose was much better than the standard drug.

Keysword: Antitussive agent, Polyherbal cough syrup, Diphenhydramine HCl, Citric acid induced.

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INTRODUCTION

Coughing is one of the common symptoms related to several respiratory diseases such as asthma, bronchitis, pneumonia, etc., though a number of synthetic drugs are available in the treatment of cough, the problem is that an effective therapy against coughing will inevitably bring side effects. [1] Northeastern India is blessed with a lot of floral diversity, abound with natural medicinal potentials. Owing to their medicinal and curing properties as well as the health boosting constituents, vegetables with medicinal property are the choice of interest in both the traditional as well as modern versions of the health care system.

Cough is a defensive reflex of the respiratory tract which is important to clear the upper airways and should not be suppressed indiscriminately. Cough is thought to be caused by a reflex. It occurs due to stimulation of mechano-or chemoreceptor in throat, respiratory passage or stretch receptor in the lungs. The sensitive receptors are located in the bronchial tree, particularly in the junction of the trachea. These receptors can be stimulated mechanically or chemically e.g. by inhalation of various irritants than nerve impulses activate the cough center in the brain. Traditionally cough is classified as either productive, *i.e.* producing mucus usually with expectoration or nonproductive (dry). Therefore, the use of an effective antitussive agent such as dextromethorphan or codeine to suppress the debilitating cough suffered by such patients seems appropriate. Non-Narcotic antitussive agents anesthetize the stretch receptor located in respiratory passages, lungs and pleura by dampening their activity and thereby reducing the cough reflex at its source. Narcotic antitussive agents depress the cough center that is located in the medulla, thereby raising its threshold for incoming cough. [2]

Lycopus europaeus is also known as Bugleweed, wolf strappkraut, bitter bugle, water horehound. *L. virginicus*: Paul's betony and water bugle. *Lycopus europaeus* is aherbaceous 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 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 [3] Other studies in rats have shown inhibition of serum thyrotropic hormone and thyroxine after oral administration [4] Cardiac

signs of hyperthyroidism were reduced in an experiment in rats treated with L.europaeus extract. [5] The plant was also reported for its antitussive activity. [6]

Traditionally *Lycopus europaeus* is being used as an astringent and sedative purposes, [7, 8] So the following study is being done to evaluate the sedative and hypnotic activities of *Lycopus europaeus* in different cough induced models in mice. An attempt to prepare a poly herbal formulation were undertaken in the present study, after reviewing various literatures for each of these promising plants with a fore mentioned activities.

Figure: 1. Aerial Parts of *Lycopus europaeus*

MATERIALS AND METHOD

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 (1kg) was subjected to maceration in 70% aqueous-methanol in amber colored bottle at room temperature for 7 days with occasional vigoous 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.

Preparation of herbal syrup

The simple syrup (66.67% w/v) was prepared as per British pharmacopoeia. 200 mg of extract of

Lycopus europaeus . Honey were dissolved in simple syrup I.P. and the volume was made up to 100 ml and finally preservatives was added.

Table 1: Physicochemical parameters of formulated polyherbal cough syrup

Colour	Reddish brown
Odour	Sweet aromatic
Taste	Sweet
Specific Gravity	1.28
Density	1.41
Refractive index	1.52
pH	4.9
Alcohol content	0.82
Acid value	0.114

Evaluation of formulated cough syrup

Physicochemical parameters like Specific gravity, Density, pH, Refractive index, Alcohol content and Acid value were analyzed as per the standard procedure mentioned in British Pharmacopoeia. The colour, odour and taste were also recorded as shown in table -1

PHARMACOLOGICAL SCREENING

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. They were kept in the departmental animal house under the conditions of light (14h light/10h dark) at 27 ± 2°C and relative humidity 44- 56%, for 1 week before and during the experiments. All animals were handled according to the approval and current guidelines of Institutional Animal Ethical Committee.

Antitussive activity

The method described by D. Marina[9] was adopted to evaluate antitussive activity.

The animal were divided into five groups

Group- **I** control group

Group -**II** received diphenhydramine hydrochloride (2.8mg/kg)

Group -**III** received 1ml formulated cough syrup

Group -**IV** received 2 ml formulated cough syrup

Group -**V** received 3 ml formulated cough syrup

The animals were placed in a cylindrical glass vessel with two tubes at either ends. One serves as the entrance of the aerosol and the other for its efflux. The latter tube has a side arm connecting to a tambour, from which change in pressure can be registered. A pinch clamp with a variable screw was placed on the efflux tube beyond the side arm permitting the regulation of sensitivity

of system so that the displacement of air in the enclosure caused by coughing of the animal was registered. The mice was exposed to the aerosol of 7.5% citric acid in water for 10 min. Each animal was tested first to obtain the control response. The number of tussive response was registered. One hour later, the standard and test substances were applied orally and 30 min later the mice was subjected to the aerosol again. The number of coughs during 10 min was recorded. [10, 11, 12]

Statistical analysis

All the data are expressed as mean ± SEM. The values obtained for the above parameters were compared with standard and control group using one way ANOVA followed by Student's test The values of $p < 0.05$ and $p < 0.001$ were considered to indicate a significant difference between the groups.

RESULTS AND DISCUSSION

Herbal formulation in the concentration range of 1, 2, 3 ml revealed significant $p < 0.05$ and $p < 0.001$ antitussive activity in a dose dependent manner in comparison to control and standard drug. It has been observed that the extract has produced 54%, 70%, 75% reduction in cough bouts at the dose level of 1, 2, 3 ml respectively after 1hr of drug administration. It is evident from the data the highest dose of 3 ml was found to be more effective. Also the antitussive activity produced by the herbal formulation in the minimum dose was much better than the standard drug. The results were tabulated in Table -2, Figure - 2.

Table 2: Effect of polyherbal cough syrup on citric acid induced cough in mice and treatment latency to citric acid induced cough

Treatment	**Latency to citric acid induced cough**		
Groups	Before in min. (mean ±SEM)	After in min. (mean ±SEM)	% reduction in cough bout against control
Control	15.1 ±2.77	14.3±1.68	-
Standard Diphenhydramine hydrochloride (2.8mg/kg)	20.7 ±1.21	8.72 ±1.31	45%
Test-I Formulated cough syrup (1ml)	18.1 ±1.76	7.21± 1.47**	54%
Test-II Formulated cough syrup (2ml)	20 ±2.74	4.51 ±1.06**	70%
Test-II Formulated cough	18 ±1.82	4.23 ±0.52**	75%

P<0.01, P<0.05 shows statistical significance compared to control

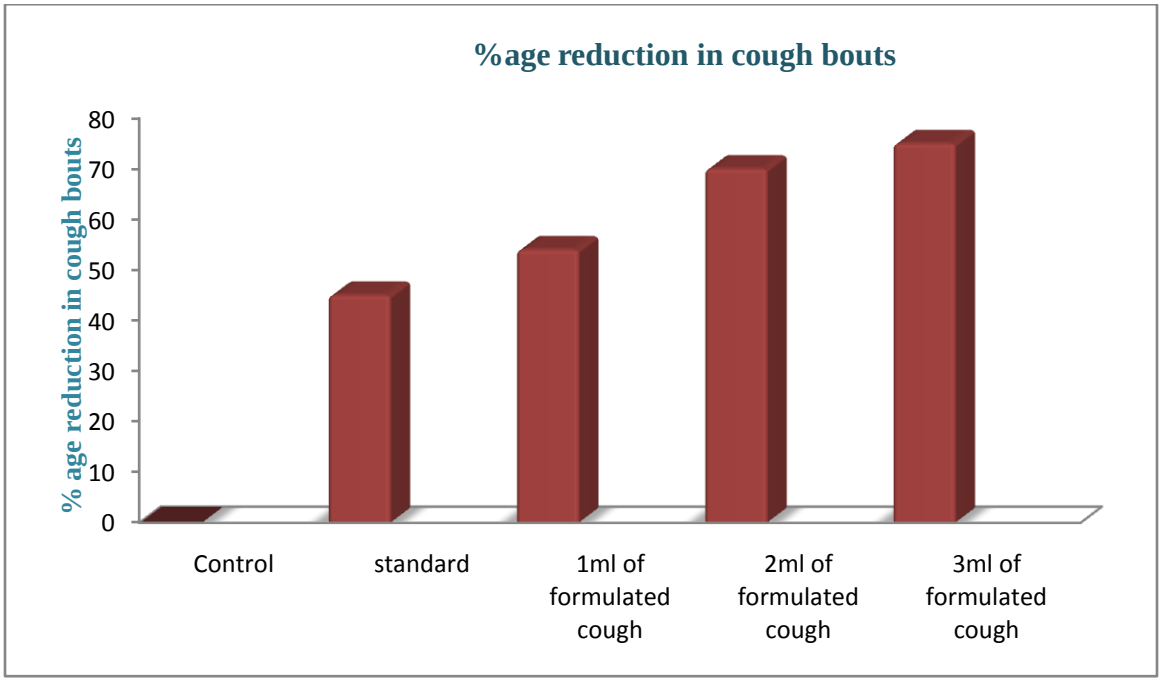

Figure:2. Graph showing % reduction in cough bouts

Although, a number of synthetic preparations have proved to be effective for managing coughing symptoms, acurative therapy for cough is lacking. The cough suppressant activity elicited by the formulated herbal syrup may also be attributed to the presence of some phyto constituents such as vasicinone and vasicinol1. Many of the currently available cough suppressants like Codeine, Ephedrine, Bromohexine, Guaifensesin etc, produce significant depression, drowsiness and addiction which makes their use unsatisfactory. [13]

CONCLUSION

The present study has provided an experimental evidence for protection against cough by the formulated poly herbal cough syrup. All the above findings support the traditional claims in Ayurveda for use of this formulation in the treatment of cough by virtue of its antitussive activity19. The Cough suppressant activity elicited by the formulated herbal syrup may also be attributed to the presence of some phyto constituents. The difference between test drugs and control group was very significant at the level of $p<0.01$.And the difference between test drugs and standard group (Diphenhydramine hydrochloride) was significant at the level of $p<0.05$.This may be suggestive of an antitussive activity of the formulation probably due to directly acting peripherally or may be due to centrally.

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