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STUDY OF ANTITUSSIVE POTENTIAL OF *GLYCYRRHIZA GLABRA* AND *ADHATODA VASICA* USING A COUGH MODEL INDUCED BY SULPHUR DIOXIDE GAS IN MICE

Yasmeen Jahan* and H.H. Siddiqui

Faculty of Pharmacy, Integral University, Kursi Road, Lucknow, Uttar Pradesh, India

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Correspondence to Author:

Yasmeen Jahan

Faculty of Pharmacy, Integral University, Kursi Road, Lucknow, Uttar Pradesh, India

ABSTRACT

Cough is the most common symptom of respiratory diseases. When cough becomes serious, opioids are effective, but they have side effects like sedation, constipation. Therefore, there is a need to have effective antitussive agent which do not have respiratory depressant activity. The present study was carried out to evaluate antitussive activity of *Glycyrrhiza glabra* and *Adhatoda vasica* using a cough model induced by sulphur dioxide gas in mice. The effect of the ethanol extracts of *Glycyrrhiza glabra* and *Adhatoda vasica* on SO_2 gas induced cough in experimental animals have very significant effects at the level of $p<0.01$ in inhibiting the cough reflex at a dose of 800 mg/kg and 200 mg/kg body wt. p.o., in comparison with the control group. Mice showed an inhibition of 35.62%, in cough on treatment with *Glycyrrhiza glabra* and 43.02% inhibition on treatment with *Adhatoda vasica* within 60 min of the experiment. The antitussive activity of the extract was comparable to that of codeine sulphate (10, 15, 20 mg/kg body wt.), a standard anti-tussive agent. Codeine sulphate, as a standard drug for suppression of cough, produced 24.80%, 32.98%, and 45.73% inhibition in cough at a dose of 10 mg/kg, 15 mg/kg and 20 mg/kg respectively, whereas, codeine sulphate (20 mg/kg) showed maximum 45.73% ($p<0.001$) inhibition at 60 min of the experiment.

INTRODUCTION: A cough (Latin: tussis) is a sudden and forceful expiration of air from the lungs caused by an involuntary contraction of the muscles controlling the process of breathing. It is a protective reflex that removes foreign material and secretions from the bronchi and bronchioles. The cough reflex consists of three phases: an inhalation, a forced exhalation against a closed glottis [1]. Ayurveda (Ayur-life, Veda-knowledge), is the knowledge of healthy living and is not merely confined to the treatment of illness. Ayurvedic medicines are largely based on herbal and herbo-mineral preparations and have specific diagnostic and therapeutic principles [2].

Glycyrrhiza glabra is one of the most commonly used medicine in Indian indigenous system of medicine.

It is used as an energy tonic, particularly for the spleen and stomach, and the root is added to many formulae. It is also used for asthmatic coughs, as an antispasmodic and ulcer remedy, and to cool 'hot' conditions.

Roots of *Glycyrrhiza glabra* being tonic, demulcent laxative emollient are used in genito-urinary diseases, coughs and sore throat [3]. The antitussive activity of glycyrrhetinic acid and its derivatives has been evaluated [4].

Adhatoda vasica (AV) is an Ayurvedic medicinal plant which is a home remedy for several diseases. It is mentioned in Vedas as an herbal remedy for treating cold, cough, whooping cough and chronic bronchitis and asthma, as sedative expectorant, antispasmodic and anthelmintic. It is an official drug and is mentioned in the Pharmacopoeia of India (1966). The drug is employed in different forms such as fresh juice, decoction, infusion and powder; also given as alcoholic extract and liquid extract or syrup. The leaf juice is stated to cure diarrhoea, dysentery and glandular tumor and the plant is an emmenagogue.

The powder is reported to be used as poultice on rheumatic joints as counter-irritant on inflammatory swelling, on fresh wounds, urticaria and in neuralgia have shown that AV consists of peganine-type alkaloids namely vasicinone, vasicine and vasicinol [6]. Vasicinone and vasicinol have elicited bronchodialating action which is attributed to the quinazol-4-one ring system. Vasicine, on the other hand has elicited bronchoconstricting action. The active alkaloid vasicine and its auto oxidation product vasicinone have shown antitussive activity [7].

The extract of leaves is traditionally used for the treatment of bronchitis. It is known to Ayurveda for 2000 years. The primary action of currently available cough suppressants on the central cough pathway. The significant side effects of these agents such as constipation, respiratory depression, dependence, drowsiness and death from this action limit their use in human and thus highly unsatisfactory [8]. So, the need of hour is to screen a number of medicinal plants for promising biological activity.

MATERIALS AND METHODS:

Plant Material: The dried part of plants was purchased from the local shop in the city (Lucknow) during the month of January 2011. Samples of plant material were given to National Botanical Research Institute (NBRI) Lucknow, India for identification and Taxonomic authentification. The test report from NBRI, Lucknow confirmed the authenticity of plant sample. Ref.No.NBRI/CIF/215/2011, Lucknow.

Extraction of *Glycyrrhiza glabra*: The roots and rhizomes (250 gm) was crushed and pulverized by mechanical grinder to make a coarse powder and extracted with ethanol (70%v/v) using Soxhlet's extractor for 24 h. The extract was concentrated under reduced pressure and then dried in air (yield -35 g). The extract stored in a refrigerator and reconstituted in distilled water before use. The yield of *Glycyrrhiza glabra* in 70% ethanol was 14% w/w with respect to the dry starting material**.**

Extraction of *Adhatoda vasica*: Leaves were crushed to a coarse powder and dried plant material (1 kg) was extracted with 80% ethanol at room temperature. The alcoholic extract was dried at 40^{o}C for 1 week and was maintained in the dark in a cool and dry place. AV extract was freshly dissolved or suspended in distilled water for oral administration. The yield of *Adhatoda vasica* in 80% ethanol was 0.4% w/w with respect to the dry starting material.

Experimental Animals used: The experiments were carried out in Albino mice of either sex weighing between 20–30 g obtained from animal house of Integral University. Animals were kept in the animal house at 26±2^{o}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 university's ethical committee approval and guidelines R.No: IU/Pharm/M.Pharm/CPCSEA/10/30.

Evaluation of Antitussive Activity:

Sulphur dioxide (SO_2) induced Cough: Anti-tussive effect against sulphur dioxide (SO_2)-induced cough was evaluated by the method as described by Miyagoshi, 1986 with slight modification [9].

A vial containing 2 ml of 500mg/ml solution of sodium hydrogen sulfite (NaHSO3; Qualigens fine chemicals) in double distilled water was placed at the base of a dessicator and covered with a porcelain porous plate to serve as a platform for placement of mice as shown in **Figure 1.**

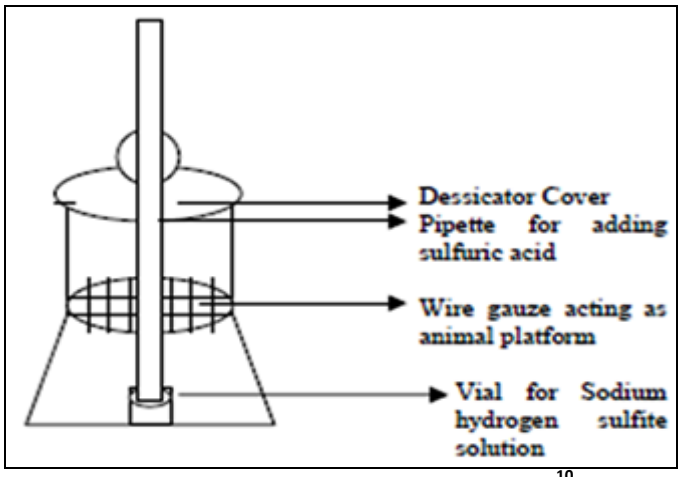

FIG. 1: SETUP FOR SO_2 INDUCED COUGH [10]

To the $NaHSO_3$ solution, 0.2 ml of sulphuric acid (H_2SO4; Qualigens fine chemicals) was added using a pipette.

The reaction involved is as follows:

$$2NaHSO_3 + H_2SO_4 \rightarrow 2SO_2 + Na_2SO_4 + H_2O$$

After 15 seconds, the mice were placed on the platform in the desiccator and exposed to SO_2 for 20 seconds. The mice were then removed from the desiccator and placed in an observation chamber for counting of bouts of cough for five minutes thereafter.

Initially the cough responses of all groups of animals were observed (0 min) by placing the animal individually in the desiccator and certain amount of SO2 gas (5 ml, which was fixed throughout the experiment) was introduced. After 20 seconds exposure of the gas, the animal was taken out of the desiccator and the frequency of cough was observed for 5 min in an unended filter funnel. In this fashion the frequency of cough were observed for all the animal groups at 0 min before the drug administration and at 60 min after the drug administration.

Scoring of Bouts of Cough: In this fashion, the frequency of cough were observed for all the animal groups at 0 min, before administration of any chemical or testing material. Since, it has been illustrated that cough response to a given stimulus varies from animal to animal but that repeated assessments within the same animals are fairly reproducible. Thus, animals having low or high cough threshold were not entertained for further studies. Number of coughs was observed for all animal groups at 60 min after drug administration by using same procedure.

Drug Treatment: All drugs were administered orally (p.o.). Animals were divided into six groups, containing 6 mice each. Treatment to be given to the animals are shown in **Table 1.** Group I served as control group and was not administered anything. Group II, Group III and Group IV was received standard drug ie. Codeine sulphate 10 mg/kg, 15 mg/kg & 20 mg/kg p.o. respectively. Group V received ethanol extract of *Glycyrrhiza glabra* in dose of 800 mg/kg & Group VI received ethanol extract of *Adhatoda vasica* in dose of 200 mg/kg.

TABLE 1: **TREATMENT TO BE GIVEN TO THE ANIMALS**

Groups	Number of Animals	Treatment to be given
I	6	Normal Control
II	6	Treated with Standard drug codeine sulphate (10mg/kg, p.o)
III	6	Treated with Standard drug codeine sulphate (15mg/kg, p.o)
IV	6	Treated with Standard drug codeine sulphate (20mg/kg, p.o)
V	6	Treated with test drug *Glycyrrhiza glabra* (800 mg/kg, p.o)
VI	6	Treated with test drug *Adhatoda vasica* (200 mg/kg, p.o)

Each animal served as its own control and was exposed to sulphur dioxide gas twice ie.before and 60 minutes after the drug treatment.

Statistical Analysis: Mean of cough bouts recorded was taken and percent inhibition in number of cough bouts calculated.The experimental results have been expressed as the mean ± SEM. Significance was evaluated by the Student's't'-test and p-value less than 0.05 vs control imply significance.[11]

RESULTS & DISCUSSION:

Standardization of Cough Induction Model: Gupta, 2009 evaluated antitussive activity of formulations by using method of Miyagoshi, 1986 with slight modification [9, 10]. He stated that 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 a 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 45 s.

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 SO_2 gas caused death.

So, there was a need to standardize the method according to the laboratory condition. Concentration of sulphuric acid and sodium bisulphite was 0.2 ml and 2 ml respectively used through all the experiment. Although in the present study, the quantification of SO_2 generated has not been attempted, it is expected that the quantity and saturation level in the chamber would be the same in all the exposures, as the other conditions were kept the same.

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 effects exhibited by exposing the animals in different time durations have been presented in **Table 2.** The graphical representation of effects exhibited by exposing the animals in different time durations have been shown in **Figure 2**.

TABLE 2: STANDARDIZATION OF COUGH INDUCTION MODEL IN LABORATORY CONDITION

Weight of animals (gm)	Exposure of SO_2 gas (sec)	Frequency of cough bouts (In 5 min)
26	5	-
22	10	4
29	15	27
23	20	79
25	25	121
26	30	173
26	35	153
24	40	309
27	45	388
28	50	death

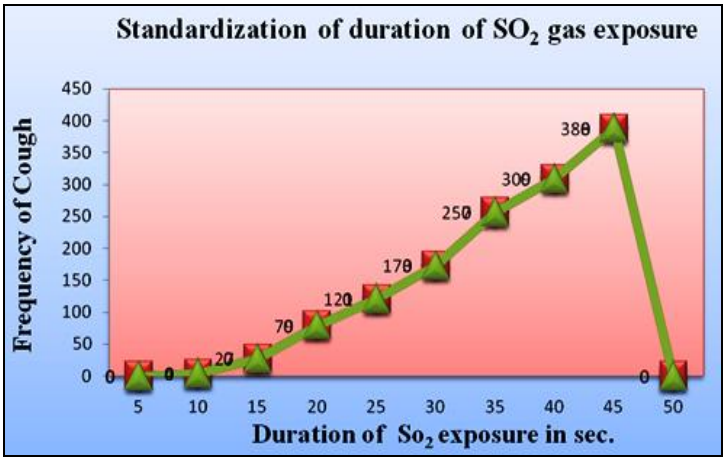

FIG. 2: STANDARDIZATION OF COUGH INDUCTION MODEL IN LABORATORY CONDITION

The effect exhibited by the entire treated group on sulphur-dioxide induced cough in experimental animals has been presented in **Table 3.**

TABLE 3: THE EFFECT EXHIBITED BY THE ENTIRE TREATED GROUP ON SULPHUR DIOXIDE INDUCED COUGH IN EXPERIMENTAL ANIMALS

Effect of drugs on the cough reflex induced by SO_2 gas in mice				
Treatment	**Dose (mg/kg)**	**No. of Animals**	**Frequency of cough (mean ± SEM)**	**Inhibition (%)**
Control group	--	6	85.66±5.31	--
Codeine sulphate	10	6	64.50±6.45 *	24.80%
	15	6	58.33±6.96 *	32.98%
	20	6	47.00±7.72 **	45.73%
Ethanol extract of *Glycyrrhiza glabra*	800	6	56.16±7.76 * #	35.62%
Ethonal extract of *Adhatoda vasica*	200	6	50.50±9.81 * #	43.02%

In normal controls, there was no significant change in the number of cough bouts, between the two exposures. The effect of the ethanol extracts of *Glycyrrhiza glabra* and *Adhatoda vasica* on SO_2 gas induced cough in experimental animals has significant effects at the level of $p<0.01$ in inhibiting the cough reflex at a dose of 800 mg/kg and 200 mg/kg body wt. p.o., in comparison with the control group.

Mice showed a inhibition of 35.62%, in cough on treatment with *Glycyrrhiza glabra* and 43.02% inhibition on treatment with *Adhatoda vasica.* Codeine sulphate used as a standard drug for suppression of cough, produced 24.80%, 32.98%, and 45.73% inhibition in cough at a dose of 10 mg/kg, 15 mg/kg and 20 mg/kg respectively**.** Whereas, codeine sulphate (20 mg/kg) showed maximum 45.73% ($p<0.001$) inhibition at 60 min of the experiment.

And the effect of the ethanol extracts of *Glycyrrhiza glabra* and *Adhatoda vasica* on SO_2 gas induced cough in experimental animals also have significant ($p<0.05$) effects in inhibiting the cough reflex at a dose of 800 mg/kg and 200 mg/kg body wt. p.o., in comparison with the Standard group.

The frequency of cough was counted for 5 min after the sulphur dioxide gas challenge each sample was dissolved in 0.3 ml distilled water. Statistical differences between plant extracts, standard (codeine) with baseline values; Significance was evaluated by Student's t-test (n = 6 animals/experiment). (**)- $P < 0.01$ as compared with control. (***) - $P < 0.001$ as compared with control. ($^{\#}$) - $P < 0.05$ as compared with codeine sulphate.

The graphical representation of results are shown in **Figure 3, Figure 4, Figure 5, Figure 6**.

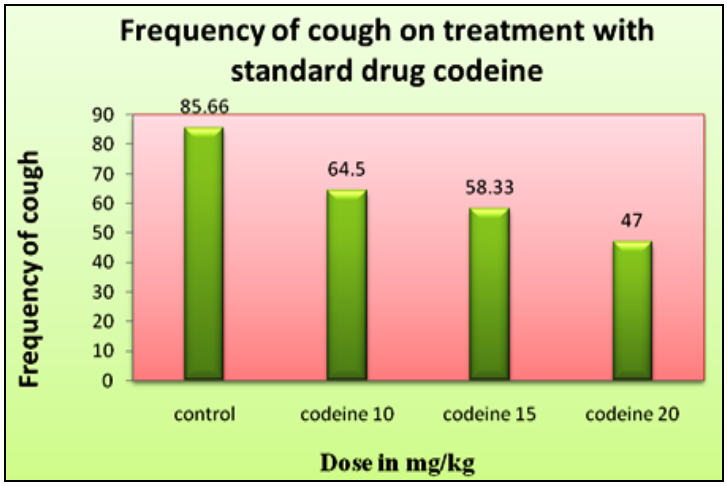

FIG. 3 : FREQUENCY OF COUGH ON TREATMENT WITH CODEINE

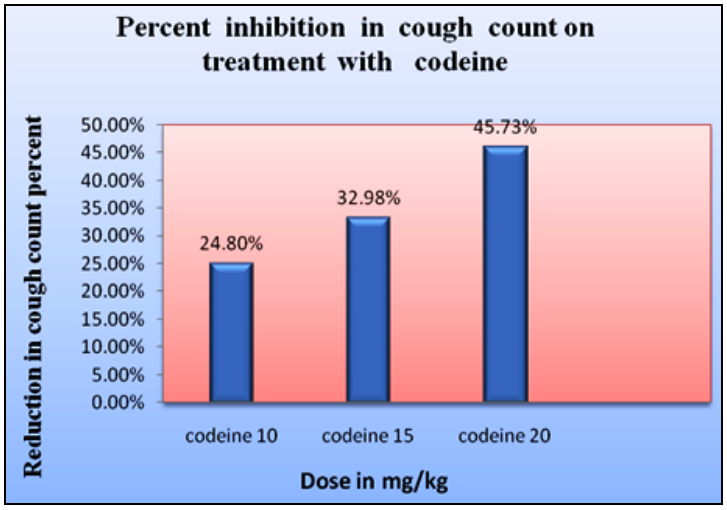

FIG 4: PERCENT INHIBITIONS IN COUGH ON TREATMENT WITH CODEINE

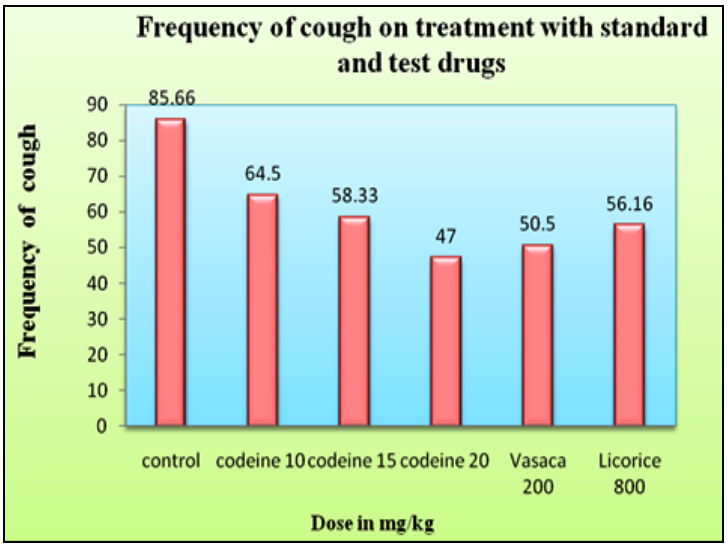

FIG. 5: FREQUENCY OF COUGH ON TREATMENT WITH ALL DRUGS

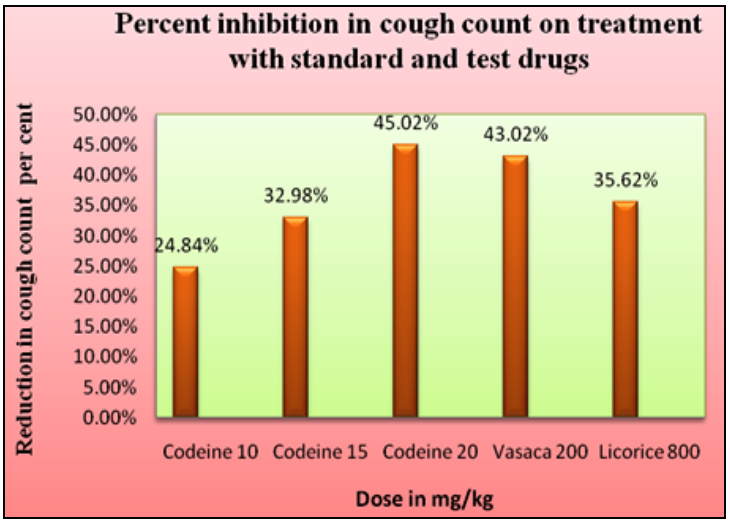

FIG. 6: COMPARATIVE STUDY OF PERCENT INHIBITION ON COUGH ON TREATMENT WITH ALL DRUGS

Herbs have been important contributors to the quality of human life for thousands of years. It has been estimated by World Health Organization (WHO) that approximately 80% of world's inhabitants, mainly residing in developing countries, rely on traditional medicine, and 85% of traditional medicine involves the use of plant extracts or their active principles [12].

Many medicinal plants have been claimed to have antitussive activity. For example - *Ocimum sanctum* [15], *Ionidium suffruticosam* [16], *Trichodesma indicum* [17], *Abies webbiana* [18], *Ficus racemosa* [19], *Lagerstroemia parviflora* [20], *Jussiaea suffruticosa* [21], *Asparagus racemosus* ,*Solanum xanthocarpum* [22] etc. *Ocimum sanctum*(tulsi), ginger, *Glycyrrhiza glabra* (licorice/ mulethi), *Voila odorata* (banafsha), *Justicia adhatoda* (vasaka) leaves and *Foeniculum vulgare* (fennel)etc.,

are major components of household cough and cold remedies worldwide, in the form of decoctions, teas etc. *Glycyrrhiza glabra* is effective as an expectorant and demulcent in inflammation of bronchi tubules. The glycyrrhetic acid interferes with mucopolysaccharid synthesis. *Adhatoda vasica* increases bronchial secretion or reduce its viscosity, facilitating its removal by coughing.

Some isolated experimental and clinical studies have also been carried out on these agents for cough. Preliminary investigation shows promising results as antitussive and expectorant activity, this aspect has been further investigated so that these herbs can be established individually as a standard antitussive and expectorant drug. The model used in this study is a modification of Gupta, 2009 [10].

In the present study, the quantification of SO_2 generated has not been attempted, it is expected that the quantity and saturation level in the chamber would be the same in all the exposures, as the other conditions were kept the same. The present data indicates that the ethanol extracts of both plant possesses obvious antitussive activity against chemically induced cough in mice. The antitussive activity of ethanol extracts of both the plant was tested and the results showed significant activity in this animal model which supports the use of the plant in traditional medicine. The ethanol extract at dose levels of 800mg/kg (*Glycyrrhiza glabra)* and 200 mg/kg (*Adhatoda vasica)* showed significant activity after 1 h as far as the frequency of cough as well as inhibition of cough reflex is concerned.

It can be concluded that the ethanol extracts exerts a significant antitussive effect in experimentally induced cough reflex in mice comparable to the standard drug codeine sulphate. The cough supressant actualy of *Glycyrrhiza glabra was* 35.62% as compared to the actually of codeine sulphate. The cough supressant actualy of *Adhatoda vasica was* 43.02% as compared to the actually of codeine sulphate. The difference between test drugs (*Glycyrrhiza glabra, Adhatoda vasica*) and control group was very significant at the level of $p<0.01$.And the difference between test drugs (*Glycyrrhiza glabra, Adhatoda vasica*) and standard group (codeine sulphate) was significant at the level of $p<0.05$.

The result of the present study provides pharma-cological evidence in support of the folklore-claim of *Glycyrrhiza glabra* and *Adhatoda vasica* as an antitussive agent.

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