

EVALUATION OF ANTITUSSIVE ACTIVITY OF FORMULATIONS WITH HERBAL EXTRACTS IN SULPHUR DIOXIDE (SO₂) INDUCED COUGH MODEL IN MICE

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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, some addiction liability and also compromise the respiratory function. Therefore, there is need to have effective anti-tussive agent which do not have respiratory suppressant activity. The present study was carried out to evaluate anti-tussive activity of combination of herbal drugs as formulations in sulphur dioxide (SO₂)-induced cough model in mice.

Albino mice of either sex, weighing 25-30 g were divided into eight groups, (n = 6). Group 1 served as normal control, group 2 mice were given distilled water, group 3 was positive control and received codeine sulphate (10 mg/kg, p.o.) and group 4, 5, 6, 7 received coded 1 formulations 1, 2, 3 and 4 respectively at a dose of 0.3 ml/mice, orally, while group VIII was the vehicle control. Thirty minutes later, the mice were exposed to sulphur dioxide again for 45 sec. The mice were then placed in an observation chamber for counting of cough bouts, by two independent observers, for five minutes. All the formulations used showed significant antitussive activity in sulphur dioxide induced cough model. Thus, these formulations can prove to be useful for alleviating cough.

Key words : cough antitussive activity sulphurdioxide – induced cough

INTRODUCTION

Cough is a defensive reflex of the

respiratory tract which is important to clear the upper airways. However, the cough sometimes may become exaggerated and

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interfere with quality of life and even cause exhaustion. Unproductive or dry cough are often associated with eosinophilic bronchitis, irritation of airways due to several environmental pollutants, airway hyperresponsiveness due to infection, gastroesophageal reflux disease and also without any associated cause, often referred to as idiopathic cough.

Hydration of respiratory tract by steam inhalation, demulscents are effective in reducing symptoms in majority of cases but, for uncontrolled cough, opioidergic central cough suppressants are used. Among opioids, codeine, pholcodeine, noscapine, dextromethorphan are effective, but they have certain inherent side effects like sedation, constipation, and also some addiction liability. Furthermore, their use in severe cough conditions like asthma is contraindicated, as they are known to further compromise the respiratory function. Therefore, there is need to have effective anti-tussive which can successfully alleviate chronic cough without side effects.

Cough suppressant and antiasthmatic activities have been claimed for many medicinal plants, in the literature. Based on this knowledge, different workers have evaluated botanicals for antitussive/cough suppressant activity. For example, *Ocimum sanctum* (1), *Passiflora incarnate* (2), *Ionidium suffruticosam* (3), *Trichodesma indicum* (4), *Abies webbiana* (5), *Ficus racemosa* (6), *Lagerstroemia parviflora* (7), *Drymaria cordata* (8), *Leucas lavandulaefolia* (9), *Jussiaea suffruticosa* (10), *Asparagus racemosus* (11), etc.

While it is apparent that the different

medicinal plants would work by different mechanisms in suppressing cough, there are very few studies available on combined activity of the different medicinal plants.. We therefore evaluated some formulations with a combination of herbals in sulphur dioxide induced cough model in mice.

MATERIAL AND METHODS

Experimental animals

The experiments were performed on male albino mice weighing between 25–30 g. The animals were procured from the central animal facility of All India Institute of Medical Sciences, New Delhi and were maintained in the departmental animal house. The mice were group housed in polyacrylic cages (38x23x10 cm) with not more than four animals per cage and maintained under standard laboratory conditions with natural dark and light cycle. They were allowed free access to standard dry rodent diet (Golden Feeds, India) and tap water *ad libitum*. Food and water were withheld only during experimentation. After acclimatization, mice were randomly divided into eight groups. Each group comprised of 6 animals. All experimental procedures described were reviewed and approved by the Institutional Animal Ethics Committee.

Plant extraction and preparation of formulations

The four test formulations (Table I) were prepared and provided by Ranbaxy Research Laboratories, Gurgaon, India. Briefly, the authenticated plants were either extracted in an aqueous medium individually or co extraction was done. The first and third

TABLE I: Composition of the different formulations used. While Formulation 1 and 3 were prepared by mixing individual extracts of the given medicinal plants, for formulation 2 and 4, the different components were co - extracted.

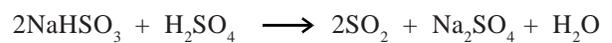
Formulation 1	Formulation 2	Formulation 3	Formulation 4
<i>Justicia adhatoda</i> (Vasaka)	<i>Justicia adhatoda</i> (Vasaka)	<i>Justicia adhatoda</i> (Vasaka)	<i>Justicia adhatoda</i> (Vasaka)
<i>Curcuma longa</i> (Haridra)	<i>Curcuma longa</i> (Haridra)	<i>Curcuma longa</i> (Haridra)	<i>Curcuma longa</i> (Haridra)
<i>Curcuma Zeodaria</i> (Shati)	<i>Curcuma Zeodaria</i> (Shati)	<i>Curcuma Zeodaria</i> (Shati)	<i>Curcuma Zeodaria</i> (Shati)
<i>Ocimum sanctum</i> (tulsi)	<i>Ocimum sanctum</i> (tulsi)	<i>Ocimum sanctum</i> (tulsi)	<i>Ocimum sanctum</i> (tulsi)
<i>Piper longum</i> (pippali)	<i>Piper longum</i> (pippali)	<i>Piper longum</i> (pippali)	<i>Piper longum</i> (pippali)
<i>Solanum surratense</i> (Kantakari)	<i>Solanum surratense</i> (Kantakari)	<i>Solanum surratense</i> (Kantakari)	<i>Solanum surratense</i> (Kantakari)
<i>Voila odorata</i> (Banafsha)	<i>Voila odorata</i> (Banafsha)	<i>Voila odorata</i> (Banafsha)	<i>Voila odorata</i> (Banafsha)
<i>Vitis vinifera</i> (Draksha),)	<i>Vitis vinifera</i> (Draksha),)	—	—
<i>Alpinia galangal</i> (Kulinjan)	<i>Alpinia galangal</i> (Kulinjan)	—	—
<i>Mentha arvensis</i> (Sat pudina)			
<i>Glycyrrhiza glabra</i> (mulethi)	<i>Glycyrrhiza glabra</i> (mulethi)	<i>Glycyrrhiza glabra</i> (mulethi)	<i>Glycyrrhiza glabra</i> (mulethi)
Ammonium chloride (Navsadar)	Ammonium chloride (Navsadar)	—	—

formulation consisted of individual extracts of the different components while, the second and fourth formulation was prepared by aqueous co-extraction of the different components.

Evaluation of antitussive activity

Sulphur dioxide (SO₂) induced cough

For evaluation of antitussive activity the method of Miyagoshi et al., 1986 (12), as modified and simplified by us was used. Briefly, a vial containing 2 ml of 500mg/ml solution of sodium hydrogen sulfite (NaHSO₃; Qualikems Fine Chemicals Pvt. Ltd.) 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 (Fig. 1). To the NaHSO₃ solution, 0.2 ml of sulphuric acid (H₂SO₄; Merck, India), was added using a pipette. The reaction involved is as follows :



After 15 seconds, the mice were placed on the platform in the dessicator and exposed to SO₂ 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.

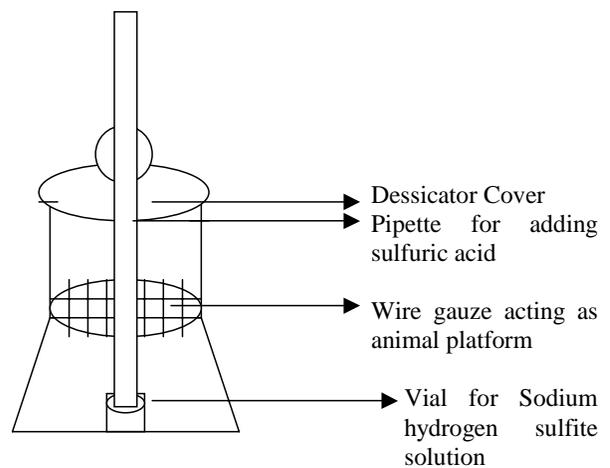


Fig. 1: Setup for SO₂-induced cough.

Scoring of bouts of cough: To avoid the observer bias, cough bouts were independently counted by two observers using digital counters and stopwatches. If for any reading the difference in count among observers was more than 10%, the experiment was discarded. Readings are mean of these two observations.

Drug treatment

All drugs were administered orally (p.o.). The mice were divided into eight groups of six animals each. Group I served as normal control and was not administered anything. Group II was given distilled water 0.3 ml/mice, group III received standard drug ie. codeine 10 mg/kg, p.o., group IV to VII were given formulations 1, 2, 3 and 4 respectively in a dose of 0.3 ml/ mice, p.o., while group VIII was administered 0.3 ml vehicle used for the formulations.

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

Data analysis

Mean of cough bouts recorded by the two observers was taken and percent inhibition in number of cough bouts calculated. The data were analysed using Kruskal Wallis test for between group analysis using SPSS software version 15.0.

RESULTS

In normal controls, there was no significant change in the number of cough bouts, between the two exposures. Mice

administered 0.3 ml distilled water showed a reduction of $20.5 \pm 2.2\%$, while those treated with vehicle for the formulations showed a change of $35.1 \pm 3.7\%$. The codeine treated animals predictably had a significantly reduced number of cough bouts ($75.3 \pm 3.1\%$). All the anti cough formulations (1, 2, 3 and 4), caused a significant reduction in cough bouts albeit not to the extent shown by codeine (Table II).

TABLE II: Percent inhibition in number of sulphurdioxide - induced cough bouts in the different treatment groups in rats. The cough bouts were counted for a period of 5 min after the irritant exposure. Each animal was exposed to irritant twice. Values are mean percent inhibition from baseline values.

Experimental group	Number of animals (n)	% Inhibition Mean \pm SE
Normal control	6	2 \pm 1.4
Distilled water	6	20.5 \pm 2.2
Codeine(10mg/kg, p.o.)	6	75.3 \pm 3.1*
Formulation 1 (0.3 ml, p.o.)	6	65.0 \pm 1.1**
Formulation 2 (0.3 ml, p.o.)	6	60.7 \pm 5.2**
Formulation 3 (0.3 ml, p.o.)	6	52.3 \pm 2.8**
Formulation 4 (0.3 ml, p.o.)	6	47.6 \pm 4.1**
Vehicle control (0.3 ml, p.o.)	6	35.1 \pm 3.7

*-P<0.05 (as compared with distilled water group)

#-P<0.05 (as compared with codeine)

While, in codeine group, the animals were sedated, in all the four test groups (mice treated with formulations), the mice were as active as the control group. There was no apparent sedation.

DISCUSSION

Many medicinal plants have been claimed to have antitussive activity and have also been screened for this purpose, singly. Of these, substances like *Ocimum sanctum* (tulsi), ginger, *Glycyrrhiza glabra* (licorice/

mulethi), *Voila odorata* (banafsha), *Justicia adhatoda* (vasaka) leaves etc. are major components of household cough and cold remedies worldwide, in the form of decoctions, teas etc. Some isolated experimental and clinical studies have also been carried out on these agents for cough (1, 13–21). We combined some of these drugs in a single formulation. To the best of our knowledge, till date there is no report describing use of polyherbal formulations as antitussive. A medline search revealed only one report with combination herbs that too for allergic asthma (22). Another group has reported development of two cough relieving herbal teas (23).

Sulphur dioxide-induced cough is widely used as a model for evaluating antitussive activity of a candidate compound. We have also used this model albeit with slight variation in methodology, in order to simplify the screening process. Although in the present study, the quantification of SO₂ 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 criteria for selection of medicinal plants for combining was primarily their

widespread use and action through different mechanisms. For example *Ocimum sanctum* (tulsi) is decongestant, *Glycyrrhiza glabra* (mulethi) is antibacterial and demulscent and sore throat reliever, *Solanum surratense* (kantakari) has some bronchodilator activity and *Voila odorata* (banafsha) is expectorant.

Since the various medicinal plants can show different activities in combination, two different methods of preparation were used ie. mixing of individual extracts of different plants and the co-extraction of plants. However, in our study the method of preparation did not cause any significant difference in the antitussive activities of the different preparations, but there was a trend towards lesser activity in co-extracted preparations. There was however a sizeable change on varying the composition of the formulations. The formulations without *Vitis vinifera* (Draksha), *Alpinia galangal* (Kulinjan) and ammonium chloride (navsadar) were less effective irrespective of method of preparation. None of the formulations were as effective as the reference standard codeine.

Thus formulations with herbal extracts are effective for cough but there is need for carrying out studies to determine additional benefits and underlying mechanisms.

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