ORIGINAL ARTICLE

Antitussive activity of *Vasa Avaleha* formulations on sulfur dioxide-induced coughing in mice

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Objective: *Vasa Avaleha* is a well-known Ayurvedic compound formulation, known for its usefulness in respiratory disorders like cough, cold, bronchitis, bronchial asthma, etc. Though *Adhatoda vasica* individually studied for antitussive activity in animals, no scientific evidence was available for *Vasa Avaleha*. This prompted us to initiate a comparative antitussive activity of *Vasa Avaleha* and granules of *Vasa Avaleha* in sulfur dioxide-induced coughing in mice. **Materials and Methods:** The test drugs were prepared as per classical guidelines and standards in the Departmental Laboratory of the Institute. The test drugs were administered orally at a dose of 1.56 g/kg and tested against sulfur dioxide-induced coughing in mice for 5 min. **Results**: *Vasa Avaleha* significantly ($P < 0.001$) inhibited the sulfur dioxide-induced cough reflexes in mice compared to control group. The effect was comparable to the standard drug Recodex, which contain codeine phosphate and chlorpheniramine maleate. Granules of *Vasa Avaleha* also produced significant ($P < 0.001$) decrease in cough reflexes compared to control group. The magnitude of the antitussive effect was more pronounced and significant in *Vasa Avaleha* treated group in comparison to granules of *Vasa Avaleha.* **Conclusions:** From the present study, it is concluded that *Vasa Avaleha* and granules of *Vasa Avaleha* may prove as useful and an effective antitussive agent which provides experimental evidence in support of the Ayurvedic ancient claim. Further, *Avaleha* form of test formulation can be converted to granule form and further evaluated in clinical studies for better human therapeutic uses.

Key words: *Adhatoda vasica* Nees., antitussive activity, asthma, *Avaleha*, cough, *vasa*

INTRODUCTION

Asthma is a disease in which the airways narrow excessively in response to various stimuli in the presence of airway hyper-responsiveness and eosinophilic airway inflammation.[1] Cough is one of the most common symptoms of asthma and is one among the symptom triad of asthma.[2] Coughing may be initiated either voluntarily or reflexively. Hydration of respiratory tract by steam inhalation, demulcents are effective in reducing symptoms in the majority of cases but, for uncontrolled cough, opioidergic central cough suppressants are used. However, their greatest disadvantage is a high rate of unwanted effects, like depression of the respiratory center, decreased secretion in the bronchioles, and inhibition of ciliary activity. Their administration can lead to increased sputum viscosity, decreased expectoration, hypotension, and constipation.[3] Further, their use in severe cough conditions like asthma is contraindicated, as they are known to further compromise the respiratory function.[4] Therefore, there is a need to have effective antitussive which can successfully alleviate chronic cough without side effects.

"*Vasa Avaleha*" is a well-known compound Ayurvedic formulation, known for its usefulness in conditions such as *Rajayakshma*, *Shwasa*, *Kasa*, and *Raktapitta*. This compound formulation is the combination of five ingredients with *Vasa* (*Adhatoda vasica* Nees.) as main ingredient along with *Pippali* (*Piper longum* Linn.), *Go-ghrira* (clarified butter), *Madhu* (honey), and *Sharkara* (sugar candy) as a base.[5] *A. vasica* individually studied for antitussive activity in animals.[6,7] But no scientific evidence was available for the antitussive activity of *Vasa Avaleha* in animals during the extensive literature search. Further Ayurvedic pharmaceutics

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named Bhaishajya Kalpana is quite extensive. Experimental studies have shown that formulation can have marked influence on the expression of biological activity. Through experimental studies, it is possible to refine the art of formulation. In view of that *Vasa Avaleha* was converted into granules as convenient in handling, dispensing, and storage. Considering above facts, the present study has been attempted to evaluate the comparative antitussive activity of both dosage forms in sulfur dioxide-induced cough reflex in mice.

MATERIALS AND METHODS

Drugs and Chemicals

The raw materials [Table 1] of the test formulation were collected from pharmacy attached to the Institute and were authenticated in Pharmacognosy Laboratory of Institute of Postgraduate Teaching and Research in Ayurveda (IPGT and RA), Gujarat Ayurved University, Jamnagar, India. *Vasa Avaleha* and granules of *Vasa Avaleha* were prepared by following the classical guidelines[5] in the Department of Rasashastra and Bhaishajya Kalpana, IPGT and RA, Jamnagar. All chemicals used in the study and for biochemical assay were of analytical grade.

Animals

Swiss albino mice of either sex weighing 30 ± 5 g were used in the study. The animals were obtained from the animal house attached to the Pharmacology Laboratory of the Institute. Animals were exposed to 12 h light and dark cycles with ideal laboratory conditions in terms of ambient temperature (23 ± 2°C) and humidity (50–60%). They were fed with Amrut brand rat pellet feed supplied by Pranav Agro Industries, Baroda, and drinking water was given *ad libitum*. The experiment was carried out in accordance with the directions of the Institutional Animal Ethics Committee (IAEC) after obtaining its permission (IAEC/14/2013/15).

Dose Calculation

Dose of the test formulations was calculated by extrapolating the human dose (12 g/day) to mice dose (1.56 g/kg) based on the body surface area ratio by referring to the standard table of Paget and Barnes (1969).[8] The test drugs were suspended in distilled water and administered orally by oral catheter.

Table 1: Composition of *Vasa Avaleha* and granules of *Vasa Avaleha*

Ingredients	Botanica/ English name	Part used	*Vasa Avaleha*	Granules of *Vasa Avaleha*
Vasa	*Adhatoda vasica* Nees.	Leaf	1 part	1 part
Sharkara	Sugar candy	-	1/2 part	1 part
Go-Ghrira	Clarified butter	-	1/8 part	1/8 part
Pippali	*Piper longum* Linn.	Fruit	1/8 part	1/8 part
Madhu	Honey	-	1/2 part	1/10 part

Experimental Design

The selected animals were divided into three groups of six animals each. Group I kept as normal control group, received vehicle as a distilled water (10 ml/kg). Group II and III were kept as drug-treated groups, received *Vasa Avaleha* and granules of *Vasa Avaleha* (1.56 g/kg), respectively. Group IV was treated with Recodex (Wockhardt Ltd., Mumbai) (5 ml/kg) and used as a positive control group. Recodex contain codeine phosphate (2 mg/ml) and chlorpheniramine maleate (0.8 mg/ml) as active constituents. The test drugs and vehicle to control group were administered 1-h before the SO_2 exposure.

The antitussive effect of the test formulations was evaluated in mice against sulfur dioxide-induced cough by following the procedure of Miyagoshi *et al*.[9] In brief, the assembly comprises a 500 ml three-necked flask containing aqueous saturated sodium hydrogen sulfite ($NaHSO_3$; Nice Chemicals Pvt., Ltd.,) solution. Into this bottle, concentrated sulfuric acid (H_2SO_4; Merck, India) was introduced drop by drop; the reaction involved is as follows:

$$2NaHSO_3 + H_2SO_4 = 2SO_2 + Na_2SO_4 + H_2O$$

SO_2 was filled previously in the column of water manometer by opening the three-way cork such that the SO_2 can enter the water manometer but without any exit way until the pressure generated reads 75 mm of water as recorded by the water manometer. Then the three-way cork was rotated in such a way that the volume of SO_2 collected in the water manometer escapes into the desiccator and not into the flask containing sodium hydrogen sulfite solution. The mouse to be tested is placed in a desiccator and covered with the lid. The desiccator was previously saturated with sulfur dioxide. Afterward, a certain amount of SO_2 (5 ml which was kept constant throughout the experiment) was introduced to the desiccators by this procedure. The mice, after exposure to SO_2 for 1-min in the desiccators, taken out of the desiccator and confined in an up-turned filter funnel. The free end of the funnel was attached to a stethoscope by the help of which the cough reflex of the mice was heard, and the number of cough episodes in 5 min was enumerated. To avoid the observer bias, cough episodes were independently counted by two observers using digital counters and stopwatches.

Statistical Analysis

The data are expressed as mean ± standard error of the mean for six mice per experimental group. The data generated during the study were subjected to Student's *t*-test for unpaired data to assess the significant difference between groups at $P < 0.05$.

RESULTS AND DISCUSSION

Cough is a normal physiological response to an irritation of the laryngo-tracheobronchial system and one of the most common symptoms of asthma. *Vasa Avaleha*, the classical Ayurvedic formulation is one of the prime drugs indicated for the treatment of asthma and the same was converted into a granule form and screened for antitussive activity in the present study. In animals, coughing has been elicited by mechanical[10] or chemical irritation[11] and by electrical stimulation[12] of tracheal mucosa or by nerve stimulation.[13] Chemical or mechanical stimulation is more similar to the physiological event and is also the most preferred experimental models. In the present study, the model adopted was sulfur dioxide-induced cough, which is widely used as a model for evaluating the antitussive activity of a candidate compound.

Exposure of the experimental animals to sulfur dioxide, the frequency of cough for the control group was 57.33 ± 1.60 for six animals [Table 2]. *Vasa Avaleha* significantly ($P < 0.001$) inhibited the sulfur dioxide-induced cough reflexes in mice compared to control group. The effect was comparable to the standard drug Recodex, which contain codeine phosphate and chlorpheniramine maleate. Granules of *Vasa Avaleha* also produced significant ($P < 0.001$) decrease in cough reflexes compared to control group. The magnitude of the antitussive effect was more pronounced and significant in *Vasa Avaleha* treated group in comparison to granules of *Vasa Avaleha*.

The observed difference in magnitude of therapeutic effects may be due to modification of dosage forms from *Avaleha* to granules. As per the pharmaceutical concept, both have different disintegration and dissolution rate which may responsible for variation in onset, magnitude, and duration of therapeutic effect of test drugs. Through experimental studies, it is possible to refine the art of formulation and recommends that *Avaleha* form of test formulation can be converted to granule form and further evaluated in clinical studies.

The present experimental animal study has revealed Ayurvedic formulations of *A. vasica* to be an effective antitussive agent. The antitussive activity may be attributed to the specific site of action of major alkaloids present in *A. vasica* as vasicinone and vasicinol which suppress coughing by its action on the cough center or it's neuronal system in the medulla.[14] *Madhura Dravyas* used in *Vasa* formulations are *Kaphavatahara* and are indicated for *Shwasa*. *Sarkara* (sugar candy) is *Vatapitta hara* and *Madhu* (honey) is *Kaphavatasamaka*[15] and showed antitussive activity.[16,17] Further, this provides sialagogue and demulcent effect and by increasing the flow of saliva which acts as a protective and soothing agent, increasing the production of respiratory tract fluid, which covers the mucosa and protects it.[18]

Table 2: Effect of *Vasa Avaleha* and granules of *Vasa Avaleha* on sulfur dioxide-induced cough reflexes in mice

Groups	Dose	Number of cough reflexes	Percentage inhibition
Control	-	57.33±1.60	-
Vasa Avaleha	1.56 mg/kg	25.83±1.04*,@	54.94↓
Granules of *Vasa Avaleha*	1.56 mg/kg	33.66±1.25*	41.28↓
Recodex	5.0 ml/kg	29.83±1.97*	

Data: Mean±SEM, ↓ – Decrease, *P<0.001 when compared to control group, @P<0.01 when compared to granules of *Vasa Avaleha* treated group.
SEM – Standard error of mean

CONCLUSION

From the present study, it is concluded that *Vasa Avaleha* and granules of *Vasa Avaleha* may prove as useful and an effective antitussive agent in sulfur dioxide-induced cough reflexes in animal study which provides experimental evidence in support of the ancient claim on Ayurvedic formulations of *A. vasica* as an antitussive agent. The study suggests that *Avaleha* form of test formulation can be converted to granule form and further evaluated in clinical studies for better human therapeutic uses.

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Conflicts of Interest

There are no conflicts of interest.

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