

Comparative evaluation of Ayurvedic nebulizing fluid for antihistaminic and antitussive activity

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Abstract

Background: Nebulizer is a drug delivery device used to administer medication in the form of a mist inhaled into the lungs. However, in Ayurveda, there is need of such types of dosage form which meet the need of hour. Hence, with this aim, an aerosol was prepared with the herbal ingredients of *Shirisharista* formulation, which is used in *Tamaka Swasa* (viz. bronchial asthma). In classics, *Twak* (stem bark) of *Shirisha* (*Albizia lebeck* Benth) is advised to take. In this study, *Sara* (heart wood) and *Patra* (leaf) of *Shirisha* were also taken along with *Twak* with the aim to know that if *Sara* can produce more efficacious result than *Twak*. Similarly, *Patra* was taken to protect the plant from any destruction. **Materials and Methods:** The three samples of aqueous extracts were prepared with *Twak*, *Sara*, and *Patra* of *Shirisha* with the rest nine herbal ingredients of *Shirisharista* in each. This was modified by adding alcohol to obtain better therapeutic effects. This study was performed to evaluate antihistaminic and antitussive activity of an Ayurvedic nebulizing fluid (ANF) in guinea pig and Swiss albino mice, respectively. To antihistaminic activity of three samples of test drug was assessed on isolated guinea pig ileum and prepared according to the standard method. Antitussive activity was studied by inducing cough with sulfur dioxide gas (SO₂) and followed by treatment with test drugs. **Result:** The result showed that stem bark aqueous extract showed the best antihistaminic and antitussive activity. Test drugs contain major active constituents from different plant sources such as piperin and curcumin, which induces a cough suppressant pharmacological effect and represents an attractive approach through an ANF. **Conclusion:** The bark extract of *Shirisha* and other nine herbals showed maximum efficacy. This study clearly proof the fact of classics that *Twak* was used by ancient scholars is relevant to get the maximum efficacy. Thus, formulation and this dosage form may be useful to alleviating asthma.

Key words: Antihistamine, antitussive herbal extract, Ayurvedic nebulizer, *Shirisharista*, *Tamak Swasa*

INTRODUCTION

Respiratory problems such as asthma are on the rise in many cities due to the dangerous levels of pollution that are prevalent in such areas.^[1] Although, contemporary medication does have a treatment for acute asthma attacks but it does not provide a long-term cure for this disease. This lack of a long-term treatment for asthma has forced scientists and researchers to look toward alternative forms of medication for the treatment of asthma.^[2] Medicinal plants used for the treatment of asthma should have anti-inflammatory, immunomodulatory, antihistaminic, smooth-muscle relaxants, and allergic activity.^[3] According to Ayurveda

antiasthmatic drug should have properties such as *Kapha-Vata Shamak* (subside the aggravated *Kapaha* and *Vata*).^[4] Ayurveda, the traditional system of medicine of India, enlisted some commonly occurring plants for treating asthma and other respiratory ailments. Some of them include *Shirisha* (*Albizia lebeck*), *Kantakari* (*Solanum xanthocarpum*), *Mulethi* (*Glycyrrhiza glabra*), and *Vasica* (*Adhatoda vasica*).^[2]

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Shirisha (*Albizia lebbek* Benth) is a drug with multidimensional activities mentioned in Ayurvedic classics for different disease conditions. The therapeutic attributes explained for the drug are *Swasahara* (antiasthmatic), *Vishahara* (antipoisonous), etc.,^[5] *Shirisharista*, this compound formulation is the combination of 12 ingredients with *Shirisha* as main ingredient and Jaggery as a base, multidimensional activities of this drug are emphasized in Ayurvedic classics for different disease conditions including bronchial asthma.^[6] The plant extract has been proven to be efficacious in cases of allergic rhinitis. Studies conducted in recent past reveals the antiallergic,^[7] antihistaminic,^[8] expectorant^[9] action and of the drug. Other ingredients of the formulation such as *Pippali* (*Piper longum* Linn), *Haridra*^[10] (*Curcuma longa* Linn), *Shunthi*^[11] (*Zingiber officinalis* Linn) have been evaluated individually for their antitussive activity.

However by considering few disadvantages of fermented form of the formulation such as long duration of preparation and less acceptability by different age groups. Here, an effort has been made to provide safer and faster relief using Ayurvedic drugs in newer form, i.e., nebulizer to meet the need of hour. In classics, *Twak* (stem bark) of *Shirisha* is advised to take. In this study, we had done work on *Sara* (heartwood) and *Patra* (leaf) also along with *Twak* with the aim to know that if *Sara* can produce more efficacious result than *Twak* and *Patra* were taken to protect the plant from any destruction. Hence, in this work, *Patra*, *Twak*, and *Sara* were taken as a main drug along with the rest of nine herbs present in formulation of *Shirisharista* in different batches of Ayurvedic nebulising fluid (ANF). For this, the three samples of dried aqueous extracts of the herbal ingredients of *Shirisharista* were taken. Three different parts leaf, bark, and heartwood of *Shirisha* were taken as main ingredient for three respective samples of nebulizing fluid having rest of the other nine herbal ingredients of *Shirisharista* in each sample. *Twak* (bark) and *Patra* (leaf) were taken in research studies so that these parts may be used in future in place of heartwood to save the plant to destroy by cutting to it for collecting heartwood. Three extracts were finally converted into nebulizing fluid in a definite ratio of distilled water and absolute alcohol to enhance the process of inhalation and this nebulizing fluid was evaluated for antihistamine activity and antitussive activity.

This paper reports the effect of aqueous extract of herbal ingredients of *Shirisharista* three samples prepared with leaves, stem bark, and heart wood parts of *Shirisha* on antihistaminic activity against isolated ileum muscles of guinea pigs and antitussive activity with aqueous extract of bark and heartwood of *Shirisha*, on sulphur dioxide gas cough induced albino mice model.

MATERIALS AND METHODS

Preparation of Test Formulation

The raw materials of Ayurvedic Nebulizer [Table 1] were collected from the pharmacy, I.P.G.T. and R.A., Jamnagar, Gujarat Ayurved University, Jamnagar, India except *Shirisha*, which were collected from the botanical garden of institute and subjected to pharmacognostical studies to evaluate the genuinity. From the raw material, three samples were made; taking leaf, bark, and heartwood as main drug and rest nine drugs were taken in all three samples in the same ratio as mentioned in classics in the preparation of *Shirisharista*. Aqueous extracts of all three samples were made from reflux method then whole water was evaporated and dried solid extracts were obtained. Final nebulizing fluid was made from dissolving solid extracts into solvent of distilled water and absolute alcohol (7:3). The three respective samples were coded [Table 2].

Antihistamine Activity

Preparation of test drug and histamine

Test drug was dissolved in distilled water and desired concentration was prepared. Histamine (HiMedia Laboratories Pvt. Ltd., Mumbai - 86, Lot. No- 0000067545, India) was dissolved in physiological saline. Physiological saline was widely recommended as it is known to be compatible with human tissue and isotonicity with body fluid.^[12,13]

Experimental Animals

Dunkin-Hartley guinea pig weighing 400-450 g were procured from the animal house attached to pharmacology laboratory, I.P.G.T. and R.A., Jamnagar, Gujarat Ayurved University, Jamnagar, India. They fed with food and water *ad libitum*. The animals acclimatized for 1 week in lab condition before commencement of the experiment in standard laboratory conditions 12 h day and night cycle, maintained at 25±3°C and 40% to 60% humidity. The animal protocol was approved by the Institutional Animal Ethics Committee with approval number; IAEC/13/2012/12.

Antihistaminic Activity: (Effect of Test Drug on the Guinea Pig Ileum - *In Vitro*)

To assess the antihistaminic activity of the test drug, the experiment was carried out on isolated guinea pig ileum and prepared according to the method described by Ghosh.^[14] Overnight fasted guinea pig was stunned by head blow, neck vessels cut, and the animal is bled out. Abdomen was opened through a midline incision, the ileocaecal junction exposed;

Table 1: Formulation compositions of aqueous extract Ayurvedic nebulizing fluid

Ingredients	Botanical name	Part used	Quantity
Shirisha	<i>Albizia lebbek</i> Benth.	Leaf/stem bark/heartwood	50 parts
Pippali	<i>Piper longum</i> Linn.	Fruit	1 part
Priyangu	<i>Callicarpa macrophylla</i> Vahl.	Flower	1 part
Kustha	<i>Saussurea lappa</i> C.B.Clarke	Root	1 part
Ela	<i>Elettaria cardamomum</i> Maton	Seed	1 part
Nilini	<i>Indigofera tinctoria</i> Linn.	Root	1 part
Haridra	<i>Curcuma longa</i> Linn.	Rhizome	1 part
Daruharidra	<i>Berberis aristata</i> DC	Stem	1 part
Naagkeasar	<i>Mesua ferrea</i> Linn.	Stamen	1 part
Shunthi	<i>Zingiber officinalis</i> Linn.	Rhizome	1 part

Z. officinalis: *Zingiber officinalis*, *M. ferrea*: *Mesua ferrea*, *B. aristata*: *Berberis aristata*, *C. longa*: *Curcuma longa*, *I. tinctoria*: *Indigofera tinctoria*, *E. cardamomum*: *Elettaria cardamomum*, *S. lappa*: *Saussurea lappa*, *C. macrophylla*: *Callicarpa macrophylla*, *P. longum*: *Piper longum*, *A. lebbek*: *Albizia lebbek*

Table 2: Coding of different group of ANF formulations

Formulation	Formulation code
Normal control	Control
Nebulizing formulation prepared by aqueous extract of bark	NF-A
Nebulizing formulation prepared by aqueous extract of heartwood	NF-B
Nebulizing formulation prepared by aqueous extract of leaf	NF-C

ANF: Ayurvedic nebulizing fluid

the terminal ileum was cut after discarding 10 cm nearest to the ileocaecal junction. Isolated ileum was placed on a Petri dish containing Tyrode solution (NaCl₂-137.0, KCl-2.70 CaCl₂-1.80, MgCl₂-0.1-1.00, NaHCO₃-11.90, NaH₂PO₄-0.40 and glucose-5.55 mm/l) at 37°C. A 2.5 cm long piece of the distal part of the ileum was used for the study. Experiments were performed in organ baths containing 40 mL Tyrode solution at 37°C and bubbled with oxygen (air, O₂, or 5% CO₂ in O₂ used for mammalian smooth muscles). The concentrations of the ileum strips to histamine were recorded on smoked kymograph paper with frontal writing lever having a 1:7 magnification and 500 mg initial tension. The preparation was allowed to equilibrate for 30 min during which the Tyrode solution was changed at intervals of 10 min. Initially, the dose responses were recorded with a standard spasmogenic drug, i.e. histamine to select a dose producing submaximal response. Standard response was taken with histamine with a dose of 15 ng/ml, 20 ng/ml, and 25 ng/ml of bath fluid. Contact time of 30 s and 15 min time cycle was followed for recording the response of histamine. The antagonistic effect of test drug per se if any and the modulatory effect on the tissue response to histamine were recorded. The drug was added to the bath 1 min before adding histamine.

ANTITUSSIVE ACTIVITY

Experimental Animals

Swiss albino mice of either sex weighing between 25 and 35 g were used for experimental study. The animals were obtained from the animal house attached to the pharmacology laboratory of I.P.G.T. & R.A., Jamnagar. Animals were exposed to 12 h day and night cycles with ideal laboratory condition in terms of ambient temperature (22±2°C) and humidity (50-60%). They were fed with Amrut brand rat pellet feed supplied by Pranav Agro Industries and drinking water given ad libitum. The dry wheat (post hulled) waste was used as bedding material and was changed every morning. The selected animals were kept under acclimatization for 7 days before dosing. The experimental protocols were approved by Institutional Animal Ethics Committee (IAEC/13/2012/12).

Dose Fixation and Schedule

The doses of nebulizing formulations (stem bark and heart wood samples) were selected based on human to experimental animal conversion through body surface area ratio using the table of Paget and Barnes (1969).

Test dose = Human therapeutic dose × body surface area ratio (convertibility factor) for mice

Human dose: 8 ml/day (as per expert opinion)

Body surface area ratio (convertibility factor) from human to mice = 0.0026 for mouse weighing 20g

Dose for ANF for Mice = 8 ml × 0.0026
= 0.0208 ml/20 g or 1.04 ml/kg body weight of mice

Route of Drug Administration

The test drugs and vehicle to control were administered according to the body weight of the animals by nasal route with the help of nebulizing chamber.

Antitussive Activity Study

Antitussive activity study was performed by *in-vivo* sulfur dioxide induced coughing reflex. For this experiment mice of either sex weighing between 25 and 35 g were selected and divided into four groups each consist of six animals. Group I received distilled water serving as control and was called control group. Calculated doses of the test samples nebulizing formulations prepared from bark and heartwood were administered, respectively, to Groups II-IV, respectively.

A 500 ml three-necked flask containing aqueous saturated sodium hydrogen sulfite (Na_2SO_3) solution is taken. Into this bottle, concentrated sulfuric acid (H_2SO_4) is introduced drop by drop, the inflow being controlled by the cork to generate sulfur dioxide gas. SO_2 is 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 is 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. These procedures are operated in a drift. The mouse to be tested is placed in 1 l desiccator and covered with the lid. A certain amount of SO_2 is introduced to the desiccator by this procedure. The mice, after exposure to SO_2 for 1 min in the desiccators, were taken out of the desiccator and confined in an up-turned filter funnel. The free end of the funnel is 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.

Statistical Analysis

Student's *t*-test for unpaired data has been used for analyzing the data generated during the study. $P < 0.05$ is considered as statistically significant, the value of $P < 0.01$ or $P < 0.001$ is considered statistically highly significant. Level of significance was noted and interpreted accordingly.

RESULTS AND DISCUSSION

Histamine is the local hormone synthesized by mast cells in the tissue and basophils in the blood. It is released as part of the inflammatory reaction increasing capillary permeability and dilation. It also causes contraction of smooth muscles of bronchi. It is considered as one of the contributors to the pathophysiology of asthma this is reflected in the

fact that mast cell degranulation inhibitors are among the important antiasthmatic drugs. The test formulations were screened for antihistaminic activity by noting their effect on histamine-induced spasm in isolated guinea pig ileum [Figure 1]. Effect of test drug on histamine-induced contraction of guinea pig ileum at the given histamine concentration (5 $\mu\text{g/ml}$ bath fluid) for contraction at the dose of 1 mg/ml bath fluid for each prepared aqueous extract shows different inhibition percentage. Bark extract is highly effective in inhibiting histamine-induced contractions by showing 61% of inhibition. However, a moderate effect was observed against histamine in heartwood extract because of inhibiting only 34% and mildly effective against histamine in leaf extract by showing inhibition to its least of only 13% [Table 3]. Bark extract produced a highly effective antihistaminic response by antagonizing the contractile response of histamine on guinea pig ileum. This shows dilation effect of test drug. One of the possible mechanisms for the dilation activity of the test drug could be mediated through the inhibition of histaminic receptors. Further, the antihistamine activity showed by the plant may be because of the chemical moieties present in it.

As in antihistamine study, leaf aqueous extract showed negligible effect (13%) compare to other test groups on kymograph so this group discontinued for further antitussive study to avoid the maximum use of animals as per expert suggestion.



Figure 1: Kymographic recording of isolated guinea pig ileum

Table 3: Effect of test drug on histamine-induced contraction of guinea pig ileum

Extract used	Histamine concentration	Drug concentration (ANF)	% inhibition
NF-A	5 $\mu\text{g/ml}$ bath fluid	5 mg/ml bath fluid	61
NF-B	5 $\mu\text{g/ml}$ bath fluid	5 mg/ml bath fluid	34
NF-C	5 $\mu\text{g/ml}$ bath fluid	5 mg/ml bath fluid	13

ANF: Ayurvedic nebulizing fluid

Pharmacological Antitussive Activity Study

Cough is one of the most common symptoms of asthma and is one among the symptom triad of asthma. Coughing may be initiated either voluntarily or reflexively. Any disorder resulting in inflammation, constriction, infiltration, or compression of airways can be associated with cough. Statistically highly significant reduction in the number of cough reflex was observed in the aqueous extract of *Shirisha* bark and heartwood formulations. The antitussive effect observed may be because of three major reasons. First by increasing the flow of saliva which acts as a protective and soothing agent, second by increasing the production of respiratory tract fluid, which covers the mucosa and protects it and third data pertaining to effect of different samples of nebulizing formulations on SO₂ induced cough reflex in albino mice have been presented in Table 4. The extract of bark and heartwood (1.04 ml/kg) exhibited significant activity 36.33% and 27.61%, respectively. In control group, it was observed and investigated that animals behaved normal. Our study indicated that the antitussive effect of bark and heartwood formulation exerted significant ($P < 0.001$) antitussive effect in experimentally induced cough reflex in mice. There was no apparent sedation and no animal mortalities in all the groups. Statistically significant decrease in cough reflexes was observed in nebulizing formulations prepared by aqueous extract of *Shirisha* bark and heartwood in comparison to control group.

CONCLUSION

Based on the above information, this study was undertaken on different samples of nebulizing formulation prepared by aqueous extract of three easily available parts of *Shirisha*, i.e., *Twak* (stem bark), *Sara* (heart wood), and *Patra* (leaf) to evaluate them for antihistaminic and antitussive activities.

Table 4: Effects of test drugs on SO₂ induced coughing reflex in Swiss albino mice for antitussive activity

Treatment	Dosage (g/kg)	Number of cough episodes per 5 min	Percentage change
Control	Q.S	57.33±1.606	-
NF-A	1.04 ml/kg	36.50±1.057***	36.33↓
NF-B	1.04 ml/kg	41.50±2.217***	27.61↓

Data: Mean±SEM, ↓: Decrease, *** $P < 0.001$

Table 5: Consolidated table showing antihistaminic and coughing reflex

Parameters	NF-A	NF-B	NF-C
Antihistaminic	Marked effect	Moderate effect	Mild effect
Coughing reflex	SD	SD	-

SD: Significant decrease

Twak and *Patra* were taken in research studies so that these parts may be used in future in place of heartwood which are not only effective in activities but also save the plant to destroy by cutting to it for collecting heartwood. The data generated during the study form the basis for the below provided conclusion.

Bark extract is having marked antihistamine activity. Nebulizing formulation prepared by aqueous extract of *Shirisha* bark at the dose of (5 mg/ml bath fluid) is highly effective in inhibiting histamine (5 µg/ml of bath fluid) induced contractions of guinea pig ileum. Moderate effect was observed in heartwood extract and mild effect was seen in leaf extract against histamine-induced ileum contraction. The therapeutic effectiveness in the treatment of bronchial asthma and use in traditional medicine of this plant could be due to its bronchodilator effect. The significant decrease in cough reflex observed more from bark aqueous extract in comparison to heartwood aqueous extract. Hence, this study clearly proof the fact of classics that *Twak* was used by ancient scholar is relevant to get the maximum efficacy. Both experiments, i.e., antihistamine and antitussive provides pharmacological evidence for the use of herbs of *Shirisharista* as a bronchodilator and antitussive agents in treatment of asthma through nebulizer [Table 5].

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