

Evaluation of Acute Toxicity and Anti-Tussive activity of Ayurvedic Sugar Free Cough Syrup

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ABSTRACT

Many of the currently available allopathic cough suppressants containing ingredients like codeine, ephedrine, bromhexine and guaefenesin are known for their side effects such as sedation, constipation, drowsiness and addiction. This makes their use limited and inadequate. Ayurvedic medicines for the most part are non-toxic and can be used by patients and healthy individuals for cure and prevention of diseases. Test drug, an Ayurvedic sugar free cough syrup is unique combination of herbal and mineral ingredients. Individual ingredients are well reported for their anti-tussive and expectorant effect but no scientific evidences were available for combination of them. The present study was carried out to evaluate acute toxicity and anti-tussive activity of test drug using sulphur dioxide induced cough model in albino mice. Acute toxicity study was carried out as per OECD guideline 423. Efficacy of test drug was evaluated at two different dose levels, TED (1.3 mL/kg) and TEDx2 (2.6 mL/kg). During acute toxicity study, animals did not manifest any signs of toxicity and mortality. Test drug showed 38.62% and 53.47% inhibition in frequency of cough in TED and TEDx2 groups respectively. TEDx2 showed more significant anti-tussive activity with respect to TED. From the available results, it can be concluded that test drug is safe and effective in the management of common cough.

Keywords: Ayurvedic sugar free cough syrup, Anti-tussive, Acute toxicity, Sulphur dioxide

INTRODUCTION

Cough is a protective reflex that helps to expel irritant matter and is necessary for preventing mechanical obstruction to breathing.^[1] It is the most common symptom affecting a large population and presenting to general practitioners. It can also be associated with the pulmonary diseases like common cold, pneumonia and chronic bronchitis.^[2] Up to 40% of patients with chronic cough remain unexplained in specialist clinics and are observed creating serious health problems.^[3] ^{4]} Both acute and chronic cough have significant impacts on health-related quality of life.

Drugs that suppress cough are designated as anti-tussives. Some of them act on the central nervous system to inhibit cough while others produce their effects locally. Centrally (codeine and ephedrine) and peripherally (bromhexine and guaefenesin) acting anti-tussives produce known side effects such as sedation, constipation, drowsiness and addiction

which make their use limited.^[5-7] Therefore, a need is felt to develop an effective anti-tussive and expectorant drug without any side effects.

In present study, selected Ayurvedic sugar free cough syrup consists extracts of *Ocimum sanctum* (Tulsi) Aerial,^[8-10] *Adhatoda vasica* (Vasa) Leaves,^[6,11-13] Trikatu [Compound of Shunthi (*Zingiber officinale*) Rhizome, Marich (*Piper nigrum*) fruit and Pippali (*Piper longum*) fruit],^[14,15] *Zingiber officinale* (Shunthi) Rhizome,^[16] *Glycyrrhiza glabra* (Yashtimadhu) Root,^[9] ^{17]} *Solanum xanthocarpum* (Kantakari) Whole plant^[9] ^{18]} along with powder of Navasar ^[9, 19] and Menthol ^[9, 20, 21]. Each of these ingredients is reported to have direct or indirect anti-tussive and expectorant activity however no scientific evidences were available to prove safety and anti-tussive activity of their combination. Hence, the present study was carried out to evaluate acute toxicity and anti-tussive activity of selected Ayurvedic sugar free cough syrup using sulphur dioxide induced cough model in albino mice.

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MATERIALS AND METHODS

Test drug and chemicals

Test drug, Ayurvedic sugar free anti-cough formulation was provided by Vasu Healthcare Pvt. Ltd., Vadodara, Gujarat. It is manufactured and

marketed by the product name - Zeal SF Cough Syrup. All chemicals used were of analytical grade.

Acute oral toxicity study

The acute oral toxicity study was carried out as per the guidelines set by Organization for Economic Cooperation and Development (OECD), revised draft guidelines no. 423, received from the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Environment and Forests, Government of India, New Delhi.^[22] Rats weighing 200-280 g (n=6) were selected. 2000mg/kg of test drug was given orally. Mortality and change in body weight were recorded after 24 hour, 48 hour, 7th day and 14th day. Since mortality and toxic signs were not observed with afore mentioned dose, 5000 mg/kg dose was given after 14 days incubation period and same parameters were recorded.

Evaluation of anti-tussive activity of Ayurvedic sugar free cough syrup in sulphur dioxide (SO₂) induced cough model in mice

The experiments were performed on male albino mice weighing between 25-40 g. The mice were housed in poly-acrylic 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 four groups. Each group was comprised of 6 animals. Experimental procedure described were reviewed and approved by the Institutional Animal Ethics Committee (IAEC), Sigma Institute of Pharmacy, Bakrol, Ajwa-Nimeta Road, Waghodia, Vadodara-390 019, Gujarat, India (Approval number: SIP/IAEC/01/2013-14). The care of the laboratory animals were according to current ethical guidelines by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India, New Delhi.

Selected mice were divided into four groups, each group having six animals. Group I served as Disease Control, Group II received standard drug i.e. Codeine Phosphate (10mg/kg) p.o., Group III was served as Therapeutic Effective Dose (TED), 1.3 mL/kg, p.o. and Group IV was served as double to Therapeutic Effective Dose (TED×2), 2.6 mL/kg, p.o. Test drug and standard drug were administered 45 minutes prior to exposure of sulphur dioxide.

For evaluation of anti-tussive activity method of Gupta *et al.* was followed.^[6] Briefly, a vial containing 2 mL of 500mg/mL solution of sodium hydrogen sulfite (NaHSO₃) in double distilled water was placed at the base of a desiccator and was covered with wire gauze to serve as a platform for placement of mice. To the NaHSO₃ solution, 0.2 mL of sulphuric acid (H₂SO₄) was added using a burette. After 15 seconds, the mice were placed on the platform in the desiccator and exposed to SO₂ for 45 seconds and then the mice were removed from the desiccator and placed in an observation chamber for counting of bouts of cough for one minute thereafter. Number of cough bouts were counted and noted. The percent inhibition in cough bouts was calculated by following equation, Percentage frequency of cough reflex = $(1-T/C) \times 100$ Where T = Cough reflex in standard or test drug treated groups; C = Cough reflex in disease control group.

Statistical Analysis

The results are expressed as Mean ± SEM. The variation present in data were analyzed using one way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. p ≤ 0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

Acute oral toxicity study

In the acute toxicity study, animals did not manifest any signs of toxicity or mortality at 2000 mg/kg and 5000 mg/kg subsequently.

Evaluation of anti-tussive activity of Ayurvedic sugar free cough syrup in sulphur dioxide (SO₂) induced cough model in mice

Test drug showed significant inhibition in cough bouts ($p < 0.001$) at dose level, 1.3 mL/kg (TED) and 2.6 mL/kg (TED×2) in comparison to disease control group. Percentage inhibition in frequency of cough at TED and TED×2 dose level was 38.62% and 53.47% respectively. The standard drug (codeine phosphate, 10mg/kg, p.o.) showed 63.00% inhibition in frequency of cough. TED×2 group showed more significant anti-tussive activity with respect to TED. (Table 1)

A significant anti-tussive activity of Ayurvedic sugar free cough syrup may be due to synergistic effect of its ingredients. *Ocimum sanctum* is reported for anti-tussive effect by central nervous system probably mediated by both opioid system & GABA-ergic system.^[8] Alkaloids of *Adhatoda vasica*, vasicine and vasicinone having potent bronchodilator effect through anti-cholinergic action on the vagal innervation of the bronchi.^[11,12] Trikatu, a classical formulation contains high content of piperine so, it acts as a bio-availability enhancer.^[15] An active constituents of *Glycyrrhiza glabra* (Glycyrrhizin) and *Zingiber officinale* (6-gingerol and 6-shogaol) are also reported for expectorant and anti-tussive activity.^[16,17] *Solanum xanthocarpum* also has anti-tussive and expectorant property.^[18] Navsar acts by altering mucociliary action and also alters the viscosity of cough by dissolving mucin through increasing bronchial gland secretions and act as an expectorant.^[19] Menthol provides cooling action to respiratory tract.^[20,21]

CONCLUSION

From the available data of acute toxicity study and anti-tussive activity, it can be concluded that, Ayurvedic sugar free cough syrup has been found safe and effective in sulphur dioxide induced cough. Mainstream centrally and peripherally anti-tussive drugs are known to produce side effects like sedation,

constipation, drowsiness and addiction. Thus, Ayurvedic sugar free cough syrup can be regarded as a safe and better alternative medication both in terms of efficacy as well as safety. It is also compatible for diabetic person who is suffering from cough.

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Table 1: Effect of ayurvedic sugar free cough syrup on sulphur dioxide induced cough in mice

Experimental group	Number of cough bouts / Frequency of cough (Mean ±SEM)	Percentage inhibition in frequency of cough (%)
Disease control	67.33 ± 4.33	---
Standard drug (Codeine Phosphate)	25.00 ± 0.58***	63.00 %
Test drug (1.3 mL/kg p.o.)	41.33 ± 1.86***	38.62 %
Test drug (2.6 mL/kg p.o.)	31.33 ± 1.86***	53.47 %

Values are represented as Mean ± S.E.M, (n=6).
*** $p < 0.001$ when compared with disease control

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