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Bronchodilatory effect of *Sphaeranthus indicus* Linn against allergen induced bronchospasm in guinea pigs

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ABSTRACT

The methanolic extract of whole plant of *Sphaeranthus indicus* Linn and its various fractions were tested for their bronchodilatory effect against histamine induced acute bronchospasm in guinea pigs. The methanolic extract and its fractions viz. petroleum ether, benzene, chloroform and ethyl acetate exhibited significant protection against bronchospasm, induced by histamine in guinea pigs. However significant ($p<0.001$) protection was exhibited by methanolic extract which was comparable with Chlorophenarmine maleate (2mg/kg) included as the standard in the study. The results of present study suggest that, the plant *Sphaeranthus indicus* shows the bronchodilatory activity.

KEYWORDS: *Sphaeranthus indicus*, Bronchodilatory activity, Histamine.

INTRODUCTION

Sphaeranthus indicus Linn (Asteraceae) commonly known as Gorakhmundi, is a herb found mostly in southern India. Dried and powdered leaves of *Sphaeranthus indicus* are useful in the treatment of chronic skin disease, urethral discharges and jaundice (1). All parts of the plant possess medicinal uses and have been reported to have beneficial effects on several ailments. The juice of the plant is styptic and diuretic and it is said to be useful against liver and gastric disorders. Roots and seeds are used as stomachic and anthelmintic (2). It is reported that flowers are highly alterative, depurative, cooling and tonic. They are also used as blood purifiers in skin disease (3). Dried and powdered leaves of *Sphaeranthus indicus* are useful in the treatment of chronic skin disease, urethral discharges and jaundice (1). Extract of *Sphaeranthus indicus* has been reported for the inhibition of hyaluronidase (4). It exhibited excellent antibacterial activity against gram positive as well as gram negative bacteria (5). It is reported that flower head of *Sphaeranthus indicus* shows immunomodulatory activity (6). The phytochemical analysis of the plant showed that it contains eudesmanolide type of sequiterpene possessing immunostimulating (7) and anti-inflammatory activities (8). Traditionally the herb was used in cough, bronchitis (1). In the light of above facts the present investigation was conducted to assess the protective effect of the methanolic extract and its various fractions against histamine induced bronchospasm in guinea pigs. Even though *Sphaeranthus indicus* was reported to be useful in a many ailments, scientific evaluation of the plant was not reported for its antiasthmatic activity. Hence, the antiasthmatic activity of *Sphaeranthus indicus* was studied using different animal models.

MATERIALS AND METHODS

Collection and authentication of Plant material:

The whole plant of *Sphaeranthus indicus* Linn was collected from Wada, Rajgurunagar, Pune. The plant was identified and authenticated from Botanical Survey of India, Pune.

Its voucher number is 1-TKDS.The whole plant was powdered and used for the preparation of extracts.

Preparation of extracts

The whole plant was dried and powered in electric grinder. The powder obtained was passed through sieve no. 85 and weighed. The powdered material (3kg) was extracted with methanol (95%) using Soxhlet apparatus. The extract obtained was dried in rotary vacuum evaporator at 40°C, which yielded a syrupy viscous mass (6.5%). The methanolic extract was then fractionated by solvents of different polarity like petroleum ether, benzene, chloroform and ethyl acetate. All the respective fractions were concentrated under vacuum and then used for the further studies.

Test Animal

For the experiment, Dunkin-Hartley Guinea pigs of either sex weighing 350- 400 gm respectively were used for studies. The Dunkin-Hartley Guinea pigs were obtained from animal house of National Chemical Laboratory, Pune. They were housed in standard conditions of temperature (22±20 °C), relative humidity (60±5 %) and light (12 h light/ dark cycle). They were housed in polypropylene cages with standard pellet chow and water ad *libitum*.

1. Histamine induced Bronchoconstriction in Guinea Pig:

Bronchospasm was induced in guinea pigs by exposing them to histamine aerosol (0.2%) produced by an ultra-sound nebulizer in an aerosol chamber (24×14×24 cm) made of Perspex glass. The time required for appearance of pre-convulsive dyspnoea caused due to histamine was recorded for each animal. The guinea pigs were overnight fasted and were randomly divided into 11 groups, (n=5). Group-I received Chlorpheniramine maleate (2 mg/ kg, p.o.), Group-II to XI received methanolic extract of *Sphaeranthus indicus* Linn and its fractions 87 and 174 mg/ kg, p.o. respectively. In prior drug treatment, each animal was placed in the histamine chamber and exposed to 0.2 % histamine aerosol. The preconvulsion time (PCT) i.e. the time of aerosol exposure to the onset of dyspnoea leading to the appearance of convulsion, was noted. The time for preconvulsion dyspnoea (PCD) was recorded from the time of aerosol exposure to the onset of dyspnoea leading to the appearance of convulsions (9). As soon as PCD commenced, animals were removed from the chamber and placed in fresh air to recover. Time taken for the onset of PCD was taken as basal value. Guinea pigs were then allowed to recover from dyspnoea for 24 hrs. After 24 hrs the animals of Group II to XI were administered the test drug of *Sphaeranthus indicus* extract and its fractions and group I received Chlorpheniramine maleate. These animals were again subjected to histamine aerosol later at an interval of 1 hr, 4 hrs and 24 hrs to determine preconvulsion time (PCT). The protection offered by the treatment was calculated by using the following formula:

$$\% \text{ Protection} = \frac{T_2 - T_1}{T_2} \times 100$$

Where,

T1 = The mean of PCT before administration of test drugs.

T2 = The mean of PCT after administration of test drugs at 1hr, 4hrs and 24 hrs (10, 11 and 12).

Statistical analysis:

Results were reported as mean + SEM, the statistical analysis was performed by using one-way analysis-of-variance (ANOVA) followed by Dunnett's test. Though the data was considered statistically significant at ($p<0.05$), when data was found to be very ($p<0.01$) or highly ($p<0.001$) significant, this is indicated in the results.

RESULTS

Ayurveda has recommended a number of plants for the treatment of asthma and other allergic disorders and has been successful in controlling the disease as well (13). Phytoconstituents like alkaloids and flavonoids are attributed to possess bronchodilatory activity (14, 15, and 16). The results of preliminary phytochemical investigation of *Sphaeranthus indicus* Linn and its various fractions are shown in Table 1. The methanolic extract revealed the presence of an array of active constituents including alkaloids, glycosides, tannins, flavonoids, steroids and sugars.

In the early stage of asthma, release of inflammatory mediators like histamine, tryptase, acetylcholine, leukotrienes, and prostaglandins are triggered by exposure to allergens, irritants, cold air or exercise (17). Some of these mediators directly cause acute bronchoconstriction. Spasmolytic drugs like beta adrenergic agonists, xanthine derivatives and anticholinergics are used as quick relief medications in such acute asthmatic attacks (18). In the present study, we have used histamine as spasmogens in the form of aerosols to cause immediate bronchoconstriction in guinea pigs. Chlorophenarmine maleate (2mg/kg) included as the reference standard against histamine induced bronchospasm respectively (19). Due to the presence of various phytoconstituents like alkaloids

Table 1: Phytochemical Analysis of various fractions of Sphaeranthus indicus Linn

Sr.no	Tests	Observation					Inference
		M.E.	P.E.	Ben.	Chloro.	E.A.	
1.	Dragandorff	+	−	−	−	−	Alkaloids present
2.	Hager	+	−	−	−	−	Alkaloids present
3.	Mayer	+	−	−	−	−	Alkaloids present
4.	Wagner	+	−	−	−	−	Alkaloids present
5.	Borntrager	−	−	−	−	−	Anthraquinone glycosides absent
6.	Shinoda	+	−	−	−	−	Flavonoids present
7.	Ruthenium red	+	−	−	−	−	Mucilage present
8.	Foam test	+	+	+	+	+	Saponin present
9.	$FeCl_3$	+	+	+	+	+	Tannins and phenolic compound present
10.	Lead acetate	+	+	+	+	+	Tannins and phenolic compound present
11.	Potassium dichromate	+	+	+	+	+	Tannin and phenolic compound present
12.	Dilute iodine	+	+	+	+	+	Tannin and phenolic compound present
13.	Dilute HNO_3	+	+	+	+	+	Tannin and phenolic compound present
14.	Dil $KMNO_4$	+	+	+	+	+	Tannin and phenolic compound present
15.	Salkowaski	+	+	+	+	+	Steroids present
16.	Molish	+	−	−	−	−	Carbohydrates present
17.	Barfoed's	+	−	−	−	−	Monosaccharides present
18.	Benedicts test	+	−	−	−	−	Reducing sugar present
19.	Sodium Picrate	−	−	−	−	−	Cyanogenetic glycoside absent

Table 2: Effect of Sphaeranthus indicus linn extract against histamine induced Bronchoconstriction in guinea pigs.

Groups	Latent period of convulsion (sec) (Mean ± SEM)			
	Before	1 hr	4 hr	24 hr
Std (2 mg/kg)	43.6 ± 1.66	71.2 ± 1.85***	86.6±1.56***	53.8±2.709*
M_1 (87 mg/kg.)	38 ± 3.082	58 ± 2.550**	59 ± 8.769**	43.4 ± 1.661
M_2 (174mg/kg.)	40 ± 1.414	61 ± 1.095**	74.2 ± 1.288***	49 ± 1.183*
PE_1 (87 mg/kg.)	38.8 ± 3.023	50.2 ± 1.685**	53.8 ± 8.464**	42.8 ± 1.934
PE_2 (174mg/kg.)	37.6 ± 0.927	56.2 ± 1.908**	58.2 ± 2.709**	42.2 ± 1.281
C_1(87 mg/kg.)	36.4 ± 2.977	54.2 ± 1.482**	55.6 ± 7.763**	41.6 ± 1.720*
C_2(174mg/kg.)	40.6 ± 1.965	62.2 ± 3.153**	73.2 ± 0.800***	47.2 ± 3.277
B1 (87 mg/kg.)	39 ± 1.549	55.6 ± 1.288**	57.2 ± 8.206**	44.4 ± 2.159
B_1 (174mg/kg.)	38.2 ± 1.281	54.4 ± 1.860**	65.4 ± 2.482**	43.8 ± 2.437
EA_1 (87 mg/kg.)	41.8 ± 2.728	57.4 ± 1.600**	59.8 ± 8.182**	48.4 ± 0.812
EA_2(174mg/kg.)	40.2 ± 1.158	57.8 ± 2.200**	65.8 ± 2.354**	47.0 ± 2.000

* $p< 0.05$,
** $p< 0.01$,
*** $p<0.001$, Compared with initial readings.

and flavonoids in the methanolic fraction of *Sphaeranthus indicus* Linn, was evaluated for its broncodilatory effect. The methanolic extract of *Sphaeranthus indicus* and its fractions Petroleum ether, Chloroform, Benzene, Ethyl acetate (87 and 174 mg/kg, p.o.) significantly prolonged the latent period of convulsions as compared to control following exposure to histamine aerosol. Effect of *Sphaeranthus indicus linn* extract against histamine induced bronchoconstriction in guinea pigs is shown in Table 2.

Methanolic extract of *Sphaeranthus indicus* at the dose of 174 mg/kg, p.o., showed maximum protection (46.36 %) as compared to other fractions. The other fractions Petroleum ether, Chloroform, Benzene, Ethyl acetate of *Sphaeranthus indicus* at the dose of 174 mg/kg, p.o., showed protection 41.59, 44.53, 41.59, 38.90 % respectively. The standard drug used, Chlorpheniramine maleate (1mg/kg, p.o.) also offered maximum protection 49.65 % in the present study.

DISCUSSION

Histamine causes very strong smooth muscle contraction, profound hypertension, and capillary dilation in cardiovascular system. The experimental bronchial asthma was induced in guinea pig by exposing it to 0.2 % histamine aerosol. The guinea pigs exposed to histamine aerosol showed preconvulsive dyspnoea leading to convulsion. The time of preconvulsive dyspnoea (PCD) was noted for each animal. The methanolic extract of *Sphaeranthus indicus* at the dose of 174 mg/kg, p.o., and showed maximum protection (46.36 %) as compared to other fractions of *Sphaeranthus indicus*. Results are comparable

Table 3: Percent protection against Histamine Induced Bronchoconstriction in Guinea Pig.

Groups	% Protection		
	1 hr.	4 hr.	24 hr.
Std (2 mg/kg.)	38.76	49.65	18.95
M_1(87 mg/kg.)	34.48	35.59	12.44
M_2(174mg/kg.)	34.42	46.36	12.44
PE_1(87 mg/kg.)	22.70	27.88	9.345
PE_2(174mg/kg.)	29.77	41.59	12.75
C_1(87 mg/kg.)	33.8	34.53	12.50
C_2(174mg/kg.)	34.72	44.53	13.98
B_1(87 mg/kg.)	29.85	31.81	12.16
B_1(174mg/kg.)	29.77	41.59	12.75
EA_1(87 mg/kg.)	27.17	30.10	13.63
EA_2(174mg/kg.)	30.44	38.90	14.46

Abbreviation

Std = Chlorpheniramine maleate (2 mg/kg, p.o.).
M_1 = Methanolic extract of *Sphaeranthus indicus* (87 mg/kg, p.o.).
M_2 = Methanolic extract of *Sphaeranthus indicus* (174 mg/kg, p.o.).
PE_1= Petroleum ether fraction of methanolic extract of *Sphaeranthus indicus* (87 mg/kg, p.o.).
PE_2= Petroleum ether fraction of methanolic extract of *Sphaeranthus indicus* (174 mg/kg, p.o.).
C_1 = Chloroform fraction of methanolic extract of *Sphaeranthus indicus* (87 mg/kg, p.o.).
C_2 = Chloroform fraction of methanolic extract of *Sphaeranthus indicus* (174 mg/kg, p.o.).
B_1 = Benzene fraction of methanolic extract of *Sphaeranthus indicus* (87 mg/kg, p.o.).
B_2 = Benzene fraction of methanolic extract of *Sphaeranthus indicus* (174 mg/kg, p.o.).
EA_1 = Ethyl Acetate fraction of methanolic extract of *Sphaeranthus indicus* (87 mg/kg, p.o.).
EA_2*;* = Ethyl Acetate fraction of methanolic extract of *Sphaeranthus indicus* (174 mg/kg, p.o.). n=5

to chlorpheniramine maleate. Further increase in the dose showed decreased activity. However, highly significant increase in preconvulsion time was observed due to treatment with methanol fraction when guinea pigs were exposed to histamine aerosol. The bronchodilatory effect of methanol fraction was found comparable to the protection offered by the reference standard drug Chlorpheniramine maleate. Thus, it can be concluded that methanolic extract and its fractions of whole plant of *Sphaeranthus indicus* Linn possess significant antihistaminic (H_1 receptor antagonist) activity. The methanolic extract and its fractions of whole plant of *Sphaeranthus indicus* Linn by virtue of its antihistaminic activity can be used in the treatment of asthma. Hence further detailed study needs to be undertaken to evaluate the clinical efficacy of *Sphaeranthus indicus* Linn extract.

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