IJAYUSH
International Journal of AYUSH
AYURVEDA, YOGA, UNANI, SIDDHA AND HOMEOPATHY
http://internationaljournal.org.in/journal/index.php/ijayush/

International Journal
Panacea
Research library
ISSN: 2349 7025

Original Research Article **Volume 11 Issue 6** **Nov-Dec 2022**

EVALUATION OF ANTI -ASTHMATIC ACTIVITY OF ETHANOLIC EXTRACT *ALPINIA CALCARATA*

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Abstract

Asthma is a chronic inflammatory lung disease that can cause repeated episodes of cough, wheezing and breathing difficulty. There are number of treatments that can help effectively control or sooth the asthmatic condition Drugs which are indicated for the treatment of asthma includes the classes of beta 2 agonists, corticosteroids, leukotriene inhibitors and xanthenes. however side effects are exhibited by all these drugs. Herbal medicine exhibit fewer side effects and they are very much safe. The plant *Alpinia calcarata* has been traditionally used for various diseases. In present study rhizomes of the plant *Alpinia calcarata* have been evaluated for the treatment of asthma. The rizhome of plant was collected and subjected to extraction & phytochemical screening. Further in vitro and in vivo anti asthmatic activity of plant was checked. The results showed that histamine induced bronchospasm the ethanolic extract of the plant *Alpinia calcarata* rhizomes showed significant activity and increase in dose of extract increase % protection. The maximum % protection shown by the plant extract was 60.79 % observed at 200 mg/kg for broncho relaxant study comparable to that of standard drug chlorpheniramine maleate 78.3 %. It was also observed that the ethanolic extract of *Alpinia calcarata* rhizomes at doses 100 and 200 mg/kg significantly decreased milk induced eosinophils count. Ethanolic extract of *Alpinia calcarata* rhizomes inhibits contraction produced by histamine in these tissue preparations. *Alpinia calcarata* rhizome exhibit significant percentage decreased contraction at concentration 0.8 mg/ml. from these results it can be concluded that the *Alpinia calcarata* exhibits potent anti -asthmatic activity.

Keywords: Asthma, Medicinal plants, *Alpinia calcarata* Broncho relaxant

Introduction

Asthma is a chronic inflammatory lung disease that can cause repeated episodes of cough, wheezing and breathing difficulty. During an acute asthma episode, the airway lining in the lungs becomes inflamed and swollen. In addition, mucus production occurs in the airway and muscles surrounding the airway spasm. Combined, these cause a reduction in air flow. Symptoms of asthma include coughing, wheezing, breathlessness, respiratory rate increased, chest tightness. Causes of asthma include allergens from nature, typically inhaled, which include waste from common household pests. Indoor air pollution from volatile organic compounds, medications, aspirin, βadrenergic antagonists (beta blockers), and penicillin, food allergies such as milk, peanuts, and eggs [1-3].

There are number of treatments that can help effectively control or sooth the asthmatic condition. Treatment is based on two important goals, which are (i) specific regimens for the treatment of acute attack by opening swollen airways that are limiting breathing and (ii) prophylactic measures to reduce the inflammation and airway resistance and to maintain airflow. Treatment and prevention involves a combination of medicines, life style advices and identifying and then avoiding potential asthma triggers [4,5].

Drugs which are indicated for the treatment of asthma includes the classes of beta2 agonists, corticosteroids, leukotriene inhibitors and xanthenes. They are available in the forms of inhalations, tablets, capsules and injections are used based on medical condition and supervision. Some side effects of beta-agonist medications have been noted to include tremor, increased nervousness and insomnia in children, nausea, fever, bronchospasm, vomiting, headache, pain, dizziness, cough, allergic reaction, dry mouth, sweating, chills, and dyspepsia [6,7].

Herbal medicine exhibit fewer side effects and they are very much safe. Plantsare gifts of natural to mankind for treating different types of diseases. Herbal medicines are cheaper and easily available. Also for certain diseases like hepatitis, herbs and herbal drugs are the only remedies. The traditional medicine is largely getting popularity over allopathic medicine because of their cost, availability and free from side effects [8].

The plant *Alpinia calcarata* has been traditionally used for various diseases. In present study rhizomes of the plant *Alpinia calcarata* have been used which traditionally indicated in the treatment of asthma [9,10].

Materials and methods

Plant material

In the present study, *Alpinia calcarata* was selected because of its traditional uses. The part used was rhizome.

Drug sample

Chlorpheniramine maleate was obtained from Abbott Laboratories pvt. Ltd. And Dexamethasone was obtained from Zydusbiogem, cadila health care Ltd.

Animals

Swiss albino mice (25-40 gm) and Guinea pig (400-600 gm) were used to carry out the activities. The animals had free access to standard commercial diet and water. Animals were housed in cages under standard conditions i.e., 12:12 hour light or dark cycle at 25±20 C. The experiments were carried out as per the guideline of CPCSEA, New Delhi, India.

Methods

Preparation of plant extract

The powdered rhizomes were extracted using ethanol by soxhlet extractor. In this process the powdered drug is placed into the extractor with ethanol as solvent. After extraction the extract was concentrated by evaporation then it was kept in a refrigerator for further use [11,12].

Preliminary phytochemical screening

The ethanolic extract of *Alpinia calcarata* rhizomes were subjected for the following chemical tests for the identification of various active constituents [13,14].

Acute toxicity studies

Acute toxicity of *Alpinia calcarata* was done as per OECD guidelines 423. The substance was administered in a single dose by gavage using specially designed mice oral tube. Animals were fasted prior to dosing with food but not water withheld overnight. Following the period of fasting, the animals were weighed and the test substance was orally at a dose of 5, 50, 300 and 2000 mg/kg. The animals are observed continuously for first three hours, four any toxic manifestations like increased motor activity, salivation, acute convulsion, coma and death. Changes in the animal behavior should be noted before and after administration for 24hours. Treated animals are to be further observed for 14 days. If the extract does not produce mortality at the highest dose, then the 1/10th or 1/20th of the dose was selected for experiment [15,16].

Evaluation of anti -asthmatic activity

Histamine aerosol induced bronchoconstriction in guinea pigs

Histamine was dissolved in distilled water to prepare 0.2% w/v solution. Experimentally bronchial asthma was induced in guinea pigs by exposing histamine aerosol by a nebulizer in an aerosol chamber. The required time for appearance of preconvulsive dyspnoea produced by the histamine was noted for each animal. 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 start of dyspnoea leading to the appearance of convulsion, was noted. As quickly as the preconvulsion dyspnoea (PCD) was recorded, the animals were removed from the chamber and positioned in fresh air for recover. This time for preconvulsive dyspnoea was recorded as basal value. Guinea pigs were then allowed to recover from dyspnoea for 2 days. After that, the animals were allotted to four different groups of 4-5 animals per group. Animals in group 1 served as control and received carboxy methyl cellulose. The animals of group 2 and 3 were given, by oral intubation, 100 and 200 mg/kg of the plant extract, respectively, while group 4 received the standard drug - Chlorpheniramine maleate, intraperitoneally. After receiving the drugs, all the animals were again exposed to histamine aerosol in the chamber, one hour, four hours and 24 hours, to determine pre convulsive time (PCT) [17-19].

Milk induced leukocytosis and eosinophilia

Mice were divided into 4 groups with six in each group. Blood samples were collected from retro-orbital plexus. Group 1 served as control and received carboxy methyl cellulose solution, groups 2-3 received plant extract at (100-200 mg/kg) group 4 received dexamethasone at 50 mg/kg i.p. All the groups injected boiled and cooled milk (4 ml/kg, s.c.) 30 min after treatments. Total leukocyte and eosinophile count was done in each group before administration of test compound and 24 hours after milk injection. Difference in total leukocytes and eosinophile count before and after 24-hour drug administration was calculated [20,21] .

Ex vivo anti-asthmatic activity

Isolated guinea pig tracheal preparation

Isolated guinea pig tracheal tissue was obtained by, Animals were sacrificed by cervical dislocation and carotid bleeding. The trachea was dissected out and transferred into a dish containing Krebs solution and cut crosswise between the section of the cartilage of the trachea and continuously ventilated and maintained at 37 + 0. 5°C.The adjourned trachea was allowed to make steady for at least 40 minutes. On equilibrium, the bath was supplied with Krebs solution for every 15 minutes Dose

response curve of histamine (10µg/ml) in plane Krebs solution and in 1 mg/ml of plant extract act in Krebs solution was taken. Percentage of maximum contractile response on ordinate and concentration of histamine on abscissa was plotted to record dose response curve of histamine, in absence and presence of plant extract [22].

Statistical analysis

The statistical analysis was carried out by using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. The results are expressed as Mean±S.E.M., n=6

Result and Discussion

The percentage yield of the product was found to be 17 % w/w. The phytochemical screening of the ethanolic extract of the *Alpinia calcarata* rhizomes indicate the presence of carbohydrate, cardiac glycoside, protein, alkaloids, steroids, flavonoids, tannins and

phenolic compounds. In this histamine induced bronchospasm the ethanolic extract of the plant *Alpinia calcarata* rhizomes showed significant activity and increase in dose of extract increase % protection. The maximum % protection shown by the plant extract was 60.79 % observed at 200 mg/kg for broncho relaxant study comparable to that of standard drug chlorpheniramine maleate 78.3 %. It was also observed that the ethanolic extract of *Alpinia calcarata* rhizomes at doses 100 and 200 mg/kg significantly decreased milk induced eosinophils count. Ethanolic extract of *Alpinia calcarata* rhizomes inhibits contraction produced by histamine in these tissue preparations. Histamine (10 µg/ml) was taken in different dose level and concentration response curve was plotted. Study revealed that the ethanolic extract of the plant *Alpinia calcarata* rhizome exhibit significant percentage decreased contraction at concentration 0.8 mg/ml.

Table 1: Results of Phytochemical screening

S. No	Constituents	Presence/absence
1	Carbohydrate	+
2	Proteins	+
3	Amino acids	_
4	Fats and oils	_
5	Steroids	+
6	Cardiac glycosides	+
7	Anthraquinone glycoside	_
8	Saponin glycosides	_
9	Cyanogenic glycosides	_
10	Coumarin glycosides	_
11	Flavonoids	+
12	Alkaloids	_
13	Tannins	+
14	Phenol	+

Table 2: Histamine aerosol induced bronchoconstriction in guinea pigs

Group	Latent period of convulsion			
	Before	**1 hour**	**4 hour**	**24 hour**
Control	16.3±2.23	18.36±0.183	18.63±0.186	18.4±0.12
Alpinia calcarata Ethanolic extract(100 mg/kg)	16.71±1.31	29.65±.28	39.38±0.05*	28.2±0.23
Alpinia calcarata Ethanolic extract(200 mg/kg)	15.71±0.77	30.5±3.08	40.36±1.04*	28.4±.35
Standard (CPM) (1 mg/kg)	18.46±0.89	60.25±0.03*	68.26±1.01*	36.5±0.55

Table No.3: % Protection of the plant *Alpinia calcarata* rhizomes against histamine induced bronchoconstriction in guinea pig

Group	%protection		
	1 hour	**4 hour**	**24 hour**
Control (carboxy methyl cellulose)	10.9	12.3	11.4
Alpinia calcarata ethanolic extract (100 mg/kg)	43.2	57.2	40.2
Alpinia calcarata ethanolic extract (200 mg/kg)	48	60.79	44.3
Standard(CPM)	69.76	78.3	50.1

Table 4: Effect of ethanolic extract of *Alpinia calcarata* rhizomes on milk induced leukocytosis

Groups	Difference in no of leukocytes beforeand after treatment(Cu.mm)
Control (Carboxy methyl cellulose)	4100±9
Alpinia calcarata ethanolic extract (100 mg/kg)	2580±8*
Alpinia calcarata ethanolic extract (200 mg/kg)	1280±12**
Standard (Dexamethasone(50 mg/kg))	600±10**

Table 5: Effect of ethanolic extract of *Alpinia calcarata* rhizomes on milk induced eosinophilia

Groups	Difference in no of eosinophilic count before and after treatment(Cu.mm)
Control (Carboxy methyl cellulose)	118±1.414
Alpinia calcarata ethanolic extract (100 mg/kg)	82±1.2*
Alpinia calcarata ethanolic extract (200 mg/kg)	53±1.434**
Standard (Dexamethasone (50 mg/kg))	38±1.13**

Table 6: Effect of ethanolic extract of *Alpinia calcarata* rhizomes on histamine induced contraction on isolated guinea pig tracheal preparation

Sr. no	Dose of histamine (10µg/ml) in ml	Control (Histamine 10 µg/ml) % maximum contraction	Test Histamine(10µg/ml)+EEAC(1 mg/ml) % maximum contraction
1	0.1	38.46 ± 1.58	30.76 ± 1.32**
2	0.2	53.48 ± 4.23	46.15 ± 2.91**
3	0.4	61.5 ± 3.89	53.48 ± 3.31**
4	0.8	73.07 ± 2.32	65.3 ± 1.76**
5	1.6	84.6 ± 2.13	69.2 ± 1.09**
6	3.2	100 ± 1.07	76.92 ± 2.11*

Conclusion

The result of the investigation showed that the ethanolic extract of *Alpinia calcarata* rhizomes possess anti asthmatic activity. Drugs effective in asthma are mostly steroidal in nature. Phytochemical analysis showed presence of flavonoid and steroids. The anti-asthmatic property showed by the plant may be because of these chemical moieties. The results obtained in the study supports the traditional and also demands further research and to isolate and characterize active principles responsible for anti-asthmatic activity.

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