



Studies on Anti-asthmatic property of *Cyamopsis tetragonoloba* (L.) Taub. leaf extracts

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Abstract

Objectives: The present study has been designed to evaluate the anti-asthmatic activity of leaves of *Cyamopsis tetragonoloba* on *in-vitro* and *in-vivo* models. **Material and methods:** The anti-asthmatic activity was carried out on isolated guinea pig ileum preparation (*in-vitro*), histamine induced bronchospasm in guinea pigs and milk-induced leukocytosis and eosinophilia in mice. **Results:** Results showed that, the aqueous extract of the leaves significantly inhibited ($p < 0.05$) the contractile effect of histamine. Both Aqueous and alcoholic extracts were found to inhibit ($p < 0.001$ and $p < 0.05$) bronchospasm induced by histamine in guinea pigs by varying degree. Treatment with aqueous as well as alcoholic extracts of the plant decrease blood eosinophilia and leukocyte count by 80% and 90% respectively. **Conclusion:** The leaves of the plant showed significant anti-histaminic effect in both *in-vitro* and *in-vivo* models and adaptogenic activity, thus can be consider being use full in treatment of asthma.

Key words: Anti-asthmatic, Bronchospasm, *Cyamopsis tetragonoloba*, eosinophil count, isolated tissue and leukocytes.

1. Introduction

Among several respiratory diseases affecting human, bronchial asthma is the most common disabling syndrome with a world wide incidence of 155 millions. It is a disease that does not have the boundaries of age, race and gender. The availability of effective medications is not withstanding with present challenges and the prevalence of asthma is continuously increasing with time [1, 2]. Moreover the side effects of these drugs are also quite disturbing. Hence there is an increasing demand for use of traditional medicines in the management of asthma.

Cyamopsis tetragonoloba (L) TAUB (Family-Fabaceae) is an annual herb. Its pods are used as vegetable throughout the India and showed high content of carbohydrate and fibers. Leaves of this plant acclaimed for its potency in treatment of asthma amongst folk areas of Mewar region of Rajasthan, India [3]. Various phytoconstituents are reported from this plant such as, flavonoids, p-coumarin, saponins, tannins and triterpenes [4]. The plant has been found to possess significant antidiabetic, hypolipidemic, laxative, anti-cholinergic, anti-

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ulcerogenic and anti secretory activity [5]. The present study was taken up as no such effort have made till time to evaluate the traditional claim for the anti-asthmatic activity of the leaves.

2. Material and Methods

2.1 Collection and preparation of plant material

Fresh leaves of *C. tetragonoloba* were collected in the month of May from surrounding field of Shimoga region of Karnataka. The plant was authenticated by Dr. S. B. Kamlakar, Head, Department of Botany, Sahyadri Science College, Shimoga, Karnataka. The collected leaves are washed with water, artificially dried at 30°C and powdered through hand grinder to make a coarse powder.

2.2 Extraction of plant material

About 400 gm of powdered leaves was extracted with alcohol by using soxhlet apparatus and then with water by cold maceration. The extracts were concentrated in a rotary flash evaporator under reduced pressure.

2.3 Phytochemical analysis

Phytochemical analysis for both extracts was done as per standard protocol [6].

2.4 Experimental animals

The experiments were initiated after approval of protocol by institutional animal ethical committee. Albino Guinea pig (350-400 gm) and Swiss albino mice (25-30 gm) were used in this study. Animals were housed under standard laboratory condition. The animals had free access to food and water.

2.5 Studies on smooth muscle preparation (In-vitro)

Guinea pigs were sacrificed by a sharp blow over the head, abdomen was opened and ileum was dissect out and small pieces of 2-3 cm were

taken from portion situated 15 cm proximal to the ileo-caecal junction and suspended in Tyrode solution (NaCl 8.0, KCl 0.2, CaCl₂ 0.2, MgCl₂ 0.1, NaHCO₃ 1.0, NaH₂PO₄ 0.05 and Glucose 1.0 gm/liter), and continuously aerated and maintained at 37 ± 0.5°C. The tissue was allowed to equilibrate for 30 min under a load of 500 mg. After equilibration period a contact time of 30 sec and 5 min time cycle was followed for recording the response of Histamine by using frontal writing liver. After obtaining a dose response curve of histamine (50 µg/ml) on ileum the standard Pheniramine maleate at a dose of 100 µg/ml was added to the reservoir containing Tyrode solution and response of tissue was recorded for the same dose of Histamine in presence of antagonist to obtains the standard inhibition curve. After several washings, the alcoholic extract of *C. tetragonoloba* was added to the reservoir containing tyrode solution with final dilution of 100 µg/ml and the same dose of histamine (5, 10, 15, 20, 25 µg/ml) were repeated in presences of alcoholic extract to obtains inhibition curve. Same procedure was followed to obtain inhibition curve of aqueous extract. Graph of percentage of maximum contractile response versus negative logarithm of molar concentration of histamine was plotted to record dose response curve of histamine in presence and absence of Pheniramine, alcoholic and aqueous extract of *C. tetragonoloba* leaves [7].

2.6 Milk-induced Leukocytosis and Eosinophila in mice (In vivo)

Swiss albino mice of either sex weighing between 25-30 gm were divided into four groups of six animals each. Animal belongs to group I treated as -ve control and received distilled water 10 ml/kg.p.o. Animals belong to group II, III and IV received boiled (boiling temp.70°C and boiling time 20 min) and cooled milk in dose of 4 ml/kg subcutaneously. Animals belongs to

Table 1. Inhibitory effect of extracts on histamine induced contraction in isolated guinea pig ileum preparation

Dose of Histamine ($\mu\text{g/ml}$)	(-ve) Log molar conc.	% Maximum response			
		Histamine	Pheniramine	Aqueous	Alcoholic
5	2.301	63.61 \pm 1.87	14.01 \pm 0.68**	22.76 \pm 2.44*	50.35 \pm 2.51
10	2.000	70.30 \pm 2.75	30.81 \pm 1.23**	34.37 \pm 1.29*	57.57 \pm 2.03
15	1.823	85.53 \pm 1.43	39.28 \pm 1.64**	50.11 \pm 1.94*	71.77 \pm 2.49
20	1.698	97.78 \pm 3.25	42.19 \pm 1.78**	68.72 \pm 2.66*	78.32 \pm 2.07
25	1.602	99.41 \pm 1.40	52.30 \pm 1.29**	66.57 \pm 3.33*	79.78 \pm 3.53

**p< 0.01, *p< 0.05 .Values are in Mean \pm SEM (n=5)

Table 2. Histamine induced bronchospasm in guinea pigs

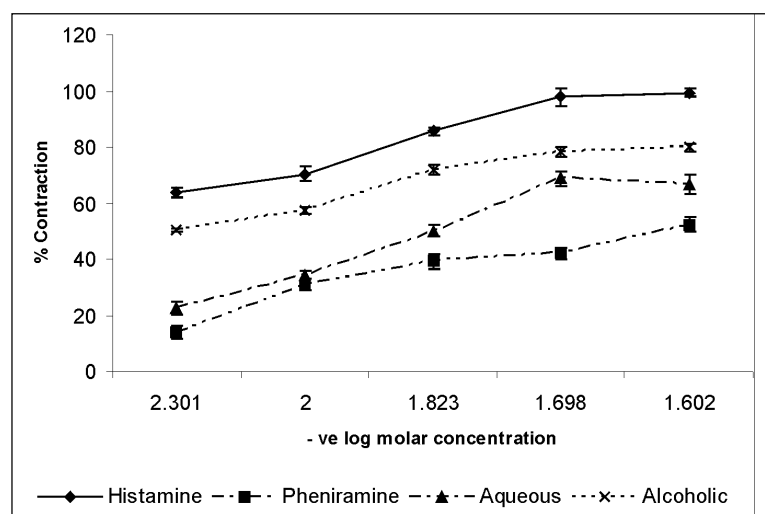
Group	Dose	Increase in mean time of PCD on histamine exposure (sec).	% Protection of bronchospasm in compare to day 0
Control (Water)	1 ml/kg	2.49 \pm 2.28	3.21
CPM	2 mg/kg	106.37 \pm 6.20**	64.22
Alcoholic extract	100 mg/kg	9.30 \pm 2.78*	14.11
Aqueous extract	100 mg/kg	28.62 \pm 3.031**	33.99

* **p<0.001, * p<0.05.Values are in Mean \pm SEM (n=6), CPM – Chlorpheniramine maleate

Table 3. Effect of *C. tetragonoloba* on milk induced leukocytosis and eosinophilia in mice before and after milk injection.

Group	Difference in no. of Leukocytes (per cu mm)	Difference in Eosinophil count
Control (Normal Animal)	213.33 \pm 95.42	51.5 \pm 2.88
Control (Untreated)	7091.33 \pm 146.84	656.66 \pm 37.77
Aqueous extract	4470.00 \pm 261.91*	120.5 \pm 4.505*
Alcoholic extract	1013.33 \pm 58.530*	80.166 \pm 3.060*

*p<0.01.Values are in Mean \pm SEM (n=6)

**Fig. 1.** Effect of extracts on histamine induced percentage contraction

group III received ethanolic, where as group IV received aqueous extract of *C. tetragonoloba* leaves orally at a dose of 100 mg/kg body weight. All the test drugs were administered 1hr before milk injection. Blood samples were collected through tail vein before and after 24 hr of milk injection. Difference in total leukocytes and eosinophil count before and after 24 hrs after drug administrations were calculated [8].

2.7 Histamine induced bronchospasm in guinea pigs

Guinea pigs of 300-400 gms were divided in four groups of six animals in each group. Bronchial asthma was induced by exposing the animal to 1% histamine aerosol (500 mg histamine in 50 ml water) at constant pressure (1 kg/cm²) in an aerosol chamber. Animal exposed to the histamine showed progressive dyspnoea which leading to the convulsion, the end point of dyspnoea was determined by the time from exposure to histamine to onset of convulsion. Pre convulsive dyspnoea was determined and animal were removed from chamber and placed in fresh air. This time was considered as day 0. A single dose treatment of alcoholic and aqueous extracts at a dose of 100 mg/kg was given for two days in their respective groups. The protection offered by test material was calculated by following formula

$$\% \text{ Protection} = (1 - T_1/T_2) \times 100$$

Where, T_1 = mean of Preconvulsive time before two days, T_2 = Mean of Preconvulsive time after two days. Chlorpheniramine meclate at a dose of 2 mg/kg was used as standard [9].

2.8 Statistical Analysis

All the results were expressed as mean + SEM. The statistical significance between groups was tested by one-way ANOVA test. A probability value less than 0.05 were considered as significant.

3. Results

3.1 Phytochemical analysis

Phytochemical analysis shows the presence of flavanoids, saponins, coumarins, Tannins, phytosterols and carbohydrates.

3.2 Effect of extracts on isolated guinea pig ileum preparation

In the present study histamine (50 µg/ml) produced dose dependent contraction of guinea pig ileum as indicated in the graph (fig.1, Table 1). The physiological salt solution containing the aqueous extract of *C. tetragonoloba* (100 µg/ml) significantly inhibited ($p < 0.05$) the contractile effect of histamine on isolated guinea pig ileum preparation, inhibition of contractile response produced by ethanolic extract is less in comparison of aqueous extract.

3.3 Effect of extracts on Milk-induced Leukocytosis in mice

Subcutaneous injection of milk in dose of 4 ml/kg produced a significant increase in leukocyte ($p < 0.001$) count after 24 hr of its administration. Mice pretreated with ethanolic and aqueous extracts of *C. tetragonoloba* exhibited inhibition of milk induced leukocytosis in comparison with +ve control ($p < 0.01$).

3.4 Effect of extracts on Milk-induced eosinophilia in mice

A Significant increase in total eosinophil count was seen in animals treated with milk 4 ml/kg (s.c.). In groups pretreated with *C. tetragonoloba* extracts at the dose of 100 mg/kg were significantly inhibited ($p < 0.01$) the increase in eosinophil counts in comparison of +ve control.

3.5 Histamine induced bronchospasm in guinea pigs

Exposure to histamine aerosol (1% histamine aerosol with a pressure of 1 kg/cm²) caused

pre convulsive dyspnoea. Animal treated with aqueous and alcoholic extracts of *C. tetragonoloba* (100 mg/kg) showed significant ($p<0.001$ and $p<0.05$) increase in mean time of PCD showed its *in-vivo* anti-histaminic effect.

4. Discussion

Bronchial asthma is characterized by increase reactivity of air way to spasmogens. An initial event in asthma appears to be the release of mediators, which cause contraction of bronchial smooth muscles. Guinea pig ileum is used for screening of anti-histaminic activity. The stimulation of H1-receptors produces graded dose related contraction of guinea pig ileum. Inhibition of contraction indicating H1-receptor antagonist activity of the drug. In the present study, ethanolic and aqueous extracts (100 µg/ml) inhibited the contraction produced by spasmogen by varying degree, showing significant antihistaminic activity.

Herbal formulations used in asthma include some anti-stress agents to enable adoption since excessive stress or nervous debility may aggravate symptoms of asthma. The normalization effect of an adaptogen can be observed in milk-induced eosinophilia after parental injection of milk [10]. After parental injection of milk showed increase in leukocyte count, where as the group treated with alcoholic and aqueous extract showed leukocyte count nearer to normal values. This indicates adaptogenic activity of *C. tetragonoloba*.

Blood eosinophilia is considered as hallmark of both allergic and non allergic asthma. There is significant association between eosinophil activation and asthma severity as well as bronchial hyper-responsiveness [11]. Activated eosinophils cause desquamation and damage to respiratory epithelial cells. The eosinophil count

increase in body fluid and tissue, emphasis is placed on number of eosinophills in blood. Eosinophilia is associated with respiratory disorders, often allergic in nature together with pulmonary infiltration that is detectable on chest film [12]. The total eosinophil count reflects asthmatic activity and is useful in management of bronchial asthma. Administration of milk at a dose of 4 ml/kg produces change in eosinophil count in animal model. In the present study it has been found that, parental administration of milk at a dose of 4 ml/kg significantly induced the eosinophil count ($p<0.001$) after 24 hr. Groups treated with aqueous and alcoholic extracts of *C. tetragonoloba* significantly inhibited increase in number of eosinophills in mice.

Thus it can be concluded from results obtained in the present study that *C. tetragonoloba* may possess significant anti-asthmatic activity. The antiasthmatic activity of *C. tetragonoloba* may be attributed due to anti-histaminic, anti-allergic and adaptogenic activity of the plant, suggesting of its potential in treatment and prophylaxis of asthma. Its anti-asthmatic activity may be contributed to its flavonoidal content especially for quercetin, which reported to have very good anti-asthmatic and anti-allergic activity [13]. Other contributors may be the saponins present in the drug.

The tradition claim of using the plant as remedy for asthma found to be appropriate on scientific evaluation parameters. Hence the further work can be carrying out to evaluate it mechanism of action and for identification of compound responsible for activity.

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