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Research Article

EVALUATION OF ANTI-ASTHMATIC ACTIVITY OF *CAPPARIS DECIDUA*

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ABSTRACT

Objective: The present study was conducted to determine the anti-asthmatic activity of *Capparis decidua.*

Methods: The acute oral toxicity study was conducted as per OECD guidelines, and the extract was proved to be safe up to the dose of 2000 mg/kg. The anti-asthmatic activity of *C. decidua* was evaluated using various experimental models such as histamine-induced bronchoconstriction in guinea pigs and milk-induced leukocytosis in mice, histamine-induced bronchospasm in guinea pigs, studies on pre convulsive time and milk-induced leukocytosis in mice, studies on blood.

Results: Antihistaminic drugs Chlorpheniramine maleate and ethanolic extract of *C. decidua* significantly protected the guinea pigs against histamine-induced bronchospasm. The ethanolic section of *C. decidua* has dramatically prolonged the latent period of convulsions compared to control. Ethanolic extract of *C. decidua* suppresses the milk-induced leukocytosis by stabilizing the oxidative stress in the surrounding tissue.

Conclusion: The results obtained in the above study suggest the ethanolic extract of *C. decidua* possesses significant anti-asthmatic activity.

Keywords: Asthma, *Capparis decidua*, Histamine, Bronchoconstriction, Leukocytosis.

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INTRODUCTION

Asthma is an allergic reaction that causes inflammation and narrowing of the respiratory tract, resulting in asthma and difficulty in breathing [1]. Asthma is the common chronic inflammatory disease of the airways characterized by frequent and recurrent symptoms, reversible airflow obstruction, and bronchospasm. Asthma is caused by various factors such as allergens, drugs, respiratory infection, dust, cold air, exercise, emotions, occupational stimuli, chemicals, histamine, etc. It is thought to be caused by a combination of genetic factors, environmental factors (such as tobacco, hygiene hypothesis, and volatile organic compounds), genetic interactions, exacerbation, social and economic factors [2-5]. Asthma is characterized by inflammatory cells in the air, including eosinophils, macrophages, mast cells, epithelial cells, and activated lymphocytes that release various cytokines, adhesion molecules, and other mediators. Inflammation results in an acute, sub-acute or chronic process that alters airway tone, modulates vascular permeability, activates nerves, increases secretion of mucus, and alters the structure of the airway by reversibly or permanently [1]. Asthma is a major public health problem worldwide [6]. There has been a dramatic increase in global prevalence, illness, death, and financial burden associated with asthma over the past 40 years, nearly 300 million people worldwide are currently suffering from asthma, and its prevalence is increasing by 50% every decade [7]. The highest rate of asthma are found in the United Kingdom (>15%), New Zealand (15.1%), Australia (14.7%), the Republic of Ireland (14.6%), Canada (14.1%), and United States (10.9%). One person in 10 has asthma in North America, Approximately, 35.5 million people [7]. The large number of drugs is used for the treatment of asthma. At present, available treatment for asthma most medications works by relaxing bronchospasm or reducing inflammation. These available treatments are not efficient for treating asthma completely as they have many toxic and side effects. The Ayurveda suggests that herbal plants have comparatively less toxic value and are more efficacious. They also have fewer chances of side effects and complications to patients compared to available synthetic drug treatments [1]. *Capparis decidua* belongs to the family Capparaceae [8,9]. The effects of the ethanolic extract of *C. decidua* may be due to the presence of phytochemicals such as flavonoids, saponins, steroids, alkaloids, glycoside, and tannins, known to have similar effects. Isocodonocarpine was found to be responsible for anti-inflammatory activity and anti-asthmatic activity [9-15].

METHODS

Plant collection and authentication

The collection of the leaves of *C. decidua* was done in January from the Jodhpur region, Rajasthan. As plants will be enriched with phytoconstituents at that time. Identification and authentication of the plants were carried out by Dr. S. L. Meena Scientist D & Botanical survey of India, Jodhpur (Raj) (No.:BSI/AZRC/I.12012/Tech./2019-20/PI.Id/235).

Preparation of plant extract

The leaves of *C. decidua* were washed and dried under shade for 25 days. The leaves were cleaned and grind with the help of a grinder. After proper milling, the weight of the powder was measured. Powder was used for the soxhlet extraction. About 130 g of dried powder was extracted with petroleum ether in soxhlet apparatus for 18–20 h at 60–80°C to the powder, and then mark was extracted with benzene (for 15–16 h at 78–80°C), chloroform (for 15–16 h at 60–62°C), and ethanol (for 16–18 h at 75–79°C). The extract at the bottom was collected, and the solvents were removed using reflex condenser and dried on the water bath. Each time, before the extraction with other solvents, the powdered substance is air-dried. Percentage (%) yield was found for petroleum ether extract was 6.69%, benzene extract was 3.44%, chloroform extract was 3.85%, and ethanolic extract was 7.75%. Obtained extracts were subjected to phytochemical investigation.

Experimental animals

Dunkin-Hartley guinea pig (350–400 g) and albino mice (20–30 g) of both sexes were housed together in a group of four in clean polypropylene cages (males separated from females). Bedding material of the cages was changed from time to time. Animals were cared for under standard environment conditions (12 h light: 12 h dark cycle, 22±3°C temperature, and 30–70% humidity). One-week time was provided to the animals for acclimatization with our laboratory

Table 1: Qualitative chemical analysis of petroleum ether, chloroform, benzene, and ethanolic extract of *Capparis decidua*

S. No.	Test for plant constituents	*Capparis decidua* root			
		Petroleum ether extract	Benzene extract	Chloroform extract	Ethanolic extract
1.	Test for carbohydrate				
	Molish test	–	+	+	+
	Fehling's test	–	+	+	+
	Benedict's test	–	+	+	+
	Barfoed's test	–	+	+	+
2.	Test for protein				
	Biuret test	–	–	–	+
	Million's test	–	–	–	+
	Ninhydrin test	–	–	–	+
3.	Test for Alkaloids				
	Mayer's test	–	–	–	+
	Dragenoff's test	–	–	–	+
	Wagner's test	–	–	–	+
4.	Test for Fats and Oils				
	Spot test	+	–	–	–
	Saponification test	+	–	–	–
5.	Test for glycoside				
	Legal test	–	–	–	+
	Baljet's test	–	–	–	+
	Borntrager's test	–	–	–	+
	Foam test	–	–	–	+
6.	Test for Flavonoids				
	Ferric chloride test	–	–	–	+
	Shinoda's test	–	–	–	+
7.	Test for Tannins and phenolic compounds				
	Ferric chloride test	–	–	–	+
	Reaction with lead acetate	–	–	–	+
8.	Steroids				
	Libermann's Burchard test	–	–	–	+

Table 2: Percentage protection against histamine-induced bronchoconstriction in guinea pigs at different time intervals

S. No.	Group N=6	Pre convulsion dyspnea (in sec) (Mean±SEM)				% Protection		
		Before treatment	After treatment			1 h	4 h	24 h
			1 h	4 h	24 h			
1.	Group-I	16.43±0.20	-	-	-	-	-	-
2.	Group-II	16.24±0.28	55.66±0.40**	69.55±0.21**	25.5±0.14**	70.83	76.65	36.32
3.	GROUP-III	14.35±0.28	30.41±0.83**	41.1±0.25**	18.11±0.42**	52.82	65.09	20.77

Data are expressed as mean±SEM. Where, n=6. Statistical analysis was done by ANOVA followed by Dunnett's test, where *p<0.01, **p<0.05 when Groups II and III were compared with Group I. Group-I (Control): Aerosolized Histamine (0.2% w/v) + Phosphate buffer (1 mL/kg, p.o.) Group-II (Std.): Aerosolized Histamine (0.2% w/v) + Chlorpheniramine maleate (2 mg/kg, i.p.). Group-III (Test): Aerosolized Histamine (0.2% w/v) + Ethanolic extract of *C. decidua* (200 mg/kg, p.o.).

Table 3: Effect of ethanolic extracts of *Capparis decidua* on milk-induced leukocytosis in mice

S. No.	Group n=6	Difference in number of leucocytes (per cumm) (Mean±SEM)
1.	Group-I	1.50±21.39
2.	Group-II	6883.34±47.85
3.	Group-III	2975±53.87**
4.	Group-IV	3700±75.22**

Data are expressed as mean±SEM Where, n=6. Statistical analysis was done by ANOVA followed by Dunnett's test, where *p<0.01, **p<0.05 when Groups II, III, and IV were compared with Group I. Group-I (Normal Control): Distilled water 10 mL/kg, p.o. Group-II (Positive control): Boiled and cooled milk (4 mL/kg, s.c.). Group-III (Std.): Dexamethasone (50 mg/kg, i.p.) + Boiled and cooled milk (4 mL/kg, s.c.). Group-IV (Test): Boiled and cooled milk (4 mL/kg, s.c.) + Ethanolic extract of *C. decidua* (200 mg/kg, p.o.)

environment. Animals were fasted 3-4 h before dosage but allowed free access to drinking water and standard pelleted diet *ad libitum*. Experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) Reg. no.16/BNCP/IAEC/2021.

Acute toxicity study

The dose was selected using an acute toxicity study (OECD, 423)**.** The acute toxicity study for ethanolic extract of *C. decidua* was performed using mice. The animals were free access to food and water before the experiment and maintained under standard conditions. Find the LD_{50} of ethanolic extract of *C. decidua*, three groups of mice, containing three in each group, were given *C. decidua* in the dose of 2000 mg/kg orally. The animals were monitored for 5 min every 30 min up to 2 h, and then at 4, 8 and 24 h after treatment for any behavioral changes/death. They were also monitored daily for 14 days for death. No deaths occurred within 14 days after the ethanolic treatment of *C. decidua* was observed and was therefore found to be safe up to a dose of 2000 mg/kg [16].

***In vivo* anti-asthmatic activity**

Histamine induced bronchoconstriction in guinea pig [17,18]

Overnight fasted guinea pigs were divided into three groups (n=6). Before drug treatment, each animal was placed in the histamine chamber and exposed to 0.2% w/v histamine aerosol. Pre convulsive time (PCT) was defined as the time of exposure to the onset of dyspnea leading to the appearance of pre convulsive dyspnea (PCD). As soon as the PCD was detected, the animal was removed from the chamber and

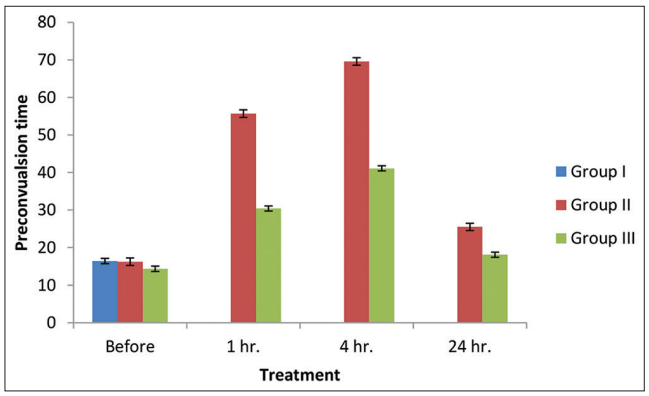

Fig. 1: Effect of ethanolic extracts of *Capparis decidua* on histamine-induced bronchoconstriction in guinea pigs. Group-I (Control): Aerosolized Histamine (0.2% w/v). Group-II (Std.): Aerosolized Histamine (0.2% w/v) + Chlorpheniramine meleate (2 mg/kg, i.p.). Group-III (Test): Aerosolized Histamine (0.2% w/v) + Ethanolic extract of *Capparis decidua* (200 mg/kg, p.o.)

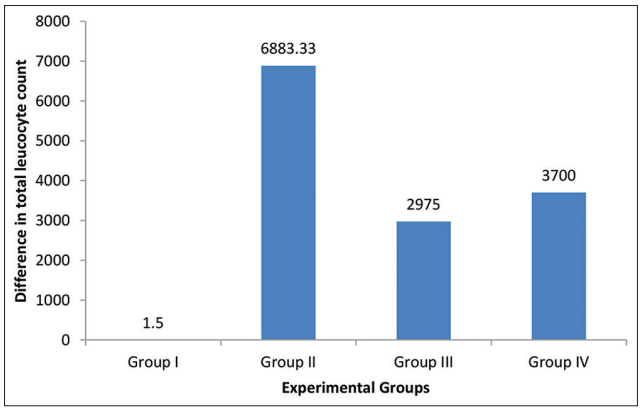

Fig. 2: Effect of ethanolic extracts of *Capparis decidua* on milk-induced leukocytosis in mice. Group-I (Normal control): Distilled water 10 mL/kg, p.o. Group-II (Positive control): Boiled and cooled milk (4 mL/kg, s.c.). Group-III (Std.): Dexamethasone (50 mg/kg, i.p.) + Boiled & cooled milk (4mL/kg, s.c.). Group-IV (Test): Boiled and cooled milk (4 mL/kg, s.c.) + Ethanolic extract of *C. decidua* (200 mg/kg, p.o.)

placed in the air. After 24 h, the animals belonging to Group I served as control and were treated with a phosphate buffer (1 mL/kg, p.o.); animals belonging to Group II were treated with Chlorpheniramine maleate (2 mg/kg, i.p.) while Group III was received a respective dose of ethanolic extract of *C. decidua.* These animals were also subjected to histamine aerosol overtime during the 1st, 4th, and 24th h of drug administration, and PCT was determined again. The protection offered by treatment was calculated using the following formula:

$$\%protection = (1–T_1/T_2) \times 100$$

T_1 = The mean of PCT before administration of test drugs.
T_2 = The mean of PCT after administering of test drugs at 1, 4, and 24 h.

Milk induced leukocytosis in mice [19,20]

Mice were divided into four groups, six animals per group. An animal part of Group-I received 10 mL/kg (p.o) of distilled water. Animal Groups II, III, and IV received an injection of boiled and cooled milk at a dose of 4 mL/kg, s.c. An animal belonging to Group III acted as standard and was received dexamethasone in a dose of 50 mg/kg, i.p. Animal belonging to Group IV acted as test group and received a respective amount of ethanolic extract of *C. decidua* and after 1 h boiled and cooled milk (4 mL/kg, s.c.) was administered to the same animals. After 24 h, blood samples were collected from all animals in their tail vein. Total leukocytes count was done in each group 24 h after injection of milk.

Statistical analysis

The results of various studies were exposed as mean±SEM and analyzed statistically using one-way ANOVA followed by Dunnett's test. *p<0.01, **p<0.05 were considered significant.

RESULTS

Phytochemical investigation

Phytochemical study of ethanolic extracts of *C. decidua* has shown the presence of carbohydrate, alkaloids, flavonoids, glycosides, tannin, polyphenols, and steroids, whereas the phytochemical analysis of chloroform, and benzene extracts of the plant *C. decidua* have shown carbohydrate and protein. Petroleum ether extract has shown fixed oils and fats.

Histamine induced bronchoconstriction in Guinea pigs

The guinea pigs, when exposed to 0.2% w/v histamine aerosol, showed signs of persistent dyspnea leading to convulsions. Guinea pigs treated with chlorpheniramine maleate (2 mg/kg, i.p.) increased the PCD at 1st, 4th, and 24th h compared to control. In the groups of guinea pigs pretreated with ethanolic extract of *C. decidua* (200 mg/kg, p.o.) increased the PCD at 1st, 4th, and 24th h compared to control. Ethanolic extract of *C. decidua* (200 mg/kg, p.o.) shows an increase in percent protection, but percent protection was found to be lower than chlorpheniramine maleate (2 mg/kg, i.p).

Milk-induced leukocytosis in mice

Subcutaneous injection of milk at a dose of 4 mL/kg produced an increase in the leukocytes count after 24 h of its administration. Mice treated with dexamethasone (50 mg/kg, i.p.), has shown inhibition of milk-induced leukocytosis as compared to positive control. In the groups of mice pretreated with ethanolic extract of *C. decidua* (200 mg/kg, p.o), there was inhibition of milk-induced leukocytosis, but inhibition of leukocytosis was found to be less than dexamethasone (50 mg/kg, i.p.).

DISCUSSION

The present study is designed to test the anti-asthmatic activity of *C. decidua*. *C. decidua* is usually very high in alkaloids, tannins, glycosides, and flavonoids, etc. *C. decidua* appears to be a good plant for the treatment of bronchial asthma due to its reported anti-allergic activity, anti-inflammatory, and anti-oxidant activity.

Phytochemical tests of *C. decidua* have shown the presence of flavonoids, alkaloids, glycosides, steroids, tannins, triterpenoids, amino acids, etc., which have anti-asthmatic activity of the plants.

Guinea pigs exposed to histamine aerosol showed persistent dyspnea, that is, difficulty in breathing, leading to convulsions. In the present study, ethanolic treatment extracted from *C. decidua* showed significant prolongation in convulsive dyspnea time, but prolongation was found to be less as compared to standard chlorpheniramine maleate. The effect may be due to its inhibition of H_1 – receptor or bronchodilating activity and may thus contribute to the regulation of asthma.

The present study of *C. decidua* was evaluated for the management of asthma using milk-induced leukocytosis in mice. Different types of mediators are involved in the pathology of asthma. It was shown that subcutaneous administration of milk produces a significant increase in leukocyte counts after 24 h of administration. Leukocyte during asthmatic inflammation release inflammatory mediators such as cytokines, histamine, and major essential protein, promoting the occurrence of inflammation. Leukocyte infiltration increases the inflammatory process through the by releasing of reactive oxygen species in the surrounding tissue, leading to increased oxidative stress and is associated with many pathogenic factors of asthma.

CONCLUSION

In the present study, various chemical tests confirmed the presence of alkaloids, flavanoids, steroids, glycosides, tannins, and saponins in the ethanolic extract of *C. decidua*. They have been the action of smooth muscle relaxant, a bronchodilator, antioxidant, anti-inflammatory, mast cell stabilizing, anti-allergic, and antihistaminic activity.

Ethanolic extract of *C. decidua* shows the anti-asthmatic activity against bronchoconstriction induced by histamine. In the present study, the antihistaminic drugs chlorpheniramine maleate and ethanolic extract from *C. decidua* protected guinea pigs against histamine-induced bronchospasm. Ethanolic extract of *C. deciduas* significantly increases the latent period of convulsions compared to control. This indicates the utility of the ethanolic extract of *C. decidua* in treating asthma by, under its H_1 – receptor blocking or bronchodilating activity.

This study shows that ethanolic extract of *C. decidua* suppresses the milk-induced leukocytosis by stabilizing the oxidative stress in the surrounding tissue. Mainly the leukocytes are responsible for releasing several inflammatory mediators such as histamine and cytokines which enhance thee inflammatory process. Infiltration of leucocytes in surrounding tissues in asthmatic inflammation causes increased oxidative stress, which is characterized as the main pathogenic feature of asthma. This study observed that the inhibition of leukocytosis was significant in mice treated with ethanolic extract of *C. decidua* as compared to the control group.

Ethanolic extract of *C. decidua* may possess anti-asthmatic activity, which may be due to antihistaminic activity, bronchodilating activity, mast cell stabilizing activity, anti-inflammatory activity, anti-allergic activity, anti-spasmodic activity, and anti-oxidant activity. All over, we can say that the ethanolic extract of *C. decidua* has significant anti-asthmatic activity.

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AUTHORS CONTRIBUTIONS

All authors have an equal share in the current research work.

CONFLICT OF INTEREST

By this, we declare that there is no conflict of interest.

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