Asthma is one of the most common problems among respiratory diseases affecting human world widely which affects to all age groups, races, and genders.¹ It is a chronic inflammatory disorder of the air pipe; characterized by narrowing of airways, frequent wheezing, dyspnea, chest tightness, morning awakening, and night coughing.² Asthma depends on the various factors such as allergens, respiratory infection, dust, cold air, exercise, emotions, occupational stimuli, certain drugs/chemicals, histamine, and heredity. These trigger factors accelerate the activation of immunoglobulin-E (IgE) mediated mast cell, release of interleukins (IL-4 and IL-5) and other inflammatory factors including eosinophils, neutrophils, β-cells, cytokines, and chemokines which lead to inflammation or obstruction in throat, bronchial hyperresponsiveness, and mucosal hypersecretions.³ Therefore, the disease statistics clearly necessitates the drugs targeting toward mast cell stabilizer, cytokine inhibitors, neutralizing antibodies directed IgE, histamine, leukotriene blockers, etc., for the management of asthma. Despite the availability of a wide range of antiasthmatic drugs, the relief offered by them is mainly symptomatic and show a poor or absent response even to high doses with more or less side effects. Hence, an ideal approach

**ABSTRACT**

**Objective:** The present study was to investigate the antiasthmatic potential from the flavonoid fraction of *Apium leptophyllum* fruit (FFALF) to validate its traditional claim. **Materials and Methods:** The antiasthmatic activity of FFALF was evaluated by histamine or acetylcholine-induced bronchospasm model in guinea pigs, compound 48/80 induced mast cell degranulation in albino rats and histamine-induced tracheal contraction in guinea pig. The preconvulsion dyspnea time at 0th and 7th day at the dose of 100 and 200 mg/kg in guinea pig’s bronchospasm model, the percentage of granulated and degranulated mast cell at the dose of 500, 750, and 1000 μg/ml in rats and tracheal contraction at the dose of 500, 750, and 1000 μg/ml in guinea pig were measured and compared with respective control groups. **Results:** The treatments of FFALF were significantly (P < 0.001) decreased the histamine/acetylcholine-induced bronchospasm, mast cell degranulation, and histamine-induced tracheal contraction as compared to inducer group. In addition, FFALF showed dose-dependent antiasthmatic activity in all the animals. **Conclusion:** Hence, this study suggested that the FFALF showed antiasthmatic activity probably by membrane stabilizing property as well as suppressing antibody production and inhibiting of antigen induced by histamine and acetylcholine.

**KEY WORDS:** *Apium leptophyllum*, asthma, bronchospasm, histamine, mast cell degranulation

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in the development of new drug toward safe and effective remedies is only from herbal sources to treat bronchial asthma. In this regard, natural compound having greater potentiality in antioxidant, anti-inflammatory, and immunomodulatory was recognized as a gold candidate for asthma.[4]

Apium leptophyllum Pers. (family- Umbelliferae), commonly known as Ajamod and was found in India, Sri Lanka, Pakistan, South America, Queensland, and tropics.[5] Traditionally, the fruit was widely used as an antinephritic, antirheumatic, carminative and was beneficial for prevention of tumor, anorexia, vomiting, colic pain, and itch.[6] The volatile oil of the leaves possesses antimicrobial and radical scavenging activity.[7] Earlier scientific investigation showed that the fruits of this plant possess antioxidant, chemopreventive, and antimutagenic activity in mice.[8,9] Phytochemically, it contains volatile oils, coumarins, terpene hydrocarbons, phenolics, alkaloids, and is a rich source of flavonoids.[6,8] Again, the fruit was useful in bronchitis, cough, and asthma by various traditional practitioners of India. In addition, the fruits possess thermogenic and antispasmodic in asthmatic patients.[10,11] On the basis of above traditional claims, this study was undertaken to investigate the antiasthmatic effect from the fruit extract of A. leptophyllum L. on various animals.

Materials and Methods

Chemicals and reagents

Histamine dihydrochloride, acetylcholine chloride, ketotifen, compound 48/80 were purchased from Sigma-Aldrich Chemical Co., USA. The solvents such as methanol, petroleum ether, diethyl ether, chloroform, ethyl acetate, n-butanol, benzene, ammonia, and formic acid were purchased from Merck, India Ltd., and other chemicals used in this study were of analytical grade.

Experimental animals

Albino rats (175–200 g) and guinea pigs (400–600 g) of either sex housed in standard conditions of temperature (22 ± 2°C), relative humidity (55 ± 5%), and light (12 h light/dark cycles) were used. They were fed with standard pellet diet and water ad libitum. The experimental protocol was approved by Institutional Animal Ethical Committee as per the guidance of CPCSEA, Ministry of Social Justice and Empowerment, Government of India (Regd. No-1693/PO/a/13/CPCSEA).

Plant collection and identification

The fruits of A. leptophyllum were collected from local region of Bhopal district, Madhya Pradesh, India. Further taxonomic identification and authentication was conducted was conducted at Department of Botany, Jiwaji University, Madhya Pradesh, India and the voucher specimen (F/HERB/2010/5405) was deposited in the herbarium for further reference.

Extraction of flavonoid fraction from Apium leptophyllum fruit

The collected fruits were cleaned, washed with distilled water and dried in shade for 4–6 days. The dried fruits were powdered using blender and then passed through 40 mesh size. The powdered material (250 g) was initially defatted with petroleum ether and then exhaustly extracted with 80% methanol into the soxhlet assembly for 48 h. The extract was separated by filtration through whatman No. 1 paper, concentrated on vacuum evaporator. The extract (Yield-16.4% w/w) was filled in plastic bottle and stored at 4°C until used.

Then, the crude methanolic extract (10 g) was subjected to column chromatography (Silica gel 120 mesh, 500 g) and eluted with n-hexane, chloroform, ethyl acetate, and n-butanol. The collected fractions were subjected to shinoda test, followed by thin-layer chromatography using benzene: methanol: ammonia (9:1:0.1) solvent system. The spot was visualized by spraying with ammonia, a reagent specific for flavonoids.[12] The fractions showing positive response for flavonoid were pooled together and considered as total flavonoid fraction. The total flavonoid fraction was concentrated (0.27% w/w) and subjected to further studies.

Preliminary phytochemical screening

Preliminary phytochemical tests were performed on methanolic extract of A. leptophyllum for the presence of various phytoconstituents as per described methods.[13]

Histamine and acetylcholine-induced bronchospasm in guinea pigs

Guinea pigs of either sex were divided into two groups, each group comprised six animals and exposed to 0.1% w/v of histamine dihydrochloride aerosol in histamine chamber. The progressive dyspnea was observed in animals when exposed to histamine aerosol. The end point, preconvulsion dyspnea (PCD) was determined from the time of aerosol exposure to the onset of dyspnea leading to the appearance of convulsion. As soon as PCD commenced, the animals were removed from chamber and placed in fresh air. PCD of this time was taken as day 0 value. Both groups of guinea pigs were given flavonoid fraction of Apium leptophyllum fruit (FFALF) at the dose of 100 mg/kg and 200 mg/kg, p.o. respectively, once a day for 7 days. On the 7th day, 2 h after the last dose, the time for the onset of PCD was recorded as on day 0. The Same procedure was followed in another set of animals (n = 6) for acetylcholine induce bronchospasm study except using 0.5% acetylcholine chloride in place of histamine dihydrochloride.[14] The percentage increased in time of PCD was calculated using the formula; Percentage increased in time of PCD = (1 – T1/T0) × 100. Where: T0 = time for PCD onset on day 0, T1 = time for PCD onset on day 7.
Mast cell degranulation study

Male albino rats were divided into six groups; each group carried six animals and sacrificed by cervical dislocation. The animals were immediately injected with 15 ml of prewarmed (37°C) buffered salt solution (NaCl 137 mM; KCl 2.7 mM; MgCl2; 1 mM; CaCl2, 0.5 mM; NaH2PO4 0.4 mM; glucose 5.6 mM; HEPES 10 mM) into the peritoneal cavity and massaged gently in this region for 90 s, to facilitate cell recovery. A midline incision was made and the peritoneum was exposed. The pale fluid was aspirated using a blunted plastic pasteur pipette and collected in a plastic centrifuge tube. The fluid was then centrifuged at 1000 rpm for 5 min, and the supernatant was discarded to reveal a pale cell pellet. The cell pellets were re-suspended in fresh buffer and re-centrifuged. The peritoneal cell suspension divided in six parts, namely, –ve control, +ve control, reference standard (ketotifen 10 μg/ml) and FFALF at different concentrations, i.e., 500, 750, and 1000 μg/ml, each containing 0.1 ml of cell suspension and incubated at constant temperature 37°C in water bath for 15 min. Then, 0.1 ml of compound 48/80 was added in all samples except in –ve control group and suspensions were further incubated for 10 min at 37°C. The cells were then stained with 10% of toluidine blue solution and observed under the higher magnification by microscope. The percent granulated and degranulated mast cells were counted in each group.[15]

Histamine induced guinea pig tracheal chain contraction

Guinea pigs of either sex (200–500 g) were divided into four groups. Each group contains six animals and was allowed to starve overnight and free access to water. The animals were killed by a blow on the head and exsanguinated. The isolated trachea was mounted in a 30 ml organ bath containing tyrode solution, maintained at 37 ± 1°C and gassed with air. The tissue was equilibrated for 45 min during which the bath solution was replaced every 10 min. At the end of the equilibration period, histamine (0.5 μg/ml) induced contraction as well as effect of FFALF extract at 500, 750, and 1000 μg/ml was recorded. A drug tissue contact time of 1 min was maintained. The percent response of each groups were calculated from the height of the peaks obtained.[15]

Statistical analysis

The results were expressed as mean ± standard error of the mean and analyzed statistically using one-way ANOVA followed by Dunnett’s test to find out the level of significance. Data were considered statistically significant at minimum level of P < 0.001.

Results

Preliminary phytochemical screening

The methanolic extract of A. leptophyllum showed the presence of carbohydrate, flavonoids, terpenoids, glycosides, tannins, volatile oil, and protein.

Effect of flavonoid fraction of Apium leptophyllum fruit on histamine and acetylcholine aerosol-induced bronchospasm in guinea pigs

The FFALF was significantly and dose-dependently increased the time of PCD following exposure to histamine (P < 0.001) and acetylcholine (P < 0.001) aerosol-induced bronchospasm in guinea pigs [Table 1]. The percentage of increase of PCD in histamine-induced bronchospasm at the dose of 100 μg/kg and 200 μg/kg body weight was found at 72.73% and 76.5%, respectively, whereas in the case of acetylcholine-induced bronchospasm at same dose level was at 59.43% and 62.14%, respectively. Hence, the percentage of increase of PCD was more against histamine-induced bronchospasm as compared to acetylcholine by the administration of A. leptophyllum.

Effect of flavonoid fraction of Apium leptophyllum fruit on compound 48/80 induced mast cell degranulation in rats

The percentage of mast cell degranulation was observed as 12.91 ± 2.41, 76.52 ± 2.17, 22.82 ± 3.13, 60.86 ± 5.31, 42.82 ± 1.14, and 28.69 ± 2.45 in groups of –ve control, +ve control, ketotifen (standard), FFALF I - 500 μg/ml, FFALF II - 750 μg/ml, and FFALF III - 1000 μg/ml, respectively as shown in Figure 1. The treated groups of FFALF, as well as the standard group, were observed significant (P < 0.001 and P < 0.01) inhibition of mast cell degranulation from rat peritoneal cell. The treated groups of FFALF were also able to dose-dependent mast cell protection against compound 48/80 as compared with positive control.

Effect of flavonoid fraction of Apium leptophyllum fruit on guinea pig tracheal chain

In isolated guinea pig tracheal studies, FFALF significantly (P < 0.001) inhibits the contraction of tracheal muscles induced by histamine as compared to control group in dose-dependent manner as shown in Figure 2. In FFALF treated groups, the

Table 1: Effect of flavonoid fraction of Apium leptophyllum fruit on histamine and acetylcholine induced bronchospasm in guinea pigs

| Treated group (mg/kg) | Histamine induce bronchospasm | Acetylcholine induce bronchospasm |
|----------------------|-------------------------------|----------------------------------|
|                      | Before treatment (control) | After treatment | Percentage of increase | Before treatment (control) | After treatment | Percentage of increase |
| FFALF (100)          | 128.53 ± 1.38                | 471.48 ± 1.31* | 72.73                 | 151.36 ± 1.18                | 373.09 ± 1.57* | 59.43                 |
| FFALF (200)          | 133.27 ± 1.49                | 567.27 ± 2.17* | 76.50                 | 161.74 ± 1.19                | 427.29 ± 1.45* | 62.14                 |

Each values were expressed as mean±SEM (n=6). *P<0.001 as compared with control by one-way ANOVA followed by Dunnett’s test.

FFALF: Flavonoid fraction of Apium leptophyllum fruit, SEM: Standard error of the mean.
percentage of inhibition at 500, 750, and 1000 μg/ml was found at 37.53%, 49.11%, and 73.41%, respectively as compared to histamine treated group.

**Discussion**

Bronchial asthma is characterized by stimulating the airway reactivity to exposure of various spasmogens. The airway stimulation leads to the release of numerous mediators such as histamine, acetylcholine, leukotrienes, and prostaglandin which cause the acute attack of bronchoconstriction. There is a very close resemblance of pulmonary responses to histamine challenge in both guinea pigs and human species, as well as the anaphylactic sensitization made this species the model of choice. Inhalation of histamine and acetylcholine is a classical model of inducing bronchoconstriction which results intense smooth muscle contractions, hypoxia, and convulsion in case of guinea pig. Bronchodilators can delay the occurrence of these symptoms. In this study, the FFALF showed a sustained inhibitory effect on preconvulsive breathing and prolonged latent period of convulsion in the guinea pigs exposed to aerosolized histamine and acetylcholine. The results of the study suggest that the FFALF was significantly increased the time of occurrence of PCD via dilatation of bronchial smooth muscles. Again, FFALF showed a dose-dependent inhibitory effect on preconvulsive breathing in sensitized guinea pigs exposed to aerosolized spray in an enclosed chamber.

Mast cell degranulation plays a pivotal role in the pathogenesis of allergic disorders. Antigen challenge in sensitized animals results in degranulation of mast cells, which is an important feature of anaphylaxis. The binding of allergen to IgE results the release of inflammatory mediators such as histamine, eosinophils, neutrophils chemotactic factors, leukotrienes, prostaglandins, and platelet-activating factor; which are responsible development of airway inflammation and bronchoconstriction. In the present investigation, the disruption of mast cells was followed due to exposure of compound 48/80 (agent having histamine releasing capacity). In this study, FFALF showed significant protection against compound 48/80 induced mast cell degranulation at dose-dependent manner, which may prevent release of various inflammatory mediators. Mast cell stabilizing the activity of FFALF may be due to the suppression of IgE antibody production, which is responsible for degranulation of mast cells.

Histamine contracts the trachea-bronchial muscle of guinea pig, goat, horse, dog, and man. The antiasthmatic drugs act on contraction of trachea-bronchial muscle by multi-mechanisms including stimulation of β-adrenergic receptors, inhibition of histamine (H1) and muscarine receptors. In the present study of the isolated guinea pig tracheal chain preparation, there is right side shift of dose response curve of histamine in the presence of FFALF indicating antiasthmatic activity. Antihistaminic activity of FFALF may be possessed due to inhibition of H1 receptor or by stimulation of β-adrenergic receptor. Phytochemical screening of A. leptophyllum showed the presence of phenolic content, terpenoids, flavonoids, etc., Flavonoid or phenolic contents are reported to possess smooth muscle relaxant, bronchodilator and spasmylytic property whereas, terpenoids were responsible for spasmylytic property by relaxing tracheal tree of lungs. Hence, the antiasthmatic activity of FFALF may be due to the presence of the above constituents.

**Conclusion**

The results of this study suggested that the FFALF possesses antihistaminic and anticholinergic activity with moderately activity in mast cell protection. These pharmacological activities collectively constitute a significant prevention against asthma. However, further studies can be carried out to evaluate its exact mechanism of action and identify active flavonoid which is responsible for antiasthmatic activity.

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Conflicts of interest

There are no conflicts of interest.

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