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Bronchodilatory and mast cell stabilising activity of *Cressa cretica* L. : Evaluation through *in vivo* and *in vitro* experimental models

Priyashree Sunita[1*], S Jha[2], Shakti Prasad Pattanayak[3]

[*1]*Govt. Pharmacy Institute, Department of Health and Family Welfare, Govt. of Jharkhand, Ranchi- 834009, India*
[2]*Division of Advanced Pharmacognosy, Birla Institute of Technology, Mesra, Ranchi - 835 215, Jharkhand, India*
[3]*Division of Pharmacology and Toxicology, Birla Institute of Technology (BIT), Ranchi - 835 215, Jharkhand, India*

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ABSTRACT

Objective: To evaluate the effect of ethylacetate fraction (Fr-Et) and methanolic fraction (Fr-Me) obtained from *Cressa cretica* L.(*C. cretica*) L. on experimental models for bronchodilatory activity and mast cell stabilising activity. **Methods:** The effect of Fr-Et and Fr-Me were studied on acetylcholine and histamine aerosol-induced broncospasm using guinea pigs as experimental animals. Also, the effects of these fractions were evaluated on the isolated guinea pig tracheal preparations. Besides this mast cell degranulation effect was assessed using egg albumin and compound 48/80 on rat peritoneal mast cells. **Results:** Significant increase in preconvulsion time was observed due to pretreatment with the fractions when guinea pigs were exposed to histamine and acetylcholine aerosol. Fr-Et and Fr-Me significantly increased the preconvulsion in a dose depended manner that suggestive of bronchodilating activity. Fr-Et and Fr-Me exhibited a significant concentration dependant relaxant effect on guinea pig trachea pre-contracted with CCh, K^+ and histamine. The results revealed that Fr-Et to be more potent than Fr-Me in relaxing histamine and K^+ and calcium induced contraction than CCh induced contractions. Studies on the fractions in protecting mast cell degranulation, which were elicited by the egg albumin as well as synthetic compound 48/80 revealed both the fractions significantly protect the mast cell degranulation, which release mediators such as histamine and proinflammatory cytokines through various stimuli in a dose depended manner. **Conclusions:** Thus our study established the bronchodilator activity, and mast cell stabilizing activity which are important mediators that provoke or sustain in asthma.

1. Introduction

Asthma is a chronic disease, involving hypersensitivity reaction of the lower respiratory system producing obstruction of the airways[1]. It is a disease that does not discriminate race, age and gender. Asthma affect about 300 million people worldwide and it has been estimated that a further 100 million will be affect by 2025[2,3]. Asthma is a complex inflammatory disease cause's airway narrowing and associated with change in the level of mast cells, lymphocytes, cytokines and other inflammatory cell products. Despite the advancements in asthma therapy, modern medicines are far from satisfactory as they provide only symptomatic relief, produce several adverse effects and may lose effectiveness on continued use. Muscle tremor and hypokalemia are major adverse effects of β 2 agonists. Theophyline has narrow therapeutic index and requires monitoring of drug levels[4]. Adverse effects of corticosteroids include fluid retention, increased cell mass, increased appetite, weight gain, osteoporosis, capillary fragility, hypertension, peptic ulceration, diabetes, cataract, and phychosis. Hence Ayurveda has recommended number of drugs from indigenous plants sources for the treatment of bronchial asthma and other allergic disorders and have been successful in controlling the disease as well. Large numbers of medicinal plant preparations have been reported to possess anti-asthmatic effects.

Cressa cretica L. (Convolulaceae) (*C. cretica*), popularly known as 'Rudanti' in Hindi and is a widely grown halophytic plant. Different parts of the plant have been claimed to be valuable in a wide spectrum of diseases. In earlier studies *C. cretica* Linn flowers exhibited cytotoxic

*Corresponding author: Priyashree Sunita, Govt. Pharmacy Institute, Department of Health and Family Welfare, Govt. Of Jharkhand, Ranchi- 834009, India.
E-mail: priyashree_lenka@yahoo.com
Tel : +91651 2541387
Fax : +91651 2275209

and anti-inflammatory activity *in vitro*. *C. cretica* is reported to be antibilous, antituberculosis, expectorant, asthma and effective against pulmonary disorder[5]. The plant produced intrusion in testosterone production and affected spermatogenesis in male albino rats[6]. Previously our research team reported anti-tussive activity of the plant[7]. More recently we demonstrated anti-inflammatory and both *in vitro* and *in vivo* antioxidant activity of the plant[8, 9]. Shahat and his co workers yielded five flavonoids (quercetin, quercetin-3-O-glucoside, kaempferol- 3-O-rhamnoglucoside, and rutin) from the aerial parts of *C. cretica*[6]. It is also reported the fruits of *C. cretica* is a potential source of edible oil. The oil of *C. cretica* was free from any undesirable components and could safely be recommended for human consumption[10]. In addition the antiviral activity from the plant was also reported. Besides this the arial parts of the plant contains scopoletin, umbelliferone, isoflavone glycoside coumaranochrome glycoside. Syringaresinol glucoside and dicaffeoyl quinic acid were also isolated[6].

In present study, we examined the bronchodilatory activity and mast cell stabiling activity of the fractions of *C. cretica*, in order to justify the use of the plant in ethno medicinal use in asthma.

2. Materials and methods

2.1. Collection of plant material

C. cretica was collected from Nalban island of Chilika lake, Orissa, India and was preliminarily identified at the Deptartment of Natural Product, Institute of Mineral and Material Technology, (formerly known as Regional Research laboratory, Bhubaneswar) India and which was later on authenticated from Botanical Survey of India, Hawrah, West Bengal, India vide access no. (CNH/I-I/32/2010/Tech.II/237-3). A voucher specimen has been kept in our laboratory for future reference.

2.2. Preparation of fraction and phytochemical screening

The plants were air-dried, pulverized to a coarse powder in a mechanical grinder, passed through a 40-mesh sieve and extracted in a soxhlet extractor with methanol. The extract was decanted, filtered with Whatman No. 1 filter paper and concentrated at reduced pressure below 40 ℃ through rota-vapor to obtain dry extract (16.73%w/w). *C. cretica* methanolic extract (CME) was adsorbed on to the 250 g of silica gel of 60-120 mesh size and fractionated using solvents of increasing polarity such as hexane (Fr-He), ethylacetate (Fr-Et), and methanol (Fr-Me). The fractions were subjected for preliminary phytochemical screening to show the presence of steroid, alkaloid, glycoside, tannin, triterpenoid, carbohydrates reducing sugar, and fatty acids.

2.3. Animals

Guinea pigs of (either sex weighing between 300 - 500 g) and wistar albino rats (either sex, weighing between 160 - 220 g) were obtained from animal house, Birla Institute of Technology, Mesra, Ranchi, India and kept in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24 ± 2 ℃ and relative humidity of 60% -70 %. A 10:14 light: dark cycle was followed. All animals were allowed to free access to water and fed with standard commercial chaw pallets (M/s. Hindustan Lever Ltd, Mumbai). All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee (Reg. No: 621/02/ac/CPCSEA) and were in accordance with the guidelines of the CPCSEA, Ministry of Forests and Environment, Government of India.

2.4. Pharmacological studies

2.4.1. Studies on acetylcholine and histamine induced bronchospasm

Experimental bronchial asthma was induced in guinea pigs by exposing them to histamine and acetylcholine aerosol. Guinea pigs were selected and divided into ten groups each containing six animals out of which groups I-V, were exposed to 0.1% w/v of histamine dihydrochloride aerosol and another groups VI -X were exposed to 0.5% w/v of acetylcholine bromide aerosol. The animals exposed to histamine and acetylcholine aerosol showed progressive dyspnoea. The end point of preconvulsion dyspnoea (PCD) was determined from the time of aerosol exposure to the onset of dyspnoea leading to the appearance of convulsion. As soon as PCD commenced, the animals were removed from chamber and placed in fresh air. This time of PCD was taken as day 0 value. Guinea pigs of all groups were treated with Fr-Et (100 and 200 mg/kg. *p.o.*) and Fr-Me (100 and 200 mg/kg. *p.o.*), once a day for 7 days. On 7th day 2 h after the last dose, the time for the onset of PCD was recorded as on day 0. The percentage increased in time of PCD was calculated using following formula[11].

Percentage increased in time of PCD $=[1- T_1 / T_2] \times 100$

Where T_1 = time for PCD onset on day 0, T_2= time for PCD onset on day 7

2.4.2. Isolated guinea pig trachea preparation

The guinea pig was sacrificed by a sharp blow to the neck and quickly exsanguinated by carotid artery transaction. The trachea was removed and put into a petridish containing Krebs-Henseleit solution with the following composition (mM): NaCl 118.2, $NaHCO_3$ 25.0, $CaCL_2$ 2.5, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2 and glucose 11.1 (pH 7.4) pre heated at 37 ℃. It was carefully cleaned of adherent fat and connective tissue, and then cut into rings, 2-3 mm wide. Each ring was opened by a longitudinal cut on the ventral side opposite to the smooth muscle layer, forming a tracheal strip with a central part of smooth muscle in between the cartilaginous portion on the edges. A tension of 1g was applied to each of the tracheal strips and was kept constant throughout the experiment[12].

2.4.3. Effect of different fractions on the histamine and acetylcholine pre-contracted guinea pig trachea

Test fractions were dissolved in dimethyl sulphoxide (DMSO). After equilibration, the strips were contracted with histamine (10^{-6} M), acetylcholine (1^{-6} M). Then their sustained contractions were obtained and relaxant effect of the fractions and standard drugs were assayed by adding in a cumulative fashion. The percentages of relaxation provoked by each concentration were calculated and its IC_{50}

was calculated. Standard antihistaminic and anticholinergic drug (mepyramine and atropine), respectively were used as positive control[13].

2.4.4. Effect of different fractions on KCl pre-contracted guinea pig trachea

After equilibration and stimulation of the organ, it was contracted with 80 mM KCl. Their sustained contractions were obtained and relaxant effect of the fractions and control drugs were assayed by adding in a cumulative fashion. The percentages of relaxation provoked by each concentration were calculated and its IC_{50} was calculated. Verapramil, was used as a positive control[13].

2.4.5 Studies on compound 48/80 and egg albumin induced rat peritoneal mast cell degranulation

Normal saline containing 5 units/mL of heparin was injected in the peritoneal cavity of male rats lightly anaesthetized with ether. After a gentle abdominal message, the peritoneal fluid containing mast cells was collected in centrifuge tubes placed over ice. Peritoneal fluid of 8 - 10 rats was collected, pooled and centrifuged at 2 000 rpm for 5 min. Supernatant solution was discarded and the cells were washed twice with saline and resuspended in 1 mL of saline. 0.1 mL of the peritoneal cell suspension was transferred to 9 test tubes and were treated as follows:

Test tube no. 1 & 2: Saline
Test tube no. 3: 0.1 mL of 0.5 mg/mL Fr-Et in saline
Test tube no. 4: 0.1mL of 1.0 mg/mL Fr-Et in saline
Test tube no. 5: 0.1mL of 2.0 mg/mL Fr-Et in saline
Test tube no. 6: 0.1 mL of 0.5 mg/mL Fr-Me in saline
Test tube no. 7: 0.1 ml of 1.0 mg/ml Fr-Me in saline
Test tube no. 8: 0.1 ml of 2.0 mg/ml Fr-Me in saline
Test tube no. 9: 0.1 ml of 10 μg/ml of Ketotifen fumarate

Each test tube was incubated for 15 min at 37 ℃ and then compound 48/80 (0.1 mL, 10 μg/mL) was added to each test tube except test tube no. 1 and 2. After further incubation for 10 min. at 37 ℃, the cells were stained with 0.1% toluidine blue solution made in distilled water and examined under the high power of light microscope. Percentage protection of the mast cells in the control group and the treated groups were calculated by counting the number of degranulated mast cells from total of at least 100 mast cells counted.

In another study, rats were sensitized by administering three doses of 350 μg of egg albumin adsorbed on 60 mg of aluminum hydroxide gel, the doses being given on the first, third and fifth day subcutaneously. The mast cells were collected on the tenth day of sensitization. The study was conducted in the same manner as above and the sensitized cells were degranulated using egg albumin (1 mg/mL). Percentage protection of the mast cells in the control group and the treated groups were calculated by counting the number of degranulated mast cells from total of at least 100 mast cells counted. Control group was consisted of positive control group in which egg albumin was added without addition of test agent and a negative control group in which neither egg albumin nor the test agent was added to correct the spontaneous degranulation of mast cells without any degranulating agent[14].

2.5. Statistical analysis

Data expressed are mean ± standard error of mean (SEM, n = number of experiment) and the minimum inhibitory concentrations (EC_{50}) with 95% confidence intervals (CI). Pharmacological data were analysed by one-way analysis of variance (ANOVA) followed by Dunnett's *t* test for comparison with equal sample size. The difference was considered significant when P value < 0.05. All the values were expressed as mean ± standard error mean (S.E.M.). Preconvulsion dyspnoea time was analyzed through student's paired t test.

3. Result

3.1. Effect of Fr-Et and Fr-Me on acetylcholine and histamine induced bronchospasm

Significant (P<0.05) in preconvulsion time was observed due to treatment with Fr-Et and Fr-Me (100 and 200 mg/kg *p.o.*), when the guinea pigs were exposed to either histamine (0.1%) or acetylcholine (0.5%) aerosol. Fr-Et at the doses of 100 and 200 mg/kg *p.o.* protected histamine induced bronchospasm animals by 41.47% and 56.32% respectively at the same time Fr-Me can protect 23.83% and 32.79% at the dose levels of 100 and 200 mg/kg respectively. Fr-Et at the doses of 100 and 200 mg/kg *p.o.* protected acetylcholine induced bronchospasm animals by 30.89% and 37.58% where as Fr-Me at the doses of 100 and 200 mg/kg *p.o.* 21.53% and 9.12% respectively. The increase in preconvulsion time was comparable to that of ketotifen(1 mg/kg *p.o.*), a standard antihistaminic drug and atropine (2 mg/kg *p.o.*), a standard anticholinergic drug and were found to protect the bronchospasm induced by acetylcholine and histamine by 66.15% and 68.41 % protection respectively. The data are shown in Table 1.

3.2. Effect of Fr-Et and Fr-Me on guinea pig trachea

Both fractions Fr-Et and Fr-Me were first tested on the resting baseline of the tissue. The fractions were found devoid of any contractile effect up to the dose of 10 mg/mL. In tracheal preparations, Fr-Et and Fr-Me caused inhibition of CCh (1 μM)-induced contractions at low concentration with IC_{50} value of 6.493 mg/mL (2.517 - 16.749, 95% Cl, n=4) and 11.507 mg/ml (3.331-39.755, 95% Cl, n=3) respectively (Figure 1A). Compared to that against K^+ (80 mM)-induced contractions with IC_{50} values of Fr-Et and Fr-Me were found to be 0.128 mg/mL (0.094 - 0.174, 95% Cl, n=3) and 0.687 mg/mL (0.210 - 2.246, 95% Cl, n=3) respectively, (Figure 1A and 1B). Verapramil was more potent against K^+ induced contractions with IC_{50} value of 0.018 μM (0.0089-0.367, 95% Cl, n=3), when compared with CCh-induced contractions with IC_{50} value 0.272 μM (0.175-0.421, 95%Cl, n=3) as shown in Figure 1C. Atropine relaxed the CCh (1 μM) induced contraction potentially with IC_{50} value of 0.003 μM (0.0015-0.0062, 95% CL, n=3), without any effect on K^+ (80mM) induced contractions. (Figure 1D).

Fr-Et and Fr-Me also caused inhibition of histamine (1 μM) induced contraction (Figure 2A and 2B) respectively. IC_{50} of Fr-Et and Fr-Me against histamine induced tracheal contraction were found to be 0.328 mg/mL (0.141-0.765, 95%Cl, n=3) and 0.686 mg/mL (0.529-0.889, 95% Cl, n=3) respectively, when compared to the standard mepyramine

with IC_{50} value of 0.0159 μM (0.0054–0.0463, 95% CI, n=3) (Figure 2C).

3.3. Effect of Fr–Et and Fr–Me on compound 48/80 and egg albumin induced rat peritoneal mast cell degranulation

Compound 48/80 (10 μg/mL) produced disruption of mast cells which was significantly inhibited in a dose–dependent manner by the treatment with Fr–Et and Fr–Me in concentrations 0.5–2.0 mg/mL, each (Table 2). Egg albumin (1 mg/mL)–induced rat mast cell degranulation was significantly inhibited by the treatment with Fr–Et and Fr–Me at concentrations 0.5–2.0 mg/mL, each. The protection was comparable to reference standard ketotifen (10 μg/mL) (Table 3).

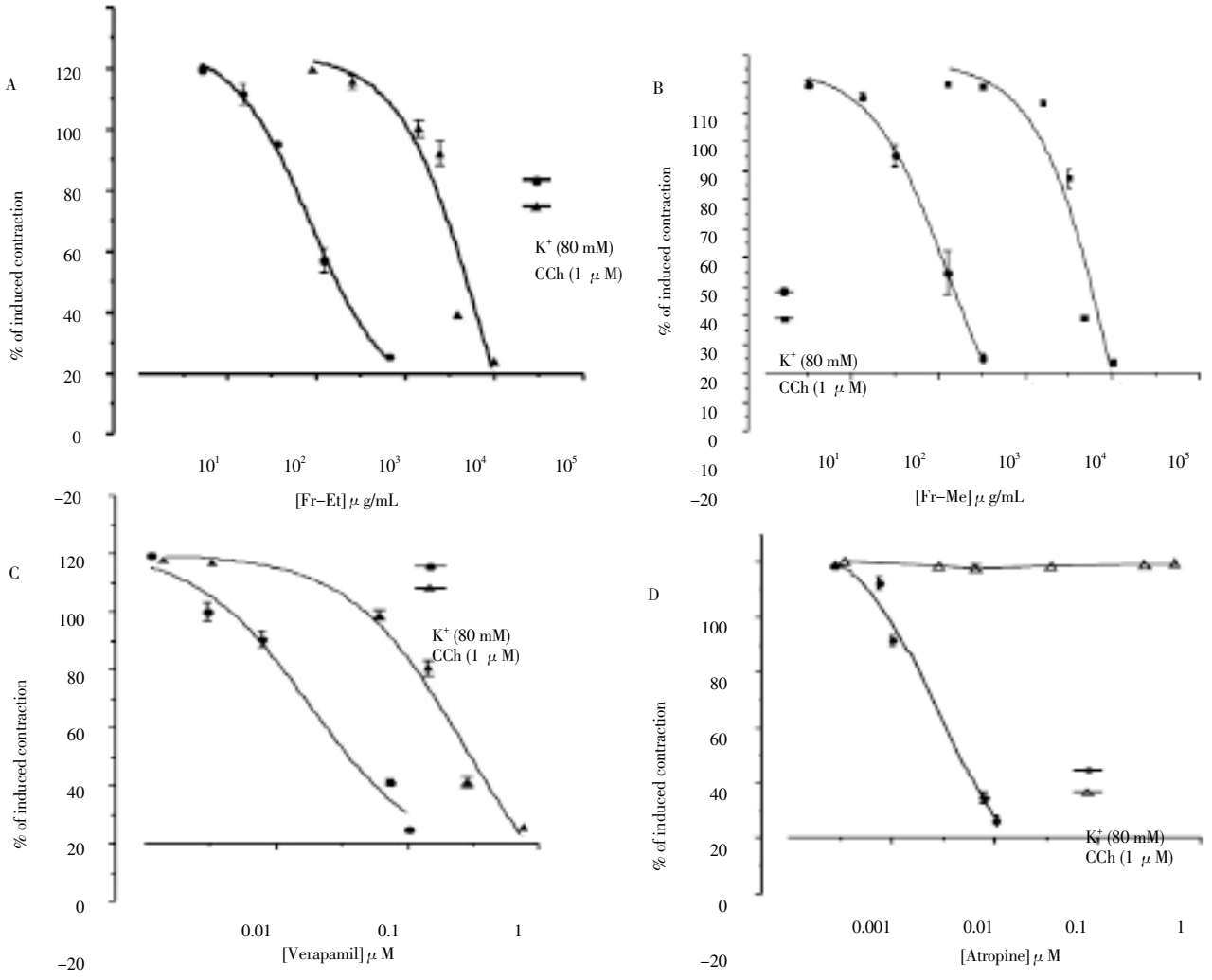

Figure 1. Concentration–response curve showing the relaxant effect of Fr–Et (A), Fr–Me (B), verapamil (C) and atropine (D) on K^+ (80 mM) and carbachol (CCh, 1 μM)–induced contractions in isolated guinea–pig tracheal preparations (values shown are expressed as mean ± SEM, n=3).

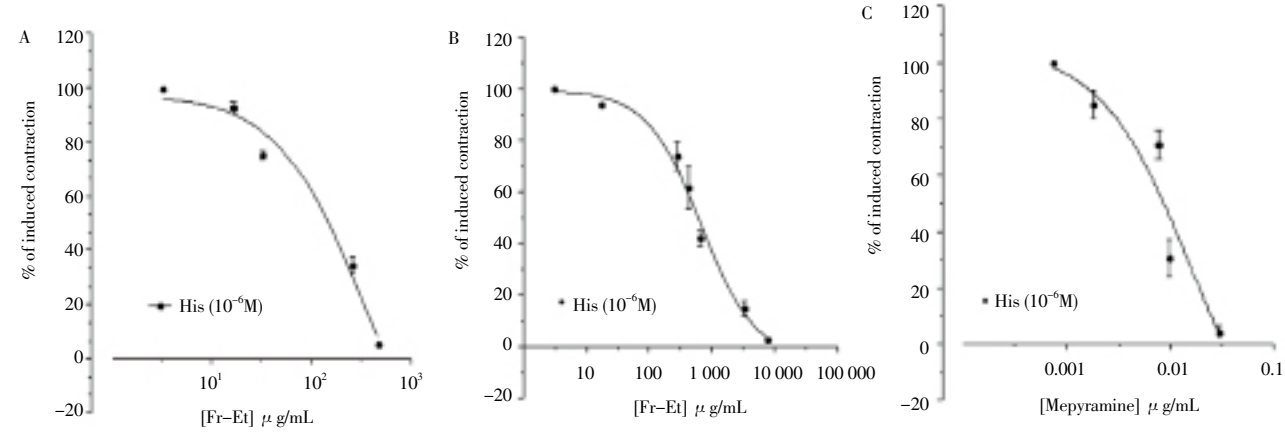

Figure 2. Concentration–response curve showing the relaxant effect of Fr–Et (A), Fr–Me (B), mepyramine (C) on histamine (His, 10–6M)–induced contractions in isolated guinea–pig tracheal preparations (values shown are expressed as mean ± SEM, n=3).

Table 1

Effect of *C. cretica* fractions (Fr-Me and Fr-Et) (p.o., for 7 days) on histamine and acetylcholine aerosol induced bronchospasm.

Treatment(mg/kg)		Preconvulsion dyspnoea (PCD) time (sec)		
		Before Treatment	After treatment	% increase in the time of PCD
Histamine aerosol (0.1% w/v)	Fr-Me (100)	112.80±7.35	148.40±6.15*	23.83±0.17
	Fr-Me (200)	122.40±8.12	182.75±7.11**	32.79±0.15
	Fr-Et (100)	127.30±6.79	217.80±9.34**	41.47±0.11
	Fr-Et (200)	125.40±4.31	291.70±5.12**	56.32±0.71
	Ketotifen (1)	115.60±2.34	349.50±7.19**	66.15±0.79
Acetylcholine aerosol (0.5% w/v)	Fr-Me (100)	129.50±8.26	143.29±3.56*	9.12±0.54
	Fr-Me (200)	131.40±6.15	168.50±5.69*	21.53±0.49
	Fr-Et (100)	126.70±4.79	183.40±7.34**	30.89±0.26
	Fr-Et (200)	129.80±7.29	208.90±2.95**	37.58±0.29
	Atropine (2)	128.60±3.52	409.70±3.68**	68.41±0.22

Values are expressed as mean±S.E.M. for six animals; Significantly *P<0.01; **P<0.001: statistically different (student's paired t-test was applied for comparison) (n=6)

Table 2

Effect of *C. cretica* fractions (Fr-Me and Fr-Et) on compound 48/80 induced rat rat peritoneal mast cell degranulation.

Treatment	Concentration(mg/mL)	% Mast cells degranulation±S.E.M.	% Inhibition of degranulation
Negative control	-	1.12±0.34	-
Positive control	-	79.85±0.46	-
Fr-Me	0.50	58.12±0.72*	27.21
Fr-Me	1.00	44.19±0.63*	44.65
Fr-Me	2.00	38.15±0.29**	52.22
Fr-Et	0.50	41.25±1.24*	48.34
Fr-Et	1.00	29.82±0.43**	62.65
Fr-Et	2.00	21.05±0.13**	73.63
Ketotifen	0.01	17.25±0.34**	78.39

Values are expressed as mean±S.E.M. Comparison: All treated (both test and standard) groups with positive control group. Significantly * P<0.01; **P<0.001. Data were analysed by one-way analysis variance (ANOVA) followed by Dunnett's t- test (n=6).

Table 3

Effect of *C. cretica* fractions (Fr-Me and Fr-Et) on egg albumin induced rat peritoneal mast cell degranulation.

Treatment	Concentration(mg/mL)	% Mast cells degranulation±S.E.M.	% inhibition of degranulation
Negative control	-	1.31±0.82	-
Positive control	-	81.23±0.45	-
Fr-Me	0.50	62.12±1.02*	23.52
Fr-Me	1.00	45.34±0.89**	44.18
Fr-Me	2.00	34.19±0.38**	57.90
Fr-Et	0.50	49.15±0.92**	39.49
Fr-Et	1.00	31.11±0.71**	61.70
Fr-Et	2.00	24.77±0.31**	69.50
Ketotifen	0.01	16.85±0.46	79.25

Values are expressed as mean±S.E.M. Comparison: All treated (both test and standard) groups with positive control group. Significantly * P<0.01; **P<0.001. Data were analysed by one-way analysis of variance (ANOVA) followed by Dunnet's t- test (n=6).

4. Discussion

Asthma, the atopic disease with the greatest clinical and economic effect[15] is an allergic and inflammatory outward sign of the respiratory disorders. It is essentially characterised by the restriction of tracheal muscles obstructing the air circulation, but upstream it is initiated by the very complicated immune process characterized by mucus secretion and influx of a variety of inflammatory cells. The partial or total reversibility of air flow obstruction in some patients indicates that the structural remodelling of the airway may occur over time. All drugs that are able to counteract the airflow obstruction could be also used to treat the pathophysiology of asthma, namely bronchodilator agents such as phosphodieterase inhibitors, antihistaminic[16] and anti-cholinergic agents, anti-inflammatory agents and calcium channel blockers[17]. In the present study, histamine and acetylcholine were used as spasmogens in the form of aerosol to cause immediate bronchoconstriction in the form of PCD in guinea pigs. Bronchodilating effect of Fr-Me and Fr-Et were evaluated by observing its effect on the time of PCD. Fr-Me and Fr-Et significantly increased in a dose depended manner that suggestive of bronchodilating activity.

The Fr-Et and Fr-Me exhibited a significant concentration dependant relaxant effect on guinea pig trachea pre-

contracted with CCh, K^+ and histamine. Fr-Et was more potent in relaxing histamine and K^+ and calcium induced contractions than CCh induced contractions in isolated guinea pig trachea preparation than Fr-Me. Its potency in relaxing the trachea is comparatively more than Fr-Me. The induced contraction shown by K^+ (80mM) is by virtue of Ca^{2+} influx into the cells from the extracellular spaces. Ca^{2+} channel blockers tend to inhibit this indced contractions as a result of their inhibitory action at the Ca^{2+} channels[18]. Similarly verapramil, a standard Ca^{2+} channel blocker also relaxed the K^+ induced contraction with specificity. Histamine and CCh are the most implicated mediators of brochoconstriction that accompanying asthma[19]. Smooth muscles contraction, in response to agonists like histamine or acetylcholine is initiated by the increase of the cytosolic-free Ca^{2+} concentration ($[ca^{2+}]_i$). These agonists, by activating phospholipase C produce inositol 1,4,5-triphosphate (IP3) which in its turn stimulates sarcoplasmic reticulum (SR) to release the internal Ca^{2+} stores[20]. This released Ca^{2+} stimulates the entrance of the external Ca^{2+} by the Ca^{2+}-induced Ca^{2+} release pathway[21, 22]. The present experimental results suggested that the inhibitory effect of the Fr-Et is mediated possibly through a combined blockade of Ca^{2+} and histaminic receptors.

In addition to bronchodilating activity, a significant number of therapeutic approaches for bronchial asthma have been designed based on the antagonism of specific mediators released from mast cells. Mast cell degranulation is important in the initiation of immediate responses following exposure to allergens. Degranulated cells liberate mediators of inflammation such as histamine, leukotrienes, platelet activating factors and chemotactic factors for eosinophils, neutrophils etc. from mast cells[23]. They play a significant role in airway inflammatory response such as airway eosinophilia, late asthmatic response and airway hyperresponsiveness as well as in immediate hypersensitivity reaction like bronchial contraction. Degranulation of mast cells has been taken as the criteria of positive anaphylaxis. Ketotifen, a well-known mast cell stabilizer, reduces synthesis of prostaglandins E2, thromboxane A2, leukotriene C4 and B4. It also inhibits release of histamine, serotonin and other inflammatory mediators from mast cells. Simultaneously it blocks H1 receptors. Mast cell degranulation can be elicited by the synthetic compound 48/80, and it has been used as a direct and convenient reagent to study the mechanism of anaphylaxis[24]. Numerous reports established that stimulation with compound 48/80 or IgE initiates the activation of signal transduction pathway which leads to histamine release. Several recent studies shown that compound 48/80 and other polybasic compounds directly activate G-proteins[25]. Compound 48/80 increases the permeability of the lipid bilayer membrane by causing the perturbation in the membrane. The intracellular calcium pathways are crucial to the degranulation of mast cells. Agents that stimulate an intracellular calcium level have been shown to induce mast cell degranulation[26]. Calcium movements in mast cells represent a major target for effective antiallergic drugs, as this is an essential event linking stimulation to secretion. Most of the studies of plant extracts and flavonoids as an antiallergic agents showed that attenuation of compound 48/80 induced intracellular calcium in mast cells was strongly speculated that decreased intracellular calcium involved in the inhibitory effect of histamine release and might have membrane stabilizing activity through inhibition of G-protein activation[27]. A significant protection of rat peritoneal mast cells from disruption by antigen and compound 48/80 by the fractions of *C. cretica* (Fr-Et and Fr-Me) points towards its ability to interfere the release and/or synthesis of mediators of inflammation, indicating its mast cell stabilizing activity in a dose depended manner.

Egg albumin is an inciting allergen which induces the release of mediators such as histamine and proinflammatory cytokines and can be elicited by various stimuli. The degranulation of mast cell occur in response to the immunological stimuli in which antigen and antibody reactions are predominant[28, 29]. The effect of Fr-Me and Fr-Et on egg albumin induced degranulation of mast cell showed significant protection of mast cell from degranulation.

To this end the fraction (Fr-Et) led to the isolation of the phytoconstituents. From that we isolated1,2benzopyrone(coumarin),7hydroxycoumarin(umbelliferone),7,8dihydroxycoumarin (daphnetin), 3,5,7,3′,4′pentahydroxy flavonoid(quercetin) and 3,5,7,4′ tetrahydroxy flavonoid(kaempferol). Quercetin-3-O-glucoside and Quercetin-3-O-glucorhamnopyranoside along with some steroidal moieties were isolated from Fr-Me (data not shown). It can be suggested that coumarin may stimulate the enzyme endothelium NO synthase (eNOS) and provokes the production of NO. It is reported that endogenous NO stimulates soluble guanylyl cyclase, which in turn generates intracellular cGMP and causes airway smooth muscle relaxation[30]. However, a bronchodilator activity of coumarin and simple derivatives may also be mediated by another mechanism, probably the redistribution of the internal calcium which may decrease the cytoplasm calcium concentration and subsequently provokes a broncho-dilation[31]. In the present study, we found also that Fr-Et had in vivo protective effect against histamine inhalation, and this is in line with a previous study in which protective anti-allergic effects of coumarin in experimental asthma was reported. Although coumarin itself was reported to have a low bioavailability in man due to extensive first-pass hepatic conversion to 7-hydroxycoumarin (umbelliferone) followed by glucuronidation[32], these metabolites might explain the observed lasting effect of *C. cretica* in the management of asthma. Moreover in Fr-Me on isolation yielded different flavonoids and steroids and that are reported to be antiallergenic and antihistaminic. Several flavonoids have been shown to possess smooth muscle relaxant and bronchodilator activity[33]. The flavonoids were known to inhibit basophil histamine release and neutrophil β-glucuronidase release, and there by possess in vivo antiallergic activity[34-36]. These flavonoids also inhibited the histamine release induced by compound 48/80. The potentiality of the plant as antitussive activity, anti-inflammatory activity and antioxidant activity along with the present findings as a bronchorelaxant and mast cell stabilizing property suggests the therapeutic efficacy of the plant in treating asthma.

Hence the plant warrants further investigations for detailed study. Our research team is engaged in identifying the presence of other constituents and the relative concentration of each constituents present in the plant and their probable mechanism of action.

Conflict of interest statement

We declare that we have no conflict of interest

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