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Bronchodilatory effect of *Myxopyrum serratulum* in animal model

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Abstract

The plant *Myxopyrum serratulum* is traditionally claimed to relieve asthma and cough. The present study was undertaken to evaluate the bronchodilatory effect of the methanolic extract of *M. serratulum* on histamine-induced bronchospasm by *in vivo* and the inhibitory effect of the extract on histamine-contracted tracheal chain and ileum by *in vitro* guinea pig model. Additionally, the relaxant effect of four cumulative concentrations of the extract (0.25, 0.5, 0.7 and 1.0 g%) was assessed using precontracted tracheal chain under different conditions. The extract (400 mg/kg) prolonged the preconvulsive time to 102.3 ± 3.8 sec when compared to saline and standard chlorpheniramine maleate as 121.3 ± 4.5 sec ($p<0.05$). The extract also possessed significant inhibitory effect on histamine-contracted guinea pig ileum and tracheal chain and also exhibited significant relaxation effect on precontracted tracheal chain of guinea pig models contracted by 60 mM KCl ($p<0.001$) and 10 µM methacholine ($p<0.001$) when compared with standard theophylline.

Introduction

Bronchial asthma is a chronic inflammatory disease of the airway. Infiltration of various inflammatory cells such as eosinophil, macrophages and lymphocytes into the airway causes asthma and bronchitis (Patel et al., 2013).

Bronchodilator drugs works by rapid reversal of the airway obstruction in asthmatics by directly acting on airway smooth muscle (Church and Hiroi, 1987). Drugs derived from natural sources are generally considered no adverse effects when compared to synthetic drugs, therefore, it might serve as better alternative for many chronic diseases such as cancer, infections and endemic diseases like asthma, bronchitis and many others (WHO, 2000). Plant extract and secondary metabolites derived from plant can directly influence the production and activation of inflammatory mediators, second-dary messengers and the expression of transcription factors (Calixto et al., 2004).

Myxopyrum serratulum belongs to large shrub mainly found in Kerala at a altitude of about 600-900 m. It is commonly known as chaturamulla and traditionally, dried and powdered leaves of the plant was mixed with ghee as a remedy for asthma, cough and nerves complaints apart from which they were also used for the treatment of fever, headache and ear diseases (Wealth of India, 2011).

According to earlier reports, the plant possesses anti-inflammatory, antiarthritic (Sheelarani et al., 2013), anti-oxidant (Sheelarani et al., 2013), antipyretic (Vanughese et al., 2015), wound healing (Gopalakrishnan and Rajameena, 2013) and antimicrobial (Gopalakrishnan et al., 2012) properties.

The phytochemical studies on *M. serratulum* revealed

the presence of flavonoids, saponins, terpenoids, carbohydrates, ursolic acid (Sudharmini and Ashalatha, 2008) and myxopyroside, its 6-o-acetyl-7-o-(E/Z)-p-methoxycinnamoyl esters (2/3) of dimethyl forsythide (Franzyk et al., 2001). There was no scientific report found regarding the bronchodilatory activity of *M. serratulum* though it has been used for allergy and inflammation. Hence, the present study was mainly focused on the evaluation of its bronchodilatory activity both *in vivo* and *in vitro* guinea pig model.

Materials and Methods

Plant collection and extract preparation

The leaves of *M. serratulum* were collected from the Western Ghats of Kerala, India in the month of September 2013, and identified by Dr. V. Chelladurai, Government Siddha Medical College, Palayamkottai, Tamilnadu, India. A voucher specimen (MS-0713/BIT) of the leaves were preserved and stored at the Department of Pharmaceutical Technology, Anna University, Tiruchirappalli, India. The dried leaves were coarsely powdered and then extracted with methanol by hot extraction method. The extract was concentrated under reduced pressure and finally viscous and dense dark green color extract was obtained with yield percentage of 22.2% w/w and stored at 4°C for further studies.

Chemicals

Histamine, methacholine, chlorpheniramine maleate, propranolol hydrochloride and theophylline were procured from the Sigma–Aldrich (Germany). All the chemicals and solvent used for the preparation of physiological salt solution and extract preparation were of analytical grade and were obtained from the Merck, Germany. Ultrapure water was used for the experiments.

Animals

Male Hartley strain guinea-pigs of 300-400 g weight and female Balb/c mice weighing 25–30 g were housed at animal house of the Bharathidasan Institute of Technology, Anna University, Tiruchirappalli, India. All the experimental animals were maintained in standard conditions with room temperature of 23-25°C and humidity of 50-60%. All the animals were given clean water *ad libitum* and standard food.

Acute toxicity test

Female Balb/c mice weighing 25–30 g were divided into 5 groups comprising of 5 mice each. The test was performed using various doses of the extract (10, 50, 300 and 2,000 mg/kg) at 10 mL/kg volume were administered orally. Another group of mice that received saline (10 mL/kg, p.o.) alone was considered as negative control. All the experimental mice models were maintained in a standard condition as mentioned above and were periodically observed for physiological parameters that includes mortality, morbidity, salivation, diarrhea, convulsions, tremors, lachrymal secretion, hair erection and loss of appetite.

In vivo bronchodilating activity

Experimentally, bronchial asthma was induced by exposing the guinea pigs to 0.5% histamine aerosol using an ultrasound nebulizer in aerosol chamber (24 x 14 x 24 cm^3) made of perspex glass. The time required for the appearance of preconvulsive dyspnoea caused by histamine was noted for each animal. The pre-convulsion time i.e. the duration of aerosol exposure for the onset of respiratory distress leading to appearance of convulsion was noted (Liu et al., 2015). The animals were removed from the perspex container and allowed to recover from the respiratory distress by exposing to fresh air for 24 hours. After 24 hours, the animals were grouped into five (n = 6), among which the animals of Group I received oral dosage of vehicle (0.95% NaCl solution); Group II received standard chlorpheniramine maleate at a dosage of 100 mg/kg; Group III, IV and V received 100, 200 and 400 mg/kg oral dose of the extract, respectively. After the administration of the vehicle, standard and test drugs, the pre-convulsion time was reassessed after 1^{st} and 4^{th} hours, and the percentage increase in pre-convulsion time was calculated as per the prescribed formula (Sheth et al., 1972).

Guinea-pig ileum preparation

The ileum was dissected out and segments of approximately 2 cm length were kept individually in a 10 mL organ bath filled with Tyrode's solution and aerated with oxygen at 37°C. A preload of 1 g tension was applied to each tissue and kept constant throughout the experiment. Following an equilibration period of 30 min, isotonic contractions to histamine (1 - 32 µM) were repeated until constant responses were obtained. The inhibitory effect of the extract (100 µg/mL) and chlorpheniramine (10 µg/mL) was determined on the resting baseline of the tissue and was assessed as percentage of the maximum effect produced by histamine (Choo and Mitchelson, 1978).

Tracheal chain preparation

Male Hartley guinea pigs (300-400 g) were killed by imposing a blow on the neck followed by the removal of tracheas. Each trachea was cut into 10 rings (each containing 2-3 cartilaginous rings). The cartilages of all rings were then cut open opposite to the tracheal muscle and sutured together to form a tracheal chain (Martin et al., 1994). The tracheal chain was then suspended in a 10 mL organ bath containing Krebs-Henseliet solution of the following composition (mM): NaCl 120, $NaHCO_3$ 25, $MgSO_4$ 0.5, KH_2PO_4 1.2, KCl 4.7, $CaCl_2$ 2.5 and dextrose 11. The Krebs solution was kept at 37°C under stream of 95% O_2 and 5% CO_2 gases.

Tissue was suspended under an isotonic tension of 1 g and allowed to equilibrate for at least 1 hour while it was washed with Krebs solution every 15 min.

Protocols

The inhibitory effect of the extract on histamine H_1-receptors was examined by producing a cumulative log concentration–response curve of histamine-induced contraction of tracheal chains 10 min after exposing tissue to extract (100 µg/mL) and 0.3 mL saline. The consecutive concentrations of histamine were added every 2 min (1 - 32 µM) and the percentage of contraction, due to each concentration in proportion to the maximum contraction obtained in the presence of saline, was plotted against the log concentration of histamine (Boskabady and Shaikhi, 2000).

In addition, the relaxant effects of four cumulative concentrations of the extract (0.25, 0.5, 0.75 and 1.0 g/100 mL), four cumulative concentrations of theophylline (0.25, 0.5, 0.75 and 1.0 mM; positive control) and saline (1.0 mL; negative control) were also examined. The above mentioned agents of specified volumes were added to 10 mL organ bath and the contracted tracheal tissues were incubated into the bath for 5 min. The post incubation, effect of the extract, theophylline and saline at different concentrations were determined. A decrease in tone was considered as relaxant (bronchodilatory) effect and expressed as positive percentage change in proportion to the maximum contraction, whereas, an increase in tone was considered as contractile (bronchoconstrictory) effect, expressed as negative percentage change (Holroyde, 1986). The relaxant effect of the extract, standard and control were evaluated with three different experimental designs as follows: a) On tracheal chains contracted by 60 mM KCl (Group I experiments); b) On non-incubated tracheal chains contracted by 10 µM methacholine hydrochloride (Group II experiments); c) On tracheal chains incubated with 1 µM propranolol hydrochloride for 30 min prior to beginning and during the evaluation of relaxation effect of different solutions. In this series of experiments tracheal chains were also contracted by 10 µM methacholine hydrochloride (Group III experiments).

The relaxant effect of theophylline was examined only on Groups I and II. Separate tracheal chains were used for all the experiments. In all experiments, responses were recorded on a kymograph and were measured after fixation. The data were expressed as mean ± standard error of the mean (SEM., n = number of experiments) and one-way analysis of variance (ANOVA) followed by Dunnett's test. $p<0.05$ is considered as significant.

Results

Acute toxicity study

It was observed that even after 14 days post administration of 2 g/kg of extract, the experimental animals did not exhibit mortality, which rules out any possibility of toxicity in the *M. serratulum*.

In vivo bronchodilating activity

Chlorpheniramine (100 mg/kg) prolonged the preconvulsive time to 121.3 ± 4.6 sec and the percentage protection was 75.3 ± 4.6 (Figure 1). Similarly, the test extract was also observed to possesses bronchodilatory activity by prolonging the preconvulsive time to 59.3 ± 3.0 sec and 102.3 ± 3.9 sec at 100 mg/kg and 400 mg/kg respectively, for which the maximum percentage protection was 68.7 ± 1.6 at higher concentration. In all the groups no significant differences were observed in preconvulsive time of guinea pigs before the drug administration. The results clearly indicated that significant difference was observed in groups treated with test extract at 400 mg/kg ($p<0.05$) and chlorpheniramine at 100 mg/kg ($p<0.05$) compared to that of control (a significant increase in preconvulsive time was also observed when compared with the results recorded before administration of the test agents).

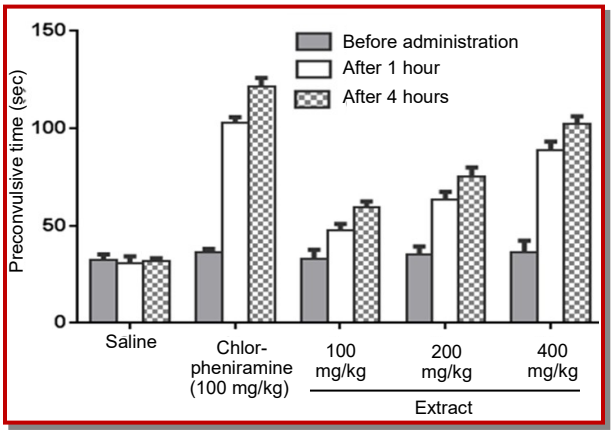

Figure 1: Effects of leaves of *M. serratulum* on the preconvulsive time of guinea pigs challenged with the solution of 0.5% histamine

Values are expressed as mean S.E.M. (n=6), $p< 0.05$, compared with the data of negative control, after drug administration $p< 0.05$ compared to before drug administration

Effect on isolated guinea pig ileum

There was inhibitory effect of the extract on histamine-induced contraction on guinea pig ileum (Figure 2). The inhibition of %response of the extract (100 µg/mL) was 19.7 ± 1.1, 33.0 ± 1.7, 41.4 ± 1.9, 42.1 ± 2.1, 58.0 ± 1.8 and 68.2 ± 1.6 at the respective concentration of 1, 2, 4, 8, 16 and 32 µg/mL of histamine when compared with the effect of chlorpheniramine (10 µg/mL) ($p<0.001$).

Effect on isolated guinea pig trachea

The relaxant efficiency of the extract (100 µg/mL) on histamine induced pre-contracted tracheal chain were as shown in Figure 3, which clearly indicates that the

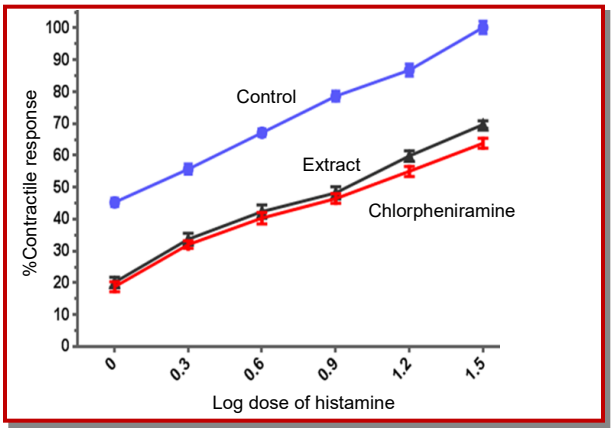

Figure 2: Log dose-response curve obtained after incubation of cumulative concentration of histamine (1 µg/mL - 32 µg/mL) in isolated guinea pig ileum preparations in the presence and absence of *M. serratulum* (100 µg/mL) and chlopheniramine. Values represent mean ± SEM, n = 6

Figure 3: Log dose-response curve obtained after incubation of cumulative concentration of histamine (1 µg/mL - 32 µg/mL) in isolated guinea pig tracheal preparations in the presence and absence of *M. serratulum* (100 µg/mL). Values represent mean ± SEM, n = 6

plant extract ($p<0.001$) significantly reduced the maximum contractile response of histamine on guinea pig tracheal chain. These above findings are also clearly stated that the extract had possess a significant bronchodilatory effect through H_1-receptor antagonistic activity as similar that of chlorpheniramine.

Relaxant effect on isolated guinea pig trachea

Theophylline and the extract (Group I) showed potent relaxant effect on precontracted guinea pig tracheal chain in concentration-dependant manner, and at higher concentration, the percentage maximum relaxant effect observed for extract and theophylline were 91.6 ± 3.2 and 88.7 ± 2.6 respectively. At all the concentrations, the relaxant effect of theophylline and test extract were significantly higher than those of the control group that received saline only (Table I).

Both the extract and theophylline (Group II) exhibited potent relaxant effect in the precontracted guinea pig tracheal chain with methacholine. At lower concentration, both theophylline and extract showed less relaxant effect and at higher concentrations the percentage relaxant effect was found to be 101.6 ± 2.3 and 92.2 ± 1.6 for test extract and theophylline respectively. In both Group 1 and 2 experiment, the extract exhibited potent relaxant effect in the dose dependant manner than the standard drug theophylline.

In Group III experiment, the extract of *M. serratulum* exhibited weak relaxant effect at all concentration when compared with saline. The relaxant effect of the extract was higher than theophylline at all concentration in Groups I and II experiments. There were positive correlations between increasing concentrations and the relaxant effects of extract in Groups I and II experiments.

Discussion

The results seem consistent with the earlier findings, that the chloroform extract of *Cynodan dactylon* exhibited bronchodilator effect by increasing preconvulsive time against acetylcholine induced bronchospasm in a dose-dependant manner at 5, 10, 50, 100 mg/kg but no significant protection against histamine-induced bronchospasm (Patel et al., 2013). In earlier studies, 70% ethanolic extract of *Elaeagnus pungens* increased preconvulsive time on combination of 0.1% histamine and 2% acetylcholine-induced bronchospasm at the dose of 1.4 g/kg after 5 days of administration (Ge et al., 2009). The protective effect of bronchodilator was demonstrated by pre-dosing of the extract, increases the pre-

Table I

Relaxant effect of extracts in tracheal chain contracted by high potassium and methacholine

Concentration of extract	High K^+		Methacholine		Propranolol and
	Extract	Theophylline	Extract	Theophylline	Extract
0.25	19.9 ± 2.1	12.3 ± 1.1	14.6 ± 1.1	11.5 ± 1.1	5.6 ± 1.2
0.5	32.4 ± 1.9	25.0 ± 1.9	39.6 ± 2.9	29.9 ± 1.6	13.8 ± 2.9
0.75	59.3 ± 2.2	47.1 ± 2.6	60.0 ± 1.9	54.2 ± 1.3	21.5 ± 2.6
1.0	91.6 ± 3.2	88.7 ± 2.6	101.6 ± 2.3	92.2 ± 1.6	26.8 ± 2.9

convulsion time after administration of spasmogenic agent, thus indicating the protective effect of the bronchodilator (Webber and Karlsson, 1996). It is evident that the H_1-receptor plays a vital role in bronchospasm, the stimulation of H_1-receptor by histamine produces smooth muscle contraction, increased vascular permeation and mucus secretion. The H_1-receptor effects are blocked generally by antihistaminic drug. In this present study, the extract (400 mg/kg) exhibited similar protective effect as that of chlorpheniramine (100 mg/kg) against histamine induced bronchospasm. The results clearly indicates that the extract at higher dose possess H_1-receptor antagonistic property similar to that of chlorpheniramine. The inhibitory effect of extract on isolated guinea pig ileum precontracted with histamine were also studied and the results clearly indicate that the phytoconstituents present in the extract exerted an antagonistic effect on H_1-receptor.

The other possible mechanisms involved in the relaxant effects of the extract from *M. serratulum* on tracheal chains of guinea pigs could be because of its β-adrenergic agonist property, antagonistic activity on H_1 -receptors. The mechanism of β-adrenergic agonist, increasing the activation of adenylate cyclase followed by increasing the concentration of intracellular cyclic adenosine 3′, 5′-monophosphaste (cAMP), accelerates the activation of specific cAMP-dependent protein kinase that causes relaxation (Popa et al., 1984). To evaluate the β-adrenergic stimulant effect of extract on its bronchodilatory effects, the effects of these extracts on tracheal chains inhibited with β-receptors by propranolol were re-examined in Group III experiment. The relaxant effects of most concentrations of methanolic extract obtained in the Group III experiment were significantly lower than those of Group I and II. These findings suggest probable mechanism of bronchodilation of the plant extract might be due to β-adrenergic stimulatory property. The potent relaxant effect of *M. serratulum* extract in Group I experiment (contracted tracheal chains by 60 mM KCl), the results clearly indicated that a calcium-channel blocking effect of this plant. It was also identified that the absence of an opening effect of this plant on potassium channel resulted in the bronchodilatory effect (Buckle et al 1993). If the extract had a potassium-channel opening effect, it would not have relaxant effects on KCl-contracted tracheal chains.

Other plants traditionally used for asthma such as *Viola odorata* (Janbaz et al., 2015) and *Buxus wallichiana* (Hussain et al., 2015) act by blocking Ca^{2+} channel. Bronchodilator activity of *Urginea indica* possibly mediates through a combination of anticholinergic and Ca^{2+} antagonist mechanism (Bashir et al., 2013).

The mechanism of bronchorelaxation of the drug theophylline, a xanthine derivative, is by inhibition of phosphodiesterase activity which leads to increased amount of intra cellular cAMP molecules causing smooth muscle relaxation, inhibition of calcium ion influx into smooth muscle (Hansel et al., 2004). The results of the above experiment suggests that the bronchodilatory activity of *M. serratulum* was similar/somewhat higher than that of theophylline, which might be due inhibition of calcium ion influx into smooth muscle by the plant extract. Apart from these, the *M. serratulum* plant extract is also found to be rich in naringenin, a predominant phenolic compound, which is known to possess excellent antiallergic property through the inhibition of iNOS and TH2 cytokine production in lung and thus reduces airway hyperresponsiveness against ovalbumin induced allergen model (Shi et al, 2009). Quercetin and rutin also found in extract at higher concentration, that are known to exhibit potent antiasthmatic effect via inhibition of the recruitment of eosinophills and neutrophils into the lung, the production of histamine, phospholipase A_2 and eosinophil peroxidase (Chan Hun et al., 2007). In addition, the plant *M. serratulum* reported to possess the bioactive compounds: Triterpenoid, ursolic acid and iridoid glycoside myxopyroside they have several pharmacological activities including antimicrobial, antiasthmatic, antiallergic and anti-inflammatory, cardiovascular and anti-cancer effect (Wozniak et al., 2015; Kim et al., 2013). The potency of several iridoid derivatives on smooth muscle were reported (Chung et al., 1980; Breschi et al., 1992; Rojas et al., 2000) which were in support of our result.

All the data derived from the present study is in correlation with these earlier findings, that suggests that plants containing the phenolic compounds including naringenin, rutin, quercetin, triterpenoids ursolic acid and iridoid glycoside may contribute the observed bronchodilatory efficiency of the plant.

Conclusion

M. serratulum possesses significant bronchodilatory effect through the underlying mechanisms such as histamine (H_1) receptor antagonistic, β-adrenoceptor stimulatory, an inhibitory effect on calcium channels. This study serves as a scientific evidence for the ethnomedicinal uses of *M. serratulum* in airway diseases.

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Ethical Issue

The animal study was performed according to the CPCSEA guidelines and approved by Institutional Animal Ethical Committee of Bharathidasan Institute of Technology, Anna

University, Tiruchirappalli. (Ref. No: AUROT/IAEC/NOV2013-0025 dt.21/11/2013).

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