Evaluation of bronchodilatory properties of fruits of *Opuntia elatior* Mill

Chauhan Sanjay P., Sheth Navin R.[a], Suhagia B. N.

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Dharmsinh Desai University, Nadiad, [a]Department of Pharmaceutical Sciences, Saurashtra University, Rajkot, Gujarat, India

Correspondence to Sanjay P. Chauhan, M. Pharm, PhD, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Dharmsinh Desai University, College road, Nadiad, 387 001, Gujarat, India,
Tel: +91 9427614966;
E-mail: sanjulmcp@rediffmail.com

Received 28 March 2014
Accepted 03 August 2014

Egyptian Pharmaceutical Journal
2015, 14:44–49

Background
Traditionally, fruits of *Opuntia elatior* Mill. were used for their immunomodulatory and antiasthmatic action. The presence of potentially active nutrients and their multifunctional properties make prickly pear a perfect candidate for the production of phytopharmaceutical products.

Objective
The objective of the present study was to evaluate the bronchodilatory properties of fruits of *O. elatior* Mill.

Materials and methods
The bronchodilatory properties were evaluated using bronchospasm induced by acetylcholine and histamine, anticholinergic action on isolated rat ileum, and antihistaminic action on isolated guinea pig ileum.

Result and discussion
Bronchodilating effect of fruit juice was dose dependent against spasm induced by acetylcholine and histamine. *O. elatior* Mill. fruits possess a significant inhibitory effect on rat and guinea pig ileum contraction through antihistaminic and antimuscarinic action.

Conclusion
This study suggested that fruits of *O. elatior* Mill. possess a significant inhibitory effect on rat and guinea pig ileum, and betacyanin, an active principle compound in prickly pear, may be responsible for the action.

Keywords:
antiasthmatic, bronchodilator, *Opuntia*, prickly pear

Egypt Pharm J 14:44–49

1687-4315

Introduction

Airway inflammation produces airflow limitation through acute bronchoconstriction, chronic mucus plug formation, and airway wall swelling or remodeling. These symptoms may be relieved either spontaneously or after treatment. Asthma can occur at any age [1]. Genetic susceptibility and personal/family history of atopy along with environmental exposures produce the clinical symptomatology of asthma. These signs and symptoms are highly variable in severity and duration [2].

Asthma has become more common in both children and adults around the world in recent decades. The increase in the prevalence of asthma has been associated with an increase in atopic sensitization and is paralleled by similar increases in other allergic disorders such as eczema and rhinitis. The rate of asthma increases as communities adopt western lifestyles and become urbanized. With the projected increase in the proportion of the world's population, that is urban from 45 to 59% in 2025, there is likely to be a marked increase in the number of asthmatics worldwide over the next two decades. It is estimated that there may be an additional 100 million persons with asthma by 2025 [3]. Asthma is thought to affect about 3% of the population in most countries.

The cactus *Opuntia* spp. (subfamily: Opuntioideae, family: Cactaceae) is a xerophytic plant producing about 200–300 species. In local parlance, cactus is called Prickly pear, Slipper thorn, Tuna (English) and has different vernacular names in India, such as Hathlo thor, Chorhthlo (Gujarati), Haththathoira, Nagphana, Nagphani (Hindi), Snuhi, Vajrakantaka, Bahushala (Sanskrit), Nagadali, Nagakkali (Tamil), Nagamulla, and Nagajemudu (Telugu). It was found that all cacti in India did not belong to one species as *Opuntia dillenii*, but three to four species are distributed over different regions in India. *O. dillenii* Haw. is found mainly in the southern parts of India, whereas *Opuntia vulgaris* Mill. (Syn *Opuntia monacantha* Haw.) is distributed mainly in the northern parts; *Opuntia elatior* Mill. is found in western India [4,5]. The presence of potentially active nutrients and their multifunctional properties make *Opuntia* spp. fruits and cladodes perfect candidates for the production of phytopharmaceutical products. Although traditionally appreciated for its pharmacological properties by the native Americans,

cactus pear is still hardly recognized because of insufficient scientific information [6]. The *Opuntia* spp. were used for their analgesic and anti-inflammatory, anticancer, antidiabetic, antihyperlipidemic and antihypercholesterolemic, antioxidant, antiulcer, antiviral, diuretics, immunomodulatory, improve platelet function, neuroprotective, wound healing, monoamino-oxidase inhibitor, and other nutritional importance [7,8].

Little is known regarding the medicinal properties of betalains and other phytoconstituents from *Opuntia* spp.; however, essentially nothing is known regarding their potential immunomodulatory properties. Of the numerous *Opuntia* spp., bioactive compounds have been isolated and characterized primarily from *Opuntia ficus-indica*, *Opuntia polycantha*, *Opuntia stricta*, and *O. dillenii* for various medicinal properties [9]. Although mast cells play an important role in allergy and asthma, the potential immunomodulatory effects of betalains from *Opuntia* spp. were not studied and reported. On the basis of previous studies and traditional use showing that prickly pear enhances immune function, we suggest that at least part of the effects of fruits of *O. elatior* Mill. on mast cell is through inhibition of degradation function. In the present study, we examined the effects of the fruits of *O. elatior* Mill. on mast cell-mediated anaphylaxis.

Materials and methods

Collection, authentication, and preparation of fruit juice (*Opuntia* fruit juice)

The fruits of *O. elatior* Mill. were collected from roadside weed near Atkot (Jasdan, Rajkot, Gujarat, India) at a latitude of 22°1′48″N, longitude of 71°12′0″E, and elevation of 193 m (633 ft) and were authenticated by Raw Materials Herbarium and Museum, National Institute of Science and Communication and Information Resources, New Delhi (NISCAIR). The herbarium (specimen voucher no. rbpmpc/museum/herbarium/07-08/01) was preserved in the museum of Department of Pharmacognosy, Smt R.B. Patel Mahila Pharmacy College, Atkot. Mature fruits of *O. elatior* Mill. were collected and immediately taken to the laboratory. Spines and glochides were removed from fruits by just heating on the wire gauge burner and then washed with water. The peel of the fruits was removed manually, and pulp was subjected to homogenization for 5 min using boss portable blender (Boss Appliances, Daman, India). After homogenization, fruits juice was filtered through glass filter G_4 (Borosil Glass Works Ltd, Mumbai, India), and filtered *Opuntia* fruit juice (OFJ) was used for mast cell degranulation test.

Phytochemical analysis

Identification of betalains by spectrophotometric, high-performance liquid chromatography, and liquid chromatography–mass spectroscopy analysis was performed and recently published by Chauhan *et al.* [10].

Antiasthmatic action

Animals

Albino Wistar rats (180–250 g body weight) and guinea pigs (350–400 g) of either sex were used for this study. They were housed at ambient temperature (22 ± 1°C), relative humidity (55 ± 5%), and 12/12-h light–dark cycle. Animals had free access to Amrut brand rat pellet diet supplied by Pranav Agro Industry (Baroda, India), and water was given *ad libitum*. The protocol of the experiment was approved by the Institutional Animal Ethical Committee (IAEC) as per the guidance of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India (vide certificate no. IAEC/RBPMPC/09-10/01 dated 18/07/2009).

Bronchospasm induced by acetylcholine and histamine in guinea pigs

To screen the sensitivity of guinea pigs toward spasmogens, guinea pigs of both sex (350–400 g) were placed in a plexiglass chamber and sprayed with 0.25% histamine and 0.5% acetylcholine chloride under the average pressure of 45 ± 5 mmHg for 15 s. The time to onset of respiratory distress (preconvulsive time) during challenge with these agents was measured; the guinea pigs with preconvulsive time of more than 120 s were considered to be insensitive and discarded [11].

The eligible guinea pigs were randomly divided into six groups, each containing six animals. The OFJ and standard drugs were administered orally. The single dose treatments were given 1½ h before the study. The following schedule of treatment was administered.

Groups	Treatment	Dose (orally)
A	Distilled water	1 ml/kg
B	Ketotifen	1 mg/kg
C	Atropine sulfate	2 mg/kg
D	OFJ	5 ml/kg
E	OFJ	10 ml/kg
F	OFJ	15 ml/kg

The method of histamine challenge was same as those of screening the sensitive guinea pigs, and time for preconvulsion state was noted for each animal. After about 15 days of washout period, the same animals were given the above treatments and time for

preconvulsion state was noted for 0.5% acetylcholine chloride aerosol spray [12].

Anticholinergic action on isolated rat ileum

Albino Wistar rats of either sex were killed by a blow to the head followed by exsanguination. A portion of ileum was removed and placed in oxygenated tyrode solution at room temperature. The connective tissue was carefully trimmed from the ileum tissue, suspended in tyrode solution at 37°C, and bubbled with 95% oxygen and 5% carbon dioxide. Intestinal segments of about 20 mm length were prepared from the terminal ileum of the rat. They were cleaned and suspended in organ bath containing tyrode solution and aerated with 95% oxygen and 5% carbon dioxide. Before the measurements, the tissue was allowed to stabilize for 30 min under a resting tension of 500 mg. The longitudinal contraction was measured according to the method of Sheth *et al.* [13]. The concentration–response curve of acetylcholine was recorded using graded dose of acetylcholine until the maximum response is obtained with contact time of 30 s. Thereafter, second concentration–response curves were obtained, in the presence of the test substance in the organ bath. A volume of 1 ml of different concentrations of OFJ test solutions (10, 50, 100 µl/ml) was added directly to the organ bath 3 min before the addition of acetylcholine.

Antihistaminic action of isolated guinea pig ileum

Guinea pigs were fasted for 24 h and later were killed, and a piece of ileum was isolated. The tissues were quickly transferred to petri dishes containing tyrode solution. The ileum was mounted in an organ bath maintained at 37°C and containing tyrode solution. A basal tension of 500 mg was applied to both the tissues throughout the experiment. After stabilization for 45 min, the tissues were exposed to graded doses of histamine, and contractions were recorded. The responses to these standard drugs were re-elicited after exposing the tissue to different concentrations of OFJ test solutions (10, 50, 100 µl/ml) for 3 min. After eliciting the response, the tissues were washed out thoroughly before proceeding for next response [13].

Statistical analysis

All the values are expressed as mean ± SEM. The data were analyzed by one-way analysis of variance followed by Tukey's multiple comparison tests. A level of P value less than 0.05 was considered statistically significant. A level of significance was noted and interpreted accordingly.

Results

Antiasthmatic action

Effect on bronchospasm induced by acetylcholine and histamine in guinea pigs

Pretreatment with OFJ (5, 10, and 15 ml/kg, orally) demonstrated significant increase ($P < 0.001$) and dose-dependent delayed onset of convulsion in guinea pigs due to acute bronchospasm induced by 0.25% histamine and 0.5% acetylcholine aerosol (Table 1). The percentage increase in preconvulsion time of OFJ (15 ml/kg)-treated animal was comparable with both ketotifen (1 mg/kg) and atropine (2 mg/kg) (Table 2).

Anticholinergic action on isolated rat ileum

Rat ileum suspended in tyrode solution with 1 g of tension stabilized for 15 min. Acetylcholine (1.83×10^{-7} to 1.46×10^{-6} mol/l) produced a concentration-dependent contraction of tissue, reaching its maximum within 30 s of tissue contact time. The OFJ at a dose of 50 and 100 µl/ml significantly ($P < 0.001$) inhibited acetylcholine-induced contraction response and caused 35 and 43% reductions to the response of 1.47 µmol/l acetylcholine, respectively. OFJ at a concentration of 10 µl/ml significantly ($P < 0.05$) inhibited the response of 1.1 µmol/l acetylcholine (Table 3 and Fig. 1).

Table 1 Effect of OFJ on preconvulsion time of guinea pig after histamine and acetylcholine aerosol exposure

Groups	Treatment (orally)	Preconvulsion time (s)	
		Histamine	Acetylcholine
A	Distilled water (1 ml/kg)	95.83 ± 5.06	95.83 ± 5.06
B	Ketotifen (1 mg/kg)	317 ± 21. 62***	–
C	Atropine sulfate (2 mg/kg)	–	230.8 ± 14.73***
D	OFJ (5 ml/kg)	156.5 ± 8.17*	169.8 ± 6.90***
E	OFJ (10 ml/kg)	219 ± 12.59***	216.2 ± 12.38***
F	OFJ (15 ml/kg)	290.2 ± 15.1***	223.3 ± 8.34***

Values are mean ± SEM ($n = 6$), analyzed by one-way ANOVA followed by Tukey's multiple comparison test. ANOVA, analysis of variance; OFJ, *Opuntia* fruit juice; *$P < 0.05$, for change difference versus vehicle control (group A). **$P < 0.01$. ***$P < 0.001$.

Table 2 Effect of OFJ on histamine and acetylcholine-induced bronchospasm in guinea pigs

Groups	Treatment (orally)	% Increase in preconvulsion time	
		Histamine	Acetylcholine
B	Ketotifen (1 mg/kg)	69.44 ± 1.64	–
C	Atropine sulfate (2 mg/kg)	–	58. 32 ± 0.89
D	OFJ (5 ml/kg)	37.5 ± 5.75	42.99 ± 4.14
E	OFJ (10 ml/kg)	55.09 ± 4.39	54.62 ± 4.19
F	OFJ (15 ml/kg)	66.82 ± 1.40	57.07 ± 1.68

Values are mean ± SEM ($n = 6$). OFJ, *Opuntia* fruit juice.

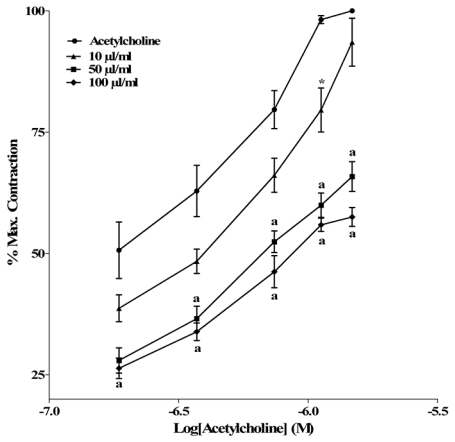
Antihistaminic action of isolated guinea pig ileum

Using the isolated guinea pig ileum as a model, the effects of OFJ (10, 50, and 100 µl/ml) on histamine-induced contractions were studied. As shown in Table 4 and Fig. 2, histamine (1.085×10^{-7} to 1.085×10^{-6} mol/l) produced dose-dependent contractions of guinea pig ileum. Pretreatment with OFJ inhibited the contractions of histamine. OFJ at concentrations of 50 and 100 µl/ml had significant ($P < 0.01$ and $P < 0.001$) inhibitory effect on the histamine concentration–response curve, reducing the maximum induced contraction, and caused 16 and 55% reductions to the response of 1.085×10^{-6} mol/l histamine.

Discussion

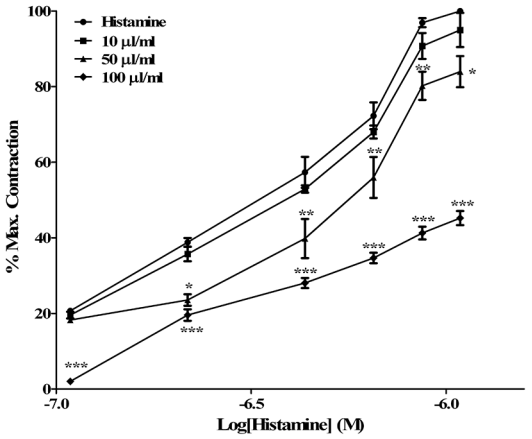
As bronchodilators, mediator-release inhibitors, and anti-inflammatory drugs are the different classes of drugs used conventionally in the treatment of bronchial asthma, various animal models and experimental

Figure 1



Effect of *Opuntia* fruit juice on tension development in isolated rat ileum. Values are mean ± SEM ($n = 6$), analyzed by one-way analysis of variance followed by Tukey's multiple comparison test ($^aP < 0.001$; $^*P < 0.05$, for change difference versus acetylcholine response).

Figure 2



Effect of *Opuntia* fruit juice on tension development in isolated guinea pig ileum. Values are mean ± SEM ($n = 6$), analyzed by one-way analysis of variance followed by Tukey's multiple comparison test ($^{***}P < 0.001$; $^{**}P < 0.01$; $^*P < 0.05$, for change versus histamine contractions).

Table 3 Effect of OFJ on acetylcholine-induced percentage maximum contraction of isolated rat ileum

Log [acetylcholine] (mol/l)	Percentage maximum contraction			
	Acetylcholine	Fruit juice of *O. elatior* Mill. (µl/ml)		
		10	50	100
−6.73	50.67 ± 5.81	38.71 ± 2.76	27.96 ± 2.59	26.34 ± 2.11[a]
−6.43	62.88 ± 5.27	48.39 ± 2.49	36.56 ± 2.59[a]	33.87 ± 1.81[a]
−6.13	79.65 ± 3.92	66.13 ± 3.50	52.42 ± 2.23[a]	46.24 ± 3.29[a]
−5.95	98.19 ± 0.82	79.57 ± 4.53*	59.95 ± 2.51[a]	55.91 ± 1.36[a]
−5.83	100 ± 0	93.55 ± 4.92	65.86 ± 3.07[a]	57.53 ± 1.93[a]

Values are mean ± SEM ($n = 6$), analyzed by one-way ANOVA followed by Tukey's multiple comparison test. ANOVA, analysis of variance; OFJ, *Opuntia* fruit juice; $^aP < 0.001$. $^*P < 0.05$, for change difference versus acetylcholine response.

Table 4 Effect of OFJ on histamine-induced percentage maximum contraction of isolated guinea pig ileum

Log [histamine] (mol/l)	Percentage maximum contraction			
	Histamine	OFJ (µl/ml)		
		10	50	100
−6.964	20.68 ± 0.72	19.57 ± 0.88	18.25 ± 0.79	2.027 ± 0.33***
−6.663	38.81 ± 1.14	35.71 ± 1.91	23.57 ± 1.52*	19.57 ± 1.51***
−6.362	57.33 ± 4.1	52.91 ± 0.97	39.81 ± 5.17**	28.04 ± 1.33***
−6.186	72.26 ± 3.54	67.98 ± 1.71	55.96 ± 5.42**	34.65 ± 1.38***
−6.061	96.91 ± 1.2	90.74 ± 3.41	80.23 ± 3.74**	41.26 ± 1.69***
−5.964	100 ± 0	94.97 ± 4.51	83.96 ± 4.12*	45.23 ± 1.86***

Values are mean ± SEM ($n = 6$), analyzed by one-way ANOVA followed by Tukey's multiple comparison test. ANOVA, analysis of variance; OFJ, *Opuntia* fruit juice; $^*P < 0.05$, for change versus histamine contractions. $^{**}P < 0.01$. $^{***}P < 0.001$.

protocols were used in the present study to evaluate antiasthmatic activity of fruit of *O. elatior* Mill.

Bronchial asthma is characterized by increased airway reactivity to spasmogens [14]. An initial event in asthma appears to be the release of inflammatory mediators (e.g. histamine, tryptase, leukotrienes, and prostaglandins). Some of these mediators directly cause acute bronchoconstriction, airway hyper-responsiveness, and bronchial airway inflammation. Spasmolytic drugs such as β-adrenergic agonists, xanthine derivatives, and anticholinergics relax the airway smooth muscles and are used as quick relief medications in acute asthmatic attacks. β-Adrenergic agonists promote bronchodilation by direct stimulation of β-adrenergic receptors in the airway smooth muscle, which lead to relaxation of bronchial smooth muscle by rapid decrease in airway resistance *in vivo*. Specific β_2-agonists such as salbutamol, salmeterol, etc. are used since long for symptomatic relief in asthma.

In present study, significant increase in preconvulsion time was observed due to pretreatment with fruit juice of *O. elatior* Mill., when the guinea pigs were exposed to either acetylcholine or histamine aerosol. This bronchodilating effect of fruit juice at high dose was comparable with ketotifen and atropine sulfate. Spasmolytic effect of *O. elatior* Mill. fruit was also evaluated by observing the effect of fruit juice (10, 50, and 100 μl/ml) on acetylcholine and histamine-induced ileum contractions to seek for scientific evidence for beneficial use of fruits in spasm produced by any means. The results showed antagonistic effects of the fruit juice against the contraction induced by the standard spasmogens. The results of this study indicated a rightward shift in the log dose–response curve of acetylcholine and histamine in the presence of the fruit juice of *O. elatior* Mill. The maximum effects of acetylcholine and histamine-induced contractions were inhibited in the presence of the fruit juice. The nonparallel rightward shift in acetylcholine and histamine log dose–response curves obtained in the presence of the fruit juice, with lowered maximum contraction effect to acetylcholine and histamine, would indicate a noncompetitive or an irreversible antagonistic effect of *O. elatior* Mill. fruits at muscarinic and histamine H_1 receptors [15]. In this case, the antagonist binds irreversibly to receptor site or to another site that inhibits response to the agonist.

Control of tension in gastrointestinal smooth muscle is dependent on the intracellular Ca^{2+} concentration. In general, there are two types of excitation–contraction coupling based on the type of mechanism responsible for changes in Ca^{2+} concentration. Electromechanical coupling requires changes in membrane potential, which in turn activate the voltage-dependent Ca^{2+} channel to trigger an influx of Ca^{2+} [16]. Acetylcholine and histamine cause contraction through specific receptors and can produce changes in tension [17]. Both acetylcholine and histamine have functional roles in natural contraction of the gastrointestinal tract. Acetylcholine is a neurotransmitter at postganglionic parasympathetic neurons that innervate the gut. The response to acetylcholine is mediated by activation of two types (M_2 and M_3) of muscarinic receptors [18,19]. Activation of these receptors results in an increase in intracellular Ca^{2+}, an effect mediated by inositol triphosphate acting on internal calcium stores [17,20,21]. Serotonin (5-HT) is also an important substance in the gastrointestinal tract and is present in both enterochromaffin cells of the mucosa and neurons of the mesenteric plexus; it affects both secretion and motor activity [22,23]. The histamine contraction is mediated by the release of acetylcholine from the cholinergic neuron as well as activation of serotonergic receptors on the smooth muscles of ileum [24]. This experiment showed that *O. elatior* Mill. fruits possess a significant inhibitory effect on rat and guinea pig ileum contraction through antihistaminic and antimuscarinic action.

Acknowledgements

Conflicts of interest

None declared.

References

1 Murthy KJR, Sastry JG. In: Byword Editorial Consultants, editor. Economic burden of asthma. *National Commission on Macroeconomics and Health (NCMH) background papers – burden of disease in India*. New Delhi, India: Ministry of Health & Family Welfare, Govt of India; 2005. 251–263.

2 Seth SD, Seth V. *Textbook of pharmacology*. 3rd ed. Noida, UP, India: Elsevier, a division of Reed Elsevier India Private Limited; 2009.

3 Masoli M, Fabian D, Holt S, Beasley R. Global burden of asthma. 2003: USA: Global Initiative for Asthma (GINA):1–20. Available at: http://www.ginasthma.com

4 Kirtikar KR, Basu BD. *Indian medicinal plants. Volume II*. 2nd ed. Dehradun, India: International Book Distributors; 1999:1173–1178.

5 Anonymous. *The wealth of India: a dictionary of Indian raw material & industrial products, raw material. Volume VIII*. New Delhi, India: National Institute of Science Communication, CSIR; 2001:100–104.

6 Feugang JM, Konarski P, Zou D, Stintzing FC, Zou C. Nutritional and medicinal use of cactus pear (*Opuntia* spp.) cladodes and fruits. Front Biosci 2006; 11:2574–2589.

7 Chauhan SP, Sheth NR, Jivani NP, Rathod IS, Shah PI. Biological actions of *Opuntia* species. Sys Rev Pharm 2010; 1:146–151.

8 Patil GG, Mali PY, Bhadane VV. Folk remedies used against respiratory disorders in Jalgoan district, Maharashtra. Nat Prod Rad 2008; 7:354– 358

9 Igor AS, Gang X, Liliya NK, Robyn AK, Mark AJ, Mark TQ. Macrophage immunomodulatory activity of polysaccharides isolated from *Opuntia polyacantha*. Int Immunopharmacol 2008; 8:1455–1466.

10 Chauhan SP, Sheth NR, Rathod IS, Suhagia BN, Maradia RB. Phytochemical screening of fruits of *Opuntia elatior* Mill. Am J Pharm Tech Res 2013; 3:1–6.

11 Chu X, Xu Z, Wu D, Zhao A, Zhou M, Qiu M, Jia W. *In vitro* and in vivo evaluation of the anti-asthmatic activities of fractions from Pheretima. J Ethnopharmacol 2007; 111:490–495.

12 Shah GB, Parmar NS. Antiasthmatic property of polyherbal preparation E-721B. Phytother Res 2003; 17:1092–1097.

13 Sheth UK, Dadkar NK, Kamat NG. *Selected topics in experimental pharmacology*. Bombay, India: Kothari Book Depot; 1972. 5–63.

14 Cockcroft DW. Mechanism of perennial allergic asthma. Lancet 1983; 2:253–256.

15 Linden A, Lofdahl CG, Ullman A, Skoogh BE. Non-adrenergic noncholinergic responses stabilize smooth muscle tone with and without parasympathetic activation in guinea-pig isolated airways. Eur Respir J 1993; 6:425–433.

16 Sadraei H, Asghari G, Hekmatti AA. Antispasmodic effect of three fractions of hydroalcoholic extract of *Pycnocycla spinosa*. J Ethnopharmacol 2003; 86:187–190.

17 Elorriaga M, Anselmi E, Hernandez JM, Docon P, Ivorra D. The source of Ca^{2+} for muscarinic receptor-induced contraction in rat ileum. J Pharm Pharmacol 1996; 48:817–819.

18 Goyal RK. Identification, localisation and classification of muscarinic receptor subtypes in the gut. Life Sci 1988; 43:2209–2220.

19 Levey AI. Immunological localization of M1–M5 muscarinic acetylcholine receptors in peripheral tissue and brain. Life Sci 1993; 52:441–448.

20 Caulfield MP. Muscarinic receptor characterization, coupling and function. Pharmacol Ther 1993; 58:319–379.

21 Eglen RM, Hedge SS, Watson N. Muscarinic receptor subtypes and smooth muscle function. Pharmacol Rev 1996; 48:531–565.

22 Briejer MR, Akkermans LM, Schuurkes JA. Gastrointestinal prokinetic bezamide: the pharmacology underlying stimulation of motility. Pharmacol Rev 1995; 47:631–657.

23 Kunze WA, Furness JB. The enteric nervous system and regulation of intestinal motility. Annu Rev Physiol 1999; 61:117–142.

24 Sander-Bush E, Mayer SE. In: Hardman JG, Limbird LE, Gilman AG, editors. 5-Hydroytryptamine (serotonine) receptor agonists and antagonists. *Goodman & Gilman's the pharmacological basis of therapeutics*. McGraw Hill, New Delhi, India, 1996; 384–385.