

Full Length Research Paper

Antiasthmatic activity of the methanolic extract of *Physalis angulata* Linn.

Chhaya Rathore^{1*}, K. R. Dutt¹, Shobharam Sahu² and Lokesh Deb³

¹BRNSS-Contract Research Center, B.R. Nahata College of Pharmacy, Mhow-Neemuch Road, Mandsaur, (MP), India-458001, India.

²Rajiv Gandhi College of Pharmacy, Nautanwa, Maharajgang, Uttar Pradesh, India-273164, India.

³Pharmacology Laboratory, Medicinal and Horticultural Plant Bioresources Division, Institute of Bioresources and Sustainable Development (IBSD), Takyelpat Institutional Area, Imphal Manipur, India-795001, India.

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In this study the methanolic leaves extract of *Physalis angulata* Linn (Solanaceae) (PAL) was investigated for anti-histaminic activity by using isolated animal smooth muscle models (guinea pig ileum preparation, guinea pig trachea and rat fundus strip), where the plants extract possess inhibitory efficacy against histamine and 5 – HT. Acute toxicity study of the plant extract was also performed to measure the safety prospective. In smooth muscle models, PAL possesses inhibitory efficacy by 100% (1 µg), 133% (2 µg) and 126% (4 µg) in guinea pig ileum preparation (GPIP) against 1 µg histamine; 86% (1 µg), 100% (2 µg) and 106% (4 µg) in guinea pig tracheal chain preparation (GPTCP) against 1 µg histamine; and 50% (1 µg), 75% (2 µg) and 100% (4 µg) in fundus strip preparation (FSP) against 1 µg 5HT. A survey by the National Asthma Campaign found that 60% of the people with moderate asthma and 70% with severe asthma used complementary and alternative medicine to treat their conditions. Herbal medicine is the third most popular choice of both adults (11%) and children (6%) suffering from asthma, although *P. angulata* is used for the treatment of antihyper glycemic, anti-inflammatory, antimicrobial, antiseptic, antiviral, diuretic, expectorant and febrifuge. In traditional systems, there was only one claim for asthma. The present study will help the industry to produce herbal drug with less side effect, less cost and more effectiveness in the treatment of asthma.

Key words: Antiasthmatic, methanolic, *Physalis angulata*.

INTRODUCTION

Asthma is a chronic inflammatory disease of the air-ways with a wide range of presentations from intermittent to mid symptoms with chronicity. Despite the fact that advancement in the treatment of asthma is on the increase, prevalence and mortality of asthma affect approximately 10% of children and 5% of adults, world-wide (Vianna, 1998; Salvi, 2001). Asthma is characterized by recurrent episodes of wheezing, breathlessness, chest tightness and cough, reversible airway obstruction and bronchial hyperresponsiveness to a variety of specific and nonspecific stimuli, including: allergen, histamines, chemical irritants, cold air and exercise

(Smith, 2001; Busse, 1993). With the understanding of the pathophysiology of asthma, the original belief that asthma is associated with the isolated acute episodes of bronchospasm resulting from wide variations in resistance to flow in the airways has changed and it is now recognized as an inflammatory disorder (Whelan, 1996). Ayurveda and other Indian literature mention the use of plants treatment for various human ailments (Grover et al., 2002). Hence, herbal therapy deserves the same respect as that of pharmaceutical drugs. Proper quantity and quality of herbal medicines are mandatory to be determined to get the maximum benefits. Since time immemorial, patients with non-insulin dependent diabetes have been treated orally in folk medicine, with a variety of plant extracts. In India, a number of plants are mentioned in ancient literature (Ayurveda) for the treatment of diabetic conditions.

*Corresponding author. E-mail: chhaya_rathod2002@yahoo.com.
Tel: 05522-235628, +919468080476.

Physalis angulata Linn. (Solanaceae) (PAL), belonging to family Solanaceae, is also known as mullaca, wild tomato, winter cherry and gooseberry, and it reaches a height found in many tropical countries. This is reported for antihyper glycemic, anti-inflammatory, antimicrobial, antiseptic, antiviral, diuretic, expectorant and febrifuge (Tropical plant database). The plant is used by some people in the state of Madhya Pradesh, Uttar Pradesh and Uttarakhand in the treatment of asthma. However, no scientific datum is available regarding the effect of *P. angulata* L. as anti-asthmatic. The present study was undertaken to explore the effect of the leaves extract of *P. angulata* L. on the isolated organ of experimental animals.

MATERIALS AND METHODS

Plant material

PAL, fresh leaves were collected from the surrounding of Ratlam M.P. India in August 2006 and were identified by Dr. H.S. Chhatree (Ex-Prof. in Botany) in the Department of Botany, Govt. P.G. College, Mandsaur, where voucher specimens BRNCP/P/005/2006 were deposited for reference to the Department of Pharmacognosy, B.R. Nahata College of Pharmacy, Mandsaur, M.P. India.

Preparation of extract

The air-dried powdered leaves were packed in Soxhlet apparatus and extracted with petroleum ether for 18 h, and the same marc was subjected to extraction for 18 h (each time) with chloroform and methanol, respectively. The extracts were dried at 45°C in vacuum distillation till they were condensed. A hot air oven was used at 45°C till solid mass was obtained. These were stored in airtight container in refrigerator below 10°C. Due to sufficient yield, methanolic extract was selected for pharmacological investigation. Extracts were dissolved in purified water and used in different pharmacological experiments.

Experimental animals

Wistar rats (150 to 200 g), guinea pig (1 to 1.5 kg) and albino mice (20 to 25 g) of either sex, provided by the Institutional Animal House of B.R. Nahata College of Pharmacy, Mandsaur, were used. Animals were maintained under standard environmental condition (Room temperature: 27 ± 3°C; relative humidity: 65° ± 10%, 12 h light / dark cycle).

The animals were fed with standard diet and water was given *ad libitum* under strict hygienic conditions. Experiments were performed in accordance with the current guidelines of CPCSEA, India (Nagappa et al., 2003). All the animal experiments were conducted according to the protocol approved by the Institutional Animal Ethics Committee.

Determination of acute toxicity (LD50)

The acute toxicity test (LD50) of the methanolic extracts of PAL was determined according to the guideline No. 420 of the organization for Economic Co-operation and Development (1997). A total of 4 groups of Albino mice (20 to 25 g) of either sex containing 6 in each group were given 2000 mg/kg of PAL. The treated animals were monitored for 14 days in terms of mortality and general behavior

and the numbers of dead animals in each group were recorded after a time period of 14 days from the 1st day of administration of the test products. No death was observed till the end of the study. The extract was found to be safe up to the dose of 2000 mg/ kg (OECD, 1997).

Phytochemical investigation

Preliminary phyto-chemical tests were carried out with leaves of methanolic extracts of PAL for the detection of constituents like carbohydrate, proteins, tannins and alkaloids (Khandelwal, 2007).

Pharmacological screening (studies on smooth muscles)

Guinea pig ileum preparation

Guinea pigs of either sex (1 to 1.5 kg), starved overnight but allowed free access to water, were used (Aka et al., 1997, 1993). The animals were killed by a blow on the head and were emarginated. A segment of the guinea pig ileum (approximately 2 cm long), removed from a freshly killed animal, was tied with a thread to the top and bottom ends without closing the lumens. It was suspended in the same way in a 30 ml organ bath containing tyrode solution maintained at 37 ± 1°C and gassed with air (Goyal, 2006, 2007). A tension of 0.5 g was applied and the tissue was allowed to equilibrate for the period of 30 min before adding any extract or drugs to the organ bath. Contractile responses were established for histamine and concentrations were recorded depending on the responses due to 1 µg histamine using writing lever. The effects of the 1, 2 and 4 µg methanolic extract of PAL on the histamine-induced contraction were investigated. Contact times of 30 s and 5 min time cycle were maintained for proper recording of the responses (Kulkarni, 2003).

Guinea pig trachea

Guinea pigs of either sex (1 to 1.5 kg), starved overnight but allowed free access to water, were used. The animals were killed by a blow on the head and were emarginated. After sacrificing the guinea pig, the trachea was dissected out and transferred into a dish containing Krebs solution. Each segment of the tracheal cartilage is cut out to give a number of rings of tracheal muscle. At least, 5 to 6 of such rings are tied together by their cartilage portion to give a tracheal chain preparation. It is mounted in the Krebs solution at 35 to 37°C under 0.5 g tension and aerated with air. The tissue was equilibrated for 30 min during which the bath solution was replaced every 10 min. (Goyal, 2006, 2007). Contractile responses were established for histamine and concentrations were recorded depending on responses due to 1 µg histamine using writing lever. The effects of the 1, 2 and 4 µg methanolic extract of PAL on the histamine-induced contraction were investigated. Contact times of 90 s and 5 min time cycle were maintained for proper recording of the responses (Kulkarni, 2003).

Rat fundus strip

Adult albino rats (150 to 200 g) of either sex were killed by a blow on the head and were exsanguinated. The fundus portion was incised from lesser curvature and was opened longitudinally. Alternate zig-zag cuts were made to make a fundal strip. The strip was suspended in Krebs solution and it was mounted in the Krebs solution at 35 to 37°C under 1 g tension and aerated with air. The tissue was equilibrated for 30 min during which the bath solution was replaced every 10 min. Contractile responses were established

Table 1. Effect of the test drug against std. agonist histamine on guinea pig ileum.

S/N	Treatment	Dose (μg)	Height of response (h) (mm)	Control height (H) (mm)	% Inhibition of response due to test drug $\text{H-h}/\text{H} \times 100$
1	Histamine	1	24	24	-----
		2	27		
		4	30		
2	PAL+ histamine	1 +1	Base line	-----	100
		2 +1	-0.5		133
		4 +1	-0.4		126

Table 2. Effect of the test drug against std. agonist histamine on guinea pig tracheal chain

S/N	Treatment	Dose (μg)	Height of response (h) (mm)	Control height (H) (mm)	% Inhibition of response due to test drug $\text{H-h}/\text{H} \times 100$
1	Histamine	1	1.5	1.5	-----
		2	3		
		4	4.5		
2	PAL+ histamine	1 +1	0.2	-----	86
		2 +1	Base line		100
		4 +1	-0.1		106

Table 3. Effect of the test drug against std. agonist 5-HT on rat fundus strip.

S/N	Treatment	Dose (μg)	Height of response (h) (mm)	Control height (H) (mm)	% Inhibition of response due to test drug $\text{H-h}/\text{H} \times 100$
1	Histamine	1	4	4	-----
		2	6		
		4	8		
2	PAL+ 5-HT	1 +1	2	-----	50
		2 +1	1		75
		4 +1	Base line		100

for 5 HT and concentrations were recorded depending on the responses due to 1 μg 5 HT using writing lever. The effects of the 1, 2 and 4 μg methanolic extracts of PAL on the 5 HT-induce contraction were investigated. Contact times of 90 s and 5 min time cycle were maintained for proper recording of the responses (Kulkarni, 2003).

RESULTS

The photochemical screening indicates the presence of alkaloids, flavonoids and steroid in methanolic extract of PAL leaves. In acute toxicity study, methanolic extract of PAL leaves showed no mortality at 2000 mg/kg, so the extracts are safe for *in vivo* studies, in that 1/20th and 1/40th (100 and 50 mg/kg) of the extract can be used for

in vivo activities. However, 1 μg histamine produces 24 and 1.5 mm height of response in GPIP and GPTCP respectively, whereas 1 μg 5-HT produces 4 mm height of response in FSP. PAL possesses inhibitory efficacy by 100% (1 μg), 133% (2 μg) and 126% (4 μg) in GPIP against 1 μg histamine; 86% (1 μg), 100% (2 μg) and 106% (4 μg) in GPTCP against 1 μg histamine; and 50% (1 μg), 75% (2 μg) and 100% (4 μg) in FSP against 1 μg 5HT (Tables 1, 2 and 3).

DISCUSSION

Histamine is an autocooid having profound physiological effect in the body. Besides the triple response caused by

it, histamine has spasmogenic response on intestinal smooth muscle by acting on H₁-histamine receptor that causes the contraction of intestinal smooth muscle. Guinea Pig is highly sensitive to histamine due to presence of histaminergic receptors in ileum and tracheal smooth muscle (Kulkarni, 2003). The smooth muscles contraction is due to increase in phosphoinositol hydrolysis and intracellular calcium (Vianna, 1998). Further, histamine is synthesized and released by mast cells in the airway wall and by circulating and infiltrating basophiles. Although, airway mast cells are likely to be the major cellular source of histamine in asthma, there is increasing evidence that basophiles may be recruited to asthmatic airways and may release histamine in response to cytokine histamine-releasing factors. Hence, Histamine has multiple effects on airway function that are mediated by specific surface receptors on target cells.

H₁ receptors have been cloned from cows, rats, guinea pigs, and humans. The published sequences suggest that there are surprisingly large differences among species, consistent with the sometimes-marked differences in the responses to histamine among species, with lower activities in rats and mice, compared with guinea pigs and humans. H₁ receptors mediate most of the effects of histamine that are relevant to asthma. H₁ receptors have been demonstrated in animal and human lung by direct receptor binding techniques. Mepyramine binding to human lung homogenates is complex, with at least three sites with different affinities.

There have been no auto radiographic mapping studies, because of the unsuitability of currently available radioligands. Antigen-induced, IgE-dependent anaphylaxis in chopped human lung causes increases in both cyclic adenosine monophosphate (AMP) and cyclic guanosine monophosphate (GMP) levels. The rise in cyclic GMP levels is blocked by an H₁ receptor antagonist, suggesting that this response is linked to H₁ receptor activation. The effect of histamine on cyclic GMP levels in guinea pig lung is dependent on L-arginine, suggesting that H₁ receptor stimulation increases the release of nitric oxide (NO), which subsequently increases cyclic GMP levels by activating soluble guanylyl cyclase. NO synthase (NOS) inhibitors, suggesting that the release of NO stimulated by histamine partially counteracts the direct bronchoconstricting action of airway smooth muscle H₁ receptors, enhance the bronchoconstricting effect of histamine.

This may not occur in human airways, because there is no increase in the bronchoconstriction response to histamine after inhalation of NOS inhibitors and no increase in the levels of exhaled NO (Peter, 1998). In present study, methanol extract of PAL, leaves was exhibited inhibition of histamine responses on guinea pig ileum preparation; indicate that, extract may be acting through H₁ receptor as antagonists. Similar kind of response exhibited by the extract in case of guinea pig

tracheal chain preparation, which support the above statement that methanol extract of PAL, leaves was acting on H₁ receptor as antagonists. Histamine is one of the major inflammatory mediators in the immediate phase of asthma, causing airway hyper responsiveness and bronchial airway inflammation. The study regarding involvement of H₁ and H₂ receptors has been done in experimental asthma in guinea pig using respiratory smooth muscle as well as histamine induced bronchoconstriction and it was confirmed that there is prominent involvement of H₁ receptors as compared to H₂ receptors especially in asthma.

Further rat fundus is a very sensitive tissue for the study of activity of several naturally occurring substances like 5-hydroxytryptamine (5-HT), histamine, acetylcholine and brodykinin. However, serotonergic receptors are more dominated in rat fundus that is sensitive to 5-HT (Kulkarni, 2003).

Serotonin [5-hydroxytryptamine (5-HT)] causes bronchoconstriction in most animal species, but interest in this mediator is minimal because it is not a constrictor of human airways and its relevance in asthma seems doubtful. Serotonin is formed by decarboxylation of tryptophan (obtained in the diet) and is stored in secretory granules. Serotonin is present in mast cell granules from rodents but not humans. The major source of serotonin in humans is platelets, but serotonin is also found in neuroendocrine cells of the respiratory tract and has been localized to peripheral nerves. Multiple serotonin receptors have now been recognized, based on the development of selective antagonists and molecular cloning. There are up to seven types of 5-HT receptors, each with several subtypes. Selective antagonists have now become available for clinical use, but few have been used in investigations of human airway cells or in the treatment of patients with asthma. Serotonin does not constrict human airway smooth muscle *in vitro* and may even have bronchodilating effects, although pulmonary vessels are constricted as expected. In animals, serotonin increases acetylcholine release from airway nerves, and this has been demonstrated in human airways.

The receptor mediating this response appears to be a 5-HT₃ receptor in guinea pig airways, serotonin inhibits noradrenergic noncholinergic (NANC), neurally induced constriction resulting from tachykinin release via a 5-HT₁-like receptor localized to sensory nerve endings. In humans, infused serotonin has no effect on airway function but may have an inhibitory effect on cough reflexes, possibly mediated by receptors on airway sensory nerves. Serotonin is a potent inducer of micro vascular leakage in rodent airways, but it is not certain whether serotonin has this property in human airways. Serotonin has a blocking effect on sodium channels in human airway epithelial cells, but the receptor subtype involved has not been established (Bertram, 2007).

In present study, methanol extract of PAL, leaves was

inhibited responses of 5-HT on rat fundus preparation, that indicate the antagonistic activity of extract on serotonergic receptor.

Conclusion

Asthma is a chronic inflammatory disease of the air-ways with a wide range of presentations from intermittent to mid symptoms with chronicity. To control such disorders with others, the conventional synthetic steroids are possibly used with the plant sources. In the present study, the methanolic extract of PAL leaves exhibited antagonistic activity on both histaminergic and serotonergic receptors. On the basis of the study's investigation, we may partially conclude that PAL could be a potent antiasthmatic agent for the next generation. Likewise, with regard to such information, further studies can be taken up on isolation and characterization of phytochemical constituents present in the methanolic extract of PAL leaves that are particularly responsible for such activity and which have the ability to give protection to mankind against such severe disease in future.

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