EVALUATION OF ANTI-ASTHMATIC ACTIVITY OF ETHANOLIC EXTRACT OF *SOLANUM XANTHOCARPUM* LEAVES

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Summary

Asthma is a chronic disease that affects approximately 300 million people worldwide. Although wide range of drug is available, the relief is mainly symptomatic and short lived. *Solanum xanthocarpum* Schrad. (Solanaceae), also known as yellow berried nightshade (English), is traditionally used to treat asthma. However, the scientific data on anti-asthmatic and anti-inflammatory actions of this plant has got little attention. An attempt has been made to evaluate anti-asthmatic, anti-inflammatory and mast cell stabilizing activities of ethanolic extract of *Solanum xanthocarpum* leaves (EESX) using acetylcholine (Ach) and histamine-induced airway constriction in guinea pigs and carrageenan-, dextran-, and histamine-induced paw oedemas, and cotton pellet-induced granuloma tests in rats. EESX showed significant bronchodilator activity on histamine-induced airway constriction and reversed the allergen-induced bronchospasm. Interestingly, EESX protected mast cells from compound 48/80-provoked degranulation and inhibited acute, sub-acute and chronic inflammation in different animal models. The LD_{50} of EESX was 2262.7 mg/kg, i.p. The present study for the first time indicates anti-asthmatic, anti-inflammatory and mast cell stabilizing activities of EESX, confirming its traditional claims.

Key Words: Anti-asthmatic, Antihistaminic, Antiinflammatory, Compound 48/80, Mast cell stabilization, *Solanum Xanthocarpum*.

Introduction

Bronchial asthma is an inflammatory disorder of the airways characterized by airway obstruction, inflammation and bronchial hyper-responsiveness and is a global health problem that results from a complex interplay between genetic and environmental factors. Among several respiratory diseases affecting man, bronchial asthma is the most common disabling syndrome. Nearly 7–10% of the world population suffers from bronchial asthma (1). A wide range of drug is available for the treatment of asthma. However, the relief offered by these drugs is mainly symptomatic, short lived and results into complex side effects. It has been reported that there has been an alarming increase in number of diseases and disorders caused by synthetic drugs prompting a switch over to traditional herbal medicine (2). The modern medicine or allopathy has gradually developed over the years by scientific and observational efforts of scientists. However, the basis of its development remains rooted in traditional medicine and therapies (3). Assessing the current status of health care system in adequacies of synthetic drugs is likely to be more glaring in the coming years. In the developing countries, herbal medicines are being used by nearly about 80% of the world population (4, 5). Ayurveda is a traditional Indian medicinal system practiced for thousands of years.

Selection of scientific and systematic approach for the biological evaluation of plant products based on their use in the traditional systems of medicine forms the basis for an ideal approach in the development of new drugs from plants. One such plant is *Solanum xanthocarpum* (SX) Schrad. (Family: Solanaceae) commonly known as yellow berried night shade (English). It is a prickly diffuse, bright green perennial herb found throughout hotter parts of India (6, 7). SX has held a place of some importance in the Hindu *Materia Medica,* primarily as an expectorant and antipyretic (7, 8). Various medicinal properties are attributed to it, particularly in the treatment of asthma, chronic cough and catarrhal fever (9). It is one of the members of the *dashamula* (ten roots) of the Ayurveda (10). A body of evidence suggests that SX is an important source of many pharmacologically and medicinally important chemicals, especially solasodine and related steroidal hormones. The plant is extensively studied for the various pharmacological activities like hepatoprotective, cardiovascular, hypoglycemic and mosquito repellent properties. The plant is traditionally prescribed for immunomodulation, hypolipidemic, antibacterial, sexual behaviour, tolerance and dependence. SX is widely used by practitioners of the Siddha system of medicine in southern India to treat respiratory diseases (11). Recently, it is suggested that treatment with SX improved the pulmonary functions to a significant level in patients suffering from mild to moderate asthma (2).

This study revealed that dose of SX was well tolerated and no untoward effects were observed. SX is a safe medicine in the traditional system and has been used by mankind over many centuries. Further, it was suggested that relief from the symptoms of bronchial asthma produced by SX may be due to: (a) a bronchodilator effect, (b) reduction in the bronchial mucosal edema, and/or (c) reduction in the secretions within the airway lumen. In the light of above background, the present study was aimed at evaluation of anti-asthmatic activity of ethanolic extract of *Solanum Xanthocarpum* (EESX) on experimental animals using different pharmacological protocols and identifying phytoconstituents present in the plant extract.

Materials and methods

Plant materials

The leaves of *Solanum xanthocarpum* were collected from the outfield of Junagadh city, Gujarat, India in Feb-March. The plant materials were identified and authenticated by Dr. P.J. Parmar, Botanical Survey of India, Jodhpur, Rajasthan, India. A voucher specimen (SU/DPS/Herb/19) of the collected sample was deposited in the Department of Pharmaceutical Sciences, Saurashtra University, Rajkot for future reference.

Drugs and Chemicals

Compound 48/80, disodium chromoglycate (DSCG), histamine dihydrochloride, acetylcholine chloride, atropine sulfate, mepyramine meleate, carrageenan, acetylsalicylic acid (ASA) and toluidine blue were purchased from Sigma Chemical Co., St. Louis, MO. Histamine solution was freshly prepared in normal saline (NaCl, 8.5 g/l). All the other drugs were of analytical grade and they were dissolved in distilled water and desired concentration was prepared. Prednisolone was gifted by Inga Laboratories Ltd., Mumbai, India.

Extraction

Leaves of SX were washed with distilled water to remove dirt and soil, and shade dried. Routine pharmacognostic studies including organoleptic tests, macroscopic and microscopic observations were carried out to confirm the identity of the material. The dried material was powdered and passed through a 10-mesh sieve. The coarsely powdered material (500 g) of SX was extracted with ethanol (95%, v/v) in Soxhlet apparatus. The extract was filtered and concentrated by distilling off the solvent and evaporated to dryness using water bath to get crude ethanol extract. The ethanolic extract was subjected to phytochemical screening. For different pharmacological tests, the extract was suspended in double distilled water containing carboxy methyl cellulose (0.5 %, w/v, CMC).

Experimental animals

Antihistaminic and anticholinergic studies were conducted on guinea pigs (350-500 g) of either sex fed on commercial pellet diet (Amrut, Pranav Agro Industries Ltd, India). They were housed in standard conditions of temperature (22 ± 20C), relative humidity (60 ± 5 %) and 12:12 light/dark cycle (lights on at 07:00 am and off at 19:00 pm). They were divided in groups of ten animals each. The saline fed group served as control and one group was treated with a standard drug. Before experimentation, the animals were kept on fast for 24 h but water was given *ad libitum*, Animals receiving different doses of plant extracts were also observed for any alteration in their general behavior.

For the anti-inflammatory paradigms, Sprague–Dawley rats weighing 140–160 g of either sex were used. Five animals were group housed in polypropylene cages (640 x 410 x 250 mm high) and kept at departmental animal house in well cross ventilated room at the same experimental condition explained earlier. They were provided with standard rodent pellet diet (Amrut, India) and tap water *ad libitum* except the food was withdrawn 18–24 h before the experiment. All the experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC), N. R. Vekaria Institute of Pharmacy and Research Centre, Junagadh (approval number NRVCP/IAEC/07/2k7/01). All the experiments and the care of the laboratory animals were according to current ethical guidelines by the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India, New Delhi.

Habituation to animals

Rats and guinea pigs were habituated to handling by holding them and injecting vehicle through oral/i.p. routes to minimize non-specific stress and to simulate the actual protocol conditions. Handling also consisted of weighing and restraining animals on platform for 1 min and gently massaging on dorsal site as was done in the actual protocols. The same platform was used during drug administration. Moreover, the animals were familiarized with the diet in their home cage-environment and laboratory environments before subjecting them to the tests. All the experiments commenced 24 hours following the final habituation and were conducted according to the protocols mentioned below.

Acute toxicity test (Determination of LD_{50})

The acute toxicity tests (LD_{50}) for the different plant extracts were determined according to the procedure described by Lorke (1983). The crude ethanolic extracts were used for the test. Albino mice (20–25 g) of either sex were used.

This method involved an initial dose finding procedure, in which the animals were divided into three groups of three animals per group. Doses of 10, 100 and 1000 mg/kg were administered intraperitoneally (i.p.), one dose for each group. The treated animals were monitored for 24 h for mortality and general behavior.

From the result of the above step, four different doses of 200, 400, 800 and 1600 mg/kg were chosen and administered (i.p.) respectively to four groups of one mouse per group. The treated animals were again monitored for 24 h. The LD_{50} was then calculated as the geometric mean of the lowest dose showing death and the highest dose showing no death (12).

Preparation of the test extracts

EESX was suspended in 1% CMC in distilled water to prepare different doses (50, 100, 200, 300 mg/kg body weight) and administered orally with the help of gastric cannula. The control animals were given an equivalent volume of CMC in vehicle. In each protocol, one of the groups is reference standard group in which various standard anti-asthmatics were injected to animals.

Histamine-induced bronchospasm in guinea pigs

The guinea pigs fasted for 24 h were exposed to an atomised fine mist of 2% histamine dihydrochloride aerosol (dissolved in normal saline) using nebulizer at a pressure of 300 mm Hg in the histamine chamber (24 x 14 x 24 cm, made of perplex glass). Guinea pigs exposed to histamine aerosol showed progressive signs of difficulty in breathing leading to convulsions, asphyxia and death. The time until signs of convulsion appeared is called pre-convulsion time (PCT). By observation, experience was gained so that the preconvulsion time can be judged accurately. As soon as PCT commenced, animals were removed from the chamber and placed in fresh air to recover. In the present experiments, the criterion used was time for onset of dysponea and percent protection was calculated. Those animals which developed typical histamine asthma within 3 min were selected out three days prior to the experiment and were given habituation practice to restrain them in the histamine chamber. They were divided in groups of ten animals each. Mepyramine 8.0 mg/kg, ip and different doses of plant extract, intraperitonially were administered 30 min prior to exposure. Animals, which did not develop typical asthma within 6 min were taken as protected (13).

Acetylcholine-induced bronchospasm in guinea pigs

Similar procedure was repeated by exposure of aerosol of 0.5% acetylcholine in another set of animals (each group having ten animals) using Atropine sulphate (2 mg/kg) as a standard (14).

Mast cell degranulation by compound 48/80

This was carried out as per the method described by Kaley and Weiner (1971) with little modification. Male albino rats were sacrificed by cervical dislocation. The animals were immediately injected with 15 ml of pre-warmed (37°C) buffered salt solution (BSS; NaCl 137 mM; KCl 2.7 mM; $MgCl_2$; 1.0 mM; $CaCl_2$ 0.5 mM; NaH_2PO_4 0.4 mM; Glucose 5.6 mM; HEPES 10 mM) into the peritoneal cavity, and massaged gently in this region for 90 s, to facilitate cell recovery. A midline incision was made and the peritoneum was exposed (Figure 4.10). The pale fluid was aspirated using a blunted plastic Pasteur pipette, and collected in a plastic centrifuge tube. The fluid was then centrifuged at 1000 rpm for 5 min, and the supernatant discarded to reveal a pale cell pellet. The cell pellets were re-suspended in fresh buffer and re-centrifuged. Aliquots of the cell suspension were incubated with the test compounds or disodium cromoglycate, before challenge with compound 48/80. The aliquots were carefully spread over glass slides and the mast cells were stained with 1% toluidine blue and counterstained with 0.1% light green. The slides were dried in air and the mast cells counted from randomly selected high power objective fields (X450). The effect of EESX on mast cell was studied by incubating the mast cells for 10 min with the above compounds in a concentration of 1, 10 or 100 µg/ml. In another set of experiments the mast cells which were pre-incubated with different plant extracts or polyherbal formulations were exposed to the mast cell degranulator, compound 48:80 (10 µg/ml) and the incubation continued for a further 10 min. Then, the mast cells were carefully spread over glass slides. The percent degranulation of the mast cells in each treatment was calculated. Disodium cromoglycate (DSCG) 20 µg/ml was also included in the study for comparison (15).

Histamine-induced hind paw edema in rats.

The method of Horakova and Moratova (1964) was followed. Oedema in one of the hind paws of the rat was induced by the subplantar injection of 0.1 ml freshly prepared 0.1% (w/v) histamine (a phlogistic agent) in normal saline. The pedal volume was measured just before (0 h) and 3 h after the phlogistic challenge. ASA (300 mg/kg, p.o.) was employed as reference standard in the experiment. EESX (50, 100, 200 and 400 mg/kg, p.o.) or ASA (30 mg/kg, p.o.) was given 1 h before the phlogistic challenge (16).

Carrageenan-induced hind paw edema in rats (acute inflammation)

The acute hind paw edema was produced by injecting 0.1 ml of carrageenan (freshly prepared as 1% suspension in 1% CMC) locally into the plantar aponeurosis of the right hind paw of rats (17). EESX (50, 100, 200 and 400

mg/kg, p.o.) was administered to four different groups while the other two groups served as negative and positive controls and received vehicle (1 ml/kg, p.o.) and standard drug, acetylsalicylic acid (ASA, 300 mg/kg, p.o.), respectively. For each treatment group six animals were used. EESX and ASA were administered 1 h prior to the injection of carrageenan. A mark was made at the ankle joint of the paw of rat and pedal volume up to this point was measured using plethysmometer (Ugo Basile, Italy) at 0 h (just before) and 1-, 2- and 3-h post-carrageenan injection. Increase in the paw edema volume was considered as the difference between 0 and 1-, 2-, or 3-h. Percent inhibition of edema volume between treated and control groups was calculated as follows:
Percent inhibition = 1 – VT/VC × 100; Where, VC and VT represent the mean increase in paw volume in control and treated groups, respectively.

All the animals were fasted for 12 h and deprived of water only during the experiment. The deprivation of water was to ensure uniform hydration and to minimize variability in oedematous response

Dextran-induced oedema in rats (subacute inflammation)

Acute oedema in one of the hind paws of the rat was induced by the subplantar injection of 0.1 ml freshly prepared 6% (w/v) dextran (a phlogistic agent) (Sigma Chemical Co., St. Louis, MO) in normal saline. The paw volume was measured before and 3 h after the phlogistic injection. Different doses of the test and reference drug dissolved in normal saline was given p.o. 1 h before the dextran injection (18).

Formaldehyde-induced hind paw volume

The test was performed according to the technique developed by Brownlee (1950). Pedal inflammation was induced by injecting 0.1 ml of 4% formaldehyde solution below the plantar aponeurosis of the right hind paw of the rats. The paw volume was recorded immediately prior to compound administration (0 h) and then at 1.5 h after formaldehyde injection. Vehicle (1 ml/kg, p.o.), EESX (50, 100, 200 and 400 mg/kg, p.o.) and standard drug, ASA (300 mg/kg, p.o.) were administered 1 h prior to formaldehyde injection (19).

Cotton pellet granuloma in rats (chronic inflammation)

The effect of EESX on chronic or proliferative phase of inflammation was assessed in cotton pellet granuloma rat model (20). Autoclaved cotton pellets weighing 10 ± 1mg each were implanted subcutaneously through small incision made along the axilla region of the rats anesthetized with thiopental sodium (45

mg/kg, i.p.). The different groups of rats were administered the EESX (50,100, 200 and 400 mg/kg, p.o.) and prednisolone (10 mg/kg, p.o.) once daily for 7 consecutive days from the day of cotton pellet insertion. The control group received vehicle (1 ml/kg, p.o.). On the eighth day, all the rats were anaesthetized and the cotton pellets covered by the granulomatous tissue were excised and dried in hot air oven at 60 0C till a constant weight was achieved. Granuloma weight was obtained by subtracting the weight of dry cotton pellet on 0 day (before start of experiment) from the weight of the dry cotton pellet on eighth day.

Data analysis

The data are presented as mean ± SEM. Statistical significance was determined using ANOVA followed by dunnett *t*-test or Chi-square test with Yate's correction factor. Differences were considered significant at $P < 0.05$.

Results and discussion

Ayurveda has recommended a number of plants for the treatment of asthma and other allergic disorders and has been successful in controlling the disease as well (21). Large numbers of medicinal plant preparations have been reported to possess bronchodilatory effects; these include *Adhatoda vasica* (11), *Albizzia lebbeck* (11), *Cissampelos sympodialis* (22) and *Sarcostemma brevistigma* (23). Phytoconstituents like alkaloids and flavonoids are attributed to possess bronchodilatory activity (11, 23).

The present study for the first time demonstrates the potent bronchodilator, mast cell stabilization and anti-inflammatory activity of the EESX in different models of inflammation, i.e., acute exudative (carrageenan-induced rat paw edema), sub acute (dextran-induced rat paw edema) and chronic proliferative inflammation (cotton pellet granuloma).

The intraperitoneal injection of carrageenan and dextran caused a time-dependent paw edema in the rat, although saline injection caused no swelling. In carrageenan and dextran-induced paw edema in rats, intraperitoneal administration of EESX (100, 200, and 300 mg/kg) significantly inhibited paw swelling at 1, 2 and 3 h after carrageenan (Table 1) and at 3 h after dextran injection (Table 2). ASA (300 mg/kg, p.o.), the reference standard drug, inhibited oedema formation at 3 h in carrageenan and dextran models. The effect of EESX was well comparable to ASA. Percent increment in paw swelling was calculated by using the values before and after the injection of these phlogistic agents.

The edema and inflammation induced by carrageenan is shown to be mediated by histamine and 5-HT during first 1 h, after which increased vascular permeability is maintained by the release of kinins up to 2.30 h and from 2.30 to 6 h, the mediators appear to be prostaglandins, the release of which is closely associated with migration of leucocytes into the inflamed site (24). The Carrageenan induced paw edema model in rats is known to be sensitive to cycloxygenase (COX) inhibitors and has been used to evaluate the effect of non-steroidal anti-inflammatory agents (NSAIAs). All NSAIAs are effective in late phase and do not inhibit early phase oedema (25). Our results are consistent with these facts.

In the early stage of asthma, release of inflammatory mediators like histamine, tryptase, acetylcholine, leukotrienes, and prostaglandins are triggered by exposure to allergens, irritants, cold air or exercise (26). Some of these mediators directly cause acute bronchoconstriction. Spasmolytic drugs like beta adrenergic agonists, xanthine derivatives and anticholinergics are used as quick relief medications in such acute asthmatic attacks (27).

The EESX at the doses 100, 200 and 300 mg/kg, p.o. significantly reduced the granuloma formation to 65.7 ± 1.93 mg, 55.8 ± 1.51 mg, and 55.4 ± 1.32 mg, respectively (percentage inhibition of granuloma formation was 40.32%, 49.31%, and 49.68%, respectively) which was highly significant ($p < 0.01$), when compared to the control group of animals (Table 3).

In the present study, we have used histamine (Table 4) and acetylcholine (Table 5) as spasmogens in the form of aerosols to cause immediate bronchoconstriction in guinea pigs. The EESX (200 and 300 mg/kg) have shown significant bronchoprotection ($p < 0.001$) against histamine but not on acetylcholine aerosol as compared to control group. The bronchodilatory effect of EESX was found comparable to the protection offered by the reference standard drug mepyramine. EESX failed to produce any significant effect in the acetylcholine aerosol test.

In the histamine-induced paw edema, EESX at the doses 100, 200 and 300 mg/kg, p.o. significantly reduced the paw edema to 0.26 ± 0.01 ml, 0.17 ± 0.02 and 0.14 ± 0.01 ml, respectively at 3 h (percentage inhibition of oedema was 27.77%, 52.77%, and 61.11%, respectively) which was highly significant ($p < 0.01$), when compared to the control group of animals (Table 6).

In the mast cell stabilization test, 33% of mast cells were degranulated in the control group. Addition of DSCG (20 µg/ml) and EESX (10-100 µg/ml) reduced the percentage of degranulation ($p < 0.01$) as compared to the control group. Compound 48/80 (10 µg/ml) produced about 76% degranulation of mast cells. Pretreatment with DSCG (20 µg/ml) and EESX (10-100 µg/ml) significantly reduced ($p < 0.01$) degranulation of mast cells as compared to compound 48/80-

tretaed control group (Table 7). The protection given by it at higher concentrations was comparable to that of DSCG, a potent mast cell stabilising agent. The LD_{50} value of EESX was 2262.7 by i.p.

Although our results demonstrate the anti-histaminic, mast cell stabilizing and anti-inflammatory (acute, sub-acute and chronic) activities of EESX, the exact mechanism of these actions could not be stated at present. The anti-inflammatory effect of the plant extract may be due to its possible antihistaminic activity or by the protecting mast cell membrane and thus inhibiting release of inflammatory mediators. Taken together, further studies on the mechanism and safety of this plant is needed in order to develop EESX as the cheaper, safer and potent anti-inflammatory and anti-asthmatic therapeutic agent.

Table 1: Effect of *S. xanthocarpum* leaves on carrageenan-induced rat paw oedema[a]

Treatment	Dose (mg/kg, p.o.)	Paw oedema volume (ml)			Inhibition (%)		
		1 h	2 h	3 h	1 h	2 h	3 h
Control	saline, 1 ml	0.72 ± 0.02	0.86 ± 0.01	0.95 ± 0.01	-	-	-
ASA	300	0.69 ± 0.04	0.78 ± 0.03	0.46 ± 0.02	4.16[ns]	9.3[ns]	51.57**
EESX	50	0.70 ± 0.04	0.82 ± 0.03	0.94 ± 0.03	2.77[ns]	4.65[ns]	1.05[ns]
EESX	100	0.55 ± 0.03	0.58 ± 0.02	0.65 ± 0.02	23.61**	32.55**	31.57**
EESX	200	0.35 ± 0.03	0.39 ± 0.02	0.51 ± 0.02	51.38**	54.65**	46.31**
EESX	300	0.33 ± 0.03	0.41 ± 0.02	0.52 ± 0.03	54.16**	52.32**	45.26**

[a]Values are mean ± S.E.M. (n=6); [ns]Not significant, $^{**}P < 0.01$ vs. control; Dunnett's *t*-test after one-way ANOVA.

Table 2: Effect of *S. xanthocarpum* leaves on dextran-induced paw oedema in rats[a]

Treatment	Dose (mg/kg, p.o.)	Paw oedema volume (ml)	Inhibition (%)
Control	saline, 1.0 ml/kg	0.95 ± 0.02	-
ASA	300	0.25 ± 0.02	73.68**
EESX	50	0.93 ± 0.02	2.1[ns]
EESX	100	0.65 ± 0.01	31.57**
EESX	200	0.45 ± 0.01	52.63**
EESX	300	0.41 ± 0.01	56.84**

[a]Values are mean ± S.E.M. (n=6); [ns]Not significant, ***P* < 0.01 vs. control; Dunnett's *t*-test after one-way ANOVA.

Table 3: Effect of ethanolic extract of *S. xanthocarpum* leaves on cotton pellet granuloma in rats[a]

Treatment	Dose (mg/kg, p.o.)	Weight of granuloma (mg)	Inhibition (%)
Control	saline, 1 ml/kg	110.1 ± 2.20	-
Prednisolone	10	63.4 ± 1.16	42.41**
EESX	50	108.5 ± 1.83	1.45[ns]
EESX	100	65.7 ± 1.93	40.32**
EESX	200	55.8 ± 1.51	49.31**
EESX	300	55.4 ± 1.32	49.68**

[a]Values are mean ± S.E.M. (n=6); [ns]Not significant, ***P* < 0.01 vs. control; Dunnett's *t*-test after one-way ANOVA.

Table 4: Effect of ethanolic extract of *S. xanthocarpum* leaves on histamine-aerosol in guinea pigs[a]

Treatment	Dose (mg/kg, i.p.)	Protection (%)
Control	saline, 1.0 ml/kg	0
Mepyramine	8	90***
EESX	50	10
EESX	100	30
EESX	200	80**
EESX	300	70**

[a]n=10 in each group; [b]Intraperitoneal injection; [ns]Not significant, ** $P < 0.01$, *** $P < 0.001$ vs. control; (χ^2 with Yate's correction)

Table 5: Effect of ethanol extract of *S. xanthocarpum* leaves on acetylcholine-aerosol-induced bronchospasm in guinea pigs[a]

Treatment	Dose (mg/kg, i.p.)	Preconvulsion time (sec)	Protection (%)
Control	saline, 1.0 ml/kg	128 ± 2.72	-
Atropine	2	460 ± 4.33	72.17**
EESX	50	130 ± 1.38	1.53[ns]
EESX	100	135 ± 2.98	5.18[ns]
EESX	200	133 ± 2.32	3.76[ns]
EESX	300	130 ± 2.02	1.53[ns]

[a]Values are mean ± S.E.M. (n=5); [ns]Not significant, ** $P < 0.01$ vs. control; Dunnett's *t*-test after one-way ANOVA.

Table 6: Effect of ethanol extract of *S. xanthocarpum* leaves on histamine-induced rat paw oedema[a]

Treatment	Dose (mg/kg, p.o.)	Paw oedema volume (ml)	Inhibition (%)
Control	saline, 1.0 ml/kg	0.36 ± 0.02	-
Mepyramine	10	0.12 ± 0.01	66.66**
EESX	50	0.31 ± 0.01	13.88[ns]
EESX	100	0.26 ± 0.01	27.77**
EESX	200	0.17 ± 0.02	52.77**
EESX	300	0.14 ± 0.01	61.11**

[a]Values are mean ± S.E.M. (n=6); [ns]Not significant, ***P* < 0.01 vs. control; Dunnett's *t*-test after one-way ANOVA.

Table 7: Effect of ethanolic extracts of *S. xanthocarpum* on mast cell degranulation[a]

Pre-treatment (µg/ml)	Degranulation after treatment (%)	
	Vehicle	Compound 48/80 (10 µg/ml)
Vehicle	33.4 ± 0.93	75.8 ± 1.36
DSCG (20)	12.8 ± 3.07**	20 ± 1.79[##]
EESX (1)	24 ± 2.91[ns]	68.4 ± 2.42[ns]
EESX (10)	20.6 ± 2.64*	32.8 ± 1.96[##]
EESX (100)	17.8 ± 1.59**	41.6 ± 3.33[##]

[a]Each value represents the mean ± S.E.M. of five observations; [ns]Not significant, **P* < 0.05, ***P* < 0.01 vs. control (vehicle treated), one-way ANOVA followed by Dunnett's *t*-test; [##]*P* < 0.01 vs. control (Compound 48/80 treated), one-way ANOVA followed by Dunnett's *t*-test.

Conclusions

The present data clearly showed that ethanol extract of *S.Xanthocarpum* exhibit acute, sub acute and chronic anti-inflammatory activity by inhibiting carrageenan and dextran-induced oedema and cotton pellet granuloma. It prevented histamine and acetylcholine, well known spasmogens, induced bronchoconstriction in guinea pigs. These data clearly show anti-asthmatic action of the plant. Further studies must be conducted in order to clarify the exact anti-inflammatory mechanism of this plant extract. Taken together, the present study clearly showed anti-asthmatic potential of EESX and validated the traditional use of this plant for treating inflammatory disorders.

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References

1. Djukanovic R, Roche WR, Wilson JW, et al. Mucosal inflammation in asthma. Am J Resp Crit Care Med 1990;142:434-457.
2. Govindan S, Viswanathan S, Vijayasekaran V, et al. A pilot study on the clinical efficacy of *Solanum xanthocarpum* and *Solanum trilobatum* in bronchial asthma. J Ethnopharmacol 1999;66:205-10.
3. Goutam MP, Purohit RM. Antimicrobial activity of essential oils of the leaves of *Murraya koenigii* Spreng. Indian J Pharm 1974;36:11-12.
4. Kirtikar KR, Basu BD. Indian Medicinal Plants. 2nd ed. Dehradun, India Oriental Enterprises. 2001:131–134.
5. Yusuf M, Chowdhury JU, Wahab MA. Medicinal Plants of Bangladesh. Dhaka, Bangladesh. Council for Scientific and Industrial Research 1994:172-1205.
6. Deshmukh SK, Jain PC, Agarwal SC. Antimicrobial activity of the essential oil of the leaves of *Solanum Xanthocarpum* (Linn) Spreng (Indian curry leaf). Fitoterapia 1986;57:295–297.
7. Pathak D, Pathak K, Singla AK. Flavonoids as medicinal agents-recent advances. Fitoterapia 1991;62:371–389.
8. Bhattacharya P, Maiti AK, Basu K, Chowdhury BK. Carbazole alkaloids from *Solanum Xanthocarpum*. Phytochemistry 1994;35:1085.

9. Wu TS, Wang ML, Wu PL, Furukawa H. Carbazole alkaloids from the leaves of *Solanum Xanthocarpum*. Phytochemistry 1996;41:14-33.
10. Shah GB, Parmar NS. Antiasthmatic property of polyherbal preparation E-721 B. Phytother Res 2003;17:1092-1097.
11. Amin AH, Mehta DR. Bronchodilator alkaloid from *Adhatoda vasica*. Nature 1959;184:1317-1318.
12. Lorke D. A new approach to acute toxicity testing. Arch Toxicol 1983;54:275–287.
13. Armitage AK, Boswood J, Large BJ. Thioxanthines with potent bronchodilator and coronary dilator properties. Br J Pharmacol Chemother 1961;16:56-76.
14. Kumar AD, Ramu P. Effect of methanolic extract of *Benincasa hispida* against histamine and acetylcholine-induced bronchospasm in guinea pigs. Indian J Pharmacol 2002;34:365-366.
15. Kaley G, Weiner R. Prostaglandin 'E' a potential mediator. Ann New York Acad Sci 1971;180:347–348.
16. Horakova Z, Moratova J. Means of influencing the oedematous component of inflammation. In: Garattini S, Dukes MNG, eds. International Symposium on Non-steroidal Anti-inflammatory Drugs, Amsterdam, Excerpta Medica Foundation, 1964:237–244.
17. Winter CA, Risley EA, Nuss GW. Carrageenan-induced edema in hind paws of rat as an assay for antiinflammatory drugs. Prog Soc Exp Biol Med 1962;11:544-547.
18. Winter CA. Anti-inflammatory testing methods: comparative evaluation of indomethacin and other agents. In: Garattini S, Dukes MNG, eds. International Symposium on Nonsteroidal Anti-inflammatory Drugs, Amsterdem, Excerpta Medica Foundation, 1964:190–202.
19. Brownlee G, Madigan DG, Griffiths LL, et al. Paraaminosalicylic acid in tuberculosis: Clinical and pharmacological aspects. The lancet 1950;255(6598):239-45.
20. Winter CA, Porter CS. Effect of alterations in side chain upon anti-inflammatory and liver glycogen activities of hydrocortisone esters. Am J Pharm Sci 1957;46:515–519.
21. Zhimmet I, Tashkin DP. Alternative medicines for allergy and asthma. J Allergy Clin Immunol 2000;106:603-614.
22. Thomas G, Araujo CC, Duarte JC, Desouza DP. Bronchodilatory activity of an aqueous fraction of an ethanol extract of the leaves of *Cissampelos sympodialis* in guinea pigs. Phytomedicine 1997;4:233-238.
23. Saraf MN, Patwardhan BK. Pharmacological studies on *Sarcostemma brevistigma*. Part II Bronchodilator activity. Indian Drugs 1988;26:54-57.
24. Di-Rosa M, Giroud JP, Willoughby DA. Studies on the mediators of acute inflammatory response induced in rats in different sites of carrageenan and turpentine. J Pathol 1971;104:15–29.
25. Rao CV, Kartik R, Ojha SK, Rao GM. Antiinflammatory and antinociceptive activity of stem juice powder of *Tinospora cordifolia* Miers in experimental animals. Hamdard Medicus 2005;48:102–106.
26. Bosquet J, Jeffery PK, Busse WW. Asthma: From bronchoconstriction to airway inflammation and remodeling. Am J Respir Crit Care Med 2000;161:1745-1749.
27. Horwitz RJ, Busse WW. Inflammation and asthma. Clin Chest Med 1995;16:583-620.