EVALUATION OF ANTI-ASTHMATIC PROPERTY OF *SOLANUM XANTHOCARPUM* FLOWER EXTRACTS.

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Summary

Kantkari (*Solanum Xanthocarpum*) is one of the members of the dashmula (ten roots) of the Ayurveda. It is a very spiny diffuse herb up to 1.2 m tall, commonly found throughout India, used in medicine in various forms, such as decoction, electuary, ghrita, etc. A decoction of the root is given with the addition of long pepper and honey, in cough and catarrh, and with rock salt and assafoetida in spasmodic cough. Plant has been investigated for much of responses and as well a pilot study on the clinical efficacy of *Solanum xanthocarpum* as a dried whole plant shown significant improvement in some respiratory diseases like bronchial asthma. The present study aimed at investigating the anti-asthmatic property of petroleum ether, ethanol (95%), water extract of flowers of *Solanum xanthocarpum*, obtained by successive extraction on i*n-vitro* and i*n-vivo* animal models. Compared to Pet. Ether and water extract only ethanolic (95%) extract (SXEX) shown promising result as relaxed the histamine precontracted isolated goat tracheal chain ($P<0.05$). A dose dependent contraction of goat tracheal chain is observed. Treatment with SXEX (100 mg/kg, i.p.) treatment significantly ($p< 0.05$) reduced milk induced eosinophilia (18.16±0.912) and SXEX (50 mg/kg, i.p.) able to control the milk induced eosinophilia (25.5± 5.71) as compared control group which receives only vehicle and milk (43.2±0.663) in mice (n=5), while mast cells were protected at a dose of (50 &100 mg/kg, i.p) by 74.39% and 78.26 % respectively by SXEX as compared to DSCG shown protection by 83.81%. Also, SXEX decreased capillary permeability by 62% in mice was evident from its effect on optical density of the dye. Phytochemical screening showed presence of phyto sterols, alkaloids, flavonoids and steroids. The result suggest that the SXEX possess antihistaminic, mast cell stabilizing and decreased capillary permeability effect and hence possesses potential role in the treatment of asthma and allergic disorders. .

Key Words: *Solanum xanthocarpum*, antihistaminic, eosinophilia, capillary permeability.

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Introduction

Asthma is a chronic inflammatory disease and pathogenesis of this disease is believed to be atopic in more than 50% adults and at least 80% of affected children (1). According to numerous epidemiological studies from different parts of the world, the prevalence of the atopic diseases has increased over the past 30–40 years (2). Atopic asthma is the most common chronic disease of childhood and childhood mortality due to bronchial asthma has doubled in the past decade (3). Bronchial asthma is a inflammatory disorder of the airways characterized by various airway obstruction, airway eosinophilic inflammation and bronchial hyperresponsiveness (4) and is a global health problem that results from a complex interplay between genetic and environmental factors (5). Bronchodilators and steroid inhalers are often effective in controlling mild to moderate asthma with minimal adverse reactions. However, the use of systemic steroid in severe and persistent cases shows only fair responses and is normally associated with serious side effects. Development of a better and safer regime for the management of asthma is therefore urgently needed, at least to target at the development of steroid sparing medication. On this behalf many drugs are originated from plant and herbal medicine should be a promising direction for the current drug search. One of such plant in the indigenous system of medicine (Kantkari) *Solanum xanthocarpum* (Solanaceae), Charaka and Sushruta used the extract of entire plant and fruits in internal prescription for bronchial asthma, tympanitis, misperistalsis, piles and dysuria and for rejuvenation. Kankari Ghrita of Charaka is specific for cough and asthma (6). Linctus prepared with stamens of kantkari flowers is prescribed for chronic cough in children (Bangasena). Roots are one of the constituents of well known Ayuervedic preparation “Dasmul Asava” (6). In ancients Ayurveda, plant is described as pungent, bitter, digestive, alternative astringent. Stems, flowers, fruits are bitter, carminative. Root decoction used as febrifuge, effective diuretic and expectorant. Aqueous extract of the fruits of SX investigated for hypoglycaemic activity (7). The methanolic extract of SX aerial parts shown to have Antinociceptive activity (8). Solasodine obtained from SX berries shown to have Antifertility (9) effects in male rats and dogs. Ethanol and Water Extract of SX dried ripe fruits studied for its Adaptogenic Activity (10) and Free radical scavenging activity (11). A pilot study on the clinical efficacy of *Solanum xanthocarpum* & *Solanum trilobatumin* bronchial asthma were also undertaken to prove the significant use of herbs in treatment of asthma (12, 13, 14). Major literature data supports use of whole plants, so the present study was undertaken as no such effort have made till time to evaluate and validate the claim the therapeutic effect i.e. asthma relieving or antihistaminic, antiallergic property of (ESX)*Solanum xanthocarpum* flowers extract i*n vitro* on isolated goat tracheal chain (15) and *in vivo* studies on milk- induced eosinophilia (16), mast cell degranulation (17) and capillary permeability (18) in mice (n=5). As huge number of studies have focused on the aetiology of asthma. Many different hypotheses have been postulated including atopy, environmental allergen exposure, hygiene hypotheses, etc. Also various derivatives from specific medicinal plants were identified as the antiasthma components and some mechanisms of action were also explored. We are focusing the present work on possible effects of these plant part extract on some of the parameters like smooth muscle relaxtion, and antagonism of asthma mediators such as histamine, eiosinophils and protection against mast cell degranulation which seems to be prominent in pathophysiology of asthma

Methods

Collection of plant material

The fresh flowers of *Solanum xanthocarpum* were collected in the month of November 2006 at Jalgaon Dist. Jalgaon, MS, India. The plant and flower material authenticated by Dr.A.K.Singhai Dept. of Pharmaceutical Sciences Dr.H.S.Gour University Sagar. A voucher specimen (No. 28676) was deposited in the Botanical Survey of India, Pune, India.

Extraction of plant material

Solanum xanthocarpum flowers were shed dried and reduced to coarse powder using pastel and mortar. The Coarse powdered flowers (2000 g) were subjected for successive extraction using Sohxlet assembly, initially with petroleum ether (40^0-60^0), then the marc dried in open air and further subjected for ethanolic extraction (95%), finally the same marc after drying rendered for extraction using purified water. Extracts is filtered and concentrated by evaporation under reduced pressure. Yield of (SXPETE) Petroleum ether extract is 12.86%w/w/, (SXEE) Ethanol (95%) yield is 11.58%w/w and (SXWE) Water extract gives max. yield of 18.20%w/w. Preliminary phytochemical screening showed presence of phyto-sterols, alkaloids, flavonoids and steroids.

Animals

Male albino mice (Swiss strain) weighing 22- 25 g were housed under standard laboratory condition in a group of five each. Animals had free access to food and water. The Institutional Animal Ethical committee (IAEC) has approved the protocol of the study.

Chemicals and reagents

Histamine Sigma USA, Clonidine Unichem, India, Disodium cromoglycate Cipla, RPMI Buffer medium 1640 of Hi Media has been purchased. Reagents like Eosin solution WBC diluting fluid purchased from Qualigens India. Toluidine blue and Evans blue were purchased from Research Lab. Bovine serum albumin and Freund's adjuvant obtained as sample from Serum Institute of Pune.

Goat tracheal chain preparation

Goat trachea brought from slaughter house were cut into individual ring and tied together in series to form a chain (15). Then suspended in a bath containing Kreb's solution (Concentration in mMoles/ L as NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.2; $NaHCO_3$, 25.0; KH_2PO_4, 1.2; Glucose, 11.1) maintained at 37 ± 1^0C a stream of CO_2 in O_2 is bubbled through the organ tube. One end tied to aerator tube and other attached to isotonic frontal writing leaver to Kymograph paper on Sherrington rotating drum. Tissue is allowed to equilibrate for 45 min. under to load of 400 mg (15). Dose response curves for histamine were taken in variant molar concentration. After obtaining a dose response curve of histamine on goat trachea aqueous solution of extract (n= 4) was added to reservoir and same dose of histamine were repeated.

Acute toxicity studies for dose selection

Healthy adult male albino mice (18- 22g) used for acute toxicity studies as per guidelines (AOT 425) suggested by the organization for economic co-operation and development (OECD-2001). The mice were observed continuously for 2 h for behavioral and autonomic profiles and for any sign of toxicity or mortality up to a period of seven days (OECD Guideline For The Testing of Chemicals: Guidance document on acute oral toxicity. Environmental Health and Safety Monograph Series on Testing and Assessment 2000). Highest dose at which no toxic signs are seen, one fifth of that should be taken as effective dose.

***In vivo* studies**

Milk- induced eosinophilia

A blood eosinophilia is hallmark of both allergic and non allergic asthma. Mice were divided into five groups, five animals each. Blood samples were collected from retro-orbital plexus under light ether anesthesia, the eosinophil count is done in each group before drug administration and 24 h after the milk injection (boiled and cooled, 4 ml/kg, s.c.). Difference in the eosinophil count before and 24 h after milk administration was noted using modified method described by Brekhman (16).

Mast cell degranulation

Mice divided in five groups, five animals each. The three days drug treatment schedules were followed. Group I received vehicle (10 ml/kg, i.p.). Group- II treated with standard drug disodium cromoglycate (DSCG, 200µg/ml, i.p.). Group-III, IV and V were treated with extract 25, 50 and 100 mg/kg, i.p. respectively. On day fourth each animal were injected with 4 ml/kg, 0.9% NaCl solution into peritoneal cavity. By gentle massage, peritoneal fluid collected after 5 min. and transferred into siliconised test tube containing 7-10 RPMI- 1640 buffer medium (pH 7.2- 7.4). This solution then centrifuged at 400-500 rpm. Pellets of mast cell were washed with same buffer medium twice by centrifugation, discarding supernatant. The cells were challenged with clonidine (50 µg) incubated at 37 ^{0}C in a water bath for 10 min. Followed by staining with 1 % toluidine blue and observed under microscope (45 X). Total 100 cells were counted from different visual area. Percent protections against degranulation were calculated using method described by Lakdawala (17).

Capillary permeability

Mice were divided into four groups of five animals each. The mice in the control group were sensitized with bovine albumin and Freund's adjuvant 0.05 ml given i.p. Three weeks later the animals were challenged with the same dose of bovine albumin. At the same time Evan's blue injected i.v. in a dose of 200mg/kg. The mice were sacrificed. 30 min. later 5 ml of saline was injected i.p. and the abdominal wall was gently massaged for a minute. The abdomen was then incised and peritoneal fluid was collected and filtered after passage through glass wool. It was centrifuged at 3000 rpm for 15 min. The group II, III and IV received test drug extract in a dose of 25, 50 and 100 mg/kg, i.p. 24 h and 2 h before the challenge. The transmittance of the dye depends on capillary permeability was determined by measuring the optical density on a spectrophotometer by modification of the method used by K. Tagoki and T. Fukao (18).

Statistical analysis

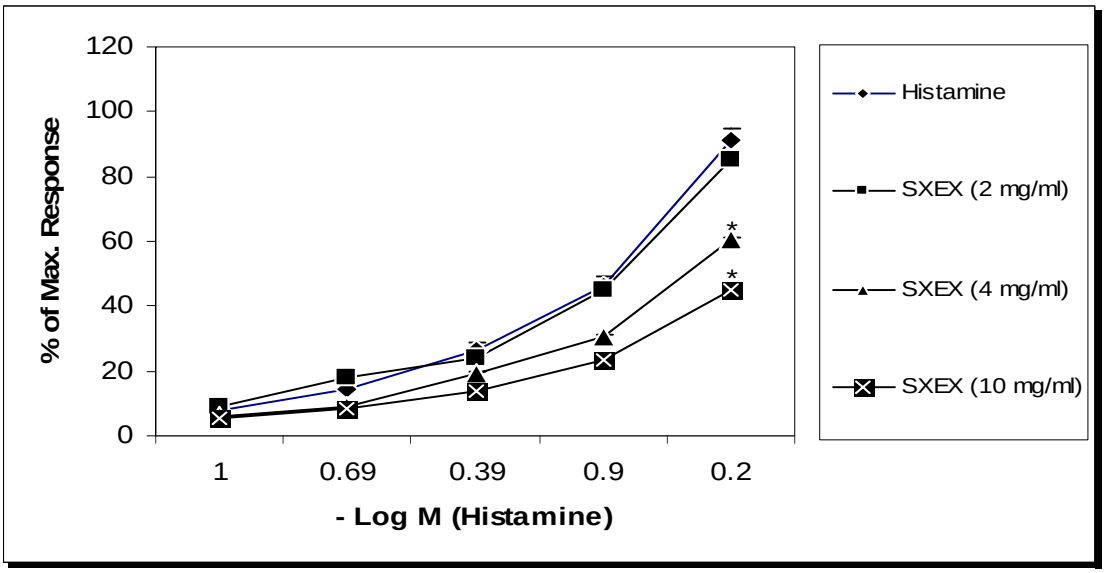
The data is presented as mean±SEM. The statistical significance between the groups has been tested by ANOVA followed by Dunnett's test. A probability value less than 0.05 were considered as significant.

Results

Effect of Pet ether (SXPETE), Ethanol (SXET) and Water extract (SXWE) on isolated goat tracheal chain

Histamine at a dose of 1.6 µg/ml able to produce notable contraction on isolated goat tracheal chain (90 mm taken as 100 %). Goat tracheal chain is known as a sensitive tissue for studying the effect of histamine. We studied the response produced by histamine in this tissue in presence of the plant extracts. Petroleum ether and Water extract exhibited no antagonistic effect while SXWE exerted antagonistic effect on histamine-induced contraction ($p< 0.05$) at a dose of 4 mg/ml (60.44±0.843) shows moderate action and 10 mg/ ml (44.71±0.947) exhibited significant antagonistic effect. Table 1 and Figure 1 is indicative of this activity might be due to relaxant effect exerted by SXEX i.e. depression of histamine H_1 receptor.

Figure 1: **Effect of SXEX on Isolated Goat Tracheal Chain**



n= 4, values are expressed in mean±SEM. *$p< 0.05$ compared with histamine induced contraction (90 mm taken as 100 %).

Effect of Pet ether (SXPETE), Ethanol (SXET) and Water extract (SXWE) on milk induced eosinophilia

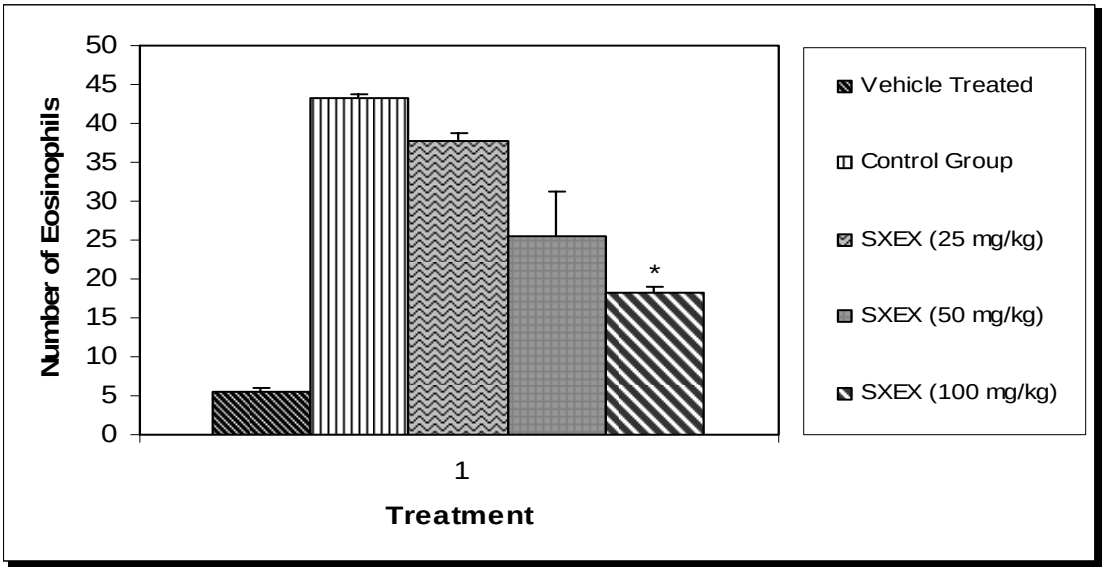
Mice treated with of milk (boiled and cooled, 4 ml/kg, s.c.) showed a significant increase in total eosinophil count. SXEX (100 mg/kg, i.p.) treatment significantly ($p< 0.05$) reduced milk induced eosinophilia (18.16±0.912) and SXEX (50 mg/kg, i.p.) able to control the milk induced eosinophilia (25.5± 5.71) as compared control group which receives only vehicle and milk (43.2±0.663). Figure 2 is suggestive of relevance of SXEX and in antagonizing the milk induced blood eosinophilia.

Table No.1:

Sr. No	Extract/Drug	Dose	Effect on the tissue	Effect on histamine- induced contraction (%)
1.	Histamine	1.6µg/ml	Contraction	91.10±3.76
2.	SXPETE	2 mg/ml	Contraction	92.77±1.72
3.	SXPETE	4 mg/ml	Contraction	91.10±1.20
4.	SXPETE	10mg/ml	Contraction	94.99±1.32
5.	SXEX	2 mg/ml	Contraction	85.27±1.657
6.	SXEX	4 mg/ml	**Relaxation**	60.44±0.843*****
7.	SXEX	10mg/ml	**Relaxation**	44.71±0.947*****
8.	SXWE	2 mg/ml	Contraction	93.88±0.716
9.	SXWE	4 mg/ml	Contraction	91.10±1.015
10	SXWE	10mg/ml	Contraction	87.49±0.950

n =4, values are expressed in Mean±SEM
*P<0.05 compared with histamine- induced contraction (90 mm taken as 100%).

Figure 2: Effect of SXEX on Milk Induced- Eosinophilia in Mice

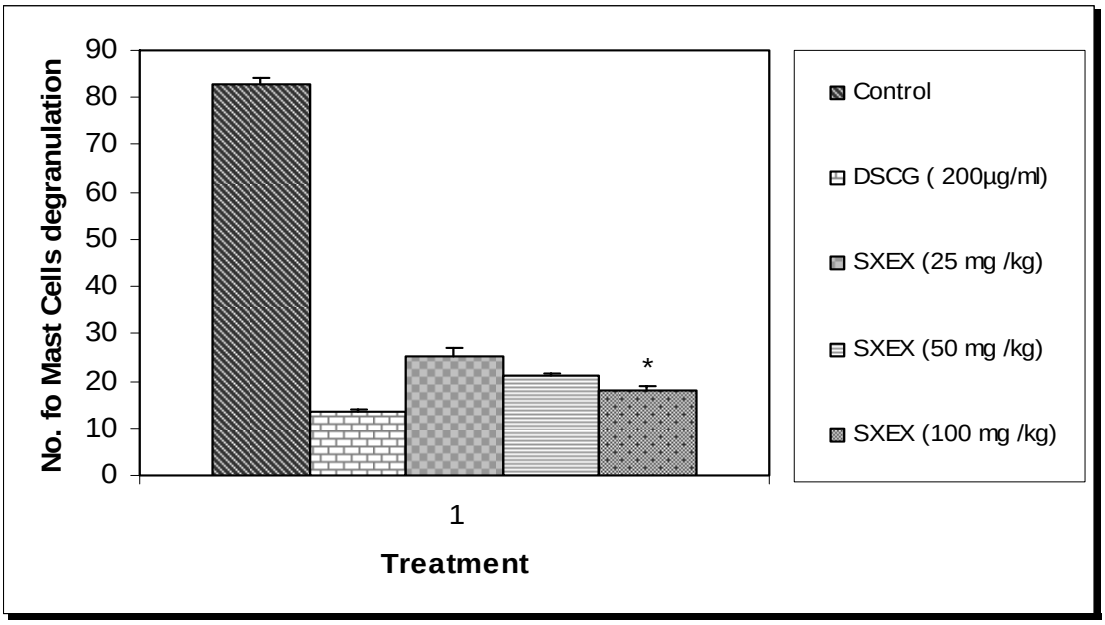


n= 5, values are expressed in mean±SEM.
*p< 0.05 compared with control group (ANOVA followed by Dunnett's test).

Effect of Pet ether (SXPETE), Ethanol (SXET) and Water extract (SXWE) on mast cell degranulation.

Clonidine challenge resulted in significant degranulation of mast cell. Pretreatment of sensitized animal with standard drug DSCG shown protection 83.81% and SXEX at a dose of (50 & 100 mg/kg, i.p) shown percentage protection of 74.39% and 78.26 % respectively as seen in figure 3.

Figure 3: Effect of SXEX on Mast Cell Degranulation in Mice

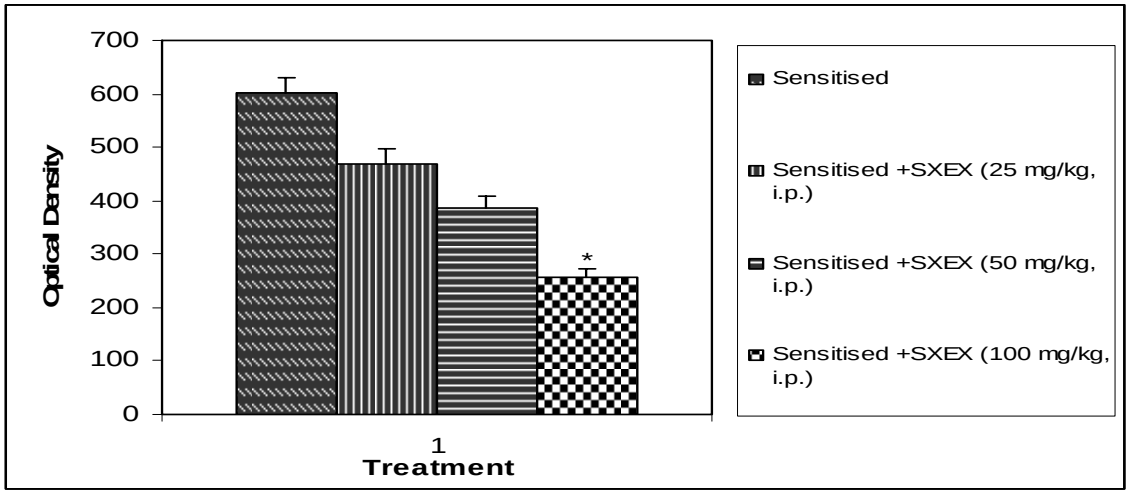


n= 5, values are expressed in mean±SEM. *p< 0.05 compared with control group (ANOVA followed by Dunnett's test).

Effect of Pet ether (SXPETE), Ethanol (SXET) and Water extract (SXWE) on capillary permeability.

The data in controlled group as well as group treated with SXPETE, SXWE shows higher percent transmittance of the dye exceptionally the treatment of SXEX (100 mg/kg, i.p) significantly decreased the percentage transmittance as is evident from its effect on optical density of the dye as shown in Fig. 4.

Figure 4: **Effect of SXEX on Capillary Permeability in Mice**



n= 5, values are expressed in mean±SEM. *p< 0.05 compared with sensitized group (ANOVA followed by Dunnett's test).

Discussion

Bronchial asthma is a chronic inflammatory disease, characterized by both bronchoconstriction and airway inflammation which leads to bronchial hyrerresponsiveness to various stimuli, in which many cell types play a role, more important being mast cells, eosinophils and T- lymphocytes (19).Histamine is an important mediator of immediate allergic (type-1) and inflammatory reactions. It causes bronchoconstriction by activating H_1–receptors. The trachea is used for the experimental purpose rather than the bronchi since it is easier to dissect and has the same reactions to spasmogenic and spasmolytic drugs. Although, the method is known for its suitability in the study of antispasmodic drugs in general, emphasis is given on its use in the testing of bronchodilators. This is because of the close anatomical and physiological association, which exists between tracheal and bronchial musculature (20). The guinea pig tracheal chain is a classical preparation, but requires some skill to prepare and is not very sensitive for many agonists (15). The goat tracheal chain is easier to handle and to prepare; it is also much more sensitive than guinea pig tracheal chain. Therefore, the dose relative contractile responses of different agonists like ACh, Histamine, 5-Hydroxytryptamine and Bradykinin can be observed in isolated goat trachea. (15). With these agonists, the concentration necessary to produce contraction is generally less with goat tracheal chain than with guinea pig tracheal chain. Histamine contracts the tracheobronchial muscle of dog, horse, guinea pig and man (21).

The dose dependent contraction by spasmogens such as histamine (0.1-102.4 mg), acetylcholine (0.1-12.8 μg), and barium chloride (0.1-51.2 mcg) using goat tracheal chain preparation demonstrated by Kulshrashtha (22). They have reported antihistaminic effect (H_1-antagonism) of mepyramine maleate by using goat tracheal chain preparation. Histamine antagonism modulated by the relaxing factors involved and may be due to the suppression of histamine H1-receptor (23). The present work aimed at justifying and investigating the potential of flowers of SX as Ethanol extract of SX shown a significant ($p< 0.05$) antihistaminic activity in histamine induced contraction in goat tracheal chain preparation. SXPETE and SXWE at a Dose of 2, 4 and 10 mg/ml showed no such significant relaxation. Thus the significant inhibition of histamine induced contractions produced by Ethanol extract of SX flower on isolated goat tracheal chain preparation indicates that the SX flower has antihistaminic (H_1-receptor antagonist) action.

A blood eosinophilia is hallmark of both allergic and non allergic asthma. Eosinophils are recruited and found to be activated during segmental allergen challenge (24). Eosinophilia is an abnormal increase in peripheral eosinophil count (25). In the late phase, especially in the development of allergic asthma, eosinophils plays role as inflammatory cells, as it secretes mediators such as eosinophil cationic protein (ECP), eosinophil derived neurotoxin (EDNT), granulocyte macrophage colony stimulating factor (GM-CSF), tumor necrosis factor (TNF), and Prostaglandin (PG), which results in epithelial shedding, bronchoconstriction and promotion of inflammation in respiratory tract (25,26). Here while screening the all three extracts of Flowers of SX, results are indicative that only SXET at a dose of 50 &100 mg / kg reduced milk-induced eosinophilia of statistical significance.

Uvnas (27) studied the mast cell degranulation and its correlation with the release of histamine after administration of compound 48/ 80, the mast cell degranulating agent. Both clonidine and compound 48/80 act through the dynamic expulsion of granules without causing any damage to the cell wall (28). Lakadawala (17) have shown that clonidine releases histamine from mast cells in a similar manner to a selective liberator like compound 48/80. It is known that disodium cromoglycate a standard mast cell stabilizer prevents degranulation of mast cells by raising the cyclic adenosine monophosphate(29). It has been known that all pharmacological agents that increase intracellular levels of AMP relax airway smooth muscle and inhibit the release of autocoids from the tissue and basophils. Present study shows statistically significant stabilization of mast cell by SXEX at a dose of (50 &100 mg/kg, i.p) shown percentage protection of 74.39% and 78.26 % respectively as Compared to standard drug DSCG 83.81%.

Asthma is a heterogeneous disorder immunologically, physiologically and biochemically and its etiology is multifactorial and in one form of allergic disorder. Anaphylaxis is included in an immediate type of allergy. Mast cells play a critical role in immediate hypersensitivity and allergic rections when activated through immunoglobulin E (IgE) by specific antigens. In this study the result of increasing leakage of dye was seen in sensitized animal while the test extract which are under investigation able to desensitize and control the immediate type of allergic which is evaluated as the transmittance of the dye (Evans blue) depends on capillary permeability was determined by measuring the optical density on spectrophotometer by modification of the method used by Tagoki and Fukao, (18). It was reported that anaphylaxis in the rat is associated with a marked increase in intestinal capillary permeability (30).The only treatment group of SXEX (100 mg/kg, i.p) able to show significant value to have promising role in allergic asthma.The marked response shown by the ethanolic extract of flowers of SX instigates further studies to explore the mechanistic approach and component responsible for the valid utility of the herbs in treatment of asthma.

References

1. World Health Organization. Prevention of allergy and allergic asthma. Geneva, (WHO), 2002: 3.
2. Von Hertzen, L.C. Haahtela T. Asthma and atopy- the price of affluence on Allergy, 2004, 59:124–137.
3 Burr M.L, Limb E.S, Andrae S, Barry D.M, Nage F. Childhood asthma in four countries: a comparative survey. International Journal of Epidemiology 1999, 23: 341–347.
4 Djukanovic R, Roche W.R, Wilson J.W. Mucosal inflammation in asthma. American journal of respiratory and critical care medicine 1990, 142: 434–457.
5 Phillip, F. Gene therapy for asthma. Molecular Therapy 2003, 7: 148–152.
6 C.P.Khare. Encyclopedia of Indian Medicinal Plants; Published by Springer 1995:432-433.
7 Kar D. M, Maharana L, Pattnaik S, Dash G. K. Studies on hypoglycaemic activity of *S.xanthocarpum* S. & W. fruit extract in rats. J. of Ethnopharmacol. 2006, 108: 251-256.

8 Rahman M.T, Ahmed M, Alimuzzaman M, Shilpi JA. Antinociceptive activity of the aerial parts of Solanum xanthocarpum. Fitoterapia 2003, 74(1-2):119-21.
9 Dixit VP. Antifertility effects of solasodine (C27 H 43 0 2 N) obtained from Solanum xanthocarpum berries in male rats and dogs. Journal of Steroid Biochemistry 1986,25: 24-27.
10 Ravindra Pandey, Anurekha Jain, V. Saini, et al. Adaptogenic Activity of Ethanol and Water Extract of *Solanum Xanthocarpum* Fruits. Seminar of Indian Society of Pharmacognosy On"Current Advances in Phytopharmaceuticals". 2006:11-12.
11 Gopal Garg, Karunakar Shukla, Swarnlata Saraf . Free radical scavenging activity of *Solanum xanthocarpum* root extracts. Seminar of Indian Society of Pharmacognosy on "Current Advances in Phytopharmaceuticals". 2006,11-12.
12 Mohan L, Sharma P, Srivastava CN. Comparative efficacy of Solanum xanthocarpum extracts alone and in combination with a synthetic pyrethroid, cypermethrin, against malaria vector, Anopheles stephensi. Southeast Asian J Trop Med Public HealthMar 2007, 38(2):256-60.
13 Govindan S, Viswanathan S, Vijayasekaran V. Further studies on the clinical efficacy of Solanum xanthocarpum and Solanum trilobatum in bronchial asthma Phytother Res. 2004, 18(10):805-9.
14 Govindan S, Viswanathan S, Vijayasekaran V, Alagappan R. A pilot study on the clinical efficacy of Solanum xanthocarpum and Solanum trilobatum in bronchial asthma. J Ethnopharmacol. ; 1999, 66(2):205-10.
15 Nag Chaudhari AK, Lahiri SC. Ind.J. Pharmacol. 1974, (6):149-151.
16 Brekhman II, Dardymov IV. Annu. Rev. Pharmacol. 1969, (9): 419-430.
17 Lakadawala AD, Dadkar MK, Dohadwala AN. J. Pharm. Pharmacol.1980, (32):790-791.
18 Tagoki K, Fukao T, Jap. J.of Pharmac.1971 (2) :455-465.
19 Bousquet, J. Jeffery, P.K., Busse, W.W. Asthma: From bronchoconstriction to airway inflammation remodeling. Am. J. Respir. Crit. Care. Med. 2000, 161: 1720-1745.
20 Castillo. J. C. & de-Beer, E. J. The tracheal chain-l. A preparation for the study of antispasmodics with particular reference to bronchodilator drugs. J. Pharmacol. Exp. Ther. 1947, 90: 104-109.
21 Ehright T, Chua S, Lim DJ. Ann.J. Allergy 1989, (62):277-83.
22 Kulshreshtha S, Misra SS, Sharma AL, Sharma P, Singhal D. Ind. J Pharmacol. 1983, (15):107-110.
23 Shi CC, Liao JF, Chen CF, Pharmacol.Taxicol. 2001, 89 (5):259-264
24 Busse, W. W., Lemanske, R. F. Jr.: Asthma. New Engl. J. Med. 2001, 344: 350-336.
25 Brigden, M.L. A Practical Workshop for Eosinophilia Postgraduate Med. 1999, 3: 105-15.
26 Osama, N.O., Joshi, K.R., Immunology: Immunology of Respiratory Disease, Agro Botanical Publishers: Bikaner. 1984: 321-329.
27 Uvnas B. Ind. J. Pharmac. 1969, (1): 23-24.
28 Stanworth DR, Immediate Hypersensitivity, North Holland Publishing Company Amersterdam, 1973:73.
29 Geetha VS, Viswanathan S, Kameswaran L, Ind.J.Pharmacol.1981, (13):199-201.
30 Benditt E.P, Holcenberg J,Lagunoff D. Ann. N. Y. Acad. Sci.1963:179.