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ANTIHISTAMINIC PROPERTY OF SOLANUM XANTHOCARPUM AND ITS PHYTOCONSTITUENTS

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Abstract

Now a days there has been steady increase in the prevalence allergic immune response involving immune cells and mediators. The key role in the immune cascade is taken by histamine. It is natural component of the body, which in the allergic inflammatory response is released by the mast cells and basophils. Compound Sx-7 isolated from alcoholic extract of S. xanthocarpum as white crystals (m.p. 164ºC) U.V., ^{1}H NMR and mass spectroscopy of this compound is similar to spectral data of stigmasterol. Active phytoconstituents Solasodine also extracted from alcoholic extract which play an important role for altering concentration in human body. In comparison with standard drug DSCG alcoholic extract protect degranulation of mast cell at dose of 50 mg & 100 mg/kg b.w. 74.39% and 78.26% respectively. Solasodine protect 79.48% and 84.38% at a dose of 1.0mg & 2.0 mg/kg b.w. significantly. Histamine causes branchoconstriction by activating H1-receptors. Eosinophilia is abnormal increase in the peripheral eosinophil count especially in the development of allergic asthma. Stabilization of mast cell by alcoholic extract of S. xanthocarpum at a dose of 50mg & 100 mg/kg b.w., i.p. shown percentage protection of 74.39% and 78.26% respectively as compared to standard drug DSCG 83.81%. Active constituent Solasodine protection percentage was found 79.48 and 84.38 at dose of 1.0mg and 2.0 mg/kg b.w. significantly.

Keywords: *Histamine, Immune response, Histamine blockers, Mastcell*

Introduction

Solanum xanthocarpum is a medicinal plant used various herbalism. The word 'herb' derived from the Latin word herba which refers to any part of plant like fruit, seed, leaves, stem, bark, flower, stigma or root as well as a non-woody plant. These medicinal plants are also used as food, flavonoid, medicine or perfume and certain spirihial activities. Medicinal plants are plants possessing nutraceutical as well as pharmaceutical properties i.e., having health boosting characteristics as well as quick and curative response against indicative/ suggestive problems. The medicinal properties of such plants are due to presence of various bioactive phytochemical constituents which produce certain physiological action or induce biological action on the human beings as well as on the animals. S. xanthocarpum is an angiospermic plant of Solanaceae family. It is an important medicinal herb commonly known as the "Indian night shade" or "Yellow berried night shade". It is used as an ingredient in many formulations like Chavanprasha, Dashmoolarishtha, Vyaghriharitaki avaleha, Vyaghritailam, Vyaghriyadi kwatha, Vyaghrighratam etc. A poly herbal formulation named "Jigrine" contains aqueous extracts of some medicinal plants and one of them is also S. xanthocarpum.

Plant contains alkaloids, sterols, saponins, flavonoids and their glycosides and also carbohydrates, fatty acids, amino acids etc. other constituents like caffeic acid Coumarins and phenolic compounds were also reported. Major chemical constituents present in plant: Solasodine, Steroidal alkaloids, -Solamargine, -Solamargine, Solasonine, Sterols viz., Cycloartenol, Norcarpesterol, Cholesterol, Solanacarpine, Carpesterol, Atropine, Diosgenin and -Sitosterol, 3-Hydroxy-3 Methyl Glutaryl COA Reductase, Solanine, Glycosides, Phenolics, Flavonoids, Steroid and Saponins[1]. S. xanthocarpum root contains solanine, solanidine a waxy substances and fatty acids. It also contains alkaloids, tannins, sugars, starch, fats, oils, proteins mucilage, lignins and calcium oxalate. A fruit contains diosgenin, solasonine, -solamargine, -solamargine, solasodine and Petals contains apigenin and stamens contain quercetin, diglycoside and -sitosterol.

Bronchial asthma is an inflammatory disorder of the airways characterized by various airway obstruction, airway eosinophilic inflammation and bronchial hyper responsiveness and is a global health problem that results from a complex interplay between genetic and environmental factors[2]. A pilot study on the clinical efficacy of S. xanthocarpum and Solanum trilobatum in bronchial asthma were undertaken to prove the significant use of herbs in treatment of asthma[3]. Major literature data supports use of whole plants. Sheth[4] evaluated the therapeutic effect of ethanolic extract of S. xanthocarpum i.e. asthma relieving or antihistaminic, antiallergic property[5].

Sheth[4] studied effects of S. xanthocarpum extract on some of the parameters like smooth muscle relaxation, and antagonism of asthma mediators such as histamine, eiosinophils and protection against mast cell degranulation which seemed to be prominent in pathophysiology of asthma[5]. Further they showed that ethanol extract of S. xanthocarpum shown a significant antihistaminic activity in histamine induced contraction in goat tracheal chain preparation. Ethanolic extract of S. xanthocarpum at a dose of 50 and 100 mg/kg reduced milk- induced eiosinophilia of statistical significance. SXE at a dose of 50-100 mg/kg, i.p showed significant mast cell stabilization as compared to standard drug Disodiumchromoglycate (DSCG). A decrease in forced expiration volume and peak expiration flow rates are indicative of both large and small airway obstruction and muscle power[6]. It was suggested that relief from the symptoms of bronchial asthma produced by S. xanthocarpum 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.

Material and Methods

The collected plant materials were brought to the laboratory for the study of phytochemical and biological study. Plant parts (stem, leaf and fruits, root) of the medicinal plant were cleaned and dried under shade. The dried plant materials were then ground well to fine powder.

Extraction: Powdered plant material was defatted with petroleum ether (60-80ºC) in a soxhelet extractor. The extract was dried and refluxed with water for 8 hrs. The aqueous extract was filtered and concentrated using rotary vacuum evaporator and the dried extract was stored in an air tight container. For alcoholic extract, 800 gm fine powder was extracted with ethanol in Soxlet apparatus for 72 hrs. Extracted solution was concentrated by distillation. Concentrated extract was dried on water bath for semi solid extract. The yield of alcoholic extract was 1.8%. It was placed at 4ºC for compound analysis and biological study.

Chromatographic Separation: The aqueous extract of plant material was subjected to thin layer chromatography using silica gel-G as stationary phase and petroleum ether: methanol (1:1) and petroleum ether: chloroform: methanol (5:2:1) as mobile phase. The chromatograms when developed provide seven and eight spots respectively that showed zones for steroidal nucleus with Liebermann – Buchard visualizing reagent.

Biological study: Acute toxicity study was performed according to Organization for Economic Co-operation and Development guidelines No. 423[7]. Swiss albino mice of either sex were divided into six groups with six animals each. SXE was administered orally as a single dose to mice at different dose levels of 250, 500, 1000, 1500 and 2000 mg/kg b.w. Animals were observed periodically for the symptoms of toxicity and death within 24 h and then proceed for 14 days.

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.

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-2000).

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.

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 37º 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 Lakdawala methods[8].

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 2h 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 Takagi, and Fukao[9].

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. SXEX at a dose of 100 to 500mg/kg and solasodine at a dose of 1, 2, 4 & 6 mg/kg, p.o. respectively were 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, Diclofenac Sodium (150 mg/kg, p.o.), respectively. For each treatment group six animals were used. Diclofenac Sodium were administered 1h 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.

Characterisation of Compound

Sx7 is a white crystalline powder (9mg) with a melting point (164ºC) was subjected to TLC using various solvent systems such as petroleum ether: chloroform: methanol (5:3:1), methylene chloride: petroleum ether (50:50) indicated it to be homogenous compound. **Sx7** was further subjected to ^{1}HNMR, ^{13}CNMR and LCMS to ascertain the chemical structure. The compound **Sx7** is a white crystalline substance with a melting point of 164ºC. IR absorptions bands appeared at 3384 cm^{-1} (OH), 3218 cm^{-1} (cyclic olefinic – HC= CH– str), 3025 (=CH str) and 2868 cm^{-1} assigned to C–H str. Other absorption frequencies include 1665 cm^{-1} as a result of C=C absorption, however, this band is weak. 1462 cm^{-1} is a bending frequency for cyclic $(CH_2)n$ and 1382 cm^{-1} for $–CH_2$ $(CH_3)_2\gamma$. The absorption frequency at 1332 cm^{-1} can be attributed to –OH group and at 1046 cm^{-1} signifies cycloalkane. These absorption frequencies resemble the absorption frequencies observed for Stigmasterol[10,11].

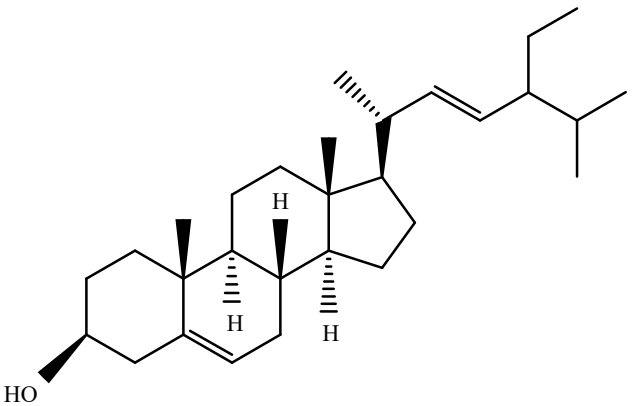


Fig.1 : Structure of isolated compounds Sx7

The Proton NMR has revealed the existence of signals for olefinic proton at 5.358 (br., s.,) Angular methyl proton at 0.68(s), 0.699 (s) and 1.01 (s) corresponding to C_{18} and C_{19} proton respectively. The ^{13}CNMR has shown recognizable signals 140.8 and 121.7 ppm which are assigned C_5 and C_6 double bonds respectively as in 5 spirostene. The value at 71.0 ppm is due to C_3 –hydroxyl group[11]. The signals at 19.4 and 11.9 ppm correspond to angular carbon atom (C_{18} and C_{19} respectively). The above IR, ^{1}HNMR, ^{13}CNMR, LCMS spectral data and a comparison of the ^{13}CNMR signal with those described in the literatures[12-15] showed the structure of Sx7 to be the Stigmasterol.

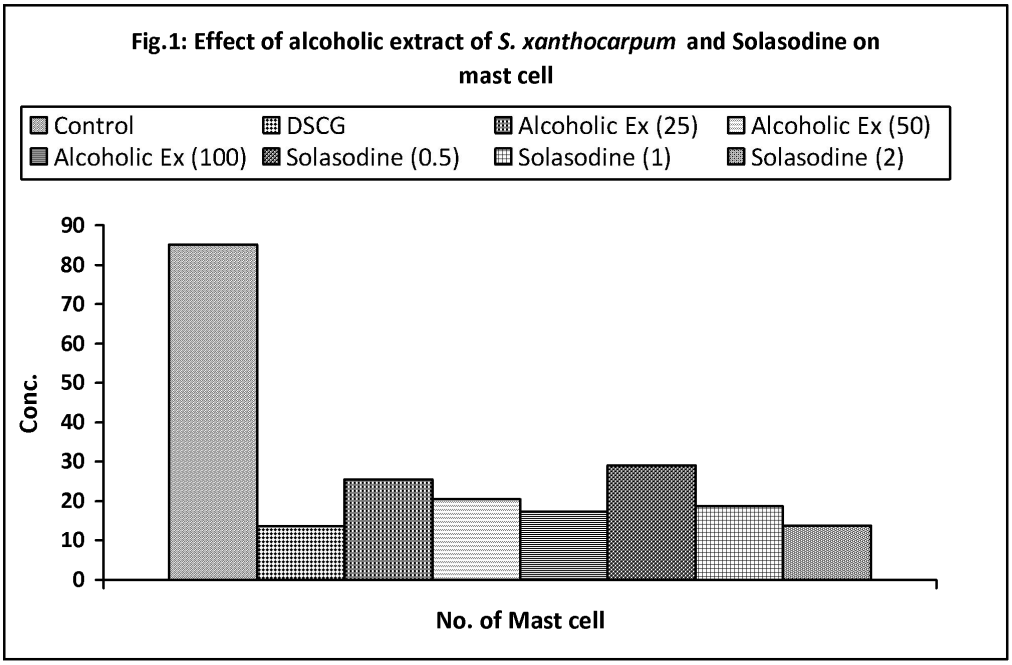
The ^{13}C–NMR has shown recognizable signals of β sitosterol and Stigmasterol. The weak molecular ions were given at m/z 414 and 412.7. The molecular weight and fragmentation pattern indicate that the isolated compound is stigmasterol.

S. xanthocarpum and its active compound possess antihistaminic, mast cell stabilizing and decreased capillary permeability effect and hence possesses potential role in the treatment of asthma and allergic disorders. Bronchial asthma is a inflammatory disorder of the airways characterized by various airway obstruction, airway eosinophilic inflammation and bronchial hyper-responsiveness and is a global health problem that results from a complex interplay between genetic and environmental factors. 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.

Histamine, one of the most intensively studied molecules in medicine[16] is a key mediator in allergic rhinitis (AR) and urticaria. Interacting with a unique group of membrane-bound receptors widely distributed across immune cell subtypes, histamine participates in intricate bidirectional messaging between cytokines and inflammatory cells or their precursors, facilitates migration of cells to inflammatory sites, stimulates lymphocyte activity, modulates aspects of eosinophil, neutrophil and mast cell behavior and is directly implicated in the generation of cardinal allergic symptoms such as rhinorrhea; sneezing; congestion; nasal, ocular, and dermal pruritus; hives; and flushing[17]. The H_1-histamine receptor is most clearly associated with modulation of proinflammatory immune cell activity and its interaction with histamine is the prime focus of suppressive therapy for AR and urticaria with second-generation H_1-antihistamines such as cetirizine, desloratadine, ebastine, fexofenadine, levocetirizine, loratadine, and rupatadine.

Table-1: Effect of alcoholic extract of S. xanthocarpum and Solasodine on mast cell degranulation in mice

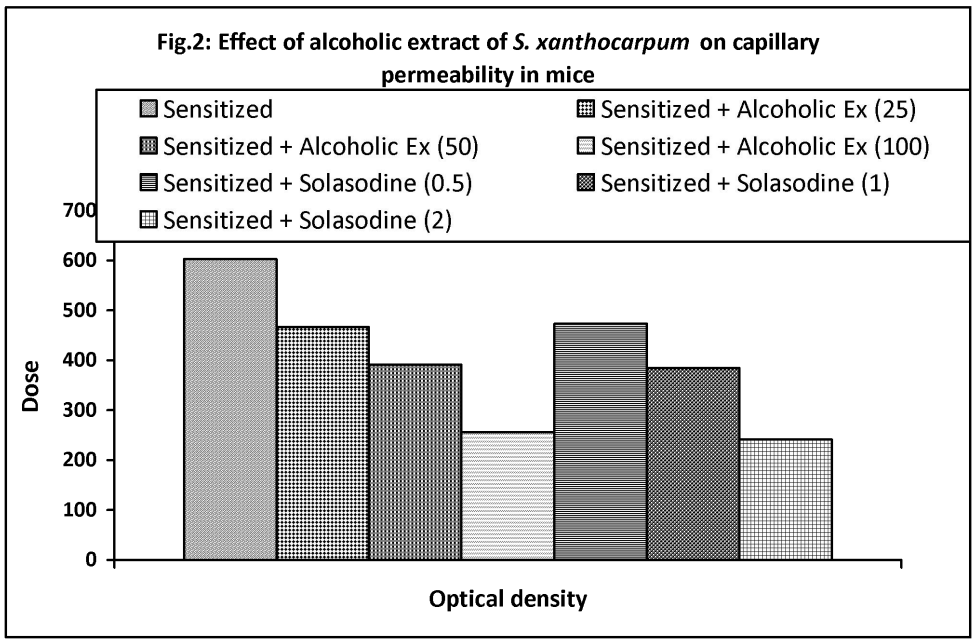
Treatment	Concentration	Number of mast cell
Control (Vehicle)	10 ml/kg	85.10
DSCG	200 g/ml	13.60
Alcoholic extract of S. xanthocarpum	25 mg/kg	25.51
Alcoholic extract of S. xanthocarpum	50 mg/kg	20.43
Alcoholic extract of S. xanthocarpum	100 mg/kg	17.32
Solasodine	0.5 mg/kg	29.13
Solasodine	1 mg/kg	18.76
Solasodine	2 mg/kg	13.87



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 presented in table-1 and Solasodine 79.48% and 84.38% at a dose of (1.0 mg and 2.0 mg/kg) respectively.

Table-2: Effect of alcoholic extract of S. xanthocarpum on capillary permeability in mice

Treatment	Dose (I.P.)	Optical density
Sensitized (bovine albumin + Freund adjuvant)	0.05 ml	602.23
Sensitized + Alcoholic extract of S. xanthocarpum	25 mg/kg	466.51
Sensitized + Alcoholic extract of S. xanthocarpum	50 mg/kg	391.30
Sensitized + Alcoholic extract of S. xanthocarpum	100 mg/kg	255.85
Sensitized + Solasodine	0.5 mg/kg	473.20
Sensitized + Solasodine	1 mg/kg	384.16
Sensitized + Solasodine	2 mg/kg	241.62

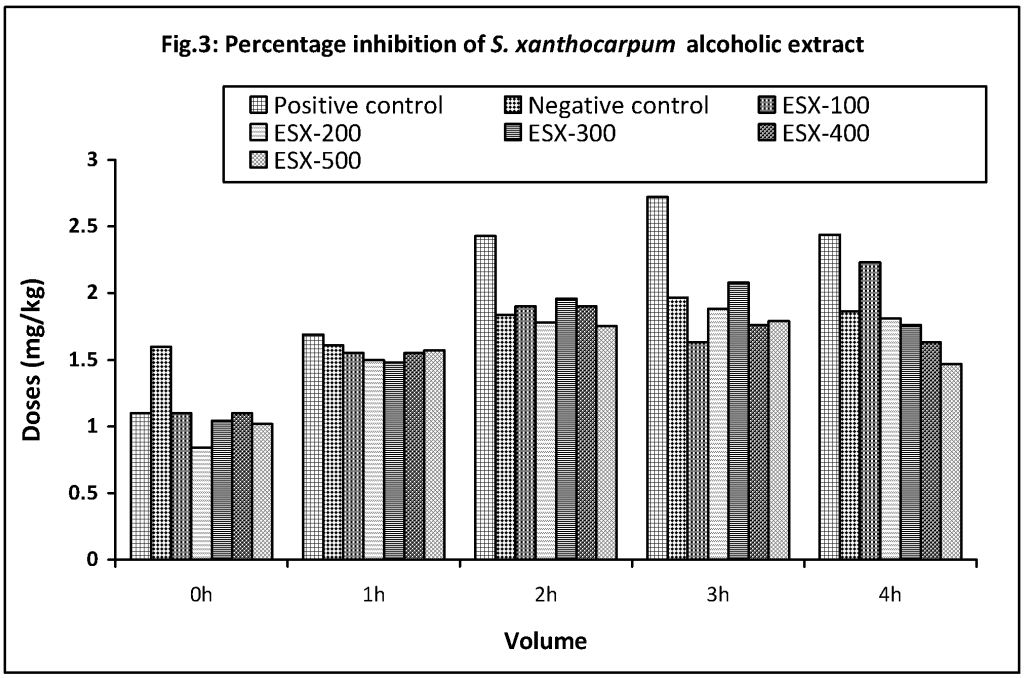


Bronchial asthma is a chronic inflammatory disease, characterized by both bronchoconstriction and airway inflammation which leads to bronchial hyper-responsiveness to various stimuli, in which many cell types play a role, more important being mast cells, eosinophils and T- lymphocytes[18]. Histamine 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. A blood eosinophilia is hallmark of both allergic and non allergic asthma. Eosinophils are recruited and found to be activated during segmental allergen challenge[19]. Eosinophilia is an abnormal increase in peripheral eosinophil count[20]. 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[21]. Here while screening the all three extracts of Flowers of SX, results indicated that only SXEX at a dose of 50 &100 mg / kg and Solasodine 1.0 mg & 2.0 mg/kg reduced milk-induced eosinophilia of statistical significance. The detail of alcoholic extract of S. xanthocarpum inhibition on inflammation presented in table-

Table-3: Percentage inhibition of Solanum xanthocarpum alcoholic extract

Treatment	Doses (mg/kg)	Average paw volume of rats					Mean difference in fourth-hour paw volume (X)
		0h	1st h	2nd h	3rd h	4th h	
Positive control		1.10	1.69	2.43	2.72	2.44	1.34
Negative control		1.60	1.61	1.84	1.97	1.86	0.26
Solanum xanthocarpum (extract)	100	1.10	1.55	1.90	1.63	2.23	1.13
	200	0.84	1.50	1.78	1.88	1.81	0.97
	300	1.04	1.48	1.96	2.08	1.76	0.72
	400	1.10	1.55	1.90	1.76	1.63	0.53

	500	1.02	1.57	1.75	1.79	1.47	0.45



Uvnas[22] 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[23]. Lakadawala[8] have shown that clonidine releases histamine from mast cells in a similar manner to a selective liberator like compound 48/80. Present study shows statistically significant stabilization of mast cell by ethanolic extract of S. xanthocarpum 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%.

Mast cells play a critical role in immediate hypersensitivity and allergic reactions when activated through immunoglobulin E (IgE) by

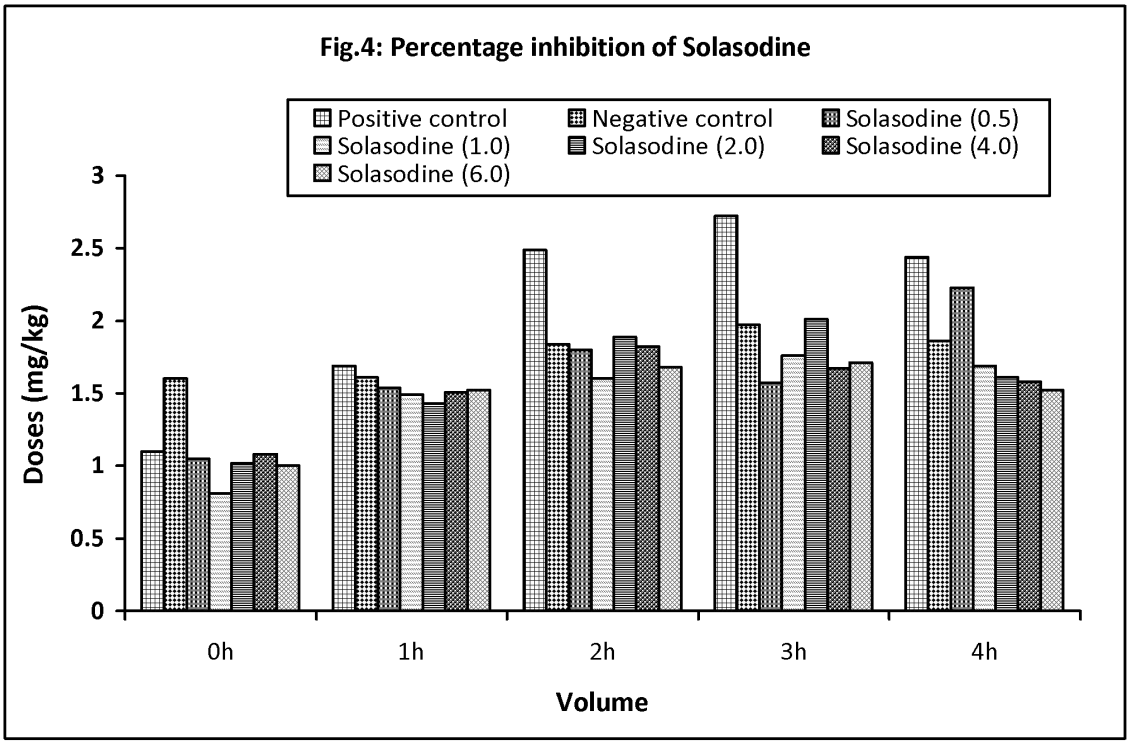
specific antigens. In this study the result of increasing leakage of dye was seen in sensitized animal while the test extract which are under investigation able to desensitize and control the immediate type of allergy which is evaluated as the transmittance of the dye (Evans blue) depends on capillary permeability was determined by measuring the optical density on spectrophotometer by modification of the method used by Takagi and

Fukao[9]. It was reported that anaphylaxis in the rat is associated with a marked increase in intestinal capillary permeability[24].

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 dextram-induced paw edema in rats, intraperitoneal administration of SXEX (100 to 500 mg/kg respectively) significantly inhibited paw swelling at 1, 2 and 3h after carrageenan (Table-3) and at 3 h after dextran injection. Diclofenac Sodium (150 mg/kg, p.o.) the reference standard drug, inhibited oedema formation at 3h in carrageenan and dextran models. The effect of SXEX was well comparable to Diclofenac Sodium. 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 1h, 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 mediations appear to be prostaglandins, the release of which is closely associated with migration of leucocytes into the inflamed site. The detail of Solasodine activity on inflammation presented in table-4.

Table-4: Percentage inhibition of Solasodine

Treatment	Doses (mg/kg)	Average paw volume of rats					Mean difference in fourth-hour paw volume (X)
		0h	1st h	2nd h	3rd h	4th h	
Positive control		1.10	1.69	2.49	2.72	2.44	1.34
Negative control		1.60	1.61	1.84	1.97	1.86	0.26
Solasodine	0.5	1.05	1.54	1.80	1.57	2.13	1.08
	1.0	0.81	1.49	1.60	1.76	1.69	0.88
	2.0	1.02	1.43	1.89	2.01	1.61	0.59
	4.0	1.08	1.51	1.82	1.67	1.58	0.50
	6.0	1.00	1.52	1.68	1.71	1.52	0.52



It was found that Solasodine inhibit more in comparison to alcoholic extract of S. xanthocarpum when inflammation developed by Carrageenan. In this process gathering of leucocytes directly countered by some site of Solasodine[6]. 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. Our results are consistent with these facts.

Conclusion

Knowledge of the mechanisms underlying the allergic reaction, records a constant growth and tells us about the complex network of cells and mediators, which are at the core of allergic inflammatory response. Through its receptors, histamine as an important chemical messenger plays an important role in the physiological response, including neurotransmission, allergic inflammation and immunomodulation. Drugs that have the histamine receptors as target for their activity can be considered as a very good choice for the treatment of allergic conditions. Pharmacodynamic and pharmacokinetic differences between the first and second generation of antihistaminics should be well known, because it can help when choosing the right drug. Cetrizine is a potent second-generation antihistamine which shows remarkable immunoregulatory properties. It influences the interaction of mediator cells with all systems. It affects the interaction of eosinophils, mast cells and fibroblasts and thus may participate in the regulation of the internal environment.

The marked anesthetic power of the antihistaminic compounds cannot be unimportant. It is probable that some effects of these compounds are due to this anesthetic activity. Burn stated: “Antihistaminic substances then join the group of other substances, which include spasmolytics like Trasentine and Syntropan, analgesies like penthidine and papaverine, local anesthetics like procaine and atropine-like substances. None of these can be sharply distinguished from one another. Probably each possesses every property in some degree”. It is due to this situation that it may remain difficult to separate more clearly the antihistaminic from the anesthetic action of the antihistaminic compounds.

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