Evaluation of Anti-Inflammatory and Immunomodulatory Effects of Aqueous Extract of Solanum Xanthocarpum in Experimental Models of Bronchial Asthma

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Abstract

The study assessed the effects of standardized extracts of Solanum xanthocarpum on airway inflammation and oxidative stress parameters in experimental model of asthma in rats. Wistar rats were immunized with ovalbumin (OVA) adsorbed on to aluminium hydroxide (i.p.) and were challenged with 1% OVA in saline aerosol from day 15 to 22. Standardized aqueous extracts of Solanum xanthocarpum (whole plant extract) were administered orally for 22 days in different treatment groups. After 24h of last challenge, rats were anesthetized and blood and bronchoalveolar lavage fluid (BALF) were collected, centrifuged and analysed for IgE levels, pro-inflammatory cytokines (TNF-α & IL-6), Th2 type cytokines (IL-4 & IFN-γ) and oxidative stress parameters malondialdehyde (MDA) and reduced glutathione (GSH). The results showed that ovalbumin specific IgE levels were elevated in the immunized rats which were reduced by 37% and 20% respectively in blood and BALF at the dose of 50 mg/kg of Solanum xanthocarpum extract as compared to control. These results were comparable with the effects of the standard drug prednisolone (10 mg/kg) which reduced IgE level by 43% and 31% respectively in blood and BALF respectively. Similarly, TNF-α, IL-6 & IL-4 were attenuated maximum at dose 100 mg/kg as compared to control and IFN-γ was elevated maximum in both blood and BALF. The oxidative stress markers, i.e. MDA levels were also attenuated while GSH levels were elevated in rats treated with Solanum xanthocarpum extract at the dose of 100 mg/kg. The results showed that aqueous extract of Solanum xanthocarpum has anti-inflammatory and immunomodulatory effects which is accompanied with reduction in oxidative stress. Thus, the results suggest that the beneficial effects of Solanum xanthocarpum in bronchial asthma could be due to balancing influence on prooxidant-antioxidant status and reducing the airway inflammation.

Keywords: Asthma; Herbal Drugs; Solanum Xanthocarpum; Ovalbumin; Oxidative Stress

Abbreviations

BALF: Bronchoalveolar Lavage Fluid; OVA: Ovalbumin; ELISA: Enzyme Linked Immunosorbent Assay; DTNB: 5,5'-Dithiobis (2-Nitrobenzoic acid); NADPH: Nicotinamide Adenine Dinucleotide Phosphate; GSH: Glutathione; MDA: Malondialdehyde; AHR: Airway Hyperresponsiveness; ROS: Reactive Oxygen Species; TNF-α: Transforming Nuclear Factor Alpha; IFN-γ: Interferon Gamma; IL: Interleukin

Introduction

Asthma is a chronic respiratory disease defined by reversible airway narrowing involving airway inflammation, airway hyper-responsiveness (AHR) and airway tissue remodelling [1]. It involves the recruitment and activation of various inflammatory and structural cells capable of synthesising and releasing inflammatory mediators which are responsible for pathophysiology of asthma disease [2,3].

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Activation of mast cells and eosinophils leads to release of proinflammatory cytokines (IL-6 & TNF-α), Th2 type cytokines (IL-4, IL-5 and IL-13) and IgE production [4]. Activated inflammatory cells like macrophages and eosinophils also produce reactive oxygen species (ROS) leading to oxidative stress [5].

Pharmacotherapy of asthma involves use of bronchodilators and anti-inflammatory agents [6]. Corticosteroids have long been used as the main therapeutic drugs for asthma. However, these treatments are not curative, and symptoms return soon after treatment termination, and prolonged use of corticosteroids may result both systemic and local side effects. In addition, increasing evidences of refractory response to these drugs is also a major issue that needs to be tackled. Thus, the development of efficient therapeutic drugs for allergic and inflammatory lung disease is urgently needed.

The World Health Organization currently encourages, recommends and promotes traditional/herbal drugs in National Health Care Programme because of their easy availability, low cost, safety and the faith of people in such remedies. In India, medicinal plants constitute the principal health care resources for the majority of population and per capita annual consumption of modern drugs is very low. The traditional medicines all over the world are nowadays revalued by an extensive activity of research on different plant species and their therapeutic principles [7]. Traditional medicinal plants reported in various ancient literatures provide rational means of treatment of various diseases [8]. Under such changed world health picture, it is quite reasonable to explore the use of less known plants as potential sources of medicines, and also to determine and identify scientifically the responsible cellular and molecular mechanisms involved in their effects.

*Solanum xanthocarpum* (Kantakari) is an important medicinal herb in Ayurvedic Medicine and used to treat respiratory disease [9]. In traditional system of medicine different parts of *Solanum xanthocarpum* like leaves, stem, flower, root, seeds and the plant as a whole is used. The herbal plant is used as anti-asthmatic, hypoglycaemic, anti-inflammatory, anti-tumor, anti-tussive, antipyretic, antispasmodic, antihistaminic and cytotoxicity activity [10]. It contains alkaloids, phenolics, flavonoids, sterol, saponins and their glycosides which have a wide range of medicinal value [11]. It has various medicinal properties particularly in treatment of asthma, chronic cough and catarrhal fever [12]. It is one of the members of the dashamula (ten roots) of the Ayurveda [13]. Vadnere., *et al*. [14] studied the anti-asthmatic property of *Solanum xanthocarpum* in animal models and found that it has antihistaminic and mast cell stabilizing property and hence possesses potential role in the treatment of asthma and allergic disorders. The present study is planned to evaluate the effects of *Solanum xanthocarpum* in experimental models of bronchial asthma and the possible cellular and molecular mechanisms involved therein.

Materials and Methods

Animals

Wistar rats of either sex (150 - 200g) were used for the study (n = 5, per group). Animals were maintained under standard laboratory conditions of natural light-dark cycle and temperature (22 ± 2°C), and had free access to food and water in the highly specialized experimental animal facility of the V.P. Chest Institute. The animal care was as per guidelines laid down by the Indian National Science Academy, New Delhi. The study protocol was approved by the Institutional Animal Ethics Committee.

Drugs and chemicals

The standardized aqueous extract of whole plant of *Solanum xanthocarpum* was procured from Natural Remedies, Bangalore. Ovalbumin (OVA) and prednisolone were procured from Sigma-Aldrich, USA. The ELISA assay kits (rat) for TNF-α, IL-4, IL-6, IFN-γ were procured from Diaclone, France, whereas assay kit for ovalbumin specific IgE was procured from KinesisDX, USA. All other routine and standard laboratory reagent was procured from Sisco research lab, New Delhi.

Experimental procedure

Rats were divided into 5 groups (n = 5 each group) viz., (I) Control [vehicle-saline]; (II, III, IV) *Solanum xanthocarpum* in doses 50, 100

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and 200 mg/kg/day respectively; (V) prednisone (10 mg/kg/day). All the drugs were administered orally for 22 days. All groups were immunized with ovalbumin (40 mg/rat, i.p.) adsorbed to 2 mg of aluminium hydroxide on day 0. Fourteen days after immunization, the animals were challenged with 1% ovalbumin aerosol, dissolved in 0.9% saline for 8 consecutive days [15].

Blood and BAL fluid collection and storage: After 24 hour of last challenge rats were anesthetized and blood was collected through cardiac puncture. Blood was centrifuged at 4 °C (3000 rpm) for 10 minutes and serum were separated and stored at -80°C. BAL fluid were collected by lavaging the lungs through a tracheal cannula with 0.9% sodium chloride solution and centrifuged at 4 °C (1500 rpm) for 10 min. the supernatant were collected and stored at -80°C for assay of various biomarkers.

**Assay for Ovalbumin specific IgE (OVA sIgE) and cytokines**

Blood and BAL fluid samples were assayed for levels of TNF-α, IL-4, IL-6 and IFN-γ and ovalbumin specific IgE using commercially available enzyme-linked immunosorbent assay (ELISA) kits. These cytokine assays were performed using sandwich ELISA. The levels of rat OVA sIgE, the kit used double antibody sandwich ELISA. The microtitre plate provided is precoated with monoclonal antibody specific to Immunoglobulin E (IgE). Samples were added to wells and after incubation OVA sIgE secondary antibody labelled with biotin was added. Streptavidin-HRPwas added to wells in order to form immune complex and was then incubated. Chromogenic solution A and B were added to develop colour and the reaction was stopped by using a stop solution. The absorbance of coloured product was measured using software based microplate reader at 450 nm and results were expressed in ng/ml. Similarly, TNF-α, IL-4, IL-6 and IFN-γ levels were also measured by using specific ELISA kits. Antigen and biotinylated polyclonal antibody specific to these cytokines were added to microtitre plate wells which were pre-coated with polyclonal antibody specific to TNF-α, IL-4, IL-6 and IFN-γ. The HRP conjugate streptavidin was added and incubated. Further, TMB substrate was added to produce a colour reaction product. After that reaction was stopped by using H2SO4 and absorbance was taken using microplate reader at 450 nm and results were expressed in pg/ml.

**Estimation of Malondialdehyde (MDA) and Reduced glutathione (GSH) levels**

Reduced glutathione (GSH) levels were estimated by using Ellman's reagent [16]. This assay was based on the enzymatic recycling procedure in which glutathione is sequentially oxidized by DTNB and is then reduced by NADPH in the presence of enzyme glutathione reductase. The extent of 2-nitro-5-benzoic acid formation was monitored as an increase in absorbance at 412 nm. Malondialdehyde (MDA), a marker of lipid peroxidation was determined by the method of Tietze method [17]. Briefly, the reaction mixture consisted of 0.2 ml of 8.1% of sodium lauryl sulphate, 1.5 ml of 20% acetic acid (pH 3.5) and 1.5 ml of 0.8% aqueous solution of thiobarbituric acid and 0.2 ml of serum sample. The mixture was made up to a volume of 4ml with distilled water and then heated at 95 °C for 60 min. After cooling with tap water, 5 ml of n-butanol and pyridine (15 : 1) and 1 ml of distilled water were added and centrifuged. The organic layer was separated and its absorbance was measured at 532 nm using a spectrophotometer.

**Statistical analysis**

All data were expressed as Mean ± SEM and analysed by one way ANOVA followed by Dunnet’s test. A p value < 0.05 was used as a level of significance in all statistical tests.

**Results**

**Effects of Solanum xanthocarpum on Ovalbumin specific IgE (OVA sIgE) levels in blood and BALF**

Sensitization with OVA on day 0 followed by challenge with OVA aerosol for 8 days resulted in significant elevation in IgE levels as compared to Normal rats which were sensitized but not challenged with OVA. The enhanced IgE levels in control (OVA) rats, reflected airway inflammation and validated the experimental model of asthma in rats. The results are depicted in Figure 1.
The aqueous extract of Solanum xanthocarpum was administered daily for 22 days and its effect was assessed on OVA sIgE levels in blood and BALF in OVA immunized rats. Solanum xanthocarpum reduced the OVA sIgE levels for all doses when compared to control group. Solanum xanthocarpum at dose 50 mg/kg reduced IgE levels by 37% and 20% in blood and BALF respectively. Solanum xanthocarpum at dose 100 mg/kg reduced IgE levels by 34% and 13% while at 200 mg/kg by 25% and 7% in blood and BALF respectively. Pretreatment with prednisolone markedly suppressed the OVA sIgE levels by 43% and 31% in blood and BALF. Analysis of data showed that these changes in different treatment groups were significantly different across the groups. \[F (4, 24) = 6.15\] for blood; \[F (4, 24) = 4.924\] for BALF; \(p < 0.05\). The results are summarized in Figure 2.

**Figure 1:** Effects of aqueous extract of *Solanum xanthocarpum* (SX) on IgE levels in blood and BALF of normal and control rats. Normal- sensitized but not challenged rats; Control- sensitized and challenged rats with ovalbumin. Data are expressed as Mean ± SEM. *\(p < 0.05\) versus normal group.

**Figure 2:** Effects of aqueous extract of *Solanum xanthocarpum* (SX) on IgE levels in blood and BALF in ovalbumin challenged rats. C(OVA)-Ova sensitized and challenged rats; PDN- Prednisolone (10 mg/kg); SX1-SX3 Solanum xanthocarpum (50, 100 and 200 mg/kg) extract orally. Data are expressed as Mean ± SEM. *\(p < 0.05\) versus control group.

**Effects of Solanum xanthocarpum on Cytokines (TNF-\(\alpha\), IL-6, IL-4 and IFN-\(\gamma\)) levels**

Effect of aqueous extract of *Solanum xanthocarpum* was assessed on cytokines levels in blood and BALF in OVA immunized rats. Assay for TNF-\(\alpha\) showed that *Solanum xanthocarpum* (50, 100 and 200 mg/kg) reduced the TNF-\(\alpha\) levels with all doses compared to control group. In blood, *Solanum xanthocarpum* at dose 50, 100 and 200 mg/kg reduced TNF-\(\alpha\) level by 29%, 31% and 30% respectively while in

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BALF by 25%, 30% and 23% respectively. Pre-treatment with prednisolone markedly suppressed the TNF-α levels by 34% and 26% in blood and BALF fluid. Analysis of data showed that changes in different treatment groups were significantly different across the groups. \[F (4, 24) = 7.41 \text{ for blood}; F (4, 24) = 7.01 \text{ for BALF}; p < 0.05\]. Further, assay for IL-6 showed that *Solanum xanthocarpum* (50, 100 and 200 mg/kg) reduced IL-6 levels in blood and BALF compared to control group. In blood *Solanum xanthocarpum* reduced IL-6 levels by 45%, 68% and 38% while in BALF IL-6 levels reduced by 17%, 25% and 6% respective in all three doses. Pre-treatment with prednisolone markedly suppressed the IL-6 levels by 63% and 37% in blood and BALF. Analysis of data showed that changes in different treatment groups were significantly different across the groups. \[F (4, 24) = 8.94 \text{ for blood}; F (4, 24) = 3.71 \text{ for BALF}; p < 0.05 \text{ for each group}\]. The results are summarized in Figure 3 (a and b).

![Figure 3a.](image)

**Figure 3a.**

**Figure 3:** Effects of aqueous extract of *Solanum xanthocarpum* (SX) on TNF-α and IL-6 levels in blood and BALF in ovalbumin-induced rats. C(OVA)-Ova sensitized and challenged rats; PDN- Prednisolone (10 mg/kg); SX1-SX3 Solanum xanthocarpum (50, 100 and 200 mg/kg) extract orally. Data are expressed as Mean ± SEM. *p < 0.05 versus control group.*

![Figure 3b.](image)

Similarly, assay for IL-4 showed that *Solanum xanthocarpum* (50, 100 and 200 mg/kg) induced suppression of IL-4 levels by 26%, 40% and 6% in blood samples in respective doses. *Solanum xanthocarpum* (50, 100 and 200 mg/kg) induced suppression of IL-4 levels by 35%, 63% and 20% in BALF sample. Pre-treatment with prednisolone were markedly suppressed the IL-6 levels by 37% and 58% in blood and BALF. Analysis of data showed that there were significant differences in IL-4 levels across the entire treatment group in blood and BALF.

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Assay for IFN-γ levels showed that *Solanum xanthocarpum* (50, 100 and 200 mg/kg) elevated IFN-γ levels in blood and BALF as compared to control group. In blood *Solanum xanthocarpum* elevated IFN-γ levels by 46%, 55% and 50% while in BALF IFN-γ levels were elevated by 76%, 80% and 75% respectively in all three doses. Pre-treatment with prednisolone markedly increased the IFN-γ levels by 99% and 73% in blood and BALF. Analysis of data showed that changes in different treatment groups were significantly different across the groups [F (4, 24) = 3.88 for blood; F (4, 24) = 11.01 for BALF; p < 0.05 for each group]. The results are summarized in Figure 4 (a and b).

**Figure 4a.**

**Figure 4b.**

**Figure 4:** Effects of aqueous extract of *Solanum xanthocarpum* (SX) on IL-4 and IFN-γ levels in blood and BALF in ovalbumin-induced rats. C(OVA)-Ova sensitized and challenged rats; PDN- Prednisolone (10 mg/kg); SX1-SX3 Solanum xanthocarpum (50, 100 and 200 mg/kg) extract orally. Data are expressed as Mean ± SEM. *p < 0.05 versus control group.

**Effects of Solanum xanthocarpum on GSH and MDA levels**

Assay of MDA showed that *Solanum xanthocarpum* (50, 100 and 200 mg/kg) induced suppression in MDA levels by 12%, 22% and 17% in blood sample in respective doses. *Solanum xanthocarpum* (50, 100 and 200 mg/kg) induced suppression of IL-4 levels by 68%, 64% and 85% in BALF samples. Pre-treatment with prednisolone were markedly found to suppress the IL-6 levels by 56% and 32% in blood and BALF respectively. Analysis of data showed that there were significant differences in MDA levels across the entire treatment
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group in blood and BALF. [F (4, 24) = 9.22 for blood; F (4, 24) = 6.77 for BALF; p < 0.05]. GSH levels in both blood and BALF showed that *Solanum xanthocarpum* (50, 100 and 200 mg/kg) elevated GSH levels in all doses as compared to control. In blood *Solanum xanthocarpum* elevated GSH levels by 50%, 20% and 15% while in BALF GSH levels increased by 25%, 128% and 50% respectively in all doses. Pre-treatment with prednisolone markedly increased the GSH levels by 160% and 133% in blood and BALF respectively. Analysis of data showed that there were significant differences in GSH levels across the entire treatment group in blood and BAL fluid samples [F (4, 24) = 18.79 for blood; F (4, 24) = 29.2 for BALF; p < 0.05 for each group]. The results are summarized in Figure 5 (a and b).

![Figure 5a](image1.png)

**Figure 5a.**

![Figure 5b](image2.png)

**Figure 5:** Effects of aqueous extract of *Solanum xanthocarpum* (SX) on MDA and GSH levels in blood and BALF in ovalbumin immunized rats. C(OVA)-Ova sensitized and challenged rats; PDN-Prednisolone (10 mg/kg); SX1-SX3 Solanum xanthocarpum (50, 100 and 200 mg/kg) extract orally. Data are expressed as Mean ± SEM. *p < 0.05 versus control group.

Discussion

Bronchial asthma is a chronic inflammatory disease characterized by both bronchoconstriction and airway inflammation which results in bronchial hyper-responsiveness to various stimuli [18]. Many cell types including mast cells, eosinophils and T lymphocytes play an important role in asthma. Mast cell plays a critical role in allergic disorders when activated through immunoglobulin E (IgE) by specific antigens [19,20]. Management of asthma requires long term treatment with bronchodilators and corticosteroids but these are associated

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with various side effects and refractory responses [21,22]. Indian traditional system of medicine may provide a better alternative strategy for the treatment of this disorder however; scientific validation is needed for medicinal plants to use them as a complementary alternative therapy [23].

*Solanum xanthocarpum* is regarded as a valuable plant in both Ayurveda and modern drug development areas for its medicinal uses. A pilot study on the clinical efficacy of *Solanum xanthocarpum* has shown that it improved the pulmonary functions to a significant level in the patients suffering from mild to moderate asthma [24]. The present study adopted a reverse pharmacology approach to evaluate the mechanism involved in anti-inflammatory response of *Solanum xanthocarpum* in the experimental model of bronchial asthma.

Inhibition of inflammatory responses mediated through inflammatory cells and their cytokines, is a basic therapeutic aim in treatment of asthma. Present study showed significant suppression of IgE responses by *Solanum xanthocarpum* administration in both blood and BALF samples as compared to control. TNF-α is a pro inflammatory cytokine in macrophages and monocytes and have an important amplifying effect on asthmatic inflammation. There are evidences of IgE triggering in sensitized lungs leading to increased expression of TNF-α in epithelial cells in both rat and human lungs. TNF-α is also present in the BAL fluid of asthmatic patients [25]. Administration of *Solanum xanthocarpum* (50, 100 and 200 mg/kg) significantly reduced the levels of TNF-α in experimental rat, thus suggesting a significant anti-inflammatory response of the *Solanum xanthocarpum*.

IL-4 is derived from Th2 derived T lymphocytes, eosinophils, mast cells and basophils. It induces IgE isotype switching of B lymphocytes and mucus production [26]. IL-6 is a small glycoprotein which is produced by cells of the innate immune system (macrophages, dendritic cells, mast cells, neutrophils) in addition to the B lymphocytes [27]. Further, there has been evidence of reduced production of IFN-γ by T cells in asthmatic patients and this can be correlated to the disease severity [28]. Administration of exogenous IFN-γ is known to prevent the airway eosinophilia and hyperresponsiveness followed by allergen exposure in mice indicating IFN-γ has a potential modulating effect on allergen responses. In this study administration of *Solanum xanthocarpum* reduced IL-4 and IL-6 and elevated IFN-γ concentration in both blood and BALF. The results clearly indicate that *Solanum xanthocarpum* produced a progressive improvement in allergic responses in experimental animals and support the anti-inflammatory role of *Solanum xanthocarpum* in bronchial asthma.

Sources of oxidative stress arise from the increased burden of inhaled oxidants, as well as elevated amounts of reactive oxygen species (ROS) released from inflammatory cells like macrophage, eosinophils, neutrophils, lymphocytes and mast cells [29,30]. ROS can initiate inflammatory responses in the lungs through the activation of redox-sensitive transcription factors [31]. Increased oxidative stress is related to disease severity that might amplify the inflammatory response. During asthma, reactive oxygen species (ROS) are produced in tissues from oxidative injury. ROS, either directly or via the formation of lipid peroxidation products, may play a role in enhancing inflammation through the activation of stress kinases. An enhanced oxidative stress might contribute to the progression of existing airway inflammation and induction of various proinflammatory chemical mediators which are related to severe asthma. Thus, ROS generation has been implicated in the pathogenesis of asthma [32]. Therefore, regulation of intracellular ROS during inflammation might have a potential advantage in case of treatment of inflammatory diseases. In the present study, administration of *Solanum xanthocarpum* markedly reduced MDA levels, a marker of lipid peroxidation and elevated GSH, an antioxidant suggesting a balancing influence on prooxidant-antioxidant status in asthma model in rats.

Therefore, it can be concluded from the above study that *Solanum xanthocarpum* has anti-inflammatory and immunomodulatory influences in the experimental model of bronchial asthma. The effects were accompanied by suppression of markers of oxidative stress suggesting reduction of oxidative damage caused by reactive oxygen species may be contributing to the beneficial effects of *Solanum xanthocarpum*.

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