Bronchodilator activity of aqueous extract of stem bark of *Ailanthus excelsa* Roxb.

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ABSTRACT

Biologically active compounds from natural sources are of interest as possible new drugs for infectious diseases. *Ailanthus excelsa* Roxb. has been used in Indian system of medicine in the treatment of asthma, bronchitis, cold, colic pain, etc. Stem bark of *A. excelsa* Roxb. has been used as a decoction in traditional claims. So, our traditional claims enforced us to evaluate its bronchodilator activity. We have evaluated its bronchodilator activity in milk-induced leukocytosis and eosinophilia, clonidine-induced mast cell degranulation, bronchoalveolar lavage fluid (BALF), and lung histopathology models. The aqueous extract of stem bark in doses of 100, 200, 400 mg/kg showed significant activity.

Key words: Bronchodilator, *Ailanthus excelsa* Roxb., bronchoalveolar lavage fluid

INTRODUCTION

Asthma is an inflammatory disease of the lungs characterized by increased infiltration of leukocytes, especially eosinophils, into the airways, and reduced respiratory function. The inflammation leads to bronchoconstriction, increased airway hyper-responsiveness (AHR), and mucus production.[1] Ailanthus is a deciduous tree belonging to the family Simarubaceae and is widely distributed in Asia and North Australia. Commonly, it is known as a plant of Heaven. Biologically active compounds from natural sources are of interest as possible new drugs for infectious diseases. The bark of this plant is used as an anthelminthic, expectorant, for treating asthma, and as an antispasmodic and antipyretic.[2-3] *Ailanthus excelsa* is a rich source of different chemical compounds with a variety of potential biological activities.[4]

MATERIALS AND METHODS

Plant material

Stem barks of *A. excelsa* Roxb. were collected in August 2008 from local area of Pimpri, Pune, India, and was identified by the Regional research institute of Ayurveda Kothrud, Pune. A voucher specimen – 899 was authenticated. Stem barks were dried, powdered, and passed through 40 mesh sieve. The powdered material was extracted with water using decoction method. The extract obtained was dried to yield a dark brown colored powdery mass (10%).

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Animals

Albino mice and rats (Wistar strain) of either sex weighing 20–25 g (mice) and 150–200 g (rats) were used for studies. The albino mice and rats were obtained from animal house of National Toxicological Centre (NTC), Pune. They were housed in polypropylene cages with standard pellet chow and water *ad libitum*. In all, five mice and five rats were used for each treatment.

Acute toxicity studies

Mice were selected for this study. They were divided into eight groups with six animals in each group. Aqueous extract of *A. excelsa* was administered orally in varying doses (0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.50 and 5 g/kg) to these animals. They were continuously observed for 2 h to detect changes in the autonomic or behavioral responses like alertness, spontaneous activity, irritability, urination, etc. Any mortality during experimentation and the following 7 days was also recorded. A group of animals treated with vehicle (distilled water) served as control. Based on the results of preliminary toxicity testing, the doses of 100, 200 and 400 mg/kg p.o. were chosen for further experiments.

Bronchodilator activity

Milk-induced leukocytosis and eosinophilia

Mice were divided into five groups with five animals in each

group.[5,6] Animals belonging to group I received distilled water (DW) 10 ml/kg (p.o.). Animals belonging to groups II, III, IV, and V received injections of boiled and cooled milk in doses of 4 ml/kg (s.c.). Animals belonging to groups III, IV, and V received test extract of *A. excelsa* Roxb. in doses of 100, 200, and 400 mg/kg p.o. respectively, 1 h before milk injection. Blood samples were collected from each mouse from the retro-orbital plexus, under light ether anesthesia. Total leukocyte and eosinophil counts were taken in each group before drug administration and 24 h after milk injection. Difference in total leukocyte and eosinophil count before and 24 h after drug administration was calculated.

Clonidine-induced mast cell degranulation

Rats were divided into five groups with five animals in each group.[7] Animals belonging to group I received vehicle 5 ml/kg (p.o.). Animals belonging to group II received sodium cromoglycate 50 mg/kg (i.p.). Animals belonging to groups III, IV, and V received aqueous extract of *A. excelsa* Roxb. in doses of 100, 200, and 400 mg/kg p.o., respectively. The treatment was continued for 7 days. On day 7, 2 h after the assigned treatment mast cells were collected from the peritoneal cavity. Ten milliliters of normal saline solution was injected into the peritoneal cavity and the abdomen was gently massaged for 90 sec. The peritoneal cavity was carefully opened and the fluid containing mast cells was aspirated and collected in siliconized test tube containing 7–10 ml of RPMI-1640 Medium (pH 7.2–7.4). The mast cells were then washed thrice by centrifugation at low speed (400–500 r.p.m.) and the pellet of mast cells was taken in the medium. The mast cell suspension (approximately 1×10^6 cells/ml) was challenged with 0.5 μg/ml of clonidine solution and stained with 1% toluidine blue and observed under high-power microscope field (400×). A total of 100 cells were counted from different visual areas and the number of intact and degranulated cells was counted. The percent protection was calculated.

Bronchoalveolar lavage and lung histology in rats

Animals were divided into five groups with each group containing five animals ($n = 5$).[8] All the animals were sensitized by an intraperitoneal injection of 1 ml alum precipitate antigen containing 20 μg of ovalbumin and 8 mg of alum suspended in 0.9% sodium chloride solution. A booster injection of this alum–ovalbumin mixture was given 7 days later. Nonsensitized animals were injected with alum only. Seven days after (15th day) the second injection, animals was exposed to aerosolized ovalbumin(1%) for 30 min. Standard and test groups received dexamethasone (1 mg/kg i.p.) as standard and aqueous extracts of 100, 200, 400 mg/kg as test drug, 5 h before antigen challenge. The rats were sacrificed at the end of study (24 h after sensitization) and tracheal catheter was inserted in trachea. Bronchoalveolar lavage fluid (BALF) was collected by lavaging the lung with two aliquots of 5 ml of 0.9% sodium chloride solution. Total recovery volume per rat was approximately 8 ml. Total leukocytes and eosinophils and neutrophils were counted under a microscope, and histopathologic evaluation of lung tissue was carried out.

Statistical analysis

All values were expressed as mean ± SEM and data were analyzed by ANOVA followed by Dunnet's test.

RESULTS

Effect of *A. excelsa* Roxb. stem bark aqueous extract (AESAq) on milk-induced leukocytosis in mice

Subcutaneous injection of milk at a dose of 4 ml/kg produced a significant (***$P < 0.001$) increase in the leukocyte and total eosinophil count, 24 h after its administration. In the groups of mice pretreated with aqueous extract of *A. excelsa* Roxb. at doses of 100, 200 and 400 mg/kg p.o., there was significant (**$P < 0.01$) inhibition of milk-induced leukocytosis at 200 and 400 mg/kg p.o. doses, and in milk-induced eosinophilia, there was significant (*$P < 0.05$,**$P < 0.01$) inhibition of milk-induced eosinophilia at all the three doses [Figures 1 and 2].

Effect of AESAq on clonidine-induced mast cell degranulation in rats

Clonidine-induced mast cell degranulation was significantly (**$P < 0.01$) inhibited by sodium cromoglycate (50 mg/kg i.p.) and percent protection was found to be 68.42%. In the groups pretreated with methanolic extract of *A. excelsa* Roxb. (100, 200, and 400 mg/kg p.o) there was significant protection (**$P < 0.01$) of mast cells and the percent protection was 17.89, 24.21, and 43.42%, respectively [Table 1].

Effect of AESAq on bronchoalveolar lavage in rats

Persistent mucosal airway inflammation, associated with an increase in T helper type 2 (Th2) cytokine levels, eosinophil infiltration into the airways, and mucus and immunoglobulin (I) E production, are the main features of allergic asthma. The infiltration of cells like eosinophils, neutrophils, monocots, macrophages, lymphocytes, etc., increases the allergic asthmatic effect. Injection of ovalbumin 20 μg + 8 mg alum in 1 ml (i.p.) on days 1 and 7 and 1% OVA aerosol on 15th day produced a significant (***$P < 0.001$) increase in the TLC i.e. 4.41 fold than the nonsensitized group and differential leukocyte count. In the groups pretreated with standard drug dexamethasone (1 mg/kg i.p.), there was a significant (**$P < 0.01$) inhibition of ovalbumin-induced TLC and differential leukocyte count. The aqueous extract of *A. excelsa* Roxb. at doses of 100, 200, and 400mg/kg showed significant decrease in

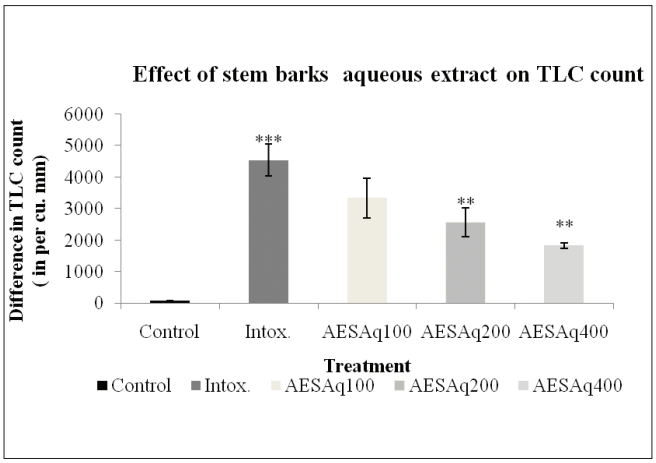

Figure 1: Effect of AESAq on milk-induced leukocytosis in mice (n = 5). Values are expressed in mean ± SEM. Control = vehicle (10 ml/kg p.o.); Intox. = milk 4 ml/kg; AESAq 100 = *A. excelsa* Roxb. stem bark aqueous extract (100 mg/kg p.o.); AESAq 200 = *A. excelsa* Roxb. stem bark aqueous extract (200 mg/kg p.o); AESAq 400 = *A. excelsa* Roxb. stem bark aqueous extract (400 mg/kg p.o.). Intox. group compared with control group using students-t test (***$P < 0.001$) and AESM compared to intox. group using statistical analysis done by ANOVA followed by Dunnet's test (**$P < 0.01$)

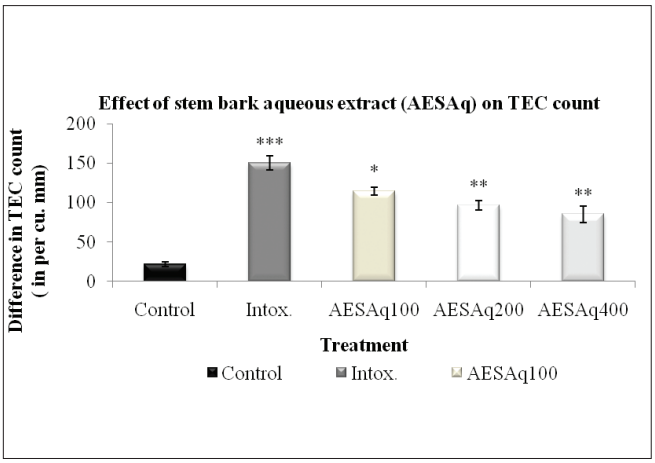

Figure 2: Effect of AESAq on milk-induced eosinophilia in mice (n = 5). Values are expressed in mean ± SEM. Control = vehicle (10 ml/kg p.o.); Intox. = milk 4 ml/kg; AESAq 100 = *A. excelsa* Roxb. stem bark aqueous extract (100 mg/kg p.o.); AESAq 200 = *A. excelsa* Roxb. stem bark aqueous extract (200 mg/kg p.o); AESAq 400 = *A. excelsa* Roxb. stem bark aqueous extract (400 mg/kg p.o.). Intox. group compared with control group using student's-t, test (***$P < 0.001$) and AESM is compared to intox. group using statistical analysis done by ANOVA followed by Dunnet's test (**$P < 0.05$,**$P < 0.01$)

Table 1: Effect of AESAq on clonidine-induced mast cell degranulation in rats

Groups	Mast cells %		% Protection
	Intact	Disrupted	
Control	24 ± 1.949	76 ± 1.949	—
Std.	78.6 ± 1.6**	21.4 ± 1.6**	68.42
AESAq100	37.6 ± 2.909**	62.4 ± 2.909**	17.89
AESAq200	42.4 ± 1.965**	57.6 ± 1.965**	24.21
AESAq400	57 ± 2.429**	43 ± 2.429**	43.42

n = 5, Values are expressed in mean ± SEM. Control = distilled water (5 ml/kg p.o.); Std. = sodium cromoglycate (50 mg/kg i.p.); AESAq100 = *A. excelsa* Roxb. stem bark aqueous extract (100 mg/kg p.o.); AESAq 200 = *A. excelsa* Roxb. stem bark aqueous extract (200 mg/kg p.o); AESAq400 = *A. excelsa* Roxb. stem bark aqueous extract (400 mg/kg p.o.); Std., AESAq100 AESAq200, AESAq400 compared with control (ANOVA followed by Dunnett's test, **$P < 0.01$)

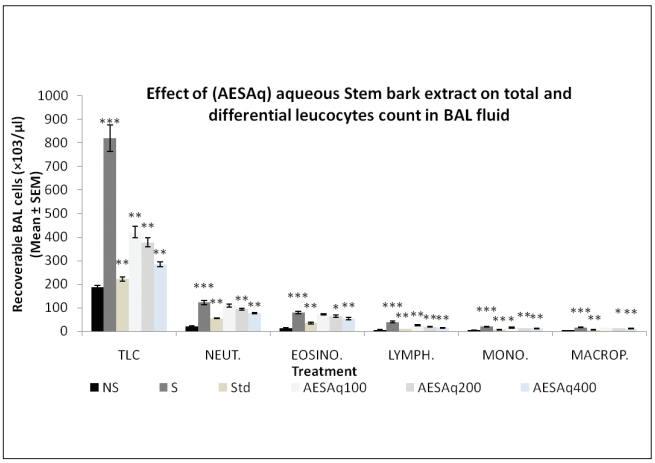

Figure 3: Effect of AESAq on bronchoalveolar lavage in rats; n = 5, values are expressed in mean ± SEM. NS = nonsensitized group, distilled water + 8 mg alum in 1 ml (i.p.); S = sensitized group, ovalbumin 20 µg + 8 mg alum in 1 ml (i.p.) on days 1 and 7 and 1% OVA - aerosol on 15th day, dose was increased by 500 folds. ; Std. = dexamethasone (1 mg/kg, i.p.); AESAq 100 = *A. excelsa* Roxb. stem bark aqueous extract (100 mg/kg p.o.); AESAq 200 = *A. excelsa* Roxb. stem bark aqueous extract (200 mg/kg p.o); AESAq400 = *A. excelsa* Roxb. stem bark aqueous extract (400 mg/kg p.o.). NS compared with S by using student's-t test (***$P < 0.001$); Std., AESAq100, AESAq200, AESAq400 compared with S by ANOVA followed by Dunnett's test (*$P < 0.05$, **$P < 0.01$)

TLC and neutrophils, lymphocytes and monocots, (*$P < 0.05$, **$P < 0.01$) but in case of other differential leukocytes like macrophages and eosinophils, it showed an inhibition at 200 and 400 mg/kg p.o. (*$P < 0.05$, **$P < 0.01$) [Figure 3].

Effect of AESAq on histopathologic evaluation of lung tissue

Histopathologic evaluation of lung tissue showed the significant bronchodilator at 400 mg/kg. (a) Absence of inflammatory cells, no edema in the lung tissue (NS). (b) Fluid accumulation along with inflammatory cells, blood cells, edema, (S). (c) Absence of fluid accumulation around the blood vessel, (Std.-dexamethasone). (d) Massive recruitment of eosinophils around the airway, blood vessels and bronchoconstuction (AESAq100). (e) Bronchodilation and blood vessels eosinophils around the airway (AESAq200). (f) Partial resolution of the tissue eosinophils around the airway and showing bronchodilator (AESAq400) [Figure 4].

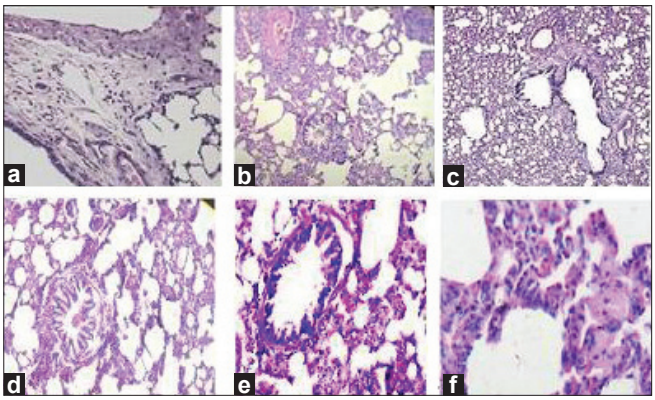

Figure 4: Effect of AESAq on histopathologic evaluation of lung tissue. Light micrograph of rat lungs collected from different treatment groups and the lungs were fixed in formalin and embedded in paraffin wax. Sections of lung tissue were cut at 5 µm thickness, mounted on glass slides and stained with hematoxylin and eosin (H × E) and cells were identified as either eosinophils, neutrophils or mononuclear cells by standard morphology and 200 cells counted under 400× magnification. a) NS - Absence of inflammatory cells, no edema in the lung tissue (25×). b) S- A low magnification lung section from an antigen-challenged animal showing evidence of Fluid accumulation along with inflammatory cells, blood cells, edema. c) A low magnification lung section from an antigen-challenged animal, received (Std.) Dexamethasone (1mg/kg, i.p.) showing absence of fluid accumulation around the blood vessel. d) A lung section from an antigen-challenged animal showing massive recruitment of eosinophils around the airway, blood vessels and bronchoconstuction, received AESAq100 (25×). e) A lung section from an antigen-challenged animal showing bronchodilation and blood vessels eosinophils around the airway, received AESAq200 (25×). f) A lung section from an antigen-challenged animal showing partial resolution of the tissue eosinophils around the airway and bronchodilation, received AESAq400

DISCUSSION

Several medicinal properties have been attributed to the plants in the traditional system of medicine. The adaptogenic properties in some plant materials is one of them, which is described to be tonics in the Ayurvedic system of medicine. According to Brahmans and Dardymov (1969) the most important characteristic of an adaptogen is that it increases resistance to adverse influences of a wide range of factors of physical, chemical, and biological nature; and its normalization action, which reveals itself irrespective of the direction of the previous pathologic shifts. Ayurveda provides a number of herbs for the treatment of asthma, and herbal formulations used for the treatment of asthma include some antistress (nervine support) herbs to enable adoption to stress, since excessive stress or nervous debility may aggravate the symptoms of asthma. After parenteral administration of milk there is an increase in TLC, and this stressful condition can be normalized by administration of an antistress or adaptogenic drug.[5] Furthermore leukocytes recruited during asthmatic inflammation release the inflammatory mediators like cytokines, histamine, and major basic protein and promote the ongoing inflammation. This model was used to evaluate the protective effect of *A. excelsa* Roxb. against milk-induced leukocytosis.

Eosinophilia is an abnormal increase in peripheral eosinophil count to more than 4% of total leukocytes. In the late phase, especially in the development of allergic asthma, eosinophils play a role as an inflammatory cell. Eosinophil 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.[9] Eosinophilia is associated with a respiratory disorder, often allergic in nature together with pulmonary infiltrates that are detectable on chest films.[10] Majority of the literature has not included a diagnostic evaluation and precise practical clinical approach to eosinophilia,[11] neither has it performed clinical studies or assessed the role of eosinophils in asthmatic response. It was also demonstrated that parental administration of milk produces a marked and significant increase in the eosinophil count after 24 h of its administration.[12]

Mast cells are widely distributed in the connective tissue, with a preferential localization adjacent to small blood vessels. The mast cells contain basophil granules literally loaded with active substances which, if allowed to escape themselves or via enzymatically formed products, cause vascular and other tissue reactions similar to those characteristic of inflammatory processes.[13] In the rat mast cell granules, the histamine concentration has been calculated to be around 0.3 M. Both clonidine and compound 48/80 act through the dynamic expulsion of granules without causing any damage to the cell wall. Clonidine releases histamine from mast cells in a similar manner to a selective liberator like compound 48/80.[7] It is known that sodium cromoglycate, a standard mast cell stabilizer prevents degranulation of mast cells by raising the cyclic adenosine monophosphate.[14] It has been known that all pharmacological agents that increase intracellular levels of cAMP relax airway smooth muscle and inhibit the release of autocoids from the tissue and basophils. The groups of animals pre-treated with methanolic extract of *A. excelsa* Roxb. resulted in a significant reduction in degranulation of mast cells and offered significant protection when challenged with clonidine indicating mast cell stabilizing activity.

Allergic inflammation associated with airway hyper reactivity is the main feature of allergic asthma. The inflammatory response is characterized by an increase in the numbers of eosinophils and mast cells, mucus hypersecretion,

and activation of T cells. Several studies have shown that T-helper type (Th2) cells play a major role in the initiation and maintenance of allergic airway inflammation and asthma through their increased production of Th2-type cytokines (IL-4, IL-5, and IL- 13). These inflammatory cytokines, also produced in the bronchial tissue by mast cells, alveolar macrophages, and epithelial cells, play a significant role in the pathogenesis of airway inflammation. This role has been highlighted in several studies using gene knockout and cytokine ablation approaches. Th2 cytokines mediate a series of events in the inflammatory cascade leading to the development of allergic asthma. Such events include B cell maturation and IgE isotype switching, activation and regulation of mast cell, eosinophil and neutrophil function, and regulation of chemokine and adhesion molecules, and mucus production.[8] Ovalbumin increases the neutrophils, eosinophils, macrophages, monocots, leukocytes, lymphocytes, epithelial cells, mucus etc., in BALF and *A. excelsa* helps to reduce all the allergic factors.

Thus, it can be concluded from the results obtained in the present investigation that methanolic extract of stem bark of *A. excelsa* Roxb. possesses significant bronchodilating, mast cell stabilizing, and adaptogenic activity, suggestive of its potential in prophylaxis and management of asthma. Hence, further detailed study needs to be conducted to evaluate the phytoconstituent responsible to produce the above result and their clinical efficacy in the treatment of asthmatic patients.

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