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## ***In vitro* and *in vivo* Activities of Stem Bark of Methanolic Extract of *Ailanthus Excelsa* Roxb. in the Management of Asthma**

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**Abstract:** The aim of study was *in vitro* and *in vivo* activities of stem bark of methanolic extract of *Ailanthus excelsa* Roxb. in the management of asthma. *Ailanthus excelsa* Roxb. used in Indian system of medicine in the treatment of asthma, bronchitis, cold colic pain etc. Methanolic extract of stem bark of *Ailanthus excelsa* was evaluated using *in vitro*- goat tracheal chain preparation model and *in vivo*- milk induced leucocytosis, milk induced eosinophilia and clonidine induced catalepsy in mice models. Dose response studies of methanolic extract were conducted at 30  $\mu\text{g mL}^{-1}$  *in vitro* and 100, 200, 400  $\text{mg kg}^{-1}$  p.o. *in vivo* models. Treatment with methanolic extract of stem bark of *Ailanthus excelsa* at 30  $\mu\text{g mL}^{-1}$  *in vitro* and 100, 200, 400  $\text{mg kg}^{-1}$  p.o. *in vivo* showed significant reduction in signs and severity of symptoms (\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ ). The findings from various studies reveal that the antihistaminic activity of methanolic extract of stem bark of *Ailanthus excelsa* may be due to the reduction in histamine induced contraction in goat tracheal chain preparation model and mast cell stabilizing potential. Inhibition of release of inflammatory mediators by decreasing total leucocytes and eosinophiles count, potentiate the antiasthmatic activity.

**Key words:** Antiasthmatic activity, *Ailanthus excelsa* roxb, clonidine induced catalepsy

### **INTRODUCTION**

Asthma is a chronic inflammatory disease that affects about 300 million people worldwide, a total that is expected to rise to about 400 million over the next 15-20 years. Most asthmatic individuals respond well to the currently available treatments of inhaled corticosteroids and  $\beta$ -adrenergic agonists; however, 5-10% has severe disease that responds poorly. Allergic asthma is a chronic airway disease with characteristic features like airway inflammation, epithelial damage, increased production of reactive oxygen species and airway hyper responsiveness to a variety of specific and nonspecific stimuli (Henricks and Nijkamp, 2001; Tobin, 2001). The supposed initial step in the allergic response is the cross linking of antigen-specific, mast cell-bound immunoglobulin (Ig) E, which upon re-exposure to allergen, leads to mast cell degranulation and the consequent release of inflammatory mediators, such as histamine and leukotrienes (Krishnaswamy *et al.*, 2001). These mediators cause airway obstruction, known as the early asthmatic response. Moreover, mast cells release a variety of chemotaxins and cytokines, which promote infiltration and activation of inflammatory cells, like

macrophages and eosinophils, into the lungs (Krishnaswamy *et al.*, 2001). These cells, together with their products, play key roles in the development and maintenance of the characteristic features of asthma (Busse and Lemanske, 2001; Saetta, 1999).

Allergy is one of the common diseases that affect mankind with diverse manifestations. The prevalence of allergy and asthma has risen in the recent years despite an improvement in the general health of the population (Gopumadhavan *et al.*, 2005). *Ailanthus* is a deciduous tree belonging to the family Simarubaceae and widely distributed in Asia and North Australia. Commonly it is known as a plant of Heaven. Tree of heaven is fast growing extensively cultivated in many parts of India towards the vicinity of villages. The tree is indigenous to Southern and Central India and distributed in Western Peninsula, Rajasthan, Bihar, Orissa, Bundelkhand, through Madhya Pradesh, Broach and Panchamal district of Gujarat, in dry deciduous forests of Maharashtra, scrae in Deccan and Karnataka, N. Circars, forest of Tamilnadu in India. It is often planted along the roads. It is exotically found in Sudan. The plant is known for its high economical and commercial importance (Anonymous 1985; Database, 2000). 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 anthelmintic, expectorant, asthma (Lavhale and Mishra, 2007), antispasmodic (Kirtikar and Basu, 1933) and antipyretic (Suresh and Dhanssekran, 1990). Indigenous preparations from *A. excelsa* were shown to be effective in the treatment of worm infections (Sharma and Ojha, 1992). *Ailanthus excelsa* is a rich source of different chemical compounds with a variety of potential biological activities (Kapoor *et al.*, 1971). The present study was designed to test the *in vitro* and *in vivo* asthmatic activity of methanolic extract of stem bark of *A. excelsa*.

## MATERIALS AND METHODS

**Plant material:** Stem barks of *Ailanthus excelsa* Roxb were collected in Aug. 2008 from local area of Pimpri, pune-18 (India) and identified by the Regional research institute of Ayurveda Kothrude, Pune (India). A voucher specimen - 899 was authenticated. Stem barks were dried, powdered pass through 40 mesh sieve. The powdered material was extracted with methanol using continuous hot extraction method (Soxhlet method) (Trease and Evans, 2002). The extract obtained was dried, yielding a dark brown colored powdery mass (5%).

**Animal:** Isolated adult goat tracheal tissue, Albino mice (Wistar Strain) of either sex weighing 20-25 g, respectively were used for studies. Isolated adult goat trachea tissue was obtained immediately after slaughter of the animal. Pieces of the trachea were collected in the ice cold oxygenated Krebs solution. The albino mice 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 experimental sets, 5 mice were used for each treatment.

**Acute toxicity studies:** Mice were selected for this study. They were divided into eight groups each containing 6 animals. Methanolic extract of *A. excelsa* was administered orally in varying doses (0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00 and 2.50 g kg<sup>-1</sup>) 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) was served as control. Based on the results of preliminary toxicity testing the doses of 100, 200 and 400 mg kg<sup>-1</sup> p.o. were chosen for further experiments (Kumar *et al.*, 2010).

## Antiasthmatic activity

### *In vitro* method

**Effect of test drug on isolated goat trachea chain preparation (Kulshrestha *et al.*, 1983):** Isolated adult Goat tracheal tissue was obtained immediately after slaughter of the animals. Trachea was cut into individual rings and tied together in series to form a chain. Trachea was suspended in bath of Kreb's solution of the composition: NaCl 6.9, KCl 0.35, CaCl<sub>2</sub> 0.28, MgSO<sub>4</sub> 0.28, NaHCO<sub>3</sub> 2.1, KH<sub>2</sub>PO<sub>4</sub> 0.16 and Glucose 2.0 g L<sup>-1</sup>, which was continuously aerated and maintained at 37±0.5°C. One end of the tracheal chain was attached to an S-shaped aerator tube and other attached to an isotonic frontal writing lever to smoked drum (magnification 10-12 folds). Tissue was allowed to equilibrate for 45 min under a load of 400 mg (NagChaudhari and Lahiri, 1974). A dose response curve for histamine was taken in variant molar concentrations, by maintaining 15 min time cycle. After obtaining a dose response curve of histamine on trachea, the *Ailanthus excelsa* extracts were added to the respective reservoir and same doses of histamine were repeated. Graph of percentage of maximum contractile response on ordinate and negative logarithm of molar concentration of histamine on abscissa were plotted to record dose response curve of histamine, in absence and in presence *Ailanthus excelsa* extracts.

### *In vivo* method

**Effect of test drug on leukocytosis (Brekhman and Dardimov, 1969; Vadnere *et al.*, 2007):** Mice were divided into five groups, five animals in each group. Animals belonging to group-I received Distilled Water (DW) 10 mL kg<sup>-1</sup>, (p.o.). Animals belonging to group II, III, IV and V received boiled and cooled milk injection in dose of 4 mL kg<sup>-1</sup>, (s.c.). Animals belonging to groups III, IV and V received test extract of *Ailanthus excelsa* Roxb. in dose 100, 200 and 400 mg kg<sup>-1</sup>, 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 count was done in each group before drug administration and 24 h after milk injection. Difference in total leukocyte count before and 24 h after drug administration was calculated.

**Effect of test drug on eosinophilia (Brekhman and Daradimov, 1969; Vadnere *et al.*, 2007):** Mice were divided into five groups, five animals in each group. Animals belonging to group-I received distilled water (DW) 10 mL kg<sup>-1</sup>, (p.o.). Animals belonging to group II, III, IV and V received boiled and cooled milk injection in dose of 4 mL kg<sup>-1</sup>, (s.c.). Animals belonging to groups III, IV and V received test extract of *Ailanthus excelsa* Roxb.

in dose 100, 200 and 400 mg kg<sup>-1</sup>, 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 Eosinophilia count was done in each group before drug administration and 24 h after milk injection. Difference in total eosinophilia count before and 24 h after drug administration was calculated.

**Effect of test drug on Clonidine-induced Catalepsy (Ferre et al., 1990; Taur et al., 2007):** Bar test was used to study the effect of test drug extracts on Clonidine induced catalepsy. Mice were divided into five groups, five animals in each group. Animals belonging to group I served as control and were administered the vehicle (10 mL kg<sup>-1</sup>, p.o.). Animals belonging to group II received standard drug Chlorpheniramine maleate (10 mg kg<sup>-1</sup>, i.p.). Animals belonging to groups III, IV and V received three doses 100, 200 and 400 mg kg<sup>-1</sup> p.o., respectively of Roxb. methanolic extract. The forepaws of mice were placed on a horizontal bar (1 cm in diameter, 3 cm above the table) and the time required to remove the paws from bar was noted for each animal. All the groups received Clonidine (1 mg kg<sup>-1</sup>, s.c.), 1 h after the test drug administration and the duration of catalepsy were measured at 15, 30, 60, 90, 120, 150 and 180 min.

**Statistical analysis:** All values were expressed as Mean±SEM and data were analyzed by ANOVAs followed by dennest.

**RESULTS**

**Effect of methanolic extract of *Ailanthus excelsa* Roxb. on histamine induced contraction of isolated goat tracheal chain preparation:** In the present study, it was observed that *Ailanthus excelsa* inhibits the contraction produced by histamine in these tissue preparations. Histamine (10 µg mL<sup>-1</sup>) was taken in different dose level and DRC was plotted in absence and in presence of *Ailanthus excelsa* extract. Study showed that *Ailanthus excelsa* extract inhibits significantly (\*p<0.05, \*\*p<0.01, \*\*\*p<0.001) percentage contraction at concentration 30 µg mL<sup>-1</sup> in goat tracheal chain preparation (Table 1).

**Effect of *Ailanthus excelsa* Roxb. stem barks methanolic extract (AESM) on Milk-Induced Leucocytosis in mice:** Subcutaneous injection of milk at dose of 4 mL kg<sup>-1</sup> produced a significant (\*\*\*p<0.001) increase in the leucocytes count after 24 h of its administration. In the groups of mice pretreated with methanolic extract of *Ailanthus excelsa* Roxb. at dose (100, 200 and

Table 1: Effect of *Ailanthus excelsa* Roxb. AESM (30 µg mL<sup>-1</sup>) on histamine induced contraction of isolated goat tracheal chain preparation

Dose (10 µg mL <sup>-1</sup> ) Histamine concn.	-Ve Log molar concn. of Histamine	Maximum contraction (%)	
		Control	AESM (30 µg mL <sup>-1</sup> )
0.1	7.08	21.85±1.232	12.58±0.7491
0.2	6.79	36.42±1.833	17.88±0.922*
0.4	6.48	47.02±1.797	22.51±0.8819*
0.8	6.18	61.59±1.945	35.09±0.9804*
1.6	5.88	84.11±3.156	42.38±1.563*
3.2	5.58	90.07±2.525	45.70±1.088**
6.4	5.23	100±2.414	54.30±1.209***

n = 5, Values are in Mean±SEM. Control = DRC of Histamine in absence of *Ailanthus excelsa* Roxb. extract. AESM = DRC of Histamine in presence of *Ailanthus excelsa* Roxb extract. (30 µg mL<sup>-1</sup>). Statistical analysis done by using Student's 't'-test. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001 significantly different from control

Table 2: Effect of *Ailanthus excelsa* Roxb. stem barks methanolic extract (AESM) on Milk-Induced Leucocytosis in mice

Dose	Difference in no. of leucocytes (cu/mm) (Mean±SEM)
Control (Vehicle (10 mL kg <sup>-1</sup> , p.o.)	80±12.247
Intox. (milk 4 mL kg <sup>-1</sup> )	4540±507.54***
AESM100	2990±486.16*
AESM200	2110±364.49**
AESM400	1580±53.852**

n = 5, values are expressed in mean±SEM. Control = Vehicle (10 mL kg<sup>-1</sup>, p.o.). Intox. = milk 4 mL kg<sup>-1</sup>. AESM100 = *Ailanthus excelsa* Roxb. Stem bark methanolic extract (100 mg kg<sup>-1</sup>, p.o.). AESM200 = *Ailanthus excelsa* Roxb. Stem bark methanolic extract (200 mg kg<sup>-1</sup>, p.o.). AESM400 = *Ailanthus excelsa* Roxb. Stem bark methanolic extract (400 mg kg<sup>-1</sup>, p.o.). \*\*\*p<0.001, Intox. group compared with control group using student't, test and \*p<0.05, \*\*p<0.01, AESM compared to intox. Group using statistical analysis done by ANOVA followed by Dunnet's test

400 mg kg<sup>-1</sup>, p.o.), there was significant (\*p<0.05, \*\*p<0.01) inhibition of milk-induced Leucocytosis (Table 2).

**Effect of *Ailanthus excelsa* Roxb. stem barks methanolic extract (AESM) on Milk-Induced Eosinophilia in mice:** Injection of Milk (4 mL kg<sup>-1</sup>, s.c.) produced a significant (\*\*\*p<0.001) increase in the total eosinophil count. In the groups pretreated with methanolic extract of *Ailanthus excelsa* Roxb. at dose (100, 200 and 400 mg kg<sup>-1</sup>, p.o.), there was significant (\*\*p<0.01) inhibition of milk-induced eosinophilia and the eosinophil count was 92.6±9.605, 89±7.969 and 64.8±6.771 cu mm, respectively (Table 3).

**Effect of *Ailanthus excelsa* Roxb. AESM on Clonidine induced catalepsy in mice:** Clonidine (1 mg kg<sup>-1</sup>, s.c.) produced catalepsy in mice, which remained for 2 h. The vehicle treated group showed maximum duration of catalepsy (80.2±3.813 sec) at 120 min after the administration clonidine. There was significant inhibition (\*p<0.05, \*\*p<0.01) of clonidine induced catalepsy in the animals pretreated with *Ailanthus excelsa* Roxb. AESM extract (100, 200 and 400 mg kg<sup>-1</sup>, p.o.) and the duration of

Table 3: Effect of *Ailanthus excelsa* Roxb. stem barks methanolic extract (AESM) on Milk-Induced Eosinophilia in mice

Dose	Difference in no. of eosinophils (cu/mm) (Mean±SEM)
Control	21.8±2.973
Intox. (milk 4 mL kg <sup>-1</sup> )	150.4±8.744***
AESM100	92.6±9.605**
AESM200	89.0±7.969**
AESM400	64.8±6.771**

n = 5, Values are expressed in mean±SEM. Control = Vehicle (10 mL kg<sup>-1</sup>, p.o.). Intox. = milk 4 mL kg<sup>-1</sup>. AESM100 = *Ailanthus excelsa* Roxb. Stem bark methanolic extract (100 mg kg<sup>-1</sup>, p.o.). AESM200 = *Ailanthus excelsa* Roxb. Stem bark methanolic extract (200 mg kg<sup>-1</sup>, p.o.). AESM400 = *Ailanthus excelsa* Roxb. Stem bark methanolic extract (400 mg kg<sup>-1</sup>, p.o.) \*\*\*p<0.001, Intox. group compared with control group using student's t, test and \*\*p<0.01, AESM compared to intox. Group using Statistical analysis done by ANOVA followed by Dunnett's test

Table 4: Effect of *Ailanthus excelsa* Roxb. (AESM) on Clonidine-induced catalepsy in mice

Group	Duration of catalepsy (Sec) at Mean±SEM						
	15	30	60	90	120	150	180
Control	23.2±1.655	41.6±2.112	49±1.871	55.2±1.356	80.2±3.813	80±6.618	66.6±2.768
Std.	13.2±1.281**	17.6±0.9274**	25.2±1.281**	32±1.949**	35.8±1.934**	56.2±1.855**	20.8±2.577**
AESM 100	20.4±1.364	36.2±2.082	37.2±2.267*	48.4±1.536*	60.8±1.934*	59.8±1.934**	56.4±3.572**
AESM 200	19±1.304	36.2±1.655	39.2±3.926*	38.4±1.327**	56.4±3.945*	50±5.422**	43.6±2.482**
AESM 400	17.8±0.8602*	22.8±1.393**	30.6±2.619**	34.8±1.356**	42.2±1.744**	41.6±2.112**	38.4±1.327**

N = 5, Control = Distilled water (10 mL kg<sup>-1</sup>, p.o.). Std. = Chlorpheniramine maleate (10 mg kg<sup>-1</sup>, i.p.). AESM 100 = *Ailanthus excelsa* Roxb. Stem bark methanolic extract (100 mg kg<sup>-1</sup>, p.o.). AESM 200 = *Ailanthus excelsa* Roxb. Stem bark methanolic extract (200 mg kg<sup>-1</sup>, p.o.). AESM 400 = *Ailanthus excelsa* Roxb. Stem bark methanolic extract (400 mg kg<sup>-1</sup>, p.o.). Statistical analysis done by ANOVA followed by Dunnett's test. \*p<0.05, \*\*p<0.01, compared to control group

catalepsy was found to be 60.8±1.934, 56.4±3.945 and 42.2±1.744 sec, respectively at 120 min after the administration clonidine. Chlorpheniramine maleate (10 mg kg<sup>-1</sup>, i.p.) significantly inhibited (\*\*p<0.01) clonidine induced catalepsy in mice at 120 min after the administration clonidine (Table 4).

## DISCUSSION

Histamine contracts the tracheobronchial muscle of guinea pig, goat, horse, dog and man. Goat tracheal chain is easier to handle and to prepare; it is also much more sensitive than guinea pig tracheal chain (Bhujbal *et al.*, 2009). Therefore, the dose relative contractile responses of different agonists like ACh, histamine, 5-hydroxytryptamine and bradykinin can be observed in isolated goat trachea. In the present study, the isolated goat tracheal chain preparation; there is right side shift of Dose Response Curve (DRC) of histamine in the presence of *Ailanthus excelsa* Roxb. extract indicating antihistaminic which help to reduce the histamine which is a mediator for asthma (Taur *et al.*, 2007). 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 increase in TLC and this stressful condition can be normalized by administration of an antistress or adaptogenic drug (Brekhman and Dardimov, 1969). 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 *Ailanthus 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 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 often allergic (Osama and Joshi, 1984). Eosinophilia is associated with respiratory disorder and nature together with pulmonary infiltrates that are detectable on chest films (Ehright and Chua, 1989). Most of the literatures do not include a diagnostic evaluation and precise practical clinical approach to eosinophilia (Karnick, 1995) performed clinical studies and assessed the role of eosinophil in asthmatic response. It was also demonstrated that parental administration of milk produces a marked and significant increase in the eosinophils count after 24 h of its administration (Bhargava and Singh, 1981). The milk induced leukocytosis and eosinophilia models helps to evaluate the stress induced asthma and *Ailanthus excelsa* helps to reduce the stressed asthma. Clonidine, a  $\alpha_2$  adrenoceptor agonist induces dose dependent catalepsy in mice, which

is inhibited by histamine (H<sub>1</sub>) receptor antagonists but not by H<sub>2</sub> receptor antagonist. It is known that clonidine releases histamine from mast cells. Brain histamine does play a definite role in the production of the extra pyramidal motor it has been suggested that the cataleptic effect of clonidine in the mouse be mediated by histamine (via H<sub>1</sub> receptors) which is released from brain mast cells in response to stimulation of  $\alpha_2$  adrenoreceptors by clonidine (Wardlaw *et al.*, 2002). The extract also significantly inhibited the clonidine induced catalepsy. The inhibition of clonidine induced catalepsy by *Ailanthus excelsa* Roxb. may be due to the potential to antagonize H<sub>1</sub> receptor or inhibition of mast cell degranulation induced by clonidine.

Thus, we know that asthma has several types and it can be concluded from the results obtained in the present investigation that methanolic extract of stem bark of *Ailanthus excelsa* Roxb. possess significant anti-asthmatic activity and can be attributed to antihistaminic (H<sub>1</sub>-antagonist), antistress 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 above result and their clinical efficacy in the treatment of asthmatic patients.

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