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ANTI-ALLERGIC AND ANTICATALEPTIC ACTIVITY OF AQUEOUS EXTRACT OF LEAVES OF *AILANTHUS EXCELSA* ROXB.

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ABSTRACT

The anti-allergic of an aqueous extract of the leaves of *Ailanthus excelsa* Roxb.(AELAq) was evaluated by using milk induced leucocytosis and eosinophilia in mice, passive paw anaphylaxis in rats models while the anticataleptic properties was evaluated by Clonidine induced catalepsy in mice mode. The extract significantly (*p<0.05, **p< 0.01) decreased the leucocytosis and eosinophilia along with passive paw anaphylaxis in the above experimental animals respectively. The extract also significantly (**p< 0.01) decreased Clonidine induced catalepsy in mice. These results suggest that aqueous extract AELAq may have the potential therapeutic value in the treatment of allergic diseases and to produce adaptogenic properties.

INTRODUCTION: Herbal medicines are being increasingly utilized to treat a wide variety of diseases, though the knowledge about their mode of action is relatively scanty. So there is a growing interest regarding the pharmacological evaluation of various plants used in traditional system of medicine [1]. Allergies occur when a hypersensitive immune system reacts to a common or unusual substance. The number of individuals suffering with allergic illnesses is increasing in the industrialized, as well as in large cities of developing countries. Allergies also have reached high prevalence and incidence in all over the world [2-3]. In Indian system of medicine *Ailanthus excelsa* Roxb. is used in the treatment of asthma, bronchatitis, cough, cold [4-5] Tribals in Nilgris region traditionally used it in antifertility, fever. Studies of stem bark extracts have shown reduced labour pain, febrifuge [6], antispasmodic [7], anticancer, antimicrobial, antiamoebic, antiprotozoal activities [8-9] and antiasthmatic activity [10]. From a pharmaceutical perspective flavonoids possess a remarkable spectrum of biochemical and pharmacological activities. The leaves were reported to contain different flavonoids like kaempferol (5, 4', 5, 7-Tetrahydroxy flavone), luteolin (3', 4', 5, 7-tetrahydroxy flavone), apigenin (4'.5, 7-trihydroxy flavone) [11]. Thus, drug development has been encouraging researchers to find strategies to treat allergic diseases and the medicinal plants have been the target of these studies and an important tool to treat immediate-type allergic response [12].

MATERIALS AND METHODS:

Plant Material: *Ailanthus excelsa* Roxb. (Simarubaceae) leaves collected in Aug. 2008 from Pune-18 nearby Hindustan antibiotic Ltd, campus, India and authenticated by Regional research Institute of Ayurveda, Pune. India. A voucher specimen – 899, has been preserved in the laboratory for future reference. Leaves were dried in shade and pulverized. The powder was subjected to decoction with water as solvent. The extract was concentrated under vacuum and dried in a vacuum desiccator (yield 18%, w/w).

Animals: Albino rats (Wistar strain) and mice (musmusculus strain) of either sex weighing 150-200gm rats and 20-25gm mice respectively were used. They were housed in microlon boxes with standard laboratory diet and water *ad libitum*. The study was conducted after obtaining Institutional Animal Ethical Committee clearance.

Acute toxicity studies: Mice (musmusculus strain) were selected for this study. They were divided into eight groups each containing six animals. Aqueous extract of leaves of *Ailanthus excelsa* Roxb. was administered orally in varying doses (0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00 2.50 and 5g/kg) to these animals. They were continuously observed for 2h to detect changes in the autonomic or behavioural 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 400mg/kg p.o were chosen for further experiments.

Antiallergic and Anticataleptic studies:

Milk induced leukocytosis and eosinophilia: Mice were divided into five groups, five animals in each group. Animals belonging to group-I received distilled water (DW) 10 ml/kg, (p.o.). Animals belonging to group II, III, IV, V received boiled and cooled milk injection in dose of 4 ml/kg, (s.c.). Animals belonging to groups III, IV and V received aqueous extract of *Ailanthus excelsa* Roxb. in dose 100, 200 and 400 mg/kg, p.

o. respectively, 1 hr before milk injection. Blood samples were collected from each mouse from the retro orbital plexus, under light ether anaesthesia. Total leukocyte count and total eosinophilia count was done in each group before drug administration and 24 hr after milk injection. Difference in Total leukocyte count and total eosinophilia count before and 24hr after drug administration was calculated [13].

Passive paw anaphylaxis in rats: Anti serum to egg albumin was raised in rats using aluminum hydroxide gel as an adjuvant [14]. Animals were given three doses of 100 mcg of egg albumin (s.c.) absorbed on 12 mg of aluminum hydroxide gel prepared in 0.5 ml of saline on 1st, 3rd, 5th day. On 10th day of sensitization, the blood was collected from the retro orbital plexus. The collected blood was allowed to clot and the serum was separated by centrifugation at 1500 rpm. Animals were divided into five groups each containing 5 animals. Animals belonging to group I served as control and were administered only the vehicle (10 ml/kg, p. o.).

Animals belonging to group II were administered Dexamethasone (0.5 mg/kg, i.p.). Whereas animals belonging to groups III, IV and V received aqueous extract of *Ailanthus excelsa* Roxb. in dose 100, 200 and 400mg/kg, p. o. respectively. The animals were passively sensitized with 0.1 ml of the undiluted serum into the left hind paw. The contra lateral paw received an equal volume of saline. Aqueous extract of *Ailanthus excelsa* Roxb. was administered 24 hr after sensitization. 1 hr. after test drug administration, the animals was challenged in the left hind paw with 10 µg of egg albumin in 0.1 ml of saline and the paw inflammation was measured by using a Plethysmometer (UGO Basile, 7140). The difference in the reading prior to and after antigen challenge represented the oedema volume and the percent inhibition of oedema was calculated by using the formula,

$$\% \text{ Inhibition} = [1-(T / C)] \times 100$$

T- Mean relative change in paw volume in test group; C- Mean relative change in paw volume in control group).

Clonidine induced catalepsy in mice: Bar test was used to study the effect of test drug extracts on Clonidine induced catalepsy [15-16]. 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, p. o.). Animals belonging to group II received standard drug Chlorpheniramine maleate (10 mg/kg, i. p.). Animals belonging to groups III, IV and V received three doses 100, 200 and 400 mg/kg p. o. respectively of aqueous extract of leaves of *Ailanthus excelsa* Roxb. 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, s. c.), 1 hr after the test drug administration and the duration of catalepsy were measured at 15, 30, 60, 90, 120, 150 and 180 min.

Statistical analysis: The statistical analysis was performed by using one-way analysis-of-variance (ANOVA) Followed by Dunnett's test for individual comparison of groups with control.

RESULTS:

Effect of aqueous extract of leaves of A. *excelsa* Roxb. (AELAq) on milk-induced leukocytosis in mice: Subcutaneous injection of milk at dose of 4 ml/kg produced a significant (***p< 0.001) increase in the leucocytes count and eosinophiles count after 24 hr of its administration. In the groups of mice pre-treated with aqueous extract

of leaves of *Ailanthus excelsa* Roxb. at dose (100, 200 and 400 mg/kg, p.o.), there was significant (*p< 0.05, **p< 0.01) inhibition of milk-induced Leucocytosis and eosinophilia .

Effect of AELAq on Milk-Induced leukocytosis in mice:

Dose	Difference in no. of leucocytes (per cu mm) (Mean ± SEM)
Control Vehicle (10 ml/kg, p. o.)	84 ± 8.12
Intox. (milk 4 ml/kg)	4850 ± 482.7***
AELAq100	3580 ± 395.16*
AELAq200	3570 ± 354.12*
AELAq400	2520 ± 332.64**

n= 5, values are expressed in mean ± SEM. Control = Vehicle (10 ml/kg, p. o.). Intox. = milk 4 ml/kg. AELAq100 = *Ailanthus excelsa* Roxb. Leaves aqueous extract (100 mg/kg, p. o.). AELAq200 = *Ailanthus excelsa* Roxb. Leaves aqueous extract (200 mg/kg, p.o). AELAq400 = *Ailanthus excelsa* Roxb. Leaves aqueous extract (400 mg/kg, p. o.) ***p< 0.001, Intox. group compared with control group using student't, test and *p< 0.05, **p< 0.01, AELAq compared to intox. Group using Statistical analysis done by ANOVA followed by Dunnet's test.

Effect of AELAq on Milk-Induced eosinophilia in mice:

Dose	Difference in no. of eosinophils (per cu mm) (Mean ± SEM)
Control	21.8 ± 3.17
Intox. (milk 4 ml/kg)	152.4 ± 9.51***
AELAq100	131.4 ± 2.48*
AELAq200	128.2 ± 1.80*
AELAq400	119.4 ± 5.44**

n= 5, values are expressed in mean ± SEM. Control = Vehicle (10 ml/kg, p.o.). Intox. = milk 4 ml/kg. AELAq100 = *Ailanthus excelsa* Roxb. Leaves aqueous extract (100 mg/kg, p.o.). AELAq200 = *Ailanthus excelsa* Roxb. Leaves aqueous extract (200 mg/kg, p.o). AELAq400 = *Ailanthus excelsa* Roxb. Leaves aqueous extract (400 mg/kg, p.o.); ***p< 0.001, Intox. group compared with control group using student't, test and *p< 0.05, **p< 0.01, AELAq compared to intox. Group using Statistical analysis done by ANOVA followed by Dunnet's test.

Effect of AELAq on passive paw anaphylaxis in rats: Antiserum to egg albumin was injected 24 hr. before administration of the test drugs or standard. Egg albumin was injected after the administration of *Ailanthus excelsa* Roxb. and Dexamethasone. In the vehicle treated group, egg albumin increased the paw volume in the sensitized animals, which was measurable up to the time period of 4 hrs. Pre-treatment with aqueous extract of leaves of *Ailanthus excelsa* Roxb. (100, 200mg/kg, p.o.) does not significantly reduced (**p< 0.01) the paw volume at 4th hr was found to be reduced maximum i.e. 10.22% & 18.28% respectively. Aqueous extract of leaves of *Ailanthus excelsa* Roxb. (400mg/kg, p.o.) significantly reduced (*p< 0.05, **p< 0.01) the paw volume at 4th hrs and the percentage inhibition was found to be 38.17% respectively. Dexamethasone (0.5 mg/kg, i.p.) significantly reduced (**p< 0.01) the paw volume at 4th hrs maximum and the percentage inhibition was found to be 46.77 % respectively (Table 2 & 3).

Effect of AELAq on passive paw anaphylaxis in rats:

Groups	Paw Edema Volume (ml), Mean ± SEM				
Control	0.48±0.02	0.44±0.03	0.41±0.02	0.39±0.01	0.37±0.02
Std. (0.5 mg/kg, i. p.)	0.24±0.01**	0.26±0.01**	0.25±0.01**	0.23±0.01**	0.19±0.01**
AELAq100	0.42±0.02	0.39±0.02	0.38± 0.01	0.362±0.01	0.33±0.02
AELAq200	0.39±0.03*	0.35±0.02*	0.33± 0.02*	0.30±0.02**	0.30±0.02*
AELAq400	0.38±0.03*	0.32±0.03**	0.30±0.02**	0.26±0.02**	0.23±0.01**

Where, n= 5, Control = Distilled water (5 ml/kg, p. o.). Std. = Dexamethasone (0.5 mg/kg, i. p.) AELAq100 = *Ailanthus excelsa* Roxb. Leaves aqueous extract (100 mg/kg, p. o.). AELAq200 = *Ailanthus excelsa* Roxb. Leaves aqueous extract (200 mg/kg, p. o). AELAq400 = *Ailanthus excelsa* Roxb. Leaves aqueous extract (400 mg/kg, p. o.). Std., AELAq100, AELAq200 & AELAq400 compared with Control (ANOVA followed by Dunnett's test), *p < 0.05, **p < 0.01..

Effect of AELAq on passive paw anaphylaxis in rats:

Groups	Percentage inhibition of Paw Edema Volume (%)				
Dose	0.5 hr	1hr	2hr	3hr	4hr
Std. (0.5 mg/kg, i. p.)	48.95	42.08	39.71	41.75	46.77
AELAq100	12.97	11.31	7.35	6.70	10.22
AELAq200	18.41	20.36	18.14	22.68	18.28
AELAq400	20.09	26.70	26.96	32.47	38.17

Where, n= 5, Control = Distilled water (5 ml/kg, p. o.). Std. = Dexamethasone (0.5 mg/kg, i. p.) AELAq100 = *Ailanthus excelsa* Roxb. Leaves aqueous extract (100 mg/kg, p.o.). AELAq200 = *Ailanthus excelsa* Roxb. Leaves aqueous extract (200 mg/kg, p. o). AELAq400 = *Ailanthus excelsa* Roxb. Leaves aqueous extract (400 mg/kg, p. o.). Std., AELAq100, AELAq200 & AELAq400 compared with Control (ANOVA followed by Dunnett's test), *p < 0.05, **p < 0.01.

Effect of AELAq on Clonidine induced catalepsy in mice:

Clonidine (1 mg/kg, s. c.) produced catalepsy in mice, which remained for 2 hr. The vehicle treated group showed maximum duration of catalepsy (80.2±3.813 sec.) at 120 minute 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. AELAq extract (100, 200, 400 mg/kg, p. o.) and the duration of catalepsy was found to be 55.40±6.33**, 49.40±3.46** and 42.20±4.42** seconds, respectively at 120 minute after the administration clonidine. Chlorpheniramine maleate (10 mg/kg, i. p.) significantly inhibited (**p< 0.01) clonidine induced catalepsy in mice at 120 minute after the administration clonidine.

Effect of AELAq on Clonidine induced catalepsy in mice:

Group	Duration of catalepsy (Sec), Mean ± SEM						
	15min.	30min	60min	90min	120min	150min	180min
Control	41.80±1.66	41.80±1.66	66.60±5.97	71.20±2.13	83.80±2.60	100.20±5.96	68.80±4.55
Std.	17.40±1.08**	17.40±1.08**	20.00±2.35**	23.60±3.54**	23.60±3.31**	13.00±2.78**	8.20±2.85**
AELAq100	27.40±2.73**	40.60±6.27	45.60±7.24**	45.60±7.24**	55.40±6.33**	52.00±6.32**	44.80±4.15
AELAq200	27.00±2.76**	33.86±6.85	40.60±5.18**	40.60±5.18**	49.40±3.46**	40.60±4.61**	34.00±2.97**
AELAq400	27.20±3.44**	30.40±3.08	39.40±2.91**	39.40±2.91**	42.20±4.42**	36.00±2.79**	33.60±5.74**

n =5, Control = Distilled water (10 ml/kg, p.o.). Std. = Chlorpheniramine maleate (10 mg/kg, i.p.) AELAq100 = *Ailanthus excelsa* Roxb. Leaves aqueous extract (100 mg/kg, p.o.). AELAq200 = *Ailanthus excelsa* Roxb. Leaves aqueous extract (200 mg/kg, p.o.). AELAq400 = *Ailanthus excelsa* Roxb. Leaves aqueous extract (400 mg/kg, p.o.). Statistical analysis done by ANOVA followed by Dunnett's test. *p<0.05, **p<0.01, compared to control group.

DISCUSSION: Allergy and anaphylaxis are the most responsible factor for diseases like asthma, rhinitis, bronchatitis, cold, cough, pain, inflammation etc. Several medicinal properties have been attributed to the plants in the traditional system of medicine. The presence of adaptogenic properties in some plant materials is being one of them, as 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.

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 [17]. Furthermore leukocytes recruited during allergic 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 eosinophile count to more than 4 % of total leukocytes.

In the late phase, especially in the development of allergic asthma, eosinophiles play role as an inflammatory cell. Eosinophil secretes mediators such as eosinophile cationic protein (ECP), eosinophile 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 [18]. Eosinophilia is associated with respiratory disorder, often allergic in nature together with pulmonary infiltrates that are detectable on chest films[19]. Immunomodulating agents are useful in the treatment of allergy by virtue of inhibiting the antigen-antibody (AG:AB) reaction thereby inhibiting release of inflammatory mediators [20]. The beneficial effect of *Ailanthus excelsa Roxb.* could be due to either inhibition AG: AB (hypersensitivity reaction-I) i.e. Producing antiallergic and anti-inflammatory properties.

Clonidine, a $\alpha_2$adrenoreceptor agonist induces dose dependent catalepsy in mice, which is inhibited by histamine (H_1) receptor antagonists but not by H_2 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_1 receptors) which is released from brain mast cells in response to stimulation of α_2 adrenoreceptors by Clonidine [21]. 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_1 receptor.

Thus, it can be concluded from the results obtained in the present investigation that aqueous extract of leaves of *Ailanthus excelsa Roxb.* possess significant antiallergic, anti-inflammatory, anticataleptic, adaptogenic activity, suggestive of its potential in prophylaxis and management of allergic diseases. 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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