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Short Communication

In-vitro and *In-vivo* Antiasthmatic Studies of *Ailanthus excelsa* Roxb. on Guinea Pigs

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Abstract

Methanolic extract of stem barks of *Ailanthus excelsa* Roxb. was evaluated for antiasthmatic activity by employing in-vivo and in-vitro screening models in Guinea pigs. The results revealed that the methanolic extract produced significant dose-dependent antiasthmatic activity.

Keywords: Mahanimb; Antiasthmatic activity; Histamine induced bronchoconstriction.

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1. Introduction

Ailanthus excelsa Roxb. (Simaroubiaceae) commonly known as Mahanimb. *Ailanthus excelsa* is a large tree originally from China, is known as the 'tree of heaven'. Different parts of this plant are used widely in traditional medicine for a variety of diseases [1]. The bark is used as bitter, refrigerant, astringent, appetizer, anthelmintic, febrifuge, in dysentery, earache, skin disease, troubles of the rectum, and fever due to tridosha and allay thirst. It is also used in gout, rheumatism, dyspepsia, bronchitis and asthma. In Ayurveda it is used to remove the bad taste of mouth [2-3]. *Ailanthus* is used to cure wounds and skin eruptions as mentioned in traditional medicine [4].The root bark has been reported to possess cytotoxic and antitumor activity both in mice and in cell cultures [5]. Stem bark extracts showed potent antibacterial and antifungal activities [6]. The alcohol extract from leaf and stem bark exhibits remarkably high antiimplantation and early abortifacient activity [7]. A recent study reveals that ethanol extracts of *A. excelsa* leaves have a significant hepatoprotective effect on experimental liver damage in rats [8] and antidiabetic activity [9]. The plant is reported to contain flavonoids, quassinoides,

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alkaloids, terpenoids, sterols and Saponins [10-13]. Based on ethno botanical practice *A. excelsa* is a rich source of different chemical compounds with a variety of potential biological activities. The vast ethnomedical uses inspired us to investigate the anti asthmatic potentials of *Ailanthus excelsa* Roxb. stem barks.

2. Materials and Methods

2.1. Plant material

The plant was collected from local areas of Pune, near by Hindustan antibiotics Ltd. and was identified and authenticated from Regional Research Institute as Voucher Specimen No. 899 by Dr. Rajesh Dabur, Research officer I/C Kothrud, Pune- 411038, India.

2.2. Preparation of extracts

The stem barks were dried under shade and coarsely powdered and passed through 40 mesh sieve. The powdered material (500g) was extracted with methanol using Soxhlet apparatus. The extract obtained was dried in rotary vacuum evaporator at 40^0 C, yielding a dark brown colored mass 10g (2.0%).

2.3. Animals

Guinea pigs (300-400g) of either sex were procured from National toxicological centre, Pune, India. The animals were housed for 2 weeks prior to the experiment for acclimatization in the animal house of Padam. Dr. D.Y. Patil Institute of Pharmaceutical Sciences and Research, Pimpri, Pune 411018, India. Animals were maintained under controlled conditions of temperature 26 ± 2°C, relative humidity 44-56%, and photo-schedule (12 h light and 12 h dark).Animals were provided with standard diet (Amrut feeds, Mumbai, India) and water *ad libitum*. The food was withdrawn 18 h, before the start of the experiment. Institutional Animal Ethics Committee approved the experimental protocol (198/99/CPCSEA). The pharmacological work was carried out as per norms of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals).

2.4. Acute toxicity studies

Mice were selected for this study. They were divided into eight groups each containing six 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 2.50g/kg) to these animals. They were continuously observed for 2h 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 400mg/kg p.o were chosen for further experiments.

2.5. Evaluation of antiasthmatic activity

2.5.1. Isolated Guinea pig ileum preparation (in-vitro)

Overnight fasted Guinea pig was sacrificed and ileum was mounted in an organ bath containing tyrode solution. The tyrode solution was continuously aerated and maintained at 37 ± 0.5ºC. The tissue was allowed to equilibrate for 30 min. under a load of 500 mg, contact time of 30 sec. and the response of histamine was recorded by 5 min time cycle. After obtaining a dose response curve of histamine (10µg/ml) on ileum, methanolic extract of stem bark of *A. excelsa* (100µg/ml) was added to the reservoir and same doses of histamine were repeated in presence of plant extract. Same procedure was repeated for standard drug (CPM 10µg/ml) as methanolic extract. Graph of percentage of maximum contractile response on ordinate and negative logarithm of molar concentration of histamine on abscissa was plotted to record dose response curve of histamine, in absence and presence of plant extract and standard drug [14-15].

2.5.2. Histamine induced bronchoconstriction in Guinea pigs (in-vivo)

Overnight fasted Guinea pigs were divided into five groups (n=5): Inducer control (IC) = histamine (0.2%, aerosol), STD received chlorpheniramine maleate (2 mg/kg, i.p.), AESM (100mg/kg), AESM (200mg/kg) and AESM (400mg/kg). received 100, 200 and 400 mg/kg, p.o. methanolic extract of stem bark of *A. excels*, respectively. Bronchospasm was induced in Guinea pigs by exposing them to histamine aerosol (0.2%) produced by an ultra-sound nebulizer in an aerosol chamber (24×14×24 cm) made of Perspex glass. The time required for appearance of pre-convulsive dyspnoea caused by the histamine was recorded for each animal. Prior to drug treatment, each animal was placed in the histamine chamber and exposed to 0.2 % histamine aerosol. The preconvulsion time (PCT), i.e. the time of aerosol exposure to the onset of dyspnoea leading to the appearance of convulsion, was noted. As soon as the preconvulsion dyspnoea (PCD) was noted, the animals were removed from the chamber and placed in fresh air to recover. This time for preconvulsive dyspnoea was recorded as basal value. Guinea pigs were then allowed to recover from dyspnoea for 24 hrs. After 24 hrs, the animals of AESM (100mg/kg), AESM (200mg/kg) and AESM (400mg/kg) received methanolic extract of *Ailanthus excelsa* Roxb. and Std. received Chlorpheniramine maleate. These animals were again subjected to histamine aerosol later at an interval of 1 hr, 4 hrs and 24 hrs to determine preconvulsion time (PCT). The protection offered by the treatment was calculated by using the following formula [16-17]:

Percentage protection = $(1 - T_1/T_2)\times 100$

where, T_1 = the mean of PCT before administration of test drugs, and
T_2 = the mean of PCT after administration of test drugs at 1 hr, 4 hr and 24 hrs.

2.6. Statistical analysis

The statistical analysis was performed by using Student's'*t*'-test, one-way analysis-of-variance (ANOVA) Followed by Dunnett's test for individual comparison of groups with control.

3. Results

3.1. Isolated Guinea pig ileum preparation

Methanolic extract of *Ailanthus excelsa* Roxb exhibited significant (**p<0.001) percent decreased contraction at 100µg/ml in isolated Guinea pig ileum preparation (Table 1).

Table 1. Effect of AESM extract on histamine induced contraction in isolated Guinea pig ileum.

Dose (ml)	Control (10µg/ml)	AESM (100µg/ml)	CPM (10µg/ml)
0.1	46.16±2.19	19.36±0.99**	19.2±1.39**
0.2	66.43±3.06	32.14±1.69**	29.4±1.72**
0.4	69.91±2.32	34.27±1.14**	42.8±2.75**
0.8	80.4±2.63	39.15±1.31**	45.4±2.43**
1.6	84.6±1.72	44.06±0.89**	54±1.98**
3.2	100±1.72	50.36±0.93**	65±2.24**

AESM = *Ailanthus excelsa* Roxb. methanolic stem bark extract.
n=5, values are mean ± SEM.
Control = DRC of histamine in absence of AESM extract.
AESM (100µg/ml) = DRC of histamine in presence of methanolic extract of *Ailanthus excelsa stem bark* {AESM (100µg/ml)}.
CPM (10µg/ml) = DRC of histamine in presence of chlorpheniramine maleate which is standard (10µg/ml).
Statistical analysis done by using Student's t-test and ANOVA followed by Dunnet's test.
* p<0.05, ** p< 0.01, *** p< 0.001 significantly different from control.

3.2. Histamine induced bronchoconstriction in Guinea pigs

The methanolic extract of stem barks of *Ailanthus excelsa* Roxb significantly prolonged the latent period of convulsions followed by exposure to histamine aerosol at the dose of 400mg/kg, p.o.and showed maximum protection of 59.4 % at 4th hour as compared to

chlorpheniramine maleate (standard) 1mg/kg, p.o. which offered maximum protection of 65.4 % at 4th hour (Tables 2 and 3).

Table 2. Effect of AESM extract on histamine induced bronchoconstriction in Guinea pigs.

Groups	Latent period of convulsion (sec) (mean ± SEM)			
	Before	1 hr.	4 hr.	24 hr.
IC (-ve control)	17.1±1.03	13.9±0.64	10.2±0.83**	16.4±1.32
STD (+ve control)	17.2±0.97	53.8±2.71**	65.4±2.14**	30 ±1.08**
AESM (100mg/kg)	17.4±0.75	28.6±1.97**	39.4±1.97**	23.8±0.86**
AESM (200mg/kg)	18.2±1.07	35.4±2.27**	45±2.17**	27.4±0.93**
AESM (400mg/kg)	19.6±0.93	42.2±1.77**	59.4±2.11**	31.4±1.50**

n=5; IC = Inducer control = histamine (0.2%, aerosol)
STD = Chlorpheniramine maleate (2 mg/kg, i.p.).
AESM100 = Methanolic extract of *Ailanthus excelsa stem bark* (100 mg/kg, p.o.).
AESM200 = Methanolic extract of *Ailanthus excelsa stem bark* (200 mg/kg, p.o.).
AESM400 = Methanolic extract of *Ailanthus excelsa stem bark* (400 mg/kg, p.o.).
Statistical analysis done by using Student's *t*-test and ANOVA followed by Dunnet's test.
**p*<0.05, ***p*<0.01, compared to normal control group.

Table 3. Percent protection against histamine induced bronchoconstriction in Guinea pig.

Groups	% protection		
	1 hr.	4 hr.	24 hr.
Std	68.02	73.7	42.66
AESM (100mg/kg)	39.16	55.84	26.89
AESM (200mg/kg)	48.58	59.55	33.57
AESM (400mg/kg)	53.55	67	37.58

n=5; STD = Chlorpheniramine maleate (2 mg/kg, i.p.).
AESM100 = Methanolic extract of *Ailanthus excelsa stem bark* (100 mg/kg, p.o.).
AESM200 = Methanolic extract of *Ailanthus excelsa stem bark* (200 mg/kg, p.o.).
AESM400 = Methanolic extract of *Ailanthus excelsa stem bark* (400 mg/kg, p.o.).
**p*<0.05, ***p*<0.01, ****p*<0.001compared to control group.
Statistical analysis done by using Student's *t*-test and ANOVA followed by Dunnet's test.

4. Discussion

Asthma is a common respiratory disease. The morbidity and mortality of the disease is increasing and making it a global concern. [18]. The syndrome of bronchial asthma is characterized by wide spread narrowing of the bronchial tree due to contraction of the smooth muscle in response to multiple stimuli resulting in the release of chemical mediators such as histamine [19]. Guinea pig ileum is used for screening of antihistaminic activity. The stimulation of H_1 receptors produces graded dose related contraction of isolated Guinea pig ileum [20-21]. In the present study, *Ailanthus excelsa* Roxb (100 µg/ml) significantly inhibited the histamine induced contraction of isolated Guinea-pig ileum preparation indicating its H_1 receptor antagonistic activity and supports the anti asthmatic properties of the plant.

Histamine induced bronchoconstriction is the traditional immunological model of antigen induced airway obstruction. Histamine when inhaled causes hypoxia and leads to convulsion in Guinea pigs and causes very strong smooth muscle contraction, profound hypotension, and capillary dilation in cardiovascular system. A prominent effect caused by histamine leads to severe bronchoconstriction in the Guinea pigs that causes asphyxia and death. Bronchodilators can delay the occurrence of these symptoms [22]. The results of the study confirmed the bronchodilator properties of the plant, justifying its traditional claim in the treatment of asthma.

Drugs effective in the asthma are mostly steroidal in nature. Phytochemical profile of the plant reveals the presence of flavonoids, steroidal nucleus in form of triterpenoids and various saponin glycosides. The antiasthmatic activity showed by the plant may be because of these chemical moieties [23]. However this claim demands for further research and the studies are infact underway to isolate and characterize the active principles responsible for the anti-asthamatic activity.

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