

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

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ABSTRACT

Background: Biologically active compounds from natural sources are of interest as possible new drugs for different diseases. Over many centuries humans have been mining the bounties of nature for discovering natural products that have been used for the treatment of all human diseases. *Ailanthus excelsa* Roxb. (Simaroubaceae) is widely used in the Indian system of medicine as an antiasthmatic, antispasmodic, bronchodilator, anticolic pain, anticancer, antidiabetic etc. The plant was also reported for its antiasthmatic, bronchodilatory, antiallegic and many more such activities. **Objective:** To evaluate the antihistaminic activity of aqueous extract of stem bark of *Ailanthus excelsa* Roxb. **Materials and Methods:** We have studied the effect of aqueous extract of stem barks of *A. excelsa* Roxb. at a doses 100 µg/mL in the isolated goat tracheal chain preparation *in vitro* and 100, 200, 400 mg/kg doses orally in passive paw anaphylaxis in rat, clonidine-induced catalepsy in mice models *in vivo* for its antihistaminic activity. **Results:** Aqueous extract of stem barks of *A. excelsa* Roxb. significantly ($***P < 0.001$) inhibits the percentage contraction at concentration of 100 µg/mL in goat tracheal chain preparation. *A. excelsa* Roxb. extract (100, 200, and 400 mg/kg oral) and dexamethasone (0.5 mg/kg, i.p.) also significantly reduced ($**P < 0.01$) the paw volume at fourth hour and the percentage inhibition was found to be 13.98%, 28.49%, 42.47% and 46.77% respectively. The aqueous extract of stem barks of *A. excelsa* Roxb. (100, 200, 400 mg/kg, p.o.) and chlorpheniramine maleate (10 mg/kg, i.p.) significantly inhibited ($*P < 0.05$, $**P < 0.01$) clonidine-induced catalepsy in mice at 150 min after the administration of clonidine. **Conclusion:** The aqueous extract of stem bark of *A. excelsa* Roxb. possess significant antihistaminic activity (H1-antagonist) and can be attributed to bronchodilating, anti-inflammatory, adaptogenic activity etc. Hence detailed study needs to be conducted to evaluate the phytoconstituent responsible for the above mentioned results and their clinical efficacy in the treatment of related diseases.

Key words: *Ailanthus excelsa* Roxb, antihistaminic activity, passive paw anaphylaxis

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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. 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] Most of the allergic diseases are due to allergens like airborne pollens (grass, trees, and weeds), house-dust, mites, animal dander, cockroaches, fungal spores, etc. Overproduction of histamine in body triggers the allergic and inflammatory responses. Drugs always exist in the nature to prevent the effect of histamine. *Ailanthus excelsa* Roxb. is a tree belonging to family Simaroubaceae, indigenous to central and southern India. Commonly it is known as a Tree of Heaven. In Indian system of medicine it is used in panic diarrhea, bronchitis, and dysenteries. In addition, *A. excelsa*

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was shown to have antipyretic activity.^[4] Even though *A. excelsa* was reported to be useful in a many ailments like bronchodilatory, antiasthmatic, antiallergic, etc., but scientific evaluation of the plant was not reported for its antihistaminic activity. Plants containing flavonoids have been reported to possess antihistaminic, antiallergic, and mast cell degranulation properties.^[5-7] Hence, in the present study, the antihistaminic activity of aqueous extract of stem bark of *A. excelsa* was studied using different animal models.

MATERIALS AND METHODS

Plant material

A. excelsa Roxb. (Simarubaceae) stem barks collected in August 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. Stem barks were dried in shade and pulverized. The powdered material was extracted with water using decoction method.^[8] The extract obtained was dried on water bath, yielding a dark brown colored powdery mass (10% w/w).

Animal

Isolated adult goat tracheal tissue, albino mice and albino rats (Wistar Strain) of either sex weighing 20–25 and 150–200 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's solution. The albino mice and albino rats were obtained from animal house of National Toxicological Laboratory, Pune. They were housed in polypropylene cages with standard pellet chow and water ad libitum. Laboratory animal handling and experimental procedures were performed in accordance with the guidelines of CPCSEA and experimental protocol was approved by Institutional Animal Ethics Committee (198/99/CPCSEA/17).

In all experimental sets, five rats and five mice 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 stem bark of *A. 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 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 in 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 p.o were chosen for further experiments.

Antihistaminic activity

Isolated goat trachea chain preparation

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 Krebs's solution and was continuously aerated at $37 \pm 0.5^\circ\text{C}$. Dose–response curve (DRC) of histamine in plain Krebs's solution and in 100 $\mu\text{g}/\text{mL}$ *A. excelsa* Roxb. extract in Krebs's solution was taken. Graph of percentage of maximum contractile response on ordinate and concentration of histamine on abscissa was plotted to record DRC of histamine, in absence and in the presence of drug extract.^[9,10]

Passive paw anaphylaxis in rats

Rats (Wistar) were given (s.c.) three doses of 100 μg of egg albumin adsorbed on 12 mg of aluminum hydroxide gel prepared in 0.5 mL of saline on 1st, 3rd, 5th day. On 10th day of sensitization, blood was collected from the retro orbital plexus and the collected blood was allowed to clot and the serum was separated by centrifugation at 1500 rpm. Animals were divided into five groups ($n = 5$). Animals belonging to group I served as control and were administered only the vehicle (10 mL/kg p.o.). Animals belonging to groups III, IV, and V received three doses (100, 200, 400 mg/kg p.o., respectively) of *A. excelsa* Roxb. extract. Animals of group II served as positive control/standard group and received dexamethasone (0.27 mg/kg p.o.). The animals were passively sensitized with 0.1 mL of the undiluted serum into the left hind paw of animals. The contralateral paw received an equal volume of saline. Drug treatment was given 24 hr after sensitization. Animals were challenged in the left hind paw with 10 μg of egg albumin in 0.1 mL of saline, and the paw inflammation was measured using a plethasmometer. The difference in the reading prior to and after antigen challenge represented the edema volume, and the percent inhibition of volume was calculated by using the following formula.

$$\text{Percent Inhibition} = 1 - (V_t / V_c) \times 100$$

where V_t = mean relative change in paw volume in test group

V_c = mean relative change in paw volume in control group.

Prior to drug treatment, animals were sensitized with serum.

24 hrs after the drug treatment, animals were again challenged with 10 μg egg albumin and edema inhibition was calculated.^[11]

Clonidine-induced catalepsy in mice

Albino mice were divided into five groups ($n = 5$). Control

group received saline (10 mL/kg) and other groups received single dose of extract (100, 200, 400 mg/kg p.o. body weight), respectively. Chlorpheniramine maleate (10 mg/kg, i.p.) was used as standard. All the groups were received clonidine (1 mg/kg s.c.) 1 hr after the drug administration, the duration of catalepsy was measured at 15, 30, 60, 90, 120, 150, and 180 min.^[12,13]

Statistical analysis

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

RESULTS

The aqueous extract of stem barks of *A. excelsa* Roxb. was

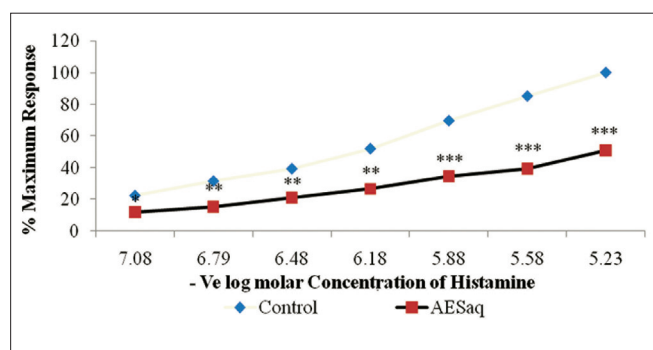


Figure 1: Effect of AESAq (100 µg/mL) on histamine-induced contraction of isolated goat tracheal chain preparation N = 5, values are in mean ± SEM. AESAq = aqueous extract of stem bark of *A. excelsa* Roxb. Control = D.R.C. of Histamine in the absence of *A. excelsa* Roxb. extract. AESAq = DRC of histamine in the presence of aqueous extract of stem bark of *A. excelsa* Roxb (100 µg/mL). Statistical analysis was done by using Student's *t*-test (**P*<0.05, ***P*<0.01, ****P*<0.001), significantly different from control

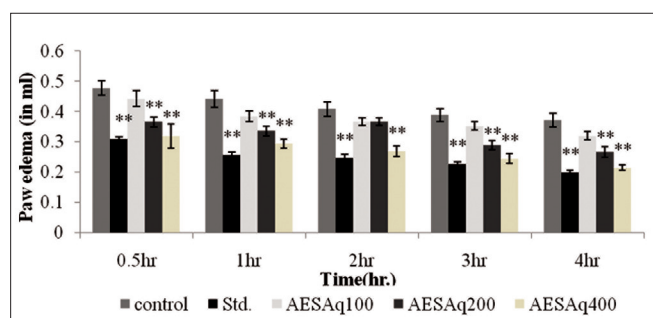


Figure 2: Effect of AESAq on passive paw anaphylaxis in rats N = 5, Values are expressed in mean ± SEM. Control = distilled water (5 mL/kg, p.o.). Std. = dexamethasone (0.5 mg/kg, i.p.). AESAq 100 = aqueous extract of stem bark of *A. excelsa* Roxb. (100 mg/kg, p.o.). AESAq 200 = aqueous extract of stem bark of *A. excelsa* Roxb. (200 mg/kg, p.o.). AESAq 400 = aqueous extract of stem bark of *A. excelsa* Roxb. (400 mg/kg, p.o.). Std., AESAq100, AESAq200, AESAq400 compared with control (ANOVA followed by Dunnett's test), ***P* < 0.01

evaluated for its antihistaminic activity and we selected 100 µg/mL doses in the isolated goat tracheal chain preparation for *in vitro* and 100, 200, 400 mg/kg for *in vivo* models.

Effect of AESAq (100 µg/mL) on histamine-induced contraction of isolated goat tracheal chain preparation

In the present study, it was observed that *A. excelsa* Roxb. inhibits the contraction produced by histamine in these tissue preparations. Histamine (50 µg/mL) was taken in different dose level and DRC was plotted in the absence and in the presence of *A. excelsa* extract. The study showed that *A. excelsa* Roxb. extract inhibits significantly (***) the percentage contraction at concentration of 100 µg/mL in goat tracheal chain preparation. Dose-dependent response relationship was observed [Figure 1].

Effect of AESAq 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 *A. 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 hr. Pretreatment with aqueous extract of stem bark of *A. excelsa* Roxb. (100, 200, and 400 mg/kg oral) significantly reduced (***P*<0.01) the paw volume at fourth hour and the percentage inhibition was found to be 13.98%, 28.49%, and 42.47%, respectively. Dexamethasone (0.5 mg/kg, i.p.) significantly reduced (***P*<0.01) the paw volume at fourth hours maximum and the percentage inhibition was found to be 46.77% [Figures 2 and 3].

Effect of AESAq on clonidine-induced catalepsy in mice

Clonidine (1 mg/kg, s.c.) produced catalepsy in mice, which remained for 2 hr. The vehicle-treated

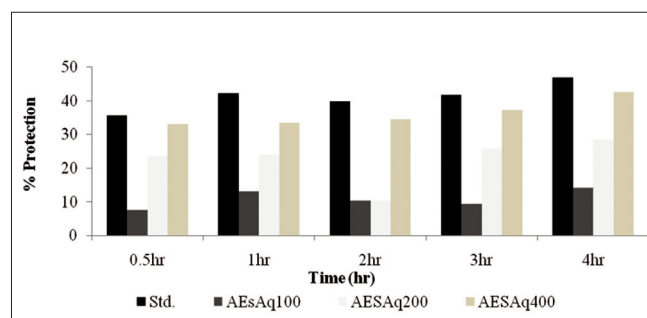


Figure 3: Percentage protection by AESAq and Std. drug against passive paw anaphylaxis in rats N = 5, Values are expressed in mean ± SEM. Control = distilled water (5 mL/kg, p.o.). Std. = dexamethasone (0.5 mg/kg, i.p.). AESAq 100 = aqueous extract of stem bark of *A. excelsa* Roxb. (100 mg/kg, p.o.). AESAq 200 = aqueous extract of stem bark of *A. excelsa* Roxb. (200 mg/kg, p.o.). AESAq 400 = aqueous extract of stem bark of *A. excelsa* Roxb. (400 mg/kg, p.o.). Std., AESAq100, AESAq200, AESAq400 compared with control (ANOVA followed by Dunnett's test), ***P* < 0.01

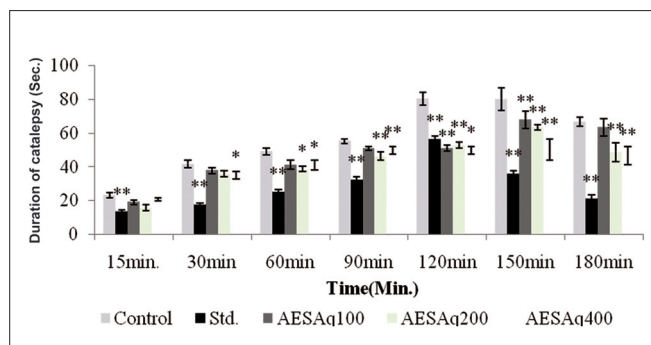


Figure 4: Effect of AESAq on clonidine-induced catalepsy in mice N=5, Values are expressed in mean \pm SEM. Control = distilled water (10 mL/kg, p.o.). Std. = chlorpheniramine maleate (10 mg/kg, i.p.). AESAq 100 = aqueous extract of stem bark of *A. excelsa* Roxb. (100 mg/kg, p.o.). AESAq 200 = aqueous extract of stem bark of *A. excelsa* Roxb. (200 mg/kg, p.o.). AESAq 400 = aqueous extract of stem bark of *A. excelsa* Roxb. (400 mg/kg, p.o.). Statistical analysis was done by ANOVA followed by Dunnett's test. * $P < 0.05$, ** $P < 0.01$, compared to control group

showed maximum duration of catalepsy (80.2 ± 3.813 sec.) at 120 min after the administration of clonidine. There was significant inhibition (* $P < 0.05$, ** $P < 0.01$) of clonidine-induced catalepsy in the animals pretreated with aqueous extract of stem bark of *A. excelsa* Roxb. (100, 200, 400 mg/kg, p.o.) and the duration of catalepsy was found to be 67.8 ± 5.09 , 63.2 ± 1.48 , and 50 ± 6.27 sec, respectively, at 150 min after the administration of clonidine. Chlorpheniramine maleate (10 mg/kg, i.p.) significantly inhibited (** $P < 0.01$) clonidine-induced catalepsy in mice at 150 min after the administration of clonidine [Figure 4].

DISCUSSION

Histamine contracts the tracheobronchial muscle of guinea pig, goat, horse, dog, and man. Goat tracheal chain is easier to handle and prepare; it is also much more sensitive than guinea pig tracheal chain. Therefore, the dose-relative contractile responses of different agonists like ACh, histamine, 5hydroxytryptamine, and bradykinin can be observed in isolated goat trachea. In the isolated goat tracheal chain preparation, there was right side shift of DRC of histamine in the presence of AESAq, which indicate antihistaminic activity.^[14] Administration of egg albumin (s.c.) to rat raises the antiserum to egg albumin in the plasma and subplantar injection of plasma containing these antibodies; then challenged with egg albumin leads to passive paw anaphylaxis in rats.^[15] The present study revealed that in the animals pretreated with *A. excelsa* Roxb. extract, there was significant reduction (** $P < 0.01$) in the paw volume. The beneficial effect of *A. excelsa* Roxb. could be due to either inhibition antigen-antibody (AG:AB) or anti-histaminic activity. Clonidine, an α_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 should 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.^[16] The extract also significantly inhibited the clonidine-induced catalepsy. The inhibition of clonidine-induced catalepsy by *A. 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 stem bark of *A. excelsa* Roxb. possess significant antihistaminic activity (H_1 -antagonist) and can be attributed to bronchodilating, anti-inflammatory, adaptogenic activity. 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 related diseases.

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