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ORIGINAL ARTICLE

Acute toxicity, anthelmintic and antispasmodic potential of bark of Ailanthus altissima (Mill)

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Abstract

Background: Medicinal plants have been gaining significant attention in recent years due to their rich repository of biologically active compounds, which serve as a foundation for new drug discoveries. Furthermore, the plant's antiviral properties underscore its importance as a valuable resource in medicinal research.

Objective: This study aims to screen and determine mode of action of relaxant activity of extract of bark of Ailanthus altissima, which is traditionally used in treatment of gut spasms. The study also focuses on anthelmintic activity against round worms and tape worms.

Material and Methods: Ailanthus altissima (A. altissima) hydro-methanolic extract was tested against tape worms at three distinct concentrations: 100 mg/ml, 200 mg/ml, and 400 mg/ml i.e.,Raillietina spiralis)and round wormsi.e,Ascaridia galli using albendazole and piperazine citrate as standard anthelmintic. A.altissimawas tested in isolated rabbits' jejunal preparations for possible antispasmodic activity in concentrations 0.01, 0.03,0.1,0.3,1.0,3.0,5.0 and 10 mg/ml. To explain its possible mode of actions, the test samples were tried in similar concentrations against 80mM KCl-induced contractions. Concentrations Response Curves (CRC)weremadewith and without test samples using verapamil as standard calcium channel blocker. Acute toxicity testing was also carried out to establish an acceptable dosage range. Preliminary phytochemical screenings were also performed.

Results: Raillietina spiralis was found more sensitive to A.altissimain concentration 40 mg/ml as compared to Ascaridia galli which has a relative index of 1.3 for paralysis. LD50 for A. altissimawas 1500 mg/kg. With EC50 values of 1.65 0.12 mg/mL & 0.82 0.13 mg/mL (n=3), A. altissima suppressed both spontaneous as well as high K+-induced contractions. A.altissimashifted the CCRCs to right like that of verapamil.

Conclusion: The study shows that Ailanthus altissimapossesses anthelmintic and antispasmodic effects through inhibition of calcium channel blockade that confirm its traditional uses as anthelmintic and antispasmodic.

Keywords: Ailanthus altissima, anthelmintic, Raillietina spiralis , Ascaridia galli, antispasmodic

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Introduction

Medicinal plants have been drawing attentions nowadays as they contain several biologically active compounds leading to the new drug discoveries[1,2]. The Malakand region especially its northern areas contain several medicinally important plants that have yet to be explored for their biological activities A ilanthusealtissimaais a medicinal plant, belonging to family Simaroubaceae. Its commonly known as

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tree of heaven. Its traditional use is for fragrance and flavoring agents[3].

Its bark juice is traditionally used for treating diarrhea and dysentery 4. Its bark is also used for anthelmintic purposes [4]. Its leaves have been reported for antioxidant, phytotoxic and antimicrobial activities [5]. The bark has been reported for presence of coumarins [6]. The importance of Ailanthus altissima cannot be ruled out as it has antiviral activity. Cytotoxicity quassinoids have been reported from Ailanthus altissima[7]. Its bark has also been reported for cyclooxygenase 2 inhibitory activity [8].

Literature search shows that bark of Ailanthus altissima is traditionally used for anthelmintic purposes that is yet to be explored at scientific grounds. More, its bark's juice is traditionally used for antispasmodic purposes in local tribe of SWAT regions[4, 9].As there are sufficient evidences for traditional use of Ailanthus altissima in gut spasms. And whereas, there is lack of scientific evidence for its use as antispasmodic. More, the peristalsis movement of the gut is due to simultaneous contractions of longitudinal and circular muscles fibers of gut preparations. An agent which relaxes the spontaneous contractions of isolated gut preparations is considered as relaxant or antispasmodic[10, 11], hence, the present research work was conducted to explore the scientific rationale for relaxant effects of bark of Ailanthus altissima, which is traditionally used in treatment of gut spasms.

Material and Method:

Collection and authentication of plant materials:

The plant materials were collected from Malakand Top regions. Renowned Taxonomist professor Dr. Jehandar Shah identified the plant. A voucher No.AA-13 specimen was presented to the Herbarium in the Department of Botany at Malakand University.

Drying and preparation of extract:

The bark was subjected to shade drying. After shade drying, 1.0 kg materials were subjected to grinding. The powdered material were steeped for three days in industrial grade methanol (85%). The menstruum system has been filtered by regular filter paper. The technique has been repeated three times. The filtrates were mixed with the vacuum pump & chiller at a temperature of 45°C using a rotating evaporator. 20.0 g of greenish brown extracts were obtained.

Drugs & related standards:

In the studies, Analyticals grade chemicals were employed. BDH in England provided the acetyl-choline. GSK, Peshawar, supplied the albendazole. The stock solutions for all test samples were created in distilled H2O, and the dilutions were produced in normal saline on the day of the experiment.

Animals and parasites:

For anthelmintic studies, rounds worms (Ascaridia galli) and tape worms (Raillietina spiralis) were isolated from the intestines of fowl's (chickens). Their intestine was obtained from the slaughter house of "Charsadda Muragh Dealer", Phase 4, Sector N2, Hayatabad, Peshawar. Earlier, intestine was maintained in normal saline. A longitudinal cut was given and the normal saline was flushed through the intestine. The parasites were obtained and maintained in normal saline. Length of the round worms (Ascaridia galli)was in range of 4-7 cm. While length of the tape worms (Raillietina spiralis) were in range of 6-7.8 cm. For antispasmodic activity, local breed rabbits, weighing 1.5-2 kg either sex were housed at the animal house of Khyber Medical University, Peshawar under controlled environment (23-25 °C). BALBs/C mice (weight = 25-30 g), were obtained from National Institute of Health (NIH), Islamabadthe toxicity studies.The research procedures were authorised by the Khyber Medical University Ethical Board through letter No. Dir/KMU-EB/RA/000131.

Preliminary phytochemical screenings:

Various phytochemicals like Alkaloids, flavonoids, saponins, tannins, glycosides, phenolics, sterols, terpenoids, proteins and carbohydrates have been confirmed [12].

A cute etoxicity:

Initially, animals, each comprising five mice, were separated into groups. The 1, 10, 1000, 2000 as well as 2500 mg/kg test dosages were intraperitoneal injection administered. One group was given simply with normal saline (negative control). The mice were supplied ad libitum food and drink for a 24-hour testing period. The animals were monitored regularly throughout the research period.

Anthelmintic screening:

Anthelmintic activity of A.altissimawas evaluated againstwere induced.A.altissimawas tested in similar Raillietina spiralis and Ascaridia gall according to the standardconcentrations i.e. 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 5.0 as methods[11, 13, 14]. Briefly describing, fresh adult tape worms andwell as 10 mg/ml after 20 minutes15. Response on KCl-round worms were used in screening for anthelmintic activity.induced contractions were noted.

Test samples of different concentrations i.e., in normal saline,**Calcium channel blocking activity:**

10 mg/ml, 20 mg/ml, and 40 mg/ml concentrations wereIt was necessary to depolarize the preparations with a generated. About 06 worms of about comparable size were puthigh concentration of K+ (80 mM. So to determine that in petri plates with 25 ml of the above-mentioned A. altissimathe test substancespasmolytic activity was mediated via test sample concentrations. Albendazole (10 mg/ml) was usedvoltage-gated calcium channel blockage[17-19], leading to as standard anthelmintic drug. A negative control containingcontractions in a sustained way. The verapamil normal saline was also run in the experiments. Time taken for(standard) in combination with A. altissimaextract was paralysis in the worms to develop was recorded using stopadministered to the tissues under study in a dose watch. Similarly, time for death was also noted in different set ofdependent manner to observe the inhibitory response . experiments. The worms were considered paralyzed their allThe relaxation of jejunal tissues, was expressed in terms movements were stopped. Similarly, death time was noted byof percent of the controlpre-contractions.

immersing the worms in warm water at 50 °C or showed noThe confirmation of plant extract's calcium channel moment upon vigorous shaking.

Isolated tissues preparations:

It was conducted in accordance with published protocols15, 16. Rabbits were subjected to fasting about 24 hours before the experiments with continuous supply of H2O. The cervical dislocation of rabbits was done and abdomens were opened for isolating jejunum. The jejunum portion was immersed in Tyrode's solution with the temperature maintained at 37○C with the continuous supply of oxygen. A tension of approximately 1.0 g of preload was implemented to the tissues and then left untouched for 30 minutes to allow for tissue stabilization. The test samples of A.altissimawas tested in concentrations 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 5.0 and 10 mg/ml at a minute interval using commutative dosing. Intestinal responses were recorded. Effects of the test samples of plant were noted.

Effects on KCl(80mM)-induced contractions:

With the use of high molar KCl concentrations(80mM) in the rabbits' jejunal preparations, sustained contractions

blocking activity was assessed after first stabilizing tissues in Tyrode's solution for a duration ofthirty minutes. Following this, the Tyrode's solution was removed and replaced with Calcium free Tyrode's solution containing 0.1 mM EDTA. Following this, the K+-rich (but Ca++-free) Tyrode's solution was substituted with EDTA solution. The control concentration-response curves of Ca++ were constructed after the tissues had been decalcified and incubated for 45 minutes. When the calcium's said curves were found super imposable mostly after 02 cycles, then the tissues which were treated with A. Altissima were tested for the possible calcium channel blocking activity. The test concentrations of the samples under study were 1.0 mg/mal & 3.0 mg/ml and0.03 & 0.1 µM verapamil.

Recording & Interpretation of data:

The intestinal responses were assessed through the Power lab(Model No: 4/26 T) A.D Instruments, Australia. While the amplification was carried out by

connecting the amplifier with power lab. The data was recorded and interpreted with lLab cChart.

Statistical Analysis:

The data was analyzed in terms of mean and standard error of the mean (SEM) and effective concentrations (EC50) were expressed in terms of 95% confidence intervals (CI)and p-value($p \leq 0.05$) using SPSS.

Results :

The photochemical screening of *Ailanthus A ltissima*indicated that it contains alkaloids, flavonoids, glycosides, phenolics, proteins, carbohydrates, sterols and terpenoids as shown in Table 1.

Table 1: Results of photochemical investigations of Ailanthus altissima bark.

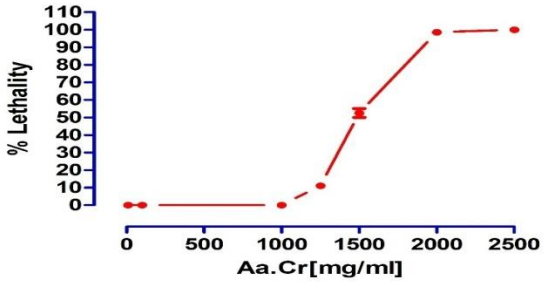
S.NO	GROUP OF PHYTOCHEMICALS	TESTS PERFORMED	OSERVATIONS
01	Alkaloids	Mayer	+
		Wagner	+
		Hager	+
02	Flavonoids	NaOH	+
		H2SO4	+
		Shinoda	+
		Lead acetate	+
03	saponins	Foaming	-
		Emulsification	-
04	Tannins	Ferric chloride	-
		Braymer's	-
05	Glycosides	Legal's test	+
06	Phenolics	Ferric chloride	+
	sterols	Liebermann-Burchard test	+
07	Terpenoids	Chloroform	+
08	CHOs	Molisch's	+
		Fehling's	+
09	Proteins	Ninhydrin	+
		Millon	+

Key: += present, -= absent

Acute toxic studies have revealed that plant extract has good safety profile (upto 1000mg/kg administered intraperitoneally) as indicated in Table 2,

Table 2: Results of acute toxicity study of methanolic extract of Ailanthus altissima in mice.

Doses (mg/kg body weight i.p.) and death toll in different Groups (n=6 in each group)			
1st stage	Group1	Group 2	Group 3
	(10 mg/kg)	(100 mg/kg)	(1000 mg/kg)
	All alive	All alive	All alive
2nd stage	Group 1 (1250 mg/kg)	Group 2 (1500 mg/kg)	Group3 (2000 mg/kg)
	1 died	3 died	All died



The figure 1 clearly depict percent mortality is expressed. LD50 of test sample was 1.5 g/kg.

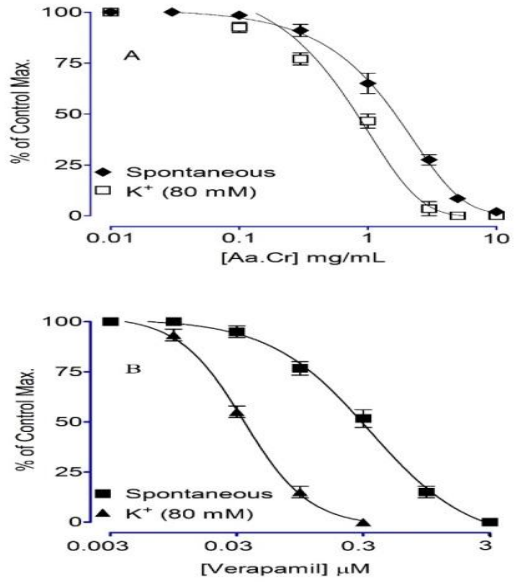
The anthelmintic activity is evident from Table 3.A.altissimahas been found to cause paralysis of parasites with the increase in concentration. The duration of paralysis can be compared to albendazole i.e. 18±1 min. However, Ascaridiagalli was more sensitive as its time for paralysis was 14 ± 1.2 min in test concentration 40 mg/ml. Similar pattern was followed for death of the test worms. Comparing the data in manner as we reported earlier [11], it is evident that relative index for paralysis of Raillietinaspiralisat a dose of 40 mg/ml, is 0.8 as compared to albendazole i.e. 1.0. This shows that Raillietinaspiralisis more sensitive to A.altissimain concentration 40 mg/ml as compared to Ascaridiagalli which has a relative index of 1.3 for paralysis in concentration of 40 mg/ml. Thus, results in Table 3 demonstrate that Ailanthus altissima has vormicidal activity against the tape worms and round worms. Hence, these findings are hoped to be useful for the guided separation of bioactive anthelmintic components. More, our findings confirmed the use of bark of Ailanthus altissima as anthelmintic.

Sample/Groups	Conc. mg/ml	**Table 3: Anthelmintic activity of Ailanthus altissima against Raillietinaspiralis and Ascaridiagalli**								
		Test Parasites								
		Raillietina spiralis(Tapeworms)					**Asc aridiagalli(Roundworms)**			
		Paralysis		Death			Paralysis			
		Time taken for paralysis	Relative Index (P)	Time taken for Death	Relative Index (D)		Time taken for Paralysis	Relative Index (P)	Time taken for death	Relative Index (D)
Ailanthus altissima.	10	31±2	1.7	85±3	1.6		24±2	2.2	75±3	2.1
	20	22±1.7	1.2	62±2.6	1.2		19±1.3	1.7	54±2.2	1.5
	40	14±1.4	0.8	55±2.5	1.1		14±1.2	1.3	47±2.5	1.3
Albendazole*	10	18±1	1.0	51±2.5	1.0		11±1	1.0	35±2	1.0
Piperazine citrate	10	17±1.2	0.9	46±2	0.9		09±1	0.8	31±2	0.9
Negative Control	0	0	0	0	0		0	0	0	0

Key: Relative Index (P) denotes the time taken for paralysis to occur using A. altissima /time taken for paralysis to occur using standard*. Relative Index (D) denotes the time taken for death to occur using A. altissima /time taken for death to occur using standard*. (Times were recorded in minutes, values are mean ±SD, n=3).

A.altissima elicited spontaneous K+-induced concentration-dependent inhibition with EC50 values of 1.65 ± 0.12 mg/mL as well as 0.82 ± 0.13 mg/ml (n=3) respectively, (Fig. 2A). Verapamil, a standard calcium channel blocker, elicited spontaneous and high potassium mediated contractions in the same manner, with E.C50 values of 0.510.07 M & 1.47 0.07 M, respectively (Fig. 2B).

Figure 2 A: Effects of Ailanthus altissima extract on spontaneous and KCl-induced contraction.



These results revealed that the antispasmodic activity may be due toinhibition of calcium channels. This may be further caused theantagonistic effect athigher concentration of potassium i.e. > 30 mM. Which is associated with the contraction of smooth musclesthat was happened by voltage-dependent L-type Ca++ channels opening, that caused the inflow of extracellular calcium ionsthat resulted a contractile

effect [20]. It has been observed that a drug that inhibits high K+-induced contraction is also thought to decrease Ca++ inflow[21].

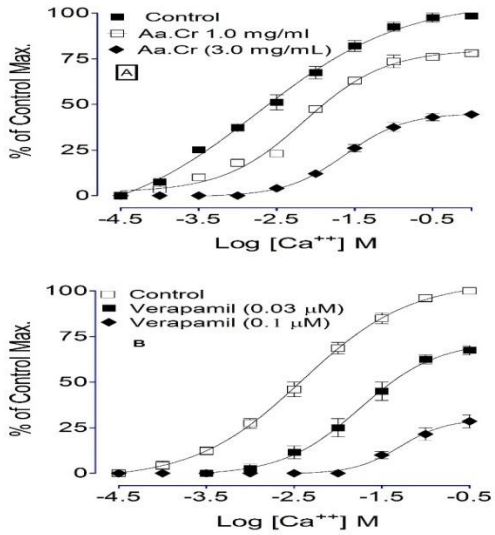


Figure 3 A: Effects of Ailanthus altissima extract on calcium chloride curves

Figure 3 B: Effects of verapamil on calcium chloride curves

It is evident figure.3A & 3B that extract of the Ailanthus altissimahas relaxede the high K+-induced contraction, in a similar way asverapamil [20, 22] that indicated its CCB effect. The Ca++ antagonistic effect of *Ailanthus* altissimawas alsofurther proved when A.altissima dose dependently (1 – 3 mg/mL) (Control EC50 -2.5 ± 0.08 and EC50 in presence of 1mg/ml=-1.9 ±0.13 log[Ca++]M ;n=3) (Fig. 3A), like that caused by verapamil (Control EC50 = -2.45 ± 0.07 and EC50 in presence of 0.03 µM= -1.37 ±0.06 log[Ca++]M ;n=3) (Fig. 3B). As a result, the Ca++ antagonistic action ofA.altissimais is thought to be advantageous in gut disorders like as diarrhea and abdominal cramps, which can be caused by intestinal hyperactivity. The presence of flavonoids as well as other photochemical that have been documented to have calcium channels blocking action as group 1 may explain the observed CCB action, which validates the therapeutic usage of Ailanthus altissima. [23, 24]. However, contribution of other compounds may not be ruled out.

In short, this study revealed that the crude extract of Ailanthus altissimapossesses anthelmintic activity against Raillietina spiralis and Ascaridiagalli. The antispasmodic activity is thought to be mediated by Ca++ channel blocking, providing a strong pharmacological basis for its use in diarrhea and gut spasms.

Discussion:

Acute toxicity:

Assessing acute toxicity is important in determining the safety of plants. Studies on Ailanthus bark revealed a wide range of window treatments. In animal models, low to moderate doses ofthe extract did not cause toxicity and no behavioral changes or deaths were observed [25].However, symptoms such as fatigue, abdominal discomfortand mild neurotoxicity were noted at higher dose[26]. These findings suggest that the bark is generally safe when used in therapy,but caution should be exercised to avoid side effects when used in larger doses. Its use issupported by in vitro and in vivo studies. Its bioactive components, including quassinins such as ailanthin, have been shown to disrupt parasite metabolism, leading to paralysis and death[27].In a comparative study ,the bark extract was compared with the standard treatmenti.e.ofalbandazole, for anthelmintic activity[28].This makes Ailanthus a potential alternative for the treatment of helminth infections, especially in underserved areas where the use of synthetic drugs is prohibited. It can control muscle tone. Studies have shown that its extract inhibits calcium influx and interacts with muscarinic receptors, thereby relaxing intestinal and uterine smooth muscles29.This effect has been experimentally confirmed with isolated tissue preparations,where the bark has been shown to have antiinflammatory properties and agonistinduced contractions.The findings revealed that it can treat conditions such as irritable bowel syndromecoldsand colic. I agree, this often leads to intestinal infections. Ailanthus bark has a dualtherapeutic effect by simultaneously eliminating parasites and reducing discomfort[30].methodsand lack of understanding of longterm safety hinder the use of medicinal products. A full toxicological evaluation, including chronic toxicity and genotoxicity studies, is necessary In addition, clinical studies on humans are needed to confirm the preliminary findingsInvestigating the combination of Ailanthus bark with traditional treatments may also reveal new treatments31 (Sharma et al., 2021).

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