ANTINOCICEPTIVE AND ANTI INFLAMMATORY ACTIVITY OF *ACHYRANTHES ASPERA* L. EXTRACTS

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Summary

The alcoholic extracts of leaves and seeds of *Achyranthes aspera* were investigated for anti inflammatory and anti nociceptive or analgesic activity at the oral dose of 250 mg/kg body weight. For evaluation of the activity in inflammatory conditions the carrageenan model served as the acute model and formalin model served as the chronic model in rats. The acetic acid induced writhing response and the hot plate technique in mice were used for study of the analgesic activity. Results show that the anti inflammatory activity was significant from day 6 onwards in the chronic model and within 3 hours in the acute model. In addition, the extracts were found to attenuate the writhing responses induced by in intraperitoneal injection of acetic acid and late phase of pain response checked by the hot plate test method.

Keywords: *Achyranthes aspera*, anti inflammatory, anti nociceptive

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Introduction

Inflammation or phlogosis is a pathophyisological response of living tissue to injuries that leads to the accumulation of plasmatic fluid and blood cells. It is the body's defense reaction in order to eliminate or limit the spread of an injurious agent. Although it is a defense mechanism, the complex events and mediators involved in the inflammatory reactions can induce, maintain or aggravate many diseases. There are various components in inflammatory reactions that can contribute to the associated symptoms of tissue injury. Edema formation, leukocyte infiltration and granuloma formation represents such components of inflammation. Edema formation in the paw is the result of a synergism between various inflammatory mediators that increase vascular permeability / or the mediators that promote vasodilation. Carrageenan induced paw edema is widely used for determining the acute phase of inflammation. Histamine, 5 HT and bradykinin are the first detectable mediators in the early phase carrageenan induced inflammation whereas prostaglandins are detected in the late phase of inflammation. Because of the side effects associated with the clinical use of the NSAID's and the opiates the search for better alternatives seems necessary and will be beneficial. To observe the effect on symptoms mimicking Rheumatoid arthritis the formalin induced edema model is used for the study of chronic phase of inflammation. The analgesic activity is studied by the hot plate method and in order to distinguish between central and peripheral analgesic action, the acetic acid writhing response in mice is used. This method is simple reliable and also affords rapid evaluation of the peripheral type of analgesic action.

The plant *Achyranthes aspera* L. var *aspera* (Family: Amaranthaceae) is an herb that grows in various parts of India. It predominantly grows as a weed in vacant agricultural land, especially in uncultivated lands and along the boundaries of the cultivated fields. It is known as 'Latjira' or 'Apamarga' in Hindi and 'Aghedo' in the Gujarati language. In English it is known as 'Prickly chaff flower'. Different parts of the plant and the whole plant are both reported to be useful in the indigenous system of medicine for the treatment of various afflictions and have a number of therapeutic uses (1-10). Phytochemical studies have shown the presence of saponins (11), alkaloids (12), a steroid & flavanol glycosides (13).

Materials and Methods

Plant Material

Collection of Plant

Plants were collected from their natural habitat from the farm areas in and around the city of Vallabh Vidyanagar, Anand in Gujarat state (GPS coordinates: Latitude 22:32:10 N, Longitude 72:54:00 E) in the months of November. The plant was authenticated by comparison with Voucher specimen numbers ARM53 and AIP3124 at the Prof. G.L. Shah Herbarium of Sardar Patel University, Vallabh Vidyanagar, Anand, Gujarat, India.

Extraction
The shade dried seeds and leaves (150 g) of *A. aspera* were grounded and were extracted successively with petroleum ether (AR Grade, SD Fine Chem, India) and alcohol (NLT 94.7% v/v and NMT 95.2% v/v) using a Soxhlet apparatus. The alcoholic extract was filtered and the solution evaporated in vacuum to give a semisolid extract.

Animals
Wistar Albino rats of either sex (weighing 200-250 g) or Swiss albino mice (22 -25 g) obtained from Cadila Pharmaceuticals Limited, Ahmedabad, Gujarat, India, were used for the study. The animals were maintained in a natural light and dark cycle. They were fed the standard pellet diet (Amrut Lab animal feed, Amrut rat and mice pellet, Sangli, Maharashtra, India) and water was given *ad libitum.* The animals were acclimatized to their environment for a week prior to experimentation. The experiments were carried out after the approval from the Institutional Animal Ethical Committee (IAEC).

Acute toxicity studies
Healthy adult swiss albino mice of either sex were divided in to four groups (n=3) and were orally administered a single dose of 10 and 15 times the effective dose of alcoholic extract of seeds and leaves of *A. aspera*. The animals were observed for gross behavioral, neurological, autonomic and toxic effects at short intervals of times for 24 h. Mortality was observed for 72 hours.

Study protocol

Acute inflammatory studies
The carrageenan induced paw edema method was used for evaluating the potential of extracts of *A. aspera* on inflammation (14). For the study, the wistar albino rats of either sex were divided into four groups (n=6). Leaf and seed extracts of *A. aspera* (250mg/kg) and Diclofenac (1 mg/kg) were administered orally one hour before the subplantar injection of 0.1 ml of 1% w/v suspension of the carrageenan in normal saline in the right hand paw of the rats. The volume of the paw was measured using the plethysmograph at 0, 3, 5 and 24 hours after the injection. Mean paw volume was measured and percent inhibition was calculated.

The rats were divided into four groups as follows-
Group I : Control- Carrageenan
Group II : Positive control - Carrageenan + Diclofenac
Group III : Test – Carrageenan + Alcoholic seed extract (250 mg/kg)
Group IV : Test – Carrageenan + Alcoholic leaf extract (250 mg/kg)

Chronic inflammatory studies
Experimental arthritis was induced and studied in rats (15). The animals were divided into four groups (n=6). A subplantar injection of 0.1 ml of 2% v/v formalin solution was administered to the right hind paw on the first and third day of experiment. Plant extracts (250 mg/kg) or Diclofenac (1 mg/kg) was administered orally once daily for 10 days. All drugs were given orally one hour prior to formalin injection. The paw thickness of each group was measured using a plethysmograph on days 0, 6 and 10.

The rats were divided into four groups as follows-
Group I : Control- Formalin
Group II : Positive control - Formalin + Diclofenac
Group III : Test – Formalin + Alcoholic seed extract (250 mg/kg)
Group IV : Test – Formalin + Alcoholic leaf extract (250 mg/kg)

Writhing test
The anti nociceptive effect was evaluated by the writhing test in mice (16) induced by acetic acid 1% v/v solution per 10 g of body weight injected ip. The animals were placed in a large glass cylinder and the intensity of nociception was quantified by counting the total number of writhes occurring between 0 and 30 min after the stimulus injection. The writhing response is characterized by a wave of contractions of the abdominal musculature followed by extension of the hind limbs. The animals were divided into four groups (n=6). The ethanolic *A. aspera* extracts (250mg/kg) were administered 30 min before the nociceptive agent. Ten minutes after the acid administration the number of writhes (constriction of abdomen, turning of trunk and extension of hind limbs) was observed for a period of 30 minutes.

The mice were divided into four groups as follows-
Group I : Control- Acetic acid
Group II : Positive control Acetic acid + Diclofenac
Group III : Test – Acetic acid + Alcoholic seed extract (250 mg/kg)
Group IV : Test – Acetic acid + Alcoholic leaf extract (250 mg/kg)

Hot Plate test
The hot plate test (17) was assessed on four groups of 6 mice. The temperature of the hot plate was maintained at 55 ± 0.2^0C. Latency to a discomfort reaction (licking paws or jumping) was determined before and after drug administration. The cut off time was 15 seconds. The latency was recorded before and 30, 60, 120 180 and 240 minutes following the oral administration of the extracts and standard.

The mice were divided into four groups as follows-
Group I : Control
Group II : Positive control- Pentazocin
Group III : Test – Alcoholic seed extract (250 mg/kg)
Group IV : Test – Alcoholic leaf extract (250 mg/kg)

Statistical analysis
The data was analyzed statistically using one way ANOVA followed by Dunnets test ‘t’ test using the SigmaStat 2.03 version.

Results and discussion

All the doses of the extracts of *A. aspera* employed for acute oral toxicity were found to be non toxic. *A. aspera* extract did not produce any mortality even at the doses employed.

Subplantar injection of carrageenan in rats showed a time dependent increase in paw thickness. The thickness was measured at 0, 3, 5 and 24 hours after the injection of Carrageenan. This increase was observed at 1 hr and was maximal at 3 hr after administration of carrageenan injection in the vehicle treated groups. However, carrageenan-induced inflammation was significantly ($p<0.05$) reduced after 3 hours in all phases of the experiment by treatment with 250 mg/kg of extracts and specified dose of Diclofenac (Table 1).

Table 1: Anti inflammatory activity of *A. aspera* extracts on carrageenan induced rat paw edema.

Groups	Rat paw volume measured at different time intervals (hours)			
	0	3	5	24
Group I	0.73 ± 0.04	1.7 ± 0.04	1.53 ± 0.06	0.8 ± 0.07
Group II	0.76 ± 0.03	0.86 ± 0.04*	0.73 ± 0.04*	0.7 ± 0.06*
Group III	0.63 ± 0.06	1.1 ± 0.04*	0.96 ± 0.03*	0.73 ± 0.06*
Group IV	0.66 ± 0.06	1.2 ± 0.07*	0.96 ± 0.03*	0.83 ± 0.06*

Values are expressed as Mean ± SEM, * $p<0.05$

Table 2 shows that the extracts were effective in significantly effective ($P<0.05$) in reducing chronic inflammation. Formalin induced pedal edema was inhibited significantly by *A. aspera* as compared to the control rats. The standard drug, Diclofenac, also exerted inhibitory action on edema formation. The percent inhibition was found to be 49.01, 35.39 and 29.41 after 3 hours and 52.17, 36.95 and 32.60 after 5 hours for Group II, Group III and Group IV respectively.

Table 2: Anti inflammatory activity of *A. aspera* extracts on formalin induced rat paw edema

Groups	Rat paw volume measured at different intervals (days)		
	0	6	10
Group I	0.66 ± 0.04	2.30 ± 0.08	0.70 ± 0.04
Group II	0.70 ± 0.04	1.26 ± 0.06*	0.66 ± 0.04
Group III	0.70 ± 0.04	1.26 ± 0.04*	0.73 ± 0.06
Group IV	0.63 ± 0.03	1.40 ± 0.05*	0.66 ± 0.04

Values are expressed as Mean ± SEM, * $p<0.05$

Table 3 shows the pain behavior of writhing response which was presented as cumulative abdominal stretching response. The treatment of animals with the extracts of *A. aspera* produced a significant ($p<0.05$) inhibition in abdominal writhes produced by acetic acid.

Table 3: Effect of Extracts of *A. aspera* on writhing response in mice.

Group	No of writhes (within 30 min)
Group I	43.6 ± 3.05
Group II	20.2 ± 2.08*
Group III	28.8 ± 1.77*
Group IV	33.4 ± 2.20*

Values are expressed as Mean ± SEM, * $p<0.05$

Table 4 shows the effectiveness of the extracts as pain suppressants. The results of the hot plate indicated a significant increase ($p<0.05$) in reaction time at 0.5, 1, 2, 3 and 4 hrs after administration of standard drug. The extracts did not show significant increase in the reaction time after administration of extracts.

Table 4: Analgesic effect of *A. aspera* extracts in the Hot Plate method

Group	Reaction time at different time intervals (hours)					
	0	0.5	1	2	3	4
Group I	6.06 ± 0.23	6.75 ± 0.07	7.13 ± 0.15	7.98 ± 0.14	8.23 ± 0.10	8.86 ± 0.12
Group II	6.12 ± 0.18	8.12 ± 0.12*	10.02 ± 0.17*	10.63 ± 0.11*	11.2 ± 0.13*	11.95 ± 0.17*
Group III	6.11 ± 0.17	6.48 ± 0.15	8.07 ± 0.39	8.82 ± 0.33	8.23 ± 0.33	9.17 ± 0.38
Group IV	6.05 ± 0.18	6.59 ± 0.19	8.08 ± 0.32	8.87 ± 0.35	8.38 ± 0.25	9.50 ± 0.30

Values are mean reaction time in seconds, expressed as Mean ± SEM, * $p<0.05$

Inhibition of the carrageenan induced inflammation in rats is one of the most reliable test procedures for screening out anti inflammatory agents. The development of carrageenan induced edema is bi phasic, the first phase is attributed to the release of histamine, 5 HT and kinins, while, the second phase is related to the release of prostaglandins (18). The alcoholic extracts of seeds and leaves of were found to contain the triterpenoid saponin, oleanolic acid. The effectiveness of oleanolic acid and other triterpenoid saponins in inflammatory conditions is well documented (19, 20).

One of the most suitable test procedures to screen anti arthritic and anti inflammatory agents is the formalin induced pedal edema as it closely resembles the human arthritis (20). Injection of formalin subcutaneously into hind paw produces localized inflammation and pain. The nociceptive effect of formalin is biphasic, an early neurogenic component followed by a later tissue mediated response (21). This shows that the formalin induced arthritis model is useful for the evaluation of an agent with probable anti proliferative activity.

The hot plate method is considered to be selective for screening of the compound acting through the opoid receptor; the extract of *A. aspera* did not increase the mean basal latency which shows that extract does not act as a centrally acting analgesic

The phytochemical screenings of the extracts of *A. aspera* show the presence of saponins and alkaloids. It may be said, in conclusion, that the study has demonstrated that the alcoholic extracts of leaves and seeds of *A. aspera* may have anti nociceptive and anti inflammatory activities.

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