Pharmacology

STUDY OF ANALGESIC AND ANTI INFLAMMATORY ACTIVITY FROM PLANT EXTRACTS OF *LACTUCA SCARIOLA* AND *ARTEMISIA ABSINTHIUM*

FAYYAZ AHMAD*
RAFEEQ A. KHAN*
SHAHID RASHEED*

SUMMARY: Seeds and samples of stems from the two medicinal plants, Lactuca scariola and Artemisia absinthium respectively were extracted in absolute methanol to determine their analgesic and anti-inflammatory activity. The analgesic activity was assessed on intact mice by tail flick latency in tail immersion method. The anti-inflammatory activity was estimated volumetrically by measuring the mean increase in hind paw volume of rat with the help of plethysmometer. Acetylsalicylic acid in the dose of 300 mg/kg is used as standard drug. Both plant extracts were given in the doses of 300, 500 and 1000 mg/kg. Control group received 0.9% NaCI (saline) solution. All the doses administered orally. Results showed that Lactuca had potent analgesic activity and Artemisia had significant analgesic and anti-inflammatory activity.

Key Words: Lactuca scariola, artemisia absinthium, methanolic extract, analgesia, anti-inflammatory.

INTRODUCTION

Drugs which are in use presently for the management of pain and inflammatory conditions are either narcotics e.g. opioids or non-narcotics e.g. salicylates and corticosteroids e.g. hydrocortisone. All of these drugs present well known side and toxic effects. Moreover synthetic drugs are very expensive to develop since, for the successful introduction of a new product approximately 3000-4000 compounds are to be synthesized, screened and tested whose cost of development ranges from 0.5 to 5 million dollars. On the contrary many medicines of plant origin had been used since long time without any adverse effects. It is therefore essential that efforts should be made to introduce new medicinal plants to develop cheaper drugs (4). Plants represent still a large untapped source of structurally novel compounds that might serve as lead for the development of novel drugs (2).

The lack of potent analgesic and anti-inflammatory drugs now actually in use prompted the present study, in which *Lactuca scariola* and *Artemisia absinthium* had been selected for their reported biological activities in indigenous system of medicine (3, 7).

* From Department of Pharmacology, Faculty of Pharmacy, University of Karachi, Karachi-75270, Pakistan.

MATERIALS AND METHODS

Plant material

L. scariola (Compositae) and *A. absinthium* (Compositae) were collected from various parts of Karachi during July to August and identified with the help of herbarium specimens.

The selected parts of these plants were then dried in shade at temperatures between 21-30°C for 15 to 30 days, after which these parts of plants were chopped and ground. Finally extraction was carried out by the following procedure.

Preparation of the extract

The ground plant materials approximately 1 kg were soaked in 500 ml absolute methanol for about six weeks. The alcoholic extracts were then evaporated under reduced pressure in rotary evaporator (Eyela) and a syrupy residue so obtained was dissolved in small quantity of water and subjected to freeze drying. Freeze-dried extracts were collected in small glass bottles and kept at -30°C for further evaluation.

Preparation of samples for bioassay

Acetylsalicylic acid in a quantity of 300 mg and extracts of *L. scariola* and *A. absinthium* in the quantities of 300, 500 and l000 mg were homogenized in 1.5% aqueous suspension of gumtragacanth. The homogenate including the insoluble fraction was administered orally to animals on the basis of mg/kg of the body weight.

DRUGS

All the drugs used in this study were of pharmaceutical grade. Carrageenan was supplied by Sigma Chemical Company, USA and acetylsalicylic acid was supplied by Aspro Nicholas Pakistan Ltd.

ANIMALS

Albino mice for analgesic studies and albino rats for anti-inflammatory studies of either sex bred at the animal house of Welcome Pakistan Ltd. were used in the present study. Weights of the mice and rats ranged from 20-25 g and from 160-210 g respectively. All animals were maintained in groups of five at 22 ± 1°C with light/dark cycle of 12:12 hours. They were starved overnight but allowed fresh water before administration of the plant extracts.

PROCEDURE FOR TESTING ANALGESIC ACTIVITY

Tail immersion method

In present study analgesia was assessed according to the method of Luiz *et al.* (6). Mice divided in the groups of five each, were held in position in a suitable restrainer with the tail extending out. 3-4 cm area of the tail was marked and immersed in the water bath thermo-statistically maintained at 51°C. The withdrawal time of the tail from hot water (in seconds) was noted as the reaction time or tail flick latency. The maximum cutoff time for immersion was 180 seconds to avoid the injury of the tissues of tail. 0.2 ml of 0.9% NaCl solution was administered to control animals, plant extracts in doses of 300, 500 and 1000 mg/kg were given orally by intubation. The initial reading was taken immediately before administration of test and standard drugs and then 60, 90, 120,150, 180 and 210 minutes after the administration.

The criterion for analgesia was postdrug latency which was greater than two times the pre-drug average latency as reported by Janssen *et al.* (5). Tail flick latency difference or mean increase in latency after drug administration was used to indicate the analgesia produced by test and standard drugs. Analgesia TFLD was calculated as follows.

Analgesia TFLD = signifies past-drug tail flick latency-
pre-drug tail flick latency.

PROCEDURE FOR TESTING ANTI-INFLAMMATORY ACTIVITY

Hind paw edema method

In present study anti-inflammatory activity was determined in albino rats of either sex according to the method of Winters *et al.* (12). Using five animals in each group. The animals were injected carrageenan (1% w/v suspension in 0.9% saline) Ocete *et al.* (8) in the right hind foot under the plantar aponeurosis.

The test groups of rats were given orally 300, 500 and l000 mg/kg of methanolic extract of plants one hour before the carrageenan injection. The controls were given the same volume of saline as in test group. Another group of rats was treated with 300 mg/kg of acetylsalicylic acid orally one hour before carrageenan injection. The inflammation was quantitated in terms of ml i.e. replacement of water by edema using a plethysmometer (Ugo Basile) immediately before carrageenan injection and then 1, 2, 3, 4 and 5 hours after carrageenan injection. The percent inhibition of edema as calculated for each group with respect to its vehicle-treated control group. The anti-inflammatory activity was calculated by using the relation

$$\frac{A - B}{A} \times 100$$

used by Planichamy (9), where A and B denote mean increase in paw volume of control and drug-treated animals respectively.

STATISTICAL ANALYSIS

Values for analgesic activity were expressed as "mean increase in latency after drug administration ±SEM" in terms of seconds whereas values for anti-inflammatory activity were expressed as "mean increase in paw volume ±SEM". The significance of difference between means was determined by student's t-test values of $p<0.05$ were considered significant and $p<0.01$ as highly significant.

All statistical procedures were performed according to the method of Alcaraz (1).

RESULTS AND DISCUSSION

The analgesic and anti-inflammatory effects of acetylsalicylic acid (standard drug) and methanolic extracts of *L. scariola* and *A. absinthium* are shown in Tables 1 and 2 respectively.

The analgesic activity was expressed as "mean increase in latency after drug administration ±SEM" relative to controls whereas the anti-inflammatory activity was expressed as "mean increase in paw volume ±SEM" in terms of ml and percentage inhibition in paw volume by different doses of the extract.

Seeds of *L. scariola* were used as potent analgesic in Unani Tibb, (10). Moreover anti-inflammatory activity of *A. absinthium* in rats had been reported by Saratikov *et al.* (11) but no work have been done to test the analgesic activity of this plant, thus to confirm the analgesic and anti-inflammatory activity of these plants pharmacological evaluation had been carried out in this study.

Table 1: Analgesic effect of methanolic extracts of *L. scariola* and *A. absinthium* in mouse tail immersion method.

Treatment	Dose/kg orally	Analgesia TFLD or mean increase in latency after drug administration ± SEM (s)					
(min)		+60	+90	+120	+150	+180	+210
Saline	0.2 ml	0.064±0.173	0.386±0.311	0.334±0.292	0.50±0.217	0.366±0.275	0.464±0.321
Lacture scariola	300 mg	0.90±0.180**	1.30±0.275*	2.90±0.290**	3.13±3.99**	3.90±0.221**	4.10±0.281**
	500 mg	1.02±0.064**	2.318±0.380**	3.166±0.302**	3.62±0.185**	4.02±0.165**	3.45±0.281**
	1000 mg	2.23±0.315**	3.33±0.34**	4.59±0.64**	5.43±0.80**	9.36±1.470**	10.7±2.02**
Artemisia absinthium	300 mg	1.40±0.360**	1.824±0.315**	2.49±0.090**	2.97±0.450**	3.33±0.339**	3.76±0.416**
	500 mg	1.06±0.201**	2.19±0.200**	2.79±0.230**	2.86±0.300**	2.89±0.290**	3.26±0.130**
	1000 mg	2.50±0.270**	3.33±0.390	2.77±0.502**	3.53±0.250**	3.74±0.310**	3.53±0.370**
Acetylsalicylic acid	300 mg	0.90±0.152**	2.22±0.195**	2.31±0.142**	3.48±0.140**	3.84±0.201**	4.14±0.239**

Significant relative to control reading: *p<0.05, **p<0.01, (n=5).
SEM = Standard error to mean.

Table 2: Anti-inflammatory effects of methanolic extracts of *L. scariola* and *A. absinthium* on carrageenan-induced rat paw edema.

Treatment	Dose/kg P.O	Mean paw volume ±SEM (ml)					
		Before carrageenan	+1 h	+2 h	+3 h	+4 h	+5 h
Saline	0.5 ml	0.80±0.03	1.23±0.07	1.60±0.09	1.82±0.07	1.95±0.05	1.80±0.04
Lactuca scariola	300 mg	0.83±0.01	1.25±0.03	1.64±0.02	1.90±0.03	2.02±0.05	2.00±0.10
	500 mg	0.82±0.03	1.24±0.04	1.65±0.06	2.00±0.14	2.06±0.11	2.12±0.19
	1000 mg	0.82±0.03	1.18±0.07	1.58±0.07	1.89±0.08	1.95±0.07	1.85±0.03
Artemisia absinthium	300 mg	0.96±0.01	1.46±0.04	1.72±0.05*	2.03±0.11	2.07±0.07*	1.81±0.05**
	500 mg	0.95±0.01	1.64±0.06	1.83±0.04	1.90±0.03*	1.94±0.06*	1.83±0.06
	1000 mg	0.92±0.02	1.51±0.05	1.63±0.05*	1.68±0.05**	1.78±0.04**	1.67±0.03**
Acetlysalicylic acid	300 mg	0.75±0.04	0.93±0.04*	0.99±0.04*	1.05±0.07**	1.11±0.07**	1.12±0.06**
		Mean increase in paw volume ±SEM (ml)					
Saline	0.5 ml	0.80±0.03	0.43±0.04	0.80±0.05	1.02±0.06	1.15±0.06	1.00±0.05
Lactuca scariola	300 mg	0.83±0.01	0.42±0.03	0.81±0.03	1.07±0.03	1.19±0.03	1.17±0.09
	500 mg	0.82±0.03	0.42±0.06	0.83±0.07	1.18±0.13	1.24±0.09	1.30±0.17
	1000 mg	0.82±0.03	0.36±0.09	0.76±0.08	1.07±0.06	1.13±0.07	1.03±0.03
Artemisia absinthium	300 mg	0.96±0.01	0.50±0.03	0.76±0.05	1.07±0.11	1.11±0.06	0.85±0.05**
	500 mg	0.95±0.01	0.69±0.07	0.88±0.04	0.95±0.03*	0.99±0.07*	0.88±0.06**
	1000 mg	0.92±0.02	0.59±0.05	0.71±0.06*	0.76±0.06**	0.86±0.03**	0.75±0.02**
Acetly-salicylic acid	300 mg	0.75±0.04	0.18±0.01**	0.24±0.02**	0.30±0.03**	0.36±0.05**	0.37±0.04**
		% inhibition in edema ±SEM (%) (ml)					
Saline	0.5 ml	0.80±0.03	-	-	-	-	-
Lactuca scariola	300 mg	0.83±0.01	1.40±7.60	-1.99±4.40	-4.60±3.70	-2.40±3.30	-16.90±9.90
	500 mg	0.82±0.03	2.33±16.10	-4.70±9.10	-16.00±13.00	-7.90±8.20	-30.00±17.80
	1000 mg	0.82±0.03	16.20±22.50	4.70±10.80	-4.80±6.14	1.38±6.14	-7.70±3.73
Artemisia absinthium	300 mg	0.96±0.01	15.70±4.95	25.90±4.85	16.80±8.76	14.60±5.00	25.70±4.40
	500 mg	0.95±0.01	11.80±0.08	14.10±4.70	26.10±2.88	23.70±5.50	22.90±5.80
	1000 mg	0.92±0.02	10.20±8.90	27.00±6.27	41.00±4.70	33.70±2.80	34.20±2.40
Acetly-salicylic acid	300 mg	0.75±0.04	54.80±1.90	61.60±4.60	56.70±4.80	58.30±5.70	54.20±5.80

Significant relative to control reading: *p<0.05, **p<0.01, (n=5).
SEM = Standard error to mean.

L. scariola exhibited potent analgesic activity at the dose levels of 300, 500 and l000 mg/kg. It is worth noting that this extract showed significant analgesic activity at low dose of 300 mg/kg even in the first hour of the test. The duration as well as the intensity of analgesia induced by *L. scariola* were dose dependent. The analgesic effect at l000 mg/kg dose level was highest at +210 minutes after which the activity began to decrease. The analgesic activity shown by *L. scariola* at 300 mg/kg was almost comparable to that produced by acetylsalicylic acid, while at the dose levels of 500 mg/kg and 1000 mg/kg. *L. scariola* showed better analgesic effect than the reference drug and at the dose level of l000 mg/kg the duration and intensity of analgesia was also greater than acetylsalicylic acid (Table 1). However methanolic extract of this plant failed to exert any inhibitory effect on "mean increase in paw volume" induced by carrageenan injection in the sub-plantar region of rat's paw (Table 2).

These results indicate that methanolic extract of *L. scariola* can produce significant analgesic activity but failed to show anti-inflammatory effects. As far as the analgesic effects are concerned our results supports the claims about this plant in folk medicine.

The methanolic extract of *A. absinthium* showed significant analgesic action at all three dose levels i.e. 300, 500 and 1000 mg/kg. The duration as well as the intensity of analgesia was dose dependent. The plant extract showed a rapid onset of analgesic action at all three doses then as compared to acetylsalicylic acid at 300 mg/kg dose. Inspite of rapid onset of analgesic action, the plant extract even at high dose showed less potent analgesia (both in terms of intensity and duration) when compared with acetylsalicylic acid (Table 1).

The methanolic extract of *A. absinthium* possessed varying degree of anti-inflammatory activity when tested at various doses i. e. 300, 500 and 1000 mg/kg. The plant extract at the dose of 1000 mg/kg showed significant anti-inflammatory activity peaked at +3h where it caused 41% inhibition in increase in paw volume, though of a short duration and intensity, as compared to that of 300 mg/kg acetylsalicylic acid (Table 2). The plant extract also showed a delayed anti-inflammatory response, this might be due to the delayed absorption of the plant extract.

On the basis of the results of this study, it is not possible to conclude that all the effects observed are true analgesic or anti-inflammatory effects. It seems safe, however to conclude that these parts do possess biological activities following oral administration. The above results need to be verified in other experimental models to be totally authentic.

Pharmacodynamics studies should be undertaken to establish the mechanism of action of the plant extracts.

Phytochemical investigation is also proposed in order to isolate the active fraction and eventually the pure compound.

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Correspondence:
Rafeeq Alam KHAN
Department of Pharmacology,
Faculty of Pharmacy,
University of Karachi,
Karachi-75270, PAKISTAN.