ORIGINAL ARTICLE

Evaluation of antinociceptive effect of *Xanthium strumarium* Linn. leaves extract in Swiss albino mice

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The aim of this study was to explore the probable antinociceptive effect of an ethanolic extract of *Xanthium strumarium* L. leaves in Swiss albino mice by using models of Eddy's hot plate test, tail immersion test and acetic acid induce writhing test. Swiss albino mice were treated i.p. with saline water (control), test group treated with 50, 100, 200 and 400 mg/kg extract per oral, standard group treated with pentazocin 3 mg/kg. The results are expressed as the mean ± SEM. and statistically analyzed using one-way analysis of variance (ANOVA) followed by a Student-Newman-keuls test for multiple comparisons using Graph Pad Instant statistical program. Values of $p < 0.05$ were considered to be significant. The ethanolic extract showed the dose-dependent antinociceptive activity in Eddy's hot plate test, tail immersion test and acetic acid induce writhing test at time intervals 15, 30, 60 and 120 min.

Key words: Antinociceptive activity, ethanolic extract, *Xanthium strumarium* Linn

INTRODUCTION

The genus *Xanthium* (family Compositae) is represented by 25 species in the world. *Xanthium strumarium* L. (Compositae) is a gregarious weed found abundantly throughout India. Historically, Xanthium species have been used as traditional herbal medicines in oriental countries. *Xanthium strumarium* L. is used in traditional Chinese medicine to treat nasal sinusitis, headache, urticaria and arthritis.[1] It has also been reported to possess curative effects against chronic bronchitis, chronic rhinitis, allergic rhinitis, lumbago and other ailments.[2] The whole plant is used as a diaphoretic, sedative, sudorific, diuretic and sialagogue, while the leaves are also used in longstanding cases of malarial fever.[3] The fruits are believed to be useful for smallpox[4] whereas the roots are used for cancer.[4] In addition, the stem has been found to possess hypoglycaemic activity in normoglycaemic rats.[5] Jawad *et al.*, reported the antimicrobial activity of methanolic extract of *X. strumarium* plant.[6] *X. strumarium* fruit extract has topical anti-inflammatory and analgesic activities.[7] Based on these properties, the present study focuses on the evaluation of antinociceptive activities of a leaves extract of *X. strumarium* and aims to establish the most active part(s) of the plant.

MATERIALS AND METHODS

Chemicals and Medicines

Pentazocin inj., Ethanol (95%), chloroform, Acetic acid (1%), Methanol and distill water.

Preparation of the Ethanol Extract

The plant *X. strumarium* linn. was collected from Kupwad MIDC, sangli (Maharashtra). The plant was authenticated on the basis of its microscopic and macroscopic characters by Dr. Mrs.Yadav, Professor and Head of Dept. Botany. Wilingdon College, Sangli (M.S.). The leaves of plant *X. strumarium* Linn. was washed and cut. The leaves were shade dried and coarse powder of leaves was prepared. The coarse powder of *X. strumarium* Linn. leaves was exhaustively extracted using (1.5L) ethanol (95%) in soxhlet extractor. The extracts were concentrated under reduced pressure and low temp. (50°C). The extract was dried and used for experimental studies.

For pharmacological studies, extract of *X. strumarium* of was suspended in a 1% aqueous solution of Tween-80.

Animals

White Swiss albino mice of either sex weighing around 18-25 g were procured from animal house of Appasaheb Birnale College of pharmacy, Sangli. The animals were housed at 25 ± 1°C followed under 12th day-night cycle and were provided free access to standard food and tap water. The animal experimentation was carried out in accordance to the guidelines mentioned in the CPCSEA, and local Institutional Animal Ethical Committee approved the experiment protocols.

Acute Toxicity Study

The acute toxicity study was carried out in adult albino rats by "fix dose" method of OECD (Organization for

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Economic Co-operation and Development) Guideline No.420.

Experimental Setup

In the treatment group, extract was administered orally as suspension (1% v/v Tween 80) in normal saline. The rats were divided into five groups consisting of six animals each, for analysis of antinociceptive study.

Group I received Normal Control treated with saline. Group II, III, IV, and V received 50 mg/kg, 100 mg/kg, 200 mg/kg, 400 mg/kg of *X strumarium* leaves extracts p.o., respectively. Group VI received 3 mg/kg pentazocin i.p.

Evaluation of Antinociception

Evaluation of antinociception was performed by following methods:

Eddy's hot plate test

The mice were divided into six groups of six animals. The basal reaction time was recorded by observing hind paw licking or jump response in animals when placed on hot plate maintained at constant temperature (55°C). Normally animals shows such response in 6-8 sec. A cut off period of 15 sec is observed to avoid damage to paws. Administered 1 % saline solution i.p. to group I, 50 mg/kg, 100 mg/kg, 200 mg/kg, 400 mg/kg of *Xanthium strumarium* leaves extract (XSE) p.o. to group II, group III, group IV and group V respectively and 3mg/kg pentazocin i.p. to group VI. The reaction time of animals on hot plate was recorded at 15, 30, 60 and 120 min after the drug administration. Percentage increase in basal reaction time (index of analgesia) at each time interval was calculated.[8]

Tail immersion test

The mice were divided into six groups of six animals. The basal reaction time was recorded by observing tail flick response in animals when tail is immersed in water maintained at constant temperature (55°C). Normally animals show such response in 3-5 sec. A cut off period of 10 sec is observed to avoid avoiding tissue damage. Administered 1% saline solution i.p. to group I, 50 mg/kg, 100 mg/kg, 200 mg/kg, 400 mg/kg of *Xanthium strumarium* leaves extract (XSE) p.o. to group II, group III, group IV and group V respectively and 3 mg/kg pentazocin i.p. to group VI. The reaction time of animals on hot plate was recorded at 15, 30, 60 and 120 min after the drug administration. Percentage increase in basal reaction time (index of analgesia) at each time interval was calculated.[9]

Acetic acid induced writhing test

The mice were divided into six groups of six animals each. one percent saline solution i.p. was administered to group I, 50 mg/kg, 100 mg/kg, 200 mg/kg, 400 mg/kg of *Xanthium strumarium* leaves extract (XSE) p.o. to group II, group III, group IV and group V respectively and 3 mg/kg pentazocin i.p. to group VI. Acetic acid (1% v/v) was administered i.p. to all the groups at the dose of 1 ml/kg of body weight. Anti-nociception was recorded at interval of 15, 30, 60 and 120 min by counting the number of writhes after the injection of acetic acid for a period of 20 min. Percentage decrease in number of writhes (index of analgesia) at each time interval was calculated. A writhe is indicated by abdominal constriction and full extension of hind limb.[10]

Statistical Analysis

All data are expressed as mean ± SEM. The results were statistically analyzed using one-way analysis of variance (ANOVA) followed by a Student-Newman-keuls test for multiple comparisons using Graph Pad Instant statistical program. Values of $P < 0.05$ were considered to be significant.

RESULTS

Evaluation of Acute Toxicity and Dose Determination

Table 1: Acute toxicity study

Treatment	Dose Mg/kg	Number of animals	Mortality			Toxicity profile
			After 3 hrs	After 6 hrs	After 24 hrs	
Sighting study	2000	1	0	0	Death	Toxic
Main Test	5	5	0	0	0	Safe
	50	5	0	0	0	Safe
	300	5	0	0	0	Safe
	1000	5	0	0	0	Safe

Table 2: Effect of *X. strumarium* leaves extract on Eddy's hot test in mice

	15 min.	30 min.	60 min.	120 min.
Control (saline)	6±0.12	6.5±0.22	6.3±0.20	6.5±0.20
XSE (50 mg/kg)	6.3±0.22	6.68±0.24	6.4±0.18	6.79±0.17
XSE (100 mg/kg)	6.3±0.24	6.8±0.12	6.9±0.21	7.0±0.18*
XSE (200 mg /kg)	6.84±0.24	8.64±0.14**	8.84±0.26***	9.56±0.18***
XSE (400 mg /kg)	7.04±0.024*	9.12±0.07***	9.7±0.30***	9.8±0.24***
Pentazocin (3 mg/kg)	7.12±0.25*	8.9±0.046***	8.9±0.51***	10.32±.28***

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ as compared to respective control; n = 6 for each experiment

Table 2a: Percentage increase in basal reaction time by Eddy's hot plate method tail immersion test

Treatment	% increase in basal reaction time			
	15 min.	30 min.	60 min.	120 min
Control (saline)	00	00	00	00
XSE (50 mg/kg)	4.7	2.69	1.56	1.47
XSE (100 mg/kg)	4.7	4.41	8.69	7.14
XSE (200 mg/kg)	12.28	24.76	28.73	32
XSE(400 mg/kg)	14.28	28.72	35.05	33.67
Pentazocin (3 mg/kg)	15.73	26.96	29.21	37.01

Table 3: Effect of *X. strumarium* leaves extract on tail immersion test in mice

Treatment	Tail flick latencies in sec. (mean ± SEM)			
	15 min.	30 min.	60 min.	120 min.
Control (saline)	3.40 ± 0.005	3.48 ± 0.003	3.41 ± 0.003	3.50 ± 0.003
XSE (50 mg/kg)	3.43 ± 0.008	3.50 ± 0.006	3.43 ± 0.003	3.55 ± 0.01
XSE (100 mg/kg)	3.45 ± 0.003	3.51 ± 0.004	3.52 ± 0.007	3.63 ± 0.008**
XSE (200 mg/kg)	3.53 ± 0.008	4.21 ± 0.005**	4.33 ± 0.017**	4.42 ± 0.02***
XSE (400 mg/kg)	4.16 ± 0.05**	4.36 ± 0.009**	5.6 ± 0.008***	5.88 ± 0.02***
Pentazocin (3 mg/kg)	4.20 ±0.45***	4.8 ±0.024***	5.8 ± 0.32***	6.3 ± 0.41***

***P* < 0 .01, ****P* < 0.001 as compared to respective control; n = 6 for each experiment

Table 3a: Percentage increase in basal reaction time by Tail immersion test Acetic acid induced writhing test

Treatment	% increase in basal reaction time			
	15 min.	30 min.	60 min.	120 min.
Control (saline)	00	00	00	00
XSE (50 mg/kg)	0.87	0.57	0.58	1.4
XSE (100 mg/kg)	1.44	0.85	3.12	3.58
XSE (200 mg/kg)	3.68	17.33	21.24	20.82
XSE (400 mg/kg)	18.26	20.18	39.10	40.76
Pentazocin (3 mg/kg)	19.04	27.5	41.20	44.44

[Tables 1-4, 2a, 3a, 4a]

The acute toxicity studies showed that the ethanolic extract was toxic at 2000 mg/kg body weight. Thus the LD_{50} value of extract is expected to be in between 300-2000 mg / kg body weight. Considering this, we had fixed 1000 as $LD_{50}$and selection doses.

DISCUSSION

The ethanolic extract of *X. strumarium* was primarily screened for analgesic activity using Eddy's hot plate test, tail immersion test and acetic acid induced writhing test. Ethanolic extract of *X. strumarium* leaves (XSE) showed significant analgesic activity at 200 mg/kg and 400 mg/ kg dose at 60 and 120 min in Eddy's hot plate test and tail immersion test respectively, with value ranging from 28.73% and 32% protection for 200 mg/kg and 35.05% and 33.67% protection for 400 mg/kg in the hot plate test, and 21.24% and 20.82% protection for 200 mg/kg and 39.10% and 40.76 % protection for 400 mg/ kg in the tail immersion test, at 60 and 120 min as compared to control (saline) and standard drug Pentazocin 3 mg/kg, respectively. Whereas 50, 100, 200, 400 mg/kg dose of extract showed significant analgesic activity at all time intervals having highest protection at 60 min (81.23%) at 400 mg/kg, as compared with control (saline) in acetic acid induced writhing test. These results provide pharmacological support for the dose dependent analgesic effect of *X. strumarium*. These results taken together indicate that extract of *X. strumarium* possesses strong analgesic activity.

Table 4: Effect of *X. strumarium* leaves extract on acetic acid induced writhing test in mice

	15 min.	30 min.	60 min.	120 min.
Control (saline)	55.4±1.720	55.4±1.720	55.4±1.720	55.4±1.720
XSE (50 mg/kg)	50.6±0.600	49.8±0.3742	48±0.9487**	51±1.720
XSE (100 mg/kg)	44.4±1.691**	45.4±2.040**	38.8±1.158***	41.8±1.068***
XSE (200 mg/kg)	36.8±2.267***	34.8±2.518***	32.4±0.9798***	35±1.844***
XSE (400 mg/kg)	16±1.304***	14.4±1.327***	10.4±0.8124***	33±1.789***
Pentazocin (3 mg/kg)	16.21±0.92***	12.45±0.54***	10±0.32***	14.34±0.23***

***P* < 0.01, ****P* < 0.001 as compared to respective control; n = 6 for each experiment

Table 4a: Percentage decrease in number of writhes by writhing method

	15 min.	30 min.	60 min.	120 min.
Control (saline)	00	00	00	00
XSE (50 mg/kg)	8.67	10.11	11.92	7.95
XSE (100 mg/kg)	19.86	18.06	29.97	24.55
XSE (200 mg/kg)	33.58	37.19	41.52	36.83
XSE (400 mg/kg)	71.12	74.01	81.23	40.44
Pentazocin (3 mg/kg)	29.25	77.53	81.86	74.12

Previous studies showed that naturally occurring phenolic acids have various pharmacological properties and can be used as cholagogues, stomach stimulants, and immunostimulants, as well as anti-tumor, antioxidant, antibacterial, and antifungal agents.[11-14] Santos *et al.*, reported that two dicaffeoylquinic acids (3,5- and 4,5-O-dicaffeoylquinic acids) in *X. strumarium* plant. The caffeoylquinic acids are phenolic acid, could partially explain the antinociceptive effect of *X. strumarium*.[15] Based on these properties of *X. strumarium*, it can be suggested that *X. strumarium* has analgesic activity. This observed activity may be associated with the presence of phenolics, as well as a probable synergistic effect of the heterocyclics components and other secondary metabolites present in plant. Further studies are required to determine the possible mechanism of actions of this compound.

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