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Research Article

EVALUATION OF ANALGESIC AND ANTI-INFLAMMATORY POTENTIAL OF *MIMOSA PUDICA LINN*

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ABSTRACT

The aim of this work to evaluate the acute toxicity, Analgesic and Anti-Inflammatory activity of Ethanolic extract of *Mimosa Pudica Linn*. In the acute toxicity study, the extracts were administered in doses of 5, 50, 300 and 2000 mg/kg p.o. and behavioral changes were observed after 24 hrs. In hot plate test the pethidine treated group, Tail flick Diclofenace treated group, and group given ethanolic extracts as 250 mg/kg and 500 mg/kg showed increase in latency time dose dependent manner. Oral administration of ethanolic extract at a dose of 500 mg/kg showed significantly reduction of writhing response induced by acetic acid as compared the dose given at 250 mg/kg.

Keywords: *Mimosa pudica Linn.* Leaves, Ethnolic extract, Analgesic activity, Antiinflammatory activity (Carrageenan induced rat hind paw oedema)

INTRODUCTION

Nature has provided a complete store house of remedies to cure all aliments of mankind [1]. This is where, nature provides us drugs in the form of herbs, plants and algae's to cure the incurable diseases without any toxic effect[2]. *Mimosa pudica* belonging to the family *fabaceae* which is a herb used in Ayurveda. *Mimosa pudica* linn. is commonly known as sensitive plant in English and lajvanti or Chhuimui in hindi, lajjalu in ayurveda, namaskari in sanskrit language [3]. The *Mimosa* plant grows mainly in tropical and subtropical parts of India, Brazil, Australia, Tanzania and South America. Sensitive plant grows on most well drained soils, even scalped or eroded sub-soils and soils with low nutrient concentrations. Sensitive plants live for about 1 to 2years. Growth of plants that survive into the second year is much slower. Potted and field grown individuals are sensitive to over watering. Collection of plant is done during Sep-March in Indian conditions. Phytochemical screening has revealed that the plant contains Mimosine, stigmasterol, leucoanthocyanidin, D-xylose, D-glucuronic acid, norepinephrine, D-pinitol, linoleic acid, oleic acid, palmitic acid, stearic acid, -sitosterol and crocetin dimethyl. Various medicinal and biological activities of mimosa pudica linn have been reported in earlier studies by various research groups like Anti-ulcer [4], Hepatoprotective [5], Hypolipidemic [6], Enzyme inhibitory [7], Anti bacterial [8], Ayperglycaemic [9], Antiimplantation, Antiestrogenic [10], Anticonvulsant[11], Antitoxin [12] Antimalarial [13],Wound healing activity[14], Anti depressant activity [15], Ovulation in female albino rat[16]. There is no scientific report on analgesic activity and anti-inflammatory activity of *Mimosa pudica,* therefore the present study was undertaken to examine the possible Analgesic and Anti-Inflammatory activity of ethanolic extract.

MATERIALS AND METHODS

Plant material

The leaves of plant *M. pudica* were collected from Jawaharlal Nehru Krishi Vishwavidyalaya Jabalpur; the plant material was identified & authenticated by Prof. (Mrs) Karuna S. Verma, Senior Botanist, Department of Post Graduate Studies & Research in Biological Sciences Rani Durgawati Vishwavidyalaya, Jabalpur.

Preparation of extract

The leaves were shade-dried and made a coarse powder which was passed through a 40-mesh sieve to get a uniform particle size and then used for extraction. A weighed quantity (200g) of the powder was then subjected to continuous hot extraction in Soxhlet apparatus with petroleum ether, Benzene, Chloroform, Acetone, Ethanol respectively and the residual marc was collected. The extract was filtered through a cotton plug, followed by whartman filter paper (No.1). After concentration and drying of each extract in vacuum desiccators the yield of the product was approximately 5.2 %. The final product was stored at 4^0C till further use.

Animals

Wistar albino rats (150–200 g) of either sex were used. The animals were housed in groups of 5 per cage (Polypropylene cages) prior to pharmacological studies animals were kept at water *ad libitum* for 2 a weeks by providing 12/12 h light/dark cycle and at a temp 25±2 °C. All animals were fasted overnight before test. The study was done with approval from the Institutional Animal Ethical Committee (IAEC) for the purpose of control and supervision of experiments on animal (CPCSEA).

Drugs and Chemicals

The following drugs were used: Pethidine, Diclofenac (Lupin limited, Mumbai) Indomethacin, Asprin (USV limited, Mumbai) Acetic acid (center drug house pvt. Ltd,New Delhi), Carrageenan (CDH Pvt. Ltd. New Delhi) Petroleum ether, Benzene, Chloroform, Acetone.

Acute toxicity study

The acute toxicity study of extract was performed using OECD (Organigation for Economic and Cultural Development) guidelines. The animals were fasted overnight prior to the experiment and fixed dose was adopted for toxicity studies (OECD Guideline No. 423). The extracts were administered in doses of 5, 50, 300 and 2000 mg/kg p.o. and behavioral change were observed after 24 hrs. The extract of *Mimosa pudica* was devoid of behavioral changes in animals at dose of 2000 mg/kg in rats by P.O. route and hence $1/8^{th}$ and $1/4^{th}$ of the same i.e. 250 mg/kg and 500 mg/kg were selected for screening dose for further studies.

Analgesic Activity

Hot plate test

Animals were divided into four groups. Five rats are studied per group. Groups I served as control group and received normal saline. Group II served as standard group and received pethidine (4 mg/kg I.P.). Groups III and IV were served as test groups and were administered with extract at 250 and 500 mg/kg, respectively. Wistar rats were placed onto an Eddy's hot plate maintained at a temperature 55°C surrounded by a Plexiglas cylinder (Height: 26 cm; Diameter: 19 cm). The latency to the first Paw-lick is measured maximum 30 seconds. The results are given in Table 1. [17]

Tail Flick Test

Wistar rat of either sex were selected and divided into four groups of five animals each. Groups I served as control group and received normal saline. Group II served as standard group and received diclofenac (5 mg/kg I.P.). Groups III and IV were served as test

groups and were administered with extract at 250 and 500 mg/kg, respectively.

The rat were kept in rat holder and middle section of tail was place on the nichrome wire (Techno, Lucknow, India) and The analgesiometer was switched on 6mA current(D amour et al 1941).The time when animal withdraw (flick) its tail from the hot wire was taken as the reaction time(maximum 30sec). Analgesic activity was measured after 15 minutes of administration of sample [18]. The results are given in Table 2.

Table 1: Effect of extract on hot plate activity in rats

Treatment	Reaction time (sec)			
	Control (2 ml/kg, dist. water)	Pethidine (4 mg/kg)	Extract Extract 1 (250 mg/kg)	Extract 2 (500 mg/kg)
Pretreatment	5.6 ± 0.09	6.25 ± 0.90	6.14 ± 0.94*	6.19 ± 0.60
30 min	5.8 ± 0.14	8.54 ± 0.32	8.43 ± 0.64	8.47 ± 0.68**
60 min	6.08 ± 0.11	10.25 ± 0.18	9.77 ± 0.34	10.14 ± 0.30
90 min	6.16 ± 0.56	14.55 ± 0.43	11.72 ± 0.48	12.64 ± 0.57*
120 min	5.44 ± 0.27	15.35 ± 0.40	12.68 ± 0.35**	13.18 ± 0.45**

Values are expressed as mean ± SEM, *P<000.1, **P<0.001; compared with control group

Table 2: Effect of extract on tail flick response in rats

Treatment	Reaction time (sec)			
	Control (2 ml/kg)	Diclofenac (5mg/kg)	Extract Extract 1 (250 mg/kg)	Extract 2 (500 mg/kg)
Pre-treatment	4.24±0.18	4.42±0.20	4.48±0.32**	4.34±0.22*
30 min	4.57±0.46	6.22±0.16	4.77±0.48*	4.72±0.52*
60 min	4.48±0.08	14.37±0.62	5.55±0.22**	5.74±0.34**
90 min	4.59±0.39	18.78±0.22	5.56±0.18*	5.86±0.46
120 min	4.49±0.68	22.28±0.36	5.68±0.24**	5.90±0.21*

Values are expressed as mean ± SEM, *P<000.1, **P<0.001; compared with control group

Acetic acid induced writhing test

Wistar rats were divided into four groups each containing five animals. Group I served as control and administered with the acetic acid and onset of wriths were observed and noted. Group II, III and IV were administered with the Aspirin (200 mg/kg, P.O.) and the extract (250 and 500 mg/kg). After 15 minutes, acetic acid was administered and onset and severity of writhing response was noted. The results are given in Table 3.

Table 3: Effect of extract on acetic acid induced writhing in rats

Control	Aspirin (200 mg/kg)	Extract	
		Extract 1 (250 mg/kg)	Extract 2 (500 mg/kg)
74.98±1.18	38.42±1.56	55.6±1.40*	42.6±1.47*
% of writing inhibition	48.75%	25.84%	43.18%

Values are expressed as mean ± SEM, *P<000.1, **P<0.001; compared with control group

Anti-inflammatory activity

Anti-Inflammatory activity was studied by:

Carrageen induced hind Paw oedema

Wistar rats of either sex weighing 150-200 g were divided into four groups containing five animals in each group. Group-I received normal saline solution (control), Group II received Indomethacin (standard 1 mg/kg, I.P.). Group-III and IV received extract (250 and 500 mg/kg, P.O.) of *Mimosa pudica,* respectively. One hour after treatment; 0.1ml of 1% suspension of carrageenin in normal saline was injected into the sub-planner region of left hind paw to induce oedema. The paw volume was measured initially at 1h, 2h, 3h, 4h after carrageenin injection using mercury displacement method (Plethysmograph). The results are given in (Table 4) (Fig 1). The % inhibition of inflammation was calculated using the formula, % Inhibition = (Vc-Vt\Vt) 100 Where 'V_t' edema volume in treated and 'V_c' edema volume in control group with extract.

Table 4: Effect of extract on carrageen induced odema in rats

Treatment	Volume of mercury displaced			
	Control 2ml/kg	Indomethacin 10 mg/kg (A)	Extract Extract 1 (250 mg/kg) (B)	Extract 2 (500 mg/kg) (C)
1h	0.224±0.014	0.113±0.028 (49.53%)	0.118±0.033* (47.32%)	0.117±0.038** (47.76%)
2h	0.382±0.042	0.121±0.036 (68.32%)	0.158±0.054* (58.63%)	0.145±0.058** (62.04%)
3h	0.375±0.037	0.108±0.064 (71.2%)	0.144±0.027* (61.6%)	0.110±0.031* (70.66%)
4h	0.354±0.048	0.076±0.053 (78.53%)	0.102±0.029** (71.18%)	0.098±0.035** (72.31%)

Values are expressed as mean ± SEM, *P<000.1, **P<0.001; compared with control group

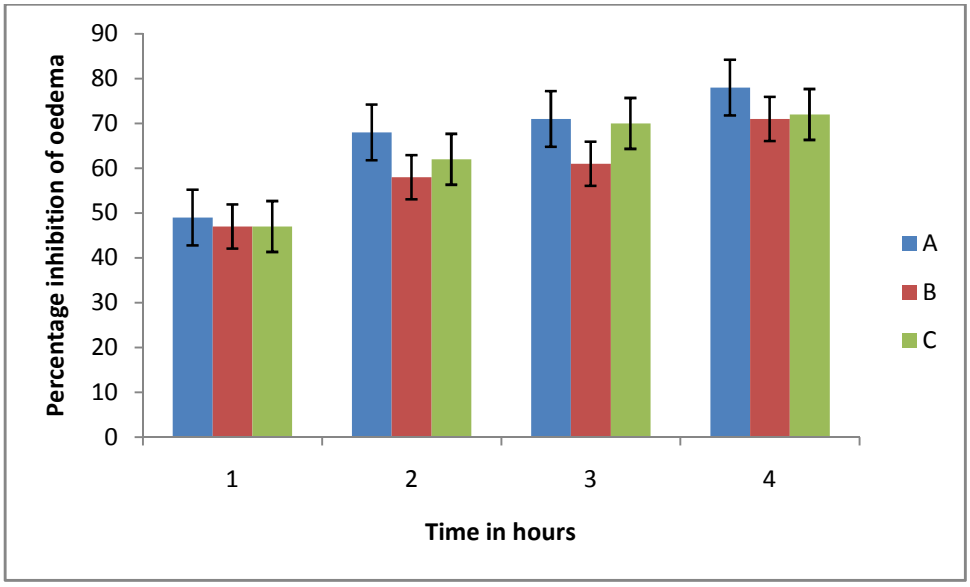

Fig. 1: Effect of *M. pudica* ethanolic extract on Carrageenan induced Paw oedema in rats

Statistical Analysis

The results are expressed as mean values and standard errors mean (SEM) or standard deviation (SD). All the activities were analyzed using one-way analysis of variance (ANOVA) (Graph Pad InStat 3) Software and all the result obtained in the study were compared with the control group)

RESULTS

Acute toxicity study

Acute toxicity testing was performed using standard method. Animals were subjected to different doses (5, 50, 300 and 2000 mg/kg). Behavioral changes were observed. At the dose of 2000 mg/kg (P.O.) the extract showed certain changes in activity and was devoid of any toxicity and hence >2000 mg/kg was taken as LD_{50} cut off value and $1/8^{th}$ and $1/4^{th}$ of the same i.e. 250 and 500 mg/kg were selected as the effective dose.

Analgesic activity

In hot plate test the Pethidine treated group and tail flick test the diclofenac treated group *Mimosa pudica* ethanol extract 250 mg/kg and 500 mg/kg showed increase in latency time dose dependent manner.

writhing responses showed that the number of acetic acid induced writhing was significantly reduced by ethanolic extract administered orally at 250 mg/kg was 55.6±1.40 writh while the same at 500 mg/kg showed writh 42.6±1.47 reduction in acetic acid induced writhing responses, which were comparable to that of standard Aspirin (200 mg/kg) that caused 74.98±1.56 wriths reduction.

Anti-inflammatory activity

The result of carrageenin induced paw oedema is shown in (Table 4). The Ethanolic extract Showed maximum inhibition of 72.31% at the dose of 500mg/kg.

DISCUSSION

Analgesic activity was studied using tail flick method, hot plate method, and acetic acid induced writhing method. All these methods have given very significant analgesic property. In the hot plate test could be a simple and sensitive procedure to evaluate analgesics reaction. This method is considered to be selective for opioid like compounds in animals [19]. Administration of extract exerts a potent anti-nociceptive action confirming the central activity of this extract. The results indicate that the oral administration of Ethanol extract of *Mimosa pudica* significantly attenuated the hot plate thermal stimulation. Hot plate is normally used to study the central analgesics effects of drugs. Therefore, it is probable that *Mimosa pudica* could be producing its effects centrally. These shows the extract increased the stress tolerances capacity of the animals by possible involvement in higher centre. The observed activity can be attributed to the overall effects of the plant constituents or the components having similar structure to NSAIDs or opioid. The acetic acid induced abdominal constriction is believed to show the involvement of peripheral mechanism whereas the hot plate test is believed to show that of central mechanisms [20]. Intraperitoneal injection of acetic acid produces pain through activation of chemo sensitive nociceptors [21]. It has been suggested that acetic acid acts by releasing endogenous inflammatory mediators or irritation of the visceral surface, which leads to the liberation of histamine, kinins, prostanoids, and serotonin. It is a sensitive procedure to evaluate peripherally- and centrally- acting analgesics. The nociceptive activity of acetic acid may be due to cytokine release, such as TNF-α , inteleukin-1β and interleukin–8, by resident peritoneal macrophages and mast cells [22].

The intraperitoneal injection of acetic acid induced an increase in the concentration of glutamate and aspartate in the cerebrospinal fluid [23]. We have reported that the *Mimosa pudica* ethanolic extract inhibited, in a dose-dependent manner, the nociception induced by acetic acid, when compared with the well-known NSAID, aspirin.

Anti- inflammatory effect of *Mimosa pudica*\ was evaluated by Carrageenin induced paw edema. Carrageenin edema is a model of acute inflammation used in the study of non-steroidal anti-inflammatory agents [24]. In conclusion, the result of the present work clearly demonstrated the anti-inflammatory.

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