

# Antinociceptive Activity of *Mimosa pudica* Linn

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## ABSTRACT

*Mimosa pudica* Linn (Mimosoideae) is a plant used in traditional medicine for various disorders. The aim of this work was to evaluate the acute toxicity and antinociceptive activity of the aqueous extract of *Mimosa pudica* in animal models. In the acute toxicity study, a single dose of aqueous extract of 2000 mg kg<sup>-1</sup> body weight p.o. was administered. For 48 h, animals showed no clinical signs and mortality. In the acetic acid-induced writhing model, the extract at a dose of 200 & 400 mg kg<sup>-1</sup> body weight showed significant ( $p < 0.001$ ) inhibition of writhing response of 46.24 and 56.0 % respectively. In the hot plate test, the extract produced a significant ( $p < 0.001$ ) increase in the latency in a dose-related manner. This study established the analgesic properties of *Mimosa pudica* Linn.

**Keywords:** *Mimosa pudica* Linn, Whole plant, Aqueous extract, Analgesic activity, Writhing test, Hot plate test

Plant still remains a major source for drug discovery in development of synthetic molecules. The use of traditional plant extract in the treatment of various diseases has been flourished. *Mimosa pudica* Linn belonging to the family *Mimosoideae* is commonly known as sensitive plant. The plant has very sensitive leaves which fold on touching and has reddish roots. The plant is being used as the drug source in ayurveda system of medicine in Kerala and in Siddha medicine. The plant is widely distributed through tropical and subtropical parts of India, common in waste place where the climate is moist and warm. The useful parts of this plant are roots, leaves and flower heads. The whole plant is used medicinally in ayurvedic folk medicine and its photochemical studies revealed the presence of minosine, orientin, isoorientin,  $\beta$ -sterol, D-pinitol, norepinephrine, crocetin, tannins and turgorins. *Mimosa pudica* is an important plant which is used for various ailments in ayurvedic system of medicine [1-7]. In previous studies, it was found that the decoction of *Mimosa pudica* leaves has anticonvulsant properties [8]. Both the ethanolic and aqueous extracts of the leaves of *Mimosa pudica* possess hyperglycemic and antidepressant activities in mice and rats respectively [9, 10]. There is no scientific report on the analgesic activity of *Mimosa pudica*, therefore the present study was undertaken to examine the possible antinociceptive activity of the aqueous extract of *Mimosa pudica* using acetic acid-induced visceral nociceptive responses and

thermal-induced nociception (chemical and thermal models) in mice.

## MATERIALS AND METHODS

### Study centre

The present study was carried out during December 2006 to June 2007 at Postgraduate Research Laboratory, Department of Pharmacology, SRM College of Pharmacy, SRM University, SRM Nagar, Kattankulathur, Kancheepuram, Tamil Nadu, India.

### Plant material

*Mimosa pudica* Linn (Mimosoideae) was collected from tropical areas in Kancheepuram district, Tamil nadu. It was identified by Prof.P.Jayaraman, Director, Plant Anatomy Research Center (PARC), Pharmacognosy Institute, West tambaram, Chennai, Tamil nadu, India. A voucher specimen (No: PARC/07/SRM/33) was deposited in the herbarium of the institute.

### Preparation of extract

The plant material was washed well with water, dried under shade and powdered to a fine grade by using laboratory scale mill. A batch of 100 gm of the whole plant powder was suspended in one liter of the distilled water and the mixture was boiled for 30 minutes. The

**Table 1.** Effect of aqueous extract of *Mimosa pudica* Linn on acetic acid induced writhing response in mice

| Treatments      | Dose<br>(mg kg <sup>-1</sup> Body weight) | Number of Writhes during 60 min <sup>a</sup> | Percentage of writhes inhibition |
|-----------------|---|--|----------------------------------|
| Control         | -   | 108.83 ± 1.851                               | -                                |
| Aqueous extract | 200                                       | 58.5 ± 1.784*                                | 46.24                            |
|                 | 400                                       | 47.83 ± 1.660*                               | 56.00                            |
| ASA             | 200                                       | 31.16 ± 1.85*                                | 71.36                            |

<sup>a</sup>Values are expressed in Mean ± SEM (n = 6); Difference between groups were statistically analyzed by one-way ANOVA; \*p < 0.001, Dunnett test as compared to control which received normal saline.

obtained decoction was centrifuged and filtered by using filter paper. The filtrate was evaporated in vacuum to give a residue. The yield of the product was approximately 4.2 % (w/w) of the whole plant of *Mimosa pudica* Linn. The final product was stored in a vacuum desiccator at room temperature until analysis.

### Animals

Male Swiss albino mice weighing 20-30 gm were procured from the inbred stock of the King's Institute, Guindy, Chennai, India. They were housed in well-ventilated polypropylene cages with a 12h light/12h dark cycle, received a standard pellet diet (Hindustan lever limited, Bangalore, India.) and water *ad libitum*. The mice were acclimatized to laboratory condition for 7 days. Animals were kept under fasting for overnight, and allowed free access to water before commencement of experiment. The study was done with approval from the Institutional Animal Ethical Committee (IAEC) of Committee for the purpose of control and supervision of experiments on animal (CPCSEA).

### Drugs and Chemicals

The following drugs and chemicals were used for the study: Acetyl Salicylic Acid (USV limited, Mumbai.), Pentazocine (Ranbaxy Laboratories Ltd, New Delhi.), Acetic acid (Ranbaxy Fine Chemicals, New Delhi.), Sodium chloride (Ranbaxy Fine Chemicals, New Delhi).

### Acute toxicity studies

Acute toxicity studies were carried out using acute toxic class-limit test dose guidelines 425 of Organization for Economic and Cultural Development (OECD). Acute toxicity of the plant extract was carried out using groups of three Swiss albino mice by administering a dose of 2000 mg kg<sup>-1</sup> body weight per o.s. (p.o.), while control group received normal saline. The toxicological effects were assessed on the basis of mortality and behavioral changes during 48 h [11].

### Analgesic activity

#### Writhing test

The peripheral analgesic activity was determined by acetic acid-induced writhing. Animals were injected intraperitoneally (i.p.) with 0.6 % acetic acid [11-14], 10 ml kg<sup>-1</sup> body weight and pretreated with *Mimosa pudica* extract at 200 & 400 mg kg<sup>-1</sup> body weight, p.o. Positive control group were received acetyl salicylic acid intramuscular (i.m.) 200 mg kg<sup>-1</sup> body weight, 30 minutes prior to the peritoneal irritation. Control group

received 10 ml kg<sup>-1</sup> body weight of 0.9 % Sodium chloride solution, i.p. The resulting writhings were observed and counted for 60 minutes after acetic acid injection.

#### Hot plate test

The method of Woolfe and McDonald [15] was used. The paw of the mice is very sensitive to heat at temperature which is not damaging the skin. The response in the form of jumping, withdrawal of the paw or the licking of the paws was defined as hot plate latency [13, 14]. The animals were placed on Eddy's hot plate kept at a temperature of 55±1°C. Those that showed a reaction time below 15 sec were placed again on the hot plate and the latency was recorded at 30, 60 and 120 minutes after administration of the test compounds. The test was terminated at 30 sec in the absence of a response. Control group received normal saline (10 ml /kg p.o.). Pentazocine was used as positive control (10 mg/kg). The test group received *Mimosa pudica* aqueous extract at the dose of 200 and 400 mg/kg p.o. The latency was recorded of the above timings. Average reaction time and percentage variation were calculated using the following ratio [16].

Percentage of protection = (Drug latency - Base line latency / Base line latency) × 100

#### Statistical analysis

The statistical analysis of all the results was carried out using one-way ANOVA followed by Dennett's multiple comparison using graph pad instat 3 software and all the results obtained in the study were compared with the control group. The *p* values < 0.05 were considered statistically significant.

## RESULTS

### Acute toxicity

The animals showed no clinical signs without any mortality recorded. About 2000 mg k<sup>-1</sup> body weight may be assumed.

### Analgesic activity

#### Writhing test

As shown in Table 1, our results showed that the number of acetic acid-induced writhing was significantly reduced by aqueous extract administered orally at 200 & 400 mg kg<sup>-1</sup> body weight in dose-related manner with 46.24 and 56.0 % of inhibition respectively. The results were statistically significance

**Table 2.** Effect of aqueous extract of *Mimosa pudica* Linn on the latency of mice exposed to hot plate

| Treatments      | Dose<br>(mg kg <sup>-1</sup> Body weight) | <sup>b</sup> Reaction time(s) |                |                |                             | Percentage protection |
|-----------------|---|-------------------------------|----------------|----------------|-----------------------------|-----------------------|
|                 |   | Basal                         | 30 min         | 60 min         | 120 min                     |                       |
| Control         | -   | 3.20 ± 0.032                  | 2.57 ± 0.047   | 3.07 ± 0.042   | 3.04 ± 0.050                | -                     |
| Aqueous extract | 200                                       | 3.48 ± 0.094*                 | 5.25 ± 0.055** | 4.42 ± 0.090** | 3.50 ± 0.112***             | 50.86                 |
|                 | 400                                       | 3.69 ± 0.061**                | 6.46 ± 0.157** | 4.87 ± 0.182** | 3.341 ± 0.102 <sup>NS</sup> | 70.06                 |
| Pentazocine     | 10  | 3.90 ± 0.056**                | 7.38 ± 0.176** | 6.51 ± 0.129** | 5.67 ± 0.074**              | 89.23                 |

<sup>b</sup>Values are expressed in Mean ± SEM (n = 6); Difference between groups were statistically analyzed by one-way ANOVA; \**p*<0.05, \*\**p*<0.001, \*\*\**p*<0.01, Dunnett test as compared to control which received normal saline.

(*p*<0.001) and similar to that of standard drug ASA with significant (*p*<0.001) inhibition at dose of 200 mg kg<sup>-1</sup> body weight.

### Hot plate test

In hot plate test (Table 2), the *Mimosa pudica* aqueous extract at doses of 200 and 400 mg/kg showed a significant increase in the latency time in a dose-dependent manner. The result was found to be statistically significant (*p*<0.01) as compared with control and is similar to that of pentazocine-treated group at a dose of 10 mg/kg.

## DISCUSSION

The results showed that the *Mimosa pudica* aqueous extract administered orally to mice produced significant antinociceptive action when assessed, using acetic acid-induced writhing and thermal-induced nociception tests in mice. In the acute toxicity study, the LD<sub>50</sub> of the aqueous extract is high because there was no death recorded, even at 10 times of the effective dose. This indicates that, the extract has high margin of safety.

The acetic acid-induced writhing test in mice is regarded as a model of inflammation pain, and it is used as screening tool for evaluation of analgesic or anti-inflammatory agents. Intraperitoneal injection of acetic acid produces pain through activation of chemosensitive nociceptors [17]. 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, serotonin and substance P. It is a sensitive procedure to evaluate peripherally- and centrally- acting analgesics [18-22]. The nociceptive activity of acetic acid may be due to cytokine release, such as TNF-α, interleukin-1β and interleukin-8, by resident peritoneal macrophages and mast cells [23]. The intraperitoneal injection of acetic acid induced an increase in the concentration of glutamate and aspartate in the cerebrospinal fluid [24]. We have reported that the *Mimosa pudica* aqueous extract inhibited, in a dose-dependent manner, the nociception induced by acetic acid, when compared with the well-known NSAID, aspirin.

In this work, we measured the nociceptive reactivity to thermal stimuli in mice using the hot plate test, which is sensitive acute pain test for detecting opiate analgesia as well as several types of hyperalgesia reactions from spinal origin. The hot plate test could be a simple and sensitive procedure to evaluate analgesics and hyperalgesics reactions in mice. This method is considered to be selective for opioid-like compounds in

animals [25]. The results indicate that the oral administration of aqueous 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. Although the underlying mechanism is unknown, the observed activity can be attributed to the overall effects of the plant constituents or the components having similar structure to NSAIDs or opioids.

In conclusion, the results of the present work clearly demonstrated the antinociceptive activity of *Mimosa pudica* aqueous extract. It also could be concluded that the aqueous extract has both peripheral and central analgesic properties the crude extract has been reported to have interaction with opioid receptors [26]. The antinociceptive effect of this extract may be a result of inhibition or reduction of proinflammatory mediators [27]. The analgesic activity of *Mimosa pudica* aqueous extract can be due to the presence of sterols. Analgesic activities of some sterols have already been shown on the models of pain induced by acetic acid and formalin [28, 29]. Detection of this class of compounds from *Mimosa pudica* may justify their antinociceptive activity. Work is in progress to isolate, characterize and find the mechanism of action of the active compounds in aqueous extract responsible for both peripheral and central analgesic activity.

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## REFERENCES

1. Sivarajan VV, Balachandran I. Ayurvedic Drugs and Their Plant Sources. Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi, 2002; pp: 271-2.
2. Chatterjee A, prakash SC. The Treatise of Indian Medicinal Plants, Publications and Information Directorate. CSIR, New Delhi, Vol 2, 2000; pp: 65-6.
3. Rastogi RP, Mehrotra BN. Compendium of Indian Medicinal Plants. Publications and Information Directorate, CSIR, New Delhi, Vol 5, 2005; p: 547.

4. Anonymous. The Wealth of India. A Dictionary of Indian Raw Materials and Industrial Products. Publications and Information Directorate. CSIR, New Delhi, Vol 4, 2001, p:134.
5. Nadkarani KM. Indian Plants and Drugs, Asiatic publish house, New Delhi, 2001; p: 233.
6. Nadkarani KM. Indian Material Medica, Popular Prakashan Private Limited, Mumbai, Vol 1, 1982; pp:799-800.
7. Chopra RN, Nayar SG, Chopra IC. Glossary of Indian Medicinal Plants, National Institute of Science Communication, New Delhi, 1<sup>st</sup> Ed, 1996; p: 167.
8. NgoBum E, Dawack DL, Schmutz M, Rakotonirina A, Rakotonirina SV, Portet C, Jeker A, Olpe HR, Herrling P. Anticonvulsant activity of *Mimosa pudica* decoction. *Fitoterapia* 2002; 75:309-14.
9. Amalraj T, Ignacimuthu S. Hyperglycemic effect of leaves of *Mimosa pudica* linn. *Fitoterapia* 2002; 73:351-2.
10. Molina M, Contreras CM, Tellez-Alcantara P. *Mimosa pudica* may possess antidepressant actions in the rat. *Fitoterapia* 1999; 6:319-23.
11. OECD Test Guideline 425, 2001. Guidelines for Testing of Chemicals, Guidelines 425, Acute Oral Toxicity–Up-and-Down Procedure ([www.oecd.org](http://www.oecd.org)).
12. Elhabazi K, Aboufatima R, Benharref A, Zyad A, Chait A, Dalal A. Study on the antinociceptive effects of *Thymus broussonetii* Boiss extracts in mice and rats. *J Ethnopharmacol* 2006; 107:406-11.
13. Vogel HG, Vogel WH. Drug Discovery and Evaluation Pharmacological Assays. Springer Verlag, Germany, 2002; pp: 670-724.
14. Kulkarni SK. Handbook of Experimental Pharmacology. Vallabh Prakashan, New Delhi, 3<sup>rd</sup> Ed, 2005; pp: 125-8.
15. Woolfee G, MacDonald AD. The evaluation of the analgesic action of pethidine hydrochloride (DEMROL). *J Pharmacol Exp Ther* 1944; 80:300-7.
16. Malairajan P, Geetha G, Narasimhan S, Veni KJK. Analgesic activity of some indian medicinal plants. *J Ethnopharmacol* 2006; 106:425-8.
17. Stai HY, Chen YF, Wu TS. Anti-inflammatory and analgesic activities of extract from roots of *Angelica pubescens*. *Planta medica* 1995; 61:1-8.
18. Murray CW, Porreca F, Cowan A. Methodological refinement in the mouse paw formalin test: an animal model of tonic pain. *J Pharmacol Method* 1988; 20:175-86.
19. Tjosen A, Berg DG, Hunskaar S, Rosland JH, Hole K. The formalin test: an evaluation of the methods. *Pain* 1992; 51:5-17.
20. Gaertner M, Muller L, Roos JF, Cani G, Santos ARS, Niero R, Calixto NB, Yunes RA, Manache FD, Cechinel-Filho V. Analgesic triterpenes from *sebastiania schottiana* roots. *Phytomed* 1999; 6:41-4.
21. Collier HOJ, Dinneen JC, Johnson CA, Schneider C. The abdominal constriction response and its suppression by analgesic drugs in mouse. *Br J Pharmacol Chemother* 1968; 32:295-310.
22. Ikeda Y, Ueno A, Naraba H, Oh-ishi S. Involvement of vanilloid receptor VR1 and prostanooids in the acid-induced writhes responses of mice. *Life Sci* 2001; 69:2911-9.
23. Ribeiro RA, Vale ML, Thomazzi SM, Paschoalato ABP, Poole S, Ferreira SH, Cunha FQ. Involvement of resident macrophages and mast cells in the writhing nociceptive response induced by zymosan and acetic acid in mice. *Eur J Pharmacol* 2000; 387:111-8.
24. Feng Y, Cui M, Willis W. Gabapentin markedly reduces acetic acid induced visceral nociception. *Anesthesiology* 2003; 98:729-33.
25. Janseen P, Niemegeers C, Dony J. The inhibitory effects of phtentanyll (R-4263) and other morphine-like analgesics on the warm water induced tail withdrawal reflex in rats. *Arzneimittel Foursching* 1963; 13:502-7.
26. Awe EO, M.Makinde J, Olajide OA, Wahkeel OK. Evaluation of anti inflammatory and analgesic activity of *Russelia Equisetiformis*. *Inflammoparmacol* 2004; 12:399-405.
27. Fernandez MA, DE Las Haras B, Gacia MD, Saen MT, Villar A. New insights into the mechanism of action of anti inflammatory triterpenes lupeol. *J pharm.pharmacol* 2001; 53:1533-9.
28. Cechinel Filho V, Santos ARS, De Campos ROP, Miguel OG, Yunes RA, Ferrai F, Messana I, Calixto JB. Chemical and pharmacological studies of *hyllanthus carolinienses* in mice. *J Phar Pharmacol* 1996; 48:1231-6.
29. Santos ARS, Neiro R, Cechinelilho V, Yunes RA, Pizzolatti MG, Delle Monache F, Calixto JB. Antinociceptive properties of steroids isolated from *phyllanthus corcovadensis*. *Planta Medica* 1995; 61:329-31.

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