

Evaluation of Anti-Inflammatory Activity of Ethanolic Extract of *Mimosa Pudica* in Swiss Albino Mice

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Abstract

Introduction: Inflammation is a common physiological event that leads to acute or chronic pain in response to tissue injury. It serves as a mechanism for initiating the elimination of noxious agents and damaged tissue. Herbaceous plants are one of the more important sources of biologically active compounds that are known to have and are being used for therapeutic benefits. In recent times, emphasis on medicinal plants has gained momentum since many studies have proved tremendous therapeutic potential in medicinal plants. *Mimosa pudica* is a creeping herb growing annually or perennially. In Ayurveda it has been identified as lajjalu and has been found to have antiasthmatic, aphrodisiac, analgesic, and antidepressant properties. Previous studies have revealed sedative, emetic, and tonic properties of *M. pudica* and traditionally it has been used in the treatment of various ailments including alopecia, diarrhea, dysentery, insomnia, tumor, and various urogenital infections. Phytochemical studies on *Mimosa pudica* have revealed the presence of constituents like alkaloids, non-protein amino acid (mimosine), flavonoids C-glycosides, sterols, terpenoids, tannins, and fatty acids. *Mimosa pudica* is a source of natural origin which has numerous medical benefits and has a tremendous future potential for research.

Objective: To evaluate the acute and subacute anti-inflammatory effects of Ethanolic Extract of *Mimosa pudica* leaf on carrageenan induced paw edema in Swiss Albino mice.

Materials and Methods: Swiss albino mice of either sex weighing 20-30 g were used. Thirty mice were divided into 5 groups each having 6 mice. Anti-inflammatory activity using Carrageenan induced paw edema method. Group 1 was the Control group and received normal saline, Group 2 received standard drug – Diclofenac at 10 mg/kg, Group 3, Group 4 and Group 5 received test drug EEMP at doses of 100 mg/kg, 200 mg/kg and 400 mg/kg respectively. Anti-inflammatory potential of EEMP was evaluated on day 1 and day 10 by measuring paw volume using plethysmograph by dipping the hind limb up to the marked level after 1 hour, 2 hours, 3 hours, 4 hours and 6 hours following the administration of drugs.

Results: Ethanolic Extract of *Mimosa pudica* showed reduction in paw edema in a dose dependent manner.

Conclusion: The study suggests that Ethanolic extract of *Mimosa pudica* (EEMP) has anti-inflammatory property and can be considered for use in treatment of pain after further testing.

Keywords: *Mimosa pudica*, Anti-Inflammatory, Diclofenac, Terpenoids, Fatty Acids

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Introduction

Inflammation is a common physiological event that leads to acute or chronic pain in response to tissue injury [1]. It serves as a mechanism for initiating the elimination of noxious agents and damaged tissue. Tissue inflammation or damage sensitizes peripheral afferents and dorsal horn neurons in inflammatory somatic or visceral pain [2]. The synaptic input in such cases is excitatory and is mainly mediated by glutamate which is the excitatory neurotransmitter [3].

Glutamate and its receptors are located in the brain, spinal cord and peripheral areas that are mainly involved in the sensation and transmission of pain. Pain is generally well accounted for in terms of nociception, since it is elicited by specific activation of nociceptors. Nociception is an excessive noxious stimulus giving rise to an intense and unpleasant sensation [4]. Roman writer Celsus enumerated the four principle signs of inflammation as dolor (pain), rubor (redness), calor (heat) and swelling (tumor). Virchow supplemented the fifth sign of inflammation as functiolaesa (loss of function) [5]. The cell damage associated with inflammation acts on cell membranes and cause leukocytes to release lysosomal enzymes.

Arachidonic acid is then liberated from its precursor compounds, and various eicosanoids are then synthesized. The cyclooxygenase pathway of arachidonate metabolism produces prostaglandins, which plays a major role in inflammation and also have a variety of effects on nerve endings and on blood vessels. Acute inflammation due to an infectious etiology is generally accompanied by fever. Prostaglandin is

secreted from vascular and perivascular cells of the hypothalamus [6].

Herbaceous plants are one of the more important sources of biologically active compounds that are known to have and are being used for therapeutic benefits. In recent times, emphasis on medicinal plants has gained momentum since many studies have proved tremendous therapeutic potential in medicinal plants. Medicinal plants have become a source of interest in therapeutics because of their potential effects in improving and maintaining quality of human health, low costs and low side effects.

Mimosa pudica is a creeping herb growing annually or perennially. In Ayurveda it has been identified as lajjalu and has been found to have antiasthmatic, aphrodisiac, analgesic, and antidepressant properties. Previous studies have revealed sedative, emetic, and tonic properties of *M pudica* and traditionally it has been used in the treatment of various ailments including alopecia, diarrhea, dysentery, insomnia, tumor, and various urogenital infections [7]. Phytochemical studies on *Mimosa pudica* have revealed the presence of constituents like alkaloids, non-protein amino acid (mimosine), flavonoids C-glycosides, sterols, terpenoids, tannins, and fatty acids [8]. *Mimosa pudica* is a source of natural origin which has numerous medical benefits and has a tremendous future potential for research.

Hence, if a drug could be prepared from medicinal plants, which has anti-inflammatory property, it can be used to treat both acute and chronic painful ailments while helping to avoid the side effects known with

the currently available drugs in the market. With this background in mind the present study was undertaken to screen for anti-inflammatory properties of Ethanolic Extract of *Mimosa pudica* (EEMP).

Materials and Methods

Ethical clearance was obtained from the Institutional Animal Ethics Committee before starting the study (Approval No. AJIMS/IAEC/18-19/03 dated 12-11-2018). The Registration number of Institutional Animal Ethics Committee is 1075/PO/Re/S/07/CPCSEA dated 27/07/2017. Swiss albino mice, 30 in number, aged around 3-4 months weighing 25 to 30gms were housed at a room temperature of $24 \pm 2^\circ\text{C}$ in tidy polypropylene cages. 12:12 hour of light and dark cycle was maintained. They were provided free access to the commercially available chow pellet and drinking water was provided in bottles ad libitum. All the experimental procedures were done within light part of their light and dark cycle.

The standard drug used was Diclofenac potassium (VOLTAFLAM 50, 50mg Tablet, Novartis) *Mimosa pudica* plant was obtained from district Udipi, Karnataka, India. The plant was authenticated by Jyothi K T, Lecturer and HOD, Department of Botany, Sri Siddhartha First Grade College, Tumkur.

The *Mimosa pudica* plants were washed, the leaves were shade dried and powdered. About 200 g of the dried leaf powder of *Mimosa pudica* was extracted with 250 ml of 99.9% Ethanol in Soxhlet extractor for about 36 hours. The ethanol was then evaporated from the mixture by placing it in a beaker and heating it over a water bath. The extract gave a yield of brownish paste like mass weighing 6 g. The yield obtained was 3% w/w with respect to dried powder [9].

On day one, 30 Swiss albino mice of either sex weighing 25-30 grams were divided into 5 groups.

Group 1 received normal saline (10 ml/kg p.o.)

Group 2 received Diclofenac (standard 10 mg/kg p.o.) [10].

Group 3, 4 and 5 received test drug in doses 100 mg/kg, 200 mg/kg, 400 mg/kg per oral respectively.

Procedure

For Acute study, on day 1, baseline paw volume measurements were taken using plethysmograph after marking the paw at the level of lateral malleolus. One hour after treatment, 0.1ml of 1% suspension of carrageenan in normal saline were injected into the sub plantar region of right hind paw to induce edema. The paw volume was measured using plethysmograph by dipping the hind limb up to the marked level after 1 hour, 2 hours, 3 hours, 4 hours and 6 hours to find the effect of acute administration of drug in acute inflammation. The administration of drug was continued for next 9 days. On day 10, one hour after treatment, 0.1ml of 1% suspension of Carrageenan in normal saline was injected into the sub plantar region of right hind paw to induce edema. The paw volume was measured using plethysmograph after 1 hour, 2 hours, 3 hours, 4 hours and 6 hours to find the effect of chronic administration of drug in inflammation.

Statistics

The recorded data was entered in Microsoft Excel. Values were expressed as Mean \pm SEM (standard error of mean). Probability 'p' values were assessed using One Way Analysis of Variance (ANOVA) followed by *post hoc* Dunnet's Multiple Comparison test (Analystat ver.1.6.50). p Value <0.05 was considered as statistically significant.

Results

Carrageenan Induced Paw Edema Model in mice on Day 1

Ethanolic Extract of *Mimosa pudica* showed reduction in paw edema in a dose dependent

manner. A significant drop in paw edema of mice administered with Diclofenac 10mg/kg was observed from 1 hour with the maximum reduction at 6 hours (0.118±0.004). A significant reduction in paw edema of mice was seen at 6 hours in the EEMP 100mg/kg test group, when compared to the normal saline group.

Significant reduction in the paw edema of mice which were administered EEMP

200mg/kg was observed from 2 hours with the maximum reduction at 6 hours (0.138±0.004). A significant reduction in the edema of paw was also observed in the EEMP 400mg/kg group from 1 hour with the maximum reduction at 6 hours (0.127±0.004). EEMP 400mg/kg group was found to be comparable to Diclofenac at reducing paw edema at all times, without significant difference between these groups.

Table 1: Carrageenan Induced Paw Edema Model in mice on Day 1

Day 1	Volume of mercury displaced (ml)				
Time	Group 1	Group 2	Group 3	Group 4	Group 5
	NS	Diclofenac 10mg/kg	EEMP 100mg/kg	EEMP 200mg/kg	EEMP 400mg/kg
0 hour	0.117 ± 0.003	0.112 ± 0.002	0.114 ± 0.004	0.123 ± 0.005	0.124 ± 0.003
1 hour	0.169 ± 0.003	0.130 ± 0.003**	0.149 ± 0.009	0.152 ± 0.006 #	0.134 ± 0.004**
2 hour	0.171 ± 0.003	0.140±0.003**	0.162 ± 0.003	0.156 ± 0.005*#	0.146 ± 0.004**
3 hour	0.171 ± 0.003	0.134 ± 0.002**	0.157 ± 0.004##	0.152 ± 0.006*#	0.146 ± 0.004**
4 hour	0.169 ± 0.004	0.131 ± 0.003**	0.152 ± 0.003#	0.147 ± 0.007*	0.132 ± 0.007**
6 hour	0.163 ± 0.003	0.118 ± 0.004**	0.128 ± 0.005**	0.138±0.004**#	0.128 ± 0.004**

Values are expressed as Mean ± SEM.

Significant at $p < 0.05$ * & $p < 0.01$ ** compared to control

Significant at $p < 0.05$ # & $p < 0.01$ ## compared to standard

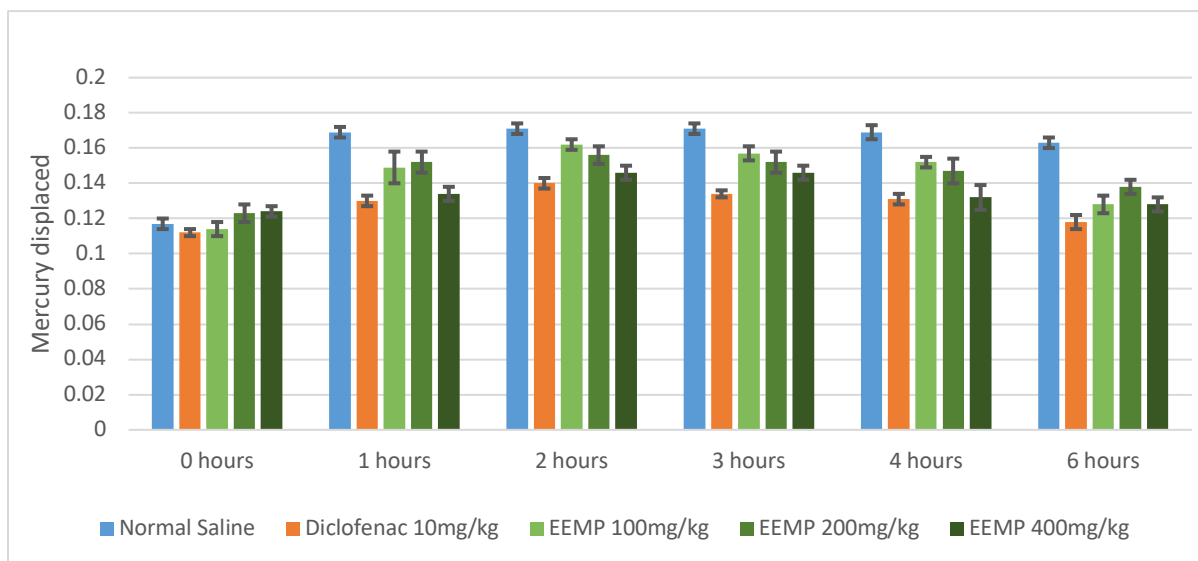


Figure 1: Carrageenan Induced Paw Edema Model in mice on Day 1

Carrageenan Induced Paw Edema Model in mice on Day 10

Ethanollic Extract of *Mimosa pudica* showed reduction in paw edema in a dose dependent manner. A significant drop in paw edema of mice administered with Diclofenac 10mg/kg was observed from 1 hour with the maximum

reduction at 6 hours (0.118±0.004). A significant reduction in paw edema of mice was seen starting in the 2nd hour in the EEMP 100mg/kg test group, with maximum reduction seen at 6th hour (0.134±0.003) when

compared to the normal saline group. Significant reduction in the paw edema of mice was seen in EEMP 200mg/kg from 3rd hour with the maximum reduction at 6 hours (0.133±0.004). A significant reduction in the paw edema was also observed in the EEMP

400mg/kg group from 2nd hour with the maximum reduction at 6 hours (0.125±0.003). All doses of EEMP were comparable with Diclofenac at reducing paw edema in the 6th hour, without significant difference between these groups.

Table 2: Carrageenan Induced Paw Edema Model in mice on Day 10

Day 10	Volume of mercury displaced (ml)				
Time	Group 1	Group 2	Group 3	Group 4	Group 5
	NS	Diclofenac 10mg/kg	EEMP 100mg/kg	EEMP 200mg/kg	EEMP 400mg/kg
0 hour	0.118 ± 0.003	0.114 ± 0.002	0.117 ± 0.004	0.121 ± 0.004	0.124 ± 0.003
1 hour	0.147 ± 0.003	0.126 ± 0.002*	0.142 ± 0.003#	0.142 ± 0.005#	0.137 ± 0.004
2 hour	0.165 ± 0.006	0.132 ± 0.002**	0.155 ± 0.003*#	0.151 ± 0.004#	0.144 ± 0.003*
3 hour	0.172 ± 0.006	0.130 ± 0.004**	0.155±0.003*##	0.151 ± 0.002*#	0.147±0.004*##
4 hour	0.168 ± 0.006	0.121 ± 0.003**	0.149±0.005*##	0.148±0.002*##	0.141±0.004*##
6 hour	0.162 ± 0.007	0.119 ± 0.004**	0.134 ± 0.003**	0.133 ± 0.004**	0.125 ± 0.003**

Values are expressed as Mean ± SEM.

Significant at $p < 0.05$ * & $p < 0.01$ ** compared to control
Significant at $p < 0.05$ # & $p < 0.01$ ## compared to standard

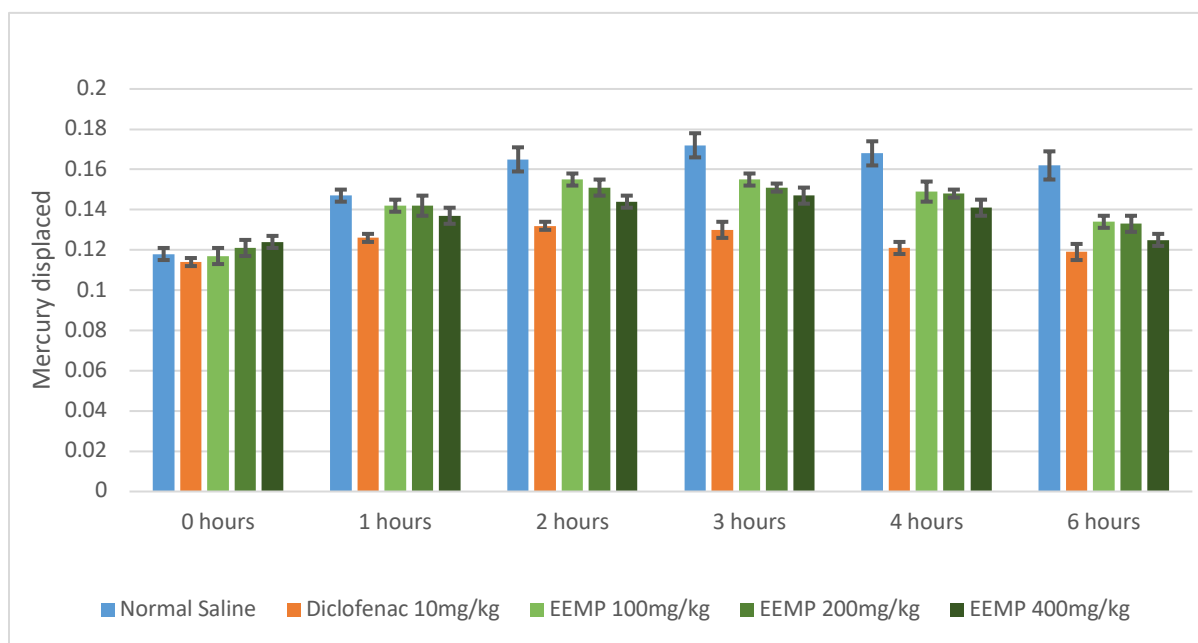


Figure 2: Carrageenan Induced Paw Edema Model in mice on Day 10

Discussion

Mimosa pudica is a widely distributed plant that grows in most tropical areas of the world. It has been used in folk lore medicines since many generations because of its many proclaimed medicinal properties. It has claimed efficacy for antiulcer, wound healing, antioxidant, antimicrobial, anticonvulsant and

antidepressant activities, to name a few. *Mimosa pudica* has been found to be useful in the treatment of edema, rheumatism, myalgia and in different painful conditions in traditional and tribal medicine. In this present study, we evaluated the analgesic and anti-

inflammatory activity of Ethanolic Extract of *Mimosa pudica* leaves in vivo.

To demonstrate the anti-inflammatory activity of EEMP, we used the carrageenan induced paw edema method. Carrageenan induces biphasic inflammation. The first phase is mediated by release of histamine, serotonin and kinin in the first hour. The second phase is related to the release of prostaglandin like substances in 2-3 hours [11]. The study showed a dose dependent reduction in paw edema with different doses of EEMP. The reduction in edema was lower in the first hour and showed increased reduction after 2 hours with a maximum reduction of paw edema in 6 hours. This could mean that *Mimosa pudica* extract might be acting by inhibiting the formation of prostaglandin and is less likely due to inhibition of histamine or serotonin.

Phytochemical analysis done in other studies showed the presence of Flavonoids, Terpenoids, Phenols, Glycosides, Alkaloids, Quinines, Tannins, Saponins, Coumarin in the ethanolic extract of leaves of *Mimosa pudica* [12]. Prostaglandins are also involved in various immunologic responses and are the end products of the cyclooxygenase and lipoxygenase pathways. They are involved in the late phase of acute inflammation. Flavonoids also inhibit both cytosolic and membranal tyrosine kinase which results in inhibition of uncontrolled cell growth and proliferation. Another anti-inflammatory property of flavonoids is their suggested ability to inhibit neutrophil degranulation. This is a direct way to diminish the release of arachidonic acid by neutrophils and other immune cells [13].

Tannins and Phenols act as primary antioxidants or free radical scavengers. These compounds exert their anti-inflammatory properties through inhibition of the production of inhibitory cytokines and chemokines, thereby suppressing the activity of cyclooxygenase (COX) and inducible nitric oxide synthase, acting as primary antioxidants

or free radical scavengers, and so decrease the production of Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) which play an important role in the pathogenesis of inflammatory diseases [14].

The above results demonstrate that leaves of *Mimosa pudica* possesses anti-inflammatory properties and can serve as lead compounds in the development of drugs to treat diseases with inflammation. This study demonstrates the possibility to develop new drugs from herbaceous source to treat chronic ailments which require prolong use of drugs. Drugs obtained from plant source such as *Mimosa pudica* may be free of multiple adverse effects associated with long term use of opioids or NSAIDs.

Conclusion

In this study, Ethanolic Extract of *Mimosa pudica* leaves (EEMP) was screened for its anti-inflammatory activity.

Anti-inflammatory screening done by Carrageenan Induced Paw edema method, EEMP showed significant anti-inflammatory activity at all doses (100, 200, 400 mg/kg) when compared to control. This could be attributed to the presence of flavonoids, tannins and phenols that work via various mechanisms. The observed anti-inflammatory property should be substantiated in further models and studies and explore the active component involved in these activities.

References

1. Stepanovic-Petrovic RM, Micov AM, Tomic MA, Kovacevic JM. and Boškovic BD. Antihyperalgesic/Antinociceptive Effects of Ceftriaxone and Its Synergistic Interactions with Different Analgesics in Inflammatory Pain in Rodents. *Anesthesiology*. 2014;120(3):737-750.
2. Salter MW. Cellular Signalling Pathways of Spinal Pain Neuroplasticity as Targets for Analgesic Development. *Curr Top Med Chem*. 2005;5(6):557-567.

3. Köles L, Kató E, Hanuska A, *et al.* Modulation of excitatory neurotransmission by neuronal/glia signalling molecules: interplay between purinergic and glutamatergic systems. *Purinergic Signal.* 2016;12(1):1-24.
4. Millan M J. The induction of pain: an integrative review, *Progress in Neurobiology.* 1999; 57(2):158-164
5. Dinarello CA. Cytokines as endogenous pyrogens. *The Journal of Infectious Diseases.* 1999;179(2): 294-304
6. Tripathi KD. Nonsteroidal Anti-inflammatory drugs And Antipyretic-Analgesics. *Essentials of Medical Pharmacology* 8th ed. Jaypee Brothers Medical Publishers; 2019:209-226
7. Ahmad H, Sehgal S, Mishra A and Gupta R. *Mimosa pudica* L. (Laajvanti): An overview; *Pharmacognosy Review.* 2012; 6(12): 115-124
8. Genest S, Kerr C, Shah A, Rahman MM, Saif-E-Naser GM, Nigam P, *et al.* Comparative bioactivity of two *Mimosa* species. *Lat Am Caribb Bull Med Aromat Plants* 2008; 7:38-43
9. Khan M, Harun N, Rehman AA, Elhussein SAA. In Vitro Antioxidant Evaluation of Extracts of Three Wild Malaysian Plants. *Procedia Engg.* 2013; 53:29–36.
10. Hosseinzadeh H, Ramezani M, Salmani GA: Antinociceptive, anti-inflammatory and acute toxicity effects of *Zataria multiflora* Boiss extracts in mice and rats. *Journal of Ethnopharmacology.* 2000;73(3):379-385
11. Nair PV, Nair BL. Anti-inflammatory activity of hydroalcoholic extract of *Mimosa pudica* whole plant in rats. *International Journal of Basic and Clinical Pharmacology.* 2017;6(3):518-522
12. Mohan SM, Pandey B, Rao SG. Phytochemical Analysis and Uses of *Mimosa pudica* Linn. in Chhattisgarh. *Journal of Environmental Science, Toxicology and Food Technology.* 2015;1(3):1-4
13. Nijveldt RJ, Nood E, Hoorn DEC, Boelens PG. Flavonoids: a review of probable mechanisms of action and potential applications. 2001;74(4):418-425
14. Bahtaa T, Karima A, Periasamy G *et al.* Analgesic, Anti-inflammatory and In-vitro Hyaluronidase Inhibitory Properties of the Leaf Extract and solvent fractions of *Otostegia fruticosa* (Forssk.) Schweinf. ex Penzig. 2020; 19:218-230.