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Original Research Article

Anti-inflammatory activity of hydroalcoholic extract of mimosa pudica whole plant in rats

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ABSTRACT

Background: Mimosa pudica is a traditionally used folk medicine to treat various disorders like infections, anxiety, depression, bleeding disorders, convulsions, rheumatoid arthritis, muscular pain, asthma, snake bite etc. We evaluated the anti-inflammatory activity of hydroalcoholic extract of Mimosa pudica whole plant (HAEMPWP) in rats.
Methods: HAEMPWP was prepared using Soxhlets apparatus. Acute toxicity tests were done with HAEMPWP given orally to albino rats in increasing doses up to 3200 mg/ kg body weight. The anti-inflammatory action was evaluated by Carrageenan induced paw edema method. Thirty albino rats were grouped into five groups and each contained six rats. Group I (control group) received distilled water orally. Group II (standard) received Aspirin orally dissolved in distilled water. Groups III, IV and V received HAEMPWP in doses of 200 mg/kg, 400 mg/kg and 800mg/kg orally dissolved in distilled water. Data analysis was done by one way ANOVA and unpaired t test using SPSS 16 for windows.
Results: HAEMPWP showed a significant anti-inflammatory activity as compared to control. There was no statistically significant dose dependent increase in the anti-inflammatory activity.
Conclusions: HAEMPWP possesses significant anti-inflammatory activity and could be an effective treatment option for various inflammatory conditions.

Keywords: Albino rats, Anti-inflammatory, Carrageenan, Mimosa pudica, Soxhlet apparatus

INTRODUCTION

Glucocorticoids and Non Steroidal Anti-inflammatory drugs (NSAIDS) comprise the main chunk of anti-inflammatory drugs in use. Glucocorticoids are having powerful anti-inflammatory and immunosuppressive effects. They inhibit all phases of inflammation including the early and late phases, whereby it reduces the redness, heat, pain and swelling along with a reduction in tissue repair and wound healing .NSAIDs act by inhibiting Cyclo-oxygenase (COX) enzyme. Most of the NSAIDs act non specifically on both COX variants (COX 1 and COX 2), but the newer NSAIDs act only on COX 2 enzyme. Action of NSAIDs is limited to the early phases of inflammation.[1]

Celsus characterized inflammation by the four Latin words Rubor, Calor, Dolor and Tumor, two thousand years ago. These four words typically depict the features of inflammation - rubor meaning redness, calor meaning heat, dolor meaning pain and tumor meaning swelling. Inflammation is divided into three phases: acute inflammation, the immune response and chronic inflammation.[2] Acute inflammation is the initial response to tissue injury mediated by the release of autacoids. Immune response occurs when immunologically competent cells are activated in response to foreign organisms or antigenic substances liberated during inflammatory response. Chronic inflammation involves the release of a number of mediators that are not prominent in the acute response and is characterized by granuloma formation.

The cyclo-oxygenase (COX) pathway of arachidonic acid metabolism produces prostaglandins that have a variety of effects on blood vessels, on nerve endings, and on cells

involved in inflammation. COX 2 is induced in cells by an inflammatory stimulus and is responsible for production of mediators of inflammation.[3] Three important aspects of inflammation that can be readily measured are erythema (local vasodilatation), edema (increased capillary permeability) and formation of granulation tissue. Compounds claimed to possess anti-inflammatory activity can be evaluated by their ability to reduce these phenomena in experimentally induced inflammation.[4] Carrageenan induced paw edema is a model of acute inflammation used in the study of NSAID.[5]

Mimosa pudica was first described from Brazil and is native to most of the New World Tropics.[6] Today it is considered to be a pantropical weed and it is common in moist grounds, open plantations and weedy thicklets.[7] The scientific name of this plant, Mimosa pudica is derived from Greek, (Mimos meaning a mimic which alludes to the sensitivity of the leaves) and Latin, (pudica, meaning bashful, retiring or shrinking).[8-10] The plant grows wildly as a rapidly growing shrub. The fern like leaves close up and droop when touched, and re-opens within minutes. Phytochemical studies had revealed the presence of alkaloids like mimosine, crocetin, tubulin, turgorines, flavanoids, tannin, sitisine.[11,12]

The medicinal use of the plant dates back to Charaka and Sushruta.[13,14] The plant is traditionally used for disorders like convulsions, anxiety, stress, depression. It is also used to treat menorrhagia, dysentery with blood and mucus, piles and fistula. It is effective in relieving the symptoms of rheumatoid arthritis, spasmodic conditions and muscular pain.[15] The roots of M. pudica are bitter, astringent and cooling and they are used in the treatment of ulcers, inflammations, asthma and diarrhea. The plant also exhibits various medicinal activities like antihistaminic, antidepressant, hyperglycaemic, muscle relaxant, hemostatic, antifertility, antibacterial, anticonvulsant, antisnake venom, antifungal, antimalarial, anticancer activities and is also an immunomodulator.[16-18] It is used in traditional medicine for the treatment of various inflammatory conditions. So we decided to evaluate the anti-inflammatory activity of HAEMPWP in albino rats.

METHODS

Plant material and extract

The whole plant of Mimosa pudica was collected locally and authenticated by the Central Pharmacognosy unit, Ayurveda Research Institute, Poojappura, Thiruvananthapuram. The fresh whole plants of Mimosa pudica were washed thoroughly in water to remove soil material. It was then cut into small pieces, shade dried and powdered. Extract was prepared as per the method of Rosenthaler using Soxhlet apparatus.[19] The solvent used was 50% water and 50% alcohol. About 2.4kg of the plant yielded about 32.3g of a sticky semisolid mass which was dark green in colour with a pungent odour. The extract was stored in a refrigerator.

Experimental animals

Wistar strain albino rats (150-200 grams) of either sex were used. The animals were housed under standard laboratory conditions in the animal house of Thiruvananthapuram Medical College. The animals were fed standard pellet diet, maintained on a natural light and dark cycle and had free access to food and water. They were acclimatized to laboratory conditions before the tests. The experimental protocols were approved by the Institutional Animal Ethics Committee (Proposal No. 65/ IAEC/ MCT/07) of Thiruvananthapuram Medical College and ethical guidelines were followed throughout the study.

Drugs and chemicals

Aspirin tablet and Carrageenan (Sigma Labs, Mumbai), Distilled water, HAEMPWP 200, 400 and 800 mg/ kg. An aqueous suspension of the extract was prepared in distilled water and used for the study.

Acute toxicity study and study on gross behavioral changes

Albino rats of either sex weighing 15-20 grams were used for the study.[20] They were divided into 5 groups of 2 rats each. The rats were observed for effects on central nervous system and autonomic changes continuously for 2 hours and then occasionally for further 4 hours and finally overnight mortality recorded. As there was no mortality with 50 mg/kg, 70 mg/kg, 100 mg/kg, 125 mg/kg, toxicity studies were repeated using 200 mg/kg, 400 mg/kg, 800mg/kg, 1600 mg/kg, 2400 mg/kg and 3200 mg/kg.[17]

Assessment of anti-inflammatory activity

Effect of the HAEMPWP on acute inflammation was studied by the method described by winter and coworkers, where paw edema was induced using carrageenan and edema measured using modified plethysmometer.[21]

Albino rats of either sex weighing between 150 and 300 grams were used for the study. The rats were weighed and divided into 5 groups containing 6 rats each. A mark was made on the skin over the lateral malleolus to ensure constant paw volume every time the paw was dipped up to the fixed mark. The rats were fasted overnight. The next morning they were given the following drugs orally. The first group (control group) received 5 ml of distilled water. The second group received the standard drug, Aspirin at a dose of 300mg/kg. The third, fourth and fifth groups received the test drug at doses of 200 mg/kg, 400 mg/kg and 800mg/kg respectively. Thirty minutes later, 0.05 ml of 1% Carrageenan suspended in sterile 0.9%

NaCl was injected through a 26G needle into the plantar tissue of the left hind paw of each animal to induce paw edema. Immediately thereafter, the volume of the injected paw was measured using modified plethysmometer. This gives the Initial Paw Volume (IPV). After 3 hours of Carrageenan administration, the paw volume was again measured to get the Final Paw Volume (FPV). The percentage inhibition of paw volume (anti-inflammatory effect) was calculated from the mean values using the formula -Percentage inhibition= (Vc-Vt)/ Vc X 100, where Vc was Mean difference in the paw volume of the control and Vt was Mean difference in the paw volume of the test group.

RESULTS

Acute toxicity studies

HAEMPWP when administered did not show any sign of toxicity, except for decreased motor activity and muscle relaxation which was evident from 1 hour after administration of the extract. These signs started appearing with a dose of 125 mg/ kg body weight. There was no mortality in any of the test groups even after 24 hours.

Anti-inflammatory activity

Figure 1 shows the anti-inflammatory activity of mimosa pudica. The mean difference in paw volume which was calculated by subtracting final paw volume (three hours after drug administration) from initial paw volume for each group. Figure 1; indicate that the mean difference in paw volume was less for all doses of the extract and standard drug as compared to control. Analysis using independent sample t test shows that these values are statistically significant (p<0.001).

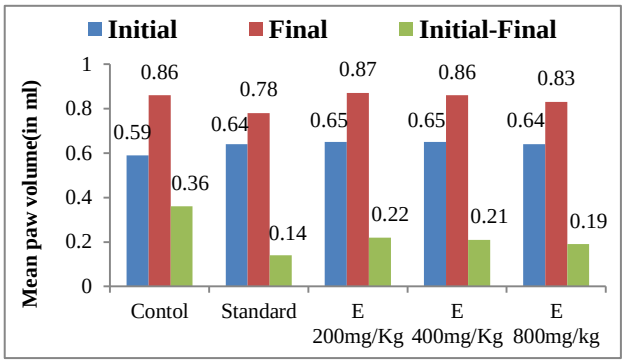

Figure 1: Antiinflammatory activity of HAEMPWP.

The final paw volume as shown in Table 1was compared using One way ANOVA (F=21.23, p<0.001). Post hoc Dunnett test revealed that there was significant difference in reduction of paw volume as compared to the control. [Standard (p<0.001), Extract 200mg/kg (p=0.002), Extract 400mg/kg (p=0.007), Extract 800mg/kg (p=0.003)].

Table 1: Effect of HAEMPWP on carragenan induced paw edema.

Treatment	Dose	Paw Volume in ml (n=6)			
		Initial (Mean± SEM)	Final (Mean± SEM)	Final-Initial (Mean)	% reduction
Control		0.59±0.01	0.86±0.01	0.36	-
Standard		0.64±0.02	0.78±0.01*	0.14	61.11
MP	200mg/kg	0.65±0.01	0.87±0.01*	0.22	38.89
MP	400mg/kg	0.65±0.01	0.86±0.01*	0.21	41.66
MP	800mg/kg	0.64±0.02	0.83±0.02*	0.19	47.22

SEM=Standard Error of Mean* Shows p <0.05 as compared to carrageenan control

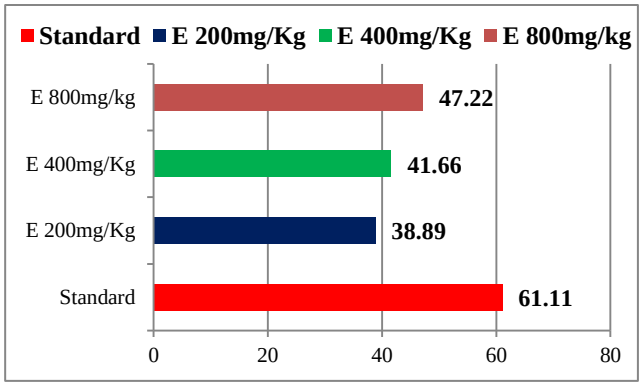

Figure 2: Percentage reduction in paw volume.

Figure 2 shows the percentage inhibition of paw volume of different doses of the extract and aspirin. At 200 mg/kg, the test drug produced inhibition of paw volume of 38.89%, at a dose of 400 mg/kg the inhibition of paw volume was 41.66% and with 800 mg/kg it was 47.22%. The extract produced a dose dependent increase in the percentage inhibition of paw volume; however it was not statistically significant. The anti-inflammatory effect exhibited by HAEMPWP was not superior to that of standard drug, acetyl salicylic acid, which showed 61.11 percent inhibition of paw volume.

DISCUSSION

Our study proves that Mimosa pudica is a very safe drug since it did not produce any mortality when doses up to 3200 mg/kg were given orally. In a study by Vikram PK et al., the extract showed certain changes in activity and was devoid of any toxicity at dose 2000mg/kg.[22]

The objective of the present study was to investigate the effect of HAEMPWP on anti-inflammatory activity in Wistar strain albino rats. Carrageenan induced paw edema method is the most prominent experimental model to evaluate acute inflammation.[23,24] 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.[25] Our study showed a dose dependent increase in inhibition of paw volume with different doses of HAEMPWP, however it was not statistically significant. In the present study HAEMPWP at doses of 200mg/kg, 400mg/kg, 800mg/kg produced significant inhibition of Carrageenan induced acute inflammation. Aspirin is a NSAID which produces anti-inflammatory effect by inhibition of prostaglandin synthesis. Further studies have to be carried out to know whether Mimosa pudica extract also act by inhibiting the formation of prostaglandins. Mimosa pudica showed significant effects on acute inflammation. Though the reduction of carrageenan induced paw edema was less when compared to aspirin, the extract produced significant inhibition of paw volume. Majority of the non-steroidal anti-inflammatory drugs produce salt and water retention which is responsible for most of the adverse effects of these very commonly needed drugs. At this point, it is very interesting to note that HAEMPWP has anti-inflammatory effect without causing salt and water retention.

A study done by Mistry et al, showed that leaves of Mimosa pudica possess anti-inflammatory activity.[26] Results of the study suggests that the extract mainly inhibits the release of prostaglandin like substances.[27] Mimosa contains flavanoids which possess anti-inflammatory activity by inhibiting phospholipase.[28] Such inhibitors are able to decrease inflammatory response to carrageenan in rats.[29] Another study done by Goli et al, demonstrated the anti-inflammatory activity of Mimosa pudica leaves.[30] In a study done by Vikram et.al the ethanolic extract of Mimosa pudica leaves showed 72.31% inhibition of paw volume at a dose of 500 mg/kg.[22]

Mimosa pudica is found to be highly effective in relieving the symptoms of rheumatoid arthritis and muscular pain. It is used for treating spasmodic conditions and fever. Many studies have substantiated the antibacterial, anti-inflammatory, analgesic and other effects of the plant. Studies done on Mimosa pudica justify the therapeutic application of this plant in indigenous system of medicine augmenting its therapeutic value.

The major limitation of the study is that the effect of the extract on chronic inflammation was not studied and phytochemical analysis was not done to identify the exact constituents. Further extensive phytochemical analysis and research is necessary to identify the exact constituents and understanding of the possible mechanism of action of anti-inflammatory activity of HAEMPWP.

CONCLUSION

The present study showed that the extract of Mimosa pudica is safe and possessed anti-inflammatory activity in experimental models. This rationalizes its use as an anti-inflammatory agent in traditional systems of medicine. Toxicity studies confirm the safety of the drug. The results of this study emphasise the need for further investigation of active principles. The plant possesses many other properties which is evident from its use in folklore which needs further evaluations. These studies will help to support the use of this plant for human and animal diseases.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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