

ANTIHYPERURICEMIA SCREENING OF *Mimosa pudica* L. HERB EXTRACT THROUGH ITS ANALGESICS AND ANTIINFLAMMATORY ACTIVITY ASSAY

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ABSTRACT

Traditionally, herbs of *Mimosa pudica* L. are being used to treat insomnia, hematuria, inflammation, emesis, dismenorrhoea, menorrhagia, arthritis rheumatoid, convulsion, depression, and diabetes. Previous *in vitro* study showed that herbs extract of *Mimosa pudica* L inhibited uric acid formation *via* xanthine oxidase inhibition of 82.11 and 62.10% for concentration of 125 and 62.5 µg/mL, respectively. Hyperuricemia is indicated by pain and oedema which are the symptoms of inflammation. This *in vivo* study was performed to screen antihyperuricemia activity of herbs extract of *Mimosa pudica* L through analgesic and antiinflammatory assays on mice. Analgesic activity of *Mimosa pudica* L herb extract at dosage of 125, 250 and 500 mg/kg of body weight was observed on mice using writhing reflex method with acetic acid 0,07 % as inducer. The results showed that all three dosages inhibited pain at the percentage of 9.58, 45.35, and 60.28% respectively. Antiinflammatory activity assay was done using carageenan-induced paw edema method on white male rats. Dosages used were 250, 500 and 1000 mg/kg of body weight. The results showed that all three dosages inhibited edema at the percentages of 35.20, 42.74, and 51.10% respectively. It is concluded that herbs extract of *Mimosa pudica* L can be proposed as an antihyperuricemia.

Keywords: Antihyperuricemia, Analgesic, Anti-inflammatory, *Mimosa pudica* L.

ABSTRAK

Secara tradisional, herbal *Mimosa pudica* L. sedang digunakan untuk mengobati insomnia, hematuria, peradangan, emesis, dismenorrhoea, menorrhagia, arthritis rheumatoid, convulsion, depresi, dan diabetes. Penelitian sebelumnya menunjukkan bahwa *in vitro* herbal ekstrak *Mimosa pudica* L. menghambat pembentukan asam urat melalui penghambatan xantin oksidase 82,11 dan 62,10% pada konsentrasi 125 dan 62,5 mg/mL, *Hyperuricemia* ditandai dengan rasa sakit dan edema yang merupakan gejala peradangan. Penelitian *in vivo* dilakukan untuk menyaring antihyperuricemia aktivitas herbal ekstrak *Mimosa pudica* L. melalui uji analgesik dan antiinflamasi pada tikus. Aktivitas analgesik *Mimosa pudica* L. ramuan ekstrak pada dosis 125, 250 dan 500 mg/kg berat badan diamati pada tikus menggunakan metode menggeliat refleksi dengan asam asetat 0,07% sebagai inducer. Hasil penelitian menunjukkan bahwa ketiga dosis menghambat rasa sakit pada persentase 9,58, 45,35, dan 60,28% masing. Uji aktivitas antiinflamasi dilakukan dengan metode edema kaki karagenan-diinduksi pada tikus putih jantan. Dosis yang digunakan adalah 250, 500 dan 1000 mg / kg berat badan. Hasil penelitian menunjukkan bahwa ketiga dosis menghambat edema pada persentase 35,20, 42,74, dan 51,10% masing. Hal ini disimpulkan bahwa herbal ekstrak *Mimosa pudica* L dapat diusulkan sebagai antihyperuricemia.

Kata kunci: Antihyperuricemia, Analgesik, Anti-inflamasi, *Mimosa pudica* L.

INTRODUCTION

NSAIDs used in the inflammatory conditions do not cure and remove the underlying cause of the disease but they only modify the inflammatory response to the disease. Large numbers of NSAIDs are available in the market with their advantages and disadvantages. Though there are standard drugs like Aspirin, Indomethacin, Phenylbutazone, etc., these drugs are not entirely free of side effects and have their own limitation. Thus there is still a need to develop newer and safer anti-inflammatory drugs. NSAIDs use is frequently limited by gastrointestinal side effects, ranging from dyspepsia to life threatening bleeding from ulceration. It is believed that NSAIDs by inhibiting COX pathway causes inhibition of prostaglandins synthesis, which are responsible for maintaining gastric mucosal integrity. Herbal medicines used in Ayurveda remain the major source of health care for the world's population.

WHO has recognized herbal medicine as an essential building block for primary health care of vast countries like India. Traditionally, herbs of *Mimosa pudica* L. are being used in insomnia, hematuria, inflammation, emesis, dismenorrhoea, menorrhagia, arthritis rheumatoid, convulsion, depression, and diabetes. It has been reported that herbs of *Mimosa pudica* L. have diureticum, anti diabetic and antioxidant activity. (Perry, 1987) Also herbs of *Mimosa pudica* are rich in mimosine (*N*-(3-alanyl) -3-hydroxy-4-piridone), norepinefrine, linoleic acid, oleic acid, palmitic acid, stearic acid, phenol, amino acid, steroid/triterpenoid, sterol, tannins, and flavonoids, 4"-hydroxymycine, dan cassiaocidentalinalin B (Bum, 2004). While in Central Java leaves of *Mimosa pudica* L. are being used to cure insomnia. Therefore, by considering the traditional claim, chemical constituents and reported activities of *Mimosa pudica* L. this study was planned to screen antihyperuricemia activity of herbs extract of *Mimosa pudica* L. by using analgesic and antiinflammatory assay methods (Bum, 2004; Dalimarta, 2003; Kasahara, 1995).

MATERIALS AND METHODS**Collection of plants**

Herbs of *Mimosa pudica* L. were collected from areas around Bandung, West Java, Indonesia. These herbs were identified and authenticated in Botanical Taxonomy Laboratory, Department of Biology, Universitas Padjadjaran, West Java, Indonesia.

Preparation of extract

After 10 days of indoor drying process, the herbs were coarsely powdered using a mixer. The powder was sieved using 40 mesh sieve. About 500 g of dried powder of the herbs were extracted with ethanol for 72 h. The extract was concentrated and dried (yield - 8% w/w). The dried ethanolic extract was kept in airtight container in desiccator and used throughout the study.

Experimental section

Male ICR mice of 15-20 g weight and Wistar albino rats of 150-200 g weight were housed in standard cages at room temperature for 1 week before the experiments. Animals were provided with standard rodent pellet diet, and water *ad libitum*. The animals were deprived of food for 24 hours before treatment, but had free access to drinking water. All experiments were performed in the morning. (Musa, et al., 2009)

Analgesic activity

Writhing test

For writhing behavior testing, 0.7% acetic acid solution 10 mL/kg of body weight was injected *i.p.*, and the number of writhing and stretching movements was counted over a 5-min period as described by Hendershot and Forsaith, 1959. Writhing is defined as contraction of the abdominal muscles accompanied by extension of the hind limbs. The extract at the dosage of 125, 250 and 500 mg/kg bw, *p.o.* was administered 60 min before acetic acid. The percentage inhibition was determined for each experimental group (Madina, 1999; Subarnas and Wagner, 2000).

Acetic Acid-Induced Writhing Test.

The writhing testing mice was conducted as described in the previous study. Male ICR mice (five per group) were fasted for 24 h before the experiment, with free access to water. The writhes were induced by intraperitoneal injection of 1.0% acetic acid in distilled water (0.1 mL/10 g body weight). Three dosages were chosen (0.125, 0.250, and 0.500 g/kg of body weight). Mice were administered orally with the extract (0.125, 0.250, and 0.500 g/kg) suspended in gum Arabic (PGA) 60 min prior to chemical induction of writhes and the same volume of PGA suspended by oral administration as the vehicle control. Aspirin (65mg/kg, *p.o.*) was administered 30 minutes prior to acetic acid injection. Mice were placed in an observation box separately and the number of writhing responses was counted within 60 minutes (Ntandou et al., 2010; Ozbek, 2005; Hajhashemi et al., 2003)

Antiinflammatory activity

Carrageenan-induced paw edema test: Inflammation was induced by administering 0.1 mL of (1%) carrageenan into sub-plantar surface of

rat hind paw. Albino rats of weighing 150-250 g were fasted overnight with *ad libitum* access to water. The animals were divided into five groups (n=3).

Group I : PGA suspension (2%)

Group II : Indomethacin (10 mg/kg, *p.o.*) in PGA suspension

Group III : Ethanolic extract of *Mimosa pudica* L. (250 mg/kg, *p.o.*) in PGA suspension

Group IV : Ethanolic extract of *Mimosa pudica* L. (500 mg/kg, *p.o.*) in PGA suspension

Group V : Ethanolic extract of *Mimosa pudica* L. (1000 mg/kg, *p.o.*) in PGA suspension

In this experiment, all drugs were given orally. One hour after drug treatment all animals were injected with 0.1 mL of 1% carrageenan solution in the sub-plantar aponeurosis of left hind paw and the paw volume was measured plethysmometrically at 1 h, 2 h, 3 h, 4 h, and 5 h. Results were expressed as,

$$\text{Edema Volume} = V_t - V_c$$

V_t = Paw volume in mL, at time t , after carrageenan administration.

V_c = Paw volume in mL, before carrageenan administration.

$$\text{Inhibition rate (\%)} = \frac{E_c - E_t}{E_c} \times 100$$

E_c = edema volume of control group.

E_t = edema volume of treated group.

RESULTS AND DISCUSSION

Analgesic Effect of Ethanolic Extract *Mimosa pudica* L. on Writhing Reflex of Mice.

The results of the analgesic activity of ethanolic extract of *Mimosa pudica* L. can be seen in Table 1.

Amount of writhing decreased in time and dose dependent manner. It can be predicted that the decreasing of writhing due to analgesic activity of some chemical compounds in the extract. Treatment of *Mimosa pudica* L. extract at 0.125, 0.250 and 0.500 g/kg and aspirin 65 mg/kg of body weight showed the decreasing of writhing compared to the negative control ($P < 0.01$) with percentage of pain protection as shown in Table 2.

Mimosa pudica L. extract at dosage 0.500 g/kg of body weight showed the best percentage of pain protection (60.28%).

Antiinflammatory Effect Ethanolic Extract of *Mimosa pudica* L. on Carrageenan Induced Hind Paw Edema on Rats

Inhibition of carrageenan induced hind paw edema on rats by indomethacin started at 1st hour and which was maintained up to 5th hour. Indomethacin at the dosage of 10 mg/kg average at 1st, 2nd, 3rd, 4th, 5th hour has shown 73.71%

Table 1. Amount of writhing in 60 minutes of observation

Group	Average amount of writhing in 60 minutes observation												Total
	5'	10'	15'	20'	25'	30'	35'	40'	45'	50'	55'	60'	
Dosage 125 mg/kg of body weight	24.8	26.4	21.2	18.6	16	15.6	12.2	11.2	9.6	10.8	8.6	8	183
Dosage 250 mg/kg of body weight	19.6	16.2	13	10.4	10	9.4	8.4	6.2	4.8	4.6	4.2	3.8	110.6
Dosage 500 mg/kg of body weight	13.4	16.8	10.6	9	6	5.8	5.6	1.8	3.2	2.8	3.2	2.4	80.4
Negative Control	39.4	27.6	23	17	15.2	15.8	14	12.8	10.2	10.4	7.8	9.2	202.4
Positive Control	13.8	16.4	12.2	11.2	10.4	7.6	7.2	4.8	3.6	2.2	2.4	3	94.8

Table 3. Average Inflammatory percentage on Rat Paw for each Test Groups After Carrageenan Induction

Time (Hour)	Inflammatory Percentage (%)				
	Negative Control PGA 2 %	Positive Control Indomethacine 10 mg/ kg of body weight	D I Extract of <i>Mimosa pudica</i> L. 1000 mg/kg of body weight	D II Extract of <i>Mimosa pudica</i> L. 500 mg/kg of body weight	D III Extract of <i>Mimosa pudica</i> L. 250 mg/kg of body weight
1	169.70	50.58	55.57	35.30	56.28
2	202.97	37.92	123.05	134.43	149
3	225.44	43.13	95.34	136.67*	166.27*
4	179.11	54.01	90.98	125.73*	132.56*
5	141.63	43.08	83.25	94.14*	90.60*

*shows significant comparison with negative control ($p < 0.01$)

Negative control: PGA Suspension 2%; Positive control: Indomethacine 10 mg/kg bw; D I: Ethanolic extract 1000 mg/kg of body weight; D II: Ethanolic extract 500 mg/kg of body weight; D III: Ethanolic extract 250 mg/kg of body weight

Table 2. Protection Percentage Towards Pain of each Test Groups of Ethanolic Extracts of *Mimosa pudica* L.

Test groups	Dosage (mg/kg of body weight)	Pain Protection (%)
Aspirin	65	53
<i>Mimosa pudica</i> L. Extract	125	9.58
<i>Mimosa pudica</i> L. Extract	250	45.35
<i>Mimosa pudica</i> L. Extract	500	60.28

inhibition, respectively. At 3rd hour, the ethanolic extract of *Mimosa pudica* L. at the dosage of 250, 500 and 1000 mg/kg has shown 35.20, 13.44 and 31.18% inhibition, respectively. The results of this work are shown in Table 3.

It can be seen that ethanolic extract of *Mimosa pudica* L. at the dosage of 500 mg/kg of body weight and 250 mg/kg of body weight has maximum percentage of inflammation at 3rd hour, while maximum percentage of inflammation at the dosage of 1000 mg/kg of body weight was at 2nd hour.

Antiinflammatory activity of the extract started to occur at the 4th hour and was stable until 5th hour. The resultant calculated inflammatory inhibition, can be seen that extract at the dosage of 1000 mg/kg of body weight have the highest inflammation inhibitory percentage which was 51.10%. Meanwhile, the positive control group showed a inhibition percentage of 73.71%. The result was shown at Table 4.

CONCLUSION

Based on analgesic (500 mg/kg of body weight inhibited 60.28%) and anti-inflammatory (1000 mg/kg of body weight inhibited 51.10%)

Table 4. Average of Inflammation Inhibitory Percentage for each Group

Test Groups	Inhibitory Percentage (%)
Indomethacine 10 mg/kg of body weight	73.71
Extract of <i>Mimosa pudica</i> L. 1000 mg/kg of body weight	51.10
Extract of <i>Mimosa pudica</i> L. 500 mg/kg of body weight	42.74
Extract of <i>Mimosa pudica</i> L. 250 mg/kg of body weight	35.20

activities showed by *Mimosa pudica* L herb extract, therefore it is concluded that herbs extract of *Mimosa pudica* L can be proposed as antihyperuricemia.

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