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Research Article

IN VIVO PHARMACOLOGICAL INVESTIGATION OF *MIMOSA PUDICA* L.

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ABSTRACT

Objective: To evaluate the anti-nociceptive, acute toxicity, gastro intestinal motility, anti-pyretic investigations of leaf extract of *Mimosa pudica L.* leaves in Swiss albino mice following oral administration.

Methods: *In-vivo* anti-nociceptive activity test was evaluated by tail immersion test. *In-vivo* acute toxicity test was conducted using acute toxic class method. *In-vivo* gastrointestinal motility was determined by charcoal feces defecation time. *In-vivo* antipyretic activity test was evaluated by brewer's yeast induced pyrexia.

Results: *In-vivo* anti-nociceptive activity test shows that methanol & ethanol extracts (250 & 500 mg/kg b.w.) performed significant activity ($p<0.05$) in mice comparing to the standard drug diclofenac Na. *In-vivo* acute toxicity test was done on mice with methanol, ethanol and chloroform extracts (2000, 1000, 500 mg/kg b.w.) of *Mimosa pudica* leaf and no reaction or death occurred in mice during two weeks of observation. *In-vivo* gastrointestinal motility test indicates significant ($p<0.01$) increase in gastrointestinal motility by ethanol extracts of (250 & 500 mg/kg b.w.) comparing to the standard drug loperamide. *In-vivo* antipyretic activity test shows that methanol (250 & 500 mg/kg b.w.), ethanol (250 & 500 mg/kg b.w.) and chloroform (250mg/kg b.w.) extracts showed significant ($p<0.05$) reduction in temperature of mice comparing to the standard drug paracetamol.

Conclusion: The result of the study indicates analgesic, antipyretic properties along with gastrointestinal motility stimulating effects. According to the acute toxicity study, the leaf extracts are safe up to 2000 mg/kg *in-vivo* concentration.

Keywords: *Mimosa pudica*, Anti-nociceptive activity, Gastro intestinal motility, Anti-pyretic activity and Acute toxicity.

INTRODUCTION

The plants which are useful for healing diseases are called medicinal plant. According to the

WHO, "A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi-synthesis." There are more than 500 medicinal plants growing in our country [1].

Mimosa pudica L. is a small, prostrate or ascending, short-lived shrub. Some authors consider it a woody herb. It is also known as dorme dorme, dormidera, humble plant, marie-honte, mayhont, morivivi, honteuse, sleeping grass, timawi, touch-me-not, and many other names [2].

It was first described from Brazil and is perhaps native to much or all of the New World Tropics [3].

It may reach 1 m in height when supported on other vegetation and more than 2 m in horizontal extension. The reddish-brown, woody stems are sparsely or densely armed with curved prickles. The root system consists of a taproot and extensive fibrous roots with nodules. The twigs are fine and flexible and support leaves with one or two pairs of pinnae and 15 to 25 pairs of oblong leaflets 3 to 12 mm long. The flowers are pink and clustered in globose heads. The legume (pod) is linear-oblong, 1.0 to 1.5 cm long and 3 mm broad, with bristles on the margins. The pods are born in groups and contain two to four brown seeds. Today, it is pan tropical in its distribution [4].

Mimosa pudica L. grows on most well drained soils, even scalped or eroded subsoils and soils with low nutrient concentrations. It requires disturbed soils to establish itself. Repeated burning may encourage its spread in pastures [5]. Sensitive plant is shade intolerant and does not compete with tall vegetation or grow under forest canopies. The species' roots produce carbon disulfide, which selectively inhibits colonization of the rhizosphere by mycorrhizal and pathogenic fungi [6]. This plant occurs in croplands, orchards, pastures, mowed areas, roadsides, and areas disturbed by construction. It may grow as a single plant or in tangled thickets. Sensitive plant grows from near sea level up to 1,300 m in elevation and in areas with annual precipitations from about 1000 to over 2000 mm. The species is frost-sensitive [1].

In the Philippines, sensitive plant flowers all year and may produce as many as 675 seeds per plant per year [1]. The species is both wind [7] and bee pollinated [8]. Air-dry seeds from Puerto Rico weighed an average of 0.0065 + 0.0002 g/seed. With no pretreatment, seeds from this collection began germinating 7 days after sowing and reached a maximum germination of 17 percent by 94 days. In another test, 80 percent germination was obtained in 4 weeks with alternating temperatures of 20°C and 40°C [1]. This species has been successfully tested and recommended for erosion control plantings using potted material at a spacing of 60 x 60 cm [9]. The preliminary Phytochemical screening of *Mimosa pudica* L. showed the presence of alkaloids, flavonoids, saponins, tannins and phenolics, steroids, carbohydrates, resins, triterpenes, glycosides mainly c-glycoside [10-11]. It also contains mimosine, mimosinamine, mimosinic acid, tyrosine 3, 4-dihydroxypiridine [12]. The seed of the plant contains mucilage composed of d -xylose and d -glucuronic acid. They yield 17% of greenish yellow fatty oil [13]. The plant contains tubulin which shows the ability to bind colchicine with its sulfhydryl groups. A new class of phytohormones-turgorines is active in the plant. These periodic leaf movement factors are derivatives of a mixture of flavonols avicularin and reynoutrin [14].

Ethanolic extract of leaves shows hypoglycemic activity in alloxan-induced diabetic rats [15]. *Mimosa pudica* root methanolic extract showed very good wound healing activity when compared to the standard drug gentamicin. The decoction of *Mimosa pudica* leaves shows anticonvulsant activity [16].

Alcoholic extract of *Mimosa pudica* significantly decreases the volume of gastric acid secretion, P^H, free acidity, total acidity and ulcer index with respect to control [17].

Extracts of the plant have been shown in scientific trials to be a moderate diuretic, depress duodenal contractions similar to atropine sulphone, promote regeneration of nerves, and reduce menorrhagia. Anitdepressant activity has been demonstrated in humans. Root extracts are reported to be a strong emetic [18].

MATERIALS AND METHODS

Plant Material

Whole plant sample of *Mimosa pudica* L.was collected during August, 2012, from Kotbari, Mainamoti, Comilla. Then the plant sample was submitted to the National Herbarium of Bangladesh, Mirpur, Dhaka. One week later its voucher specimen was collected after its identification (Accession No. 37879) which was identified and authenticated by taxonomist of the National Herbarium of Bangladesh, Mirpur, Dhaka. The leaves were sun dried for seven days. The plant roots were then oven dried for 24 h at considerably low temperature for better grinding. The dried plant was then ground in coarse powder using high capacity grinding machine.

Preparation of Extracts

Hot solvent extraction process was used for extraction of the plant material. Soxhlet extractor was used for the extraction procedure. Plant material was extracted by three solvents- methanol, ethanol and chloroform. After extraction, the extracts methanol extract (ME), ethanol extract (EE) and chloroform extract (CHE) respectively were kept at petri dishes and dried at room temperature. After drying, extracts were stored in petri dishes and kept in refrigerator for further use.

Preparation of Animals

Adult Swiss albino mice (BALB/c) weighing between (12-30) gm of either sex were used for the studies. The animals were maintained under normal laboratory condition & kept in standard cages at room temperature of 30°C±2°C and 60% to 65% relative humidity and provided with standard diet & water *ad libitum.* The experimental protocols were approved by institutional Animal Ethical Committee to carry out and complete this study.

Anti-nociceptive Activity Test

Anti-nociceptive activity was evaluated by tail immersion test according to Janssen *et al.*, 1963 [19]. Prior to analgesic experiments, the animals were screened for the sensitivity test by immersing the tail of the mice gently in hot water maintained at 55°C – 55.5°C. The animal immersing the tail from hot water within 5 second was selected for the study. The selected mice (either sex) were then divided in to 8 groups of 5 mice each (total 40 mice). Control group received normal saline & standard drug was Diclofenac Na. After administration of the doses by gavages, the reaction time was measured at 0, 15, 30, 45 and 60 minutes.

Acute Toxicity Test

The purposes of acute toxicity testing are to obtain information on the biologic activity of an extract. The acute toxic class method is a stepwise procedure with the use of 3 animals of a single sex (female) per step. The animals were administered with methanol, ethanol, chloroform extracts of dose 2000 mg/kg, 1000 mg/kg, 500 mg/kg by subcutaneous injection. 0.9% saline was used for control group. After administration of the extract solutions mortality or sign of any toxicity was observed for one hour. Then the test animals were observed every 1 hour for next 5-6 hours. The mice were kept under observation for 14 days.

This procedure is reproducible, uses very few animals and is able to rank substances in a similar manner to the other acute toxicity testing methods. The acute toxic class method of Ecobichon is based on biometric evaluations with fixed doses, adequately separated to enable a substance to be ranked for classification purposes and hazard assessment. The method as adopted in 1996 was extensively validated *in vivo* against LD_{50} data [20].

Gastrointestinal Motility Determination

This new protocol of Marona & Lucchesi intended to conform to the 3Rs (replacement, reduction, and refinement) principle, using animals (40 mice of either sex) fasted for 3 h to control intestinal motility, which reduced stress in the animals. In this new protocol, mice are deprived of food for a short time (3 h) and are not killed. 250 mg/kg and 500 mg/kg doses of methanol, ethanol and chloroform extract were used. Control group was administered with 0.9% saline and standard group with loperamide. 90 min later all the mice were administered with 0.3 ml 10% charcoal solution. Both doses and charcoal was administered by oral gavages. The mice were observed until evacuation containing charcoal is observed, and the results are based on the charcoal evacuation time [21].

Antipyretic Activity Test

Anti-pyretic activity of *Mimosa pudica* was evaluated using Brewer's yeast-induced pyrexia in mice [22]. Mice were weighed and randomized into eight groups of five mice per group (total 40 mice of female sex). The baseline body temperatures of the mice were taken by inserting a digital thermometer into their rectal cavities for about 1 min. The steady temperature readings obtained were recorded as the pre-temperatures. 20% suspension of Brewer's yeast was used for pyrexia induction. Mice were administered with 10 µl/g b.w. of Brewer's yeast suspension subcutaneously into the animal's dorsum region and 18 h later of yeast administration, the rectal temperatures were measured again. Mice that did not show a minimum increase of 0.5°C were discarded from the study [23].

Statistical analysis

Data was expressed as Mean ± SEM (Standard error of Mean). The results were analyzed statistically by ANOVA followed by Dunnet's test. Results below $p<0.05$ and $p<0.01$ are considered statistically significant.

RESULTS

Anti-nociceptive Activity Test

In-vivo anti-nociceptive activity test was done on 250mg/kg and 500mg/kg doses of methanol, ethanol and chloroform extracts of *Mimosa pudica* leaf. According to **figure 1** both methanol extracts of (250 mg/kg & 500 mg/kg) with ($p<0.05$ at 60 min) & ethanol extracts (250 mg/kg & 500 mg/kg) with showed significantly higher activity in mice comparing to the standard drug diclofenac Na at 60 & 90 minute. Chloroform extract 250 mg/kg showed higher activity at 60 min than the standard drug. The highest activity was 6.622 second shown by ethanol extract 500mg/kg at 60 min ($p<0.01$). According to **figure 1**, both methanol and ethanol extracts started their antinociceptive effect at 60 minute which gradually declined at 90 min and 120 min.

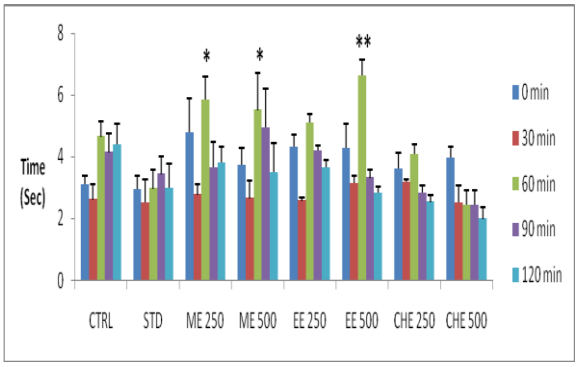

Fig. 1: Results of tail immersion test after administration of doses of control, standard and extracts. Data represent the Mean ± SEM (n=5)

(Methanol Extract = ME, Ethanol Extract = EE, Chloroform Extract = CHE, SEM= Standard Error of Mean) * Significant ($P < 0.05$), ** moderately significant ($P < 0.01$) when compared with the corresponding value of the Standard (Diclofenac Na).

Acute Toxicity Test

In-vivo acute toxicity test was done on mice with methanol, ethanol and chloroform extracts of *Mimosa pudica* leaf. Every extract had three doses of 2000 mg/kg, 1000 mg/kg, 500 mg/kg. All the doses were administered subcutaneously. No reaction or death occurred in mice during 14 day observation. This indicates that all the extract doses are safe upto 2000 mg/kg.

Gastrointestinal Motility Determination

In-vivo gastrointestinal motility test was conducted on methanol, ethanol and chloroform extracts on the doses of 250 mg/kg and 500 mg/kg. The duration between charcoal administration and charcoal defecation is measured for gastrointestinal motility determination. According to **figure 2**, methanol extracts and ethanol extracts showed significant ($p<0.01$) increase in gastrointestinal motility comparing the standard drug loperamide. Among all the test groups, the lowest defecation time was shown by methanol extract 500 mg/kg (69 min) & ethanol extract 500 mg/kg (66.672 min).

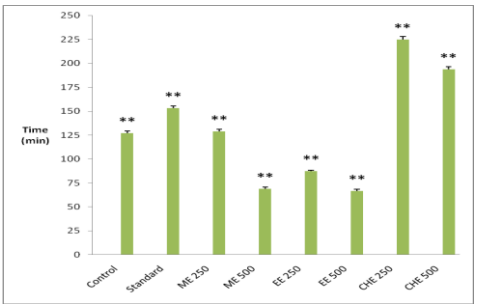

Fig. 2: Time of defecation at Gastro intestinal motility activity after administration of doses of control, standard and extracts. Data represent the Mean ± SEM (n=5)

(Methanol Extract = ME, Ethanol Extract = EE, Chloroform Extract = CHE, SEM= Standard Error of Mean)* Significant ($P < 0.05$), ** moderately significant ($P < 0.01$) when compared with the corresponding value of the Standard (Loperamide).

Antipyretic Activity Test

In-vivo Antipyretic activity was tested on methanol, ethanol and chloroform extracts. According to **figure 3**, Methanol extracts (250 mg/kg & 500 mg/kg), ethanol extracts 500 mg/kg & chloroform extract 500 mg/kg showed significant reduction of temperature in mice comparing to the standard drug Paracetamol at 1st & 2nd hour. Ethanol extract 250 mg/kg at 2nd h (92.58°F) & 3rd h (91.52°F) & methanol extract 500 mg/kg at 1st h (92.72°F) showed maximum temperature reduction.

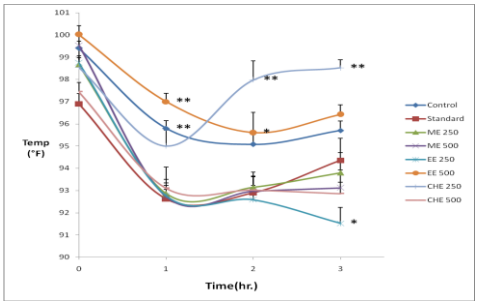

Fig.3: Anti pyretic activity after administration of doses of control, standard and extracts. Data represent the Mean ± SEM (n=5)

(Methanol Extract = ME, Ethanol Extract = EE, Chloroform Extract = CHE, SEM= Standard Error of Mean) * Significant ($P < 0.05$), ** moderately significant ($P < 0.01$) when compared with the corresponding value of the Standard (Paracetamol).

Percent Reduction

Table 1 shows the percentage reduction in body temperature of mice against experimental time in hour. Here, the basal body temperature of mice after induction of pyrexia prior to treatment was used as their control. It could be observed from **table 1** that highest reduction in body temperature was by ethanol extract 250 mg/kg (10.818%) which is higher compared to paracetamol at the 3rd hour. Methanol extract 500 mg/kg also showed significant reduction (10.098%) at 1st hour.

The major body temperature reduction activity of the extract and paracetamol could be seen in 1h. The loss of temperature is measured in % reduction according to the following formula by Makonnen *et al.*, 2003 [24].

$$\% \text{ reduction} = \frac{\text{Yeast induced pyrexia} - \text{post treatment temperature}}{\text{Yeast induced pyrexia}} \times 100$$

Table 1: Reduction in body temperature (%) of mice against experimental time (hr)

Treatment	Dose	1st hour	2nd hour	3rd hour
Control	0.9% NaCl	5.341	6.41	5.489
Standard	Paracetamol 50 mg/kg	6.595	6.163	3.915
Methanol extract	250 mg/kg	8.701	8.281	7.291
Methanol extract	500 mg/kg	10.098	9.742	9.505
Ethanol extract	250 mg/kg	8.961	9.23	10.818
Methanol extract	500 mg/kg	4.145	6.498	5.292
Chloroform extract	250 mg/kg	5.320	0.842	0.081
Chloroform extract	500 mg/kg	6.631	6.754	6.998

DISCUSSION

The tail immersion test indicates potent analgesic activity by the leaf extracts. According to phytochemical screening, this anti-nociceptive effect can be caused the presence of alkaloid, quinine, coumarin which are known to give analgesic effects *in-vivo*.

The acute toxicity test shows that the extract is safe up to 2000 mg/kg b.w. The absence of toxic material in the leaf is responsible for its non-toxic nature at this high dose.

Gastrointestinal motility determination shows that the extracts (methanol & ethanol) have stimulating effect on the gastrointestinal motility. Result indicates that the stimulating effect of the extract is dependent on its concentration. This increase of GI motility can be caused by the presence of Alkaloid in the *Mimosa pudica* leaf. Coumarin, quinine is present in *Mimosa pudica* leaf extracts which are known for analgesic & anti-inflammatory effects. Both coumarin & quinine are freely soluble in ethanol & chloroform. The antipyretic activity of ethanol and chloroform extracts also indicate their effects [25].

CONCLUSION

Mimosa pudica L. has been used widely in traditional medicine. Results of the present study made the statement about the plant justified. Further phytochemical studies can be done for isolation of pure compounds responsible for the specific pharmacological action of this study.

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