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RESEARCH ARTICLE

Muscle relaxant activity of hydroalcoholic extract of *Mimosa pudica* whole plant in mice

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ABSTRACT

Background: *Mimosa pudica* is a traditionally used folk medicine to treat various disorders such as bleeding disorders, dysentery, piles, convulsions, rheumatoid arthritis, muscular pain, and snake bite. **Aims and Objectives:** To evaluate the muscle relaxant activity of hydroalcoholic extract of *M. pudica* whole plant (HAEMPWP) in albino mice. **Materials and Methods:** HAEMPWP was prepared using Soxhlet apparatus. Acute toxicity tests were done using the extract which was given orally to albino mice in increasing doses up to 3200 mg/kg body weight. For pharmacological study, the extract in doses of 200, 400, and 800 mg/kg was orally administered to albino mice. The muscle relaxant action was evaluated by Chimney test and Rotarod method. Data analysis was done by unpaired *t*-test, one-way ANOVA, Chi-square test, and log-probit analysis using SPSS software version 16. **Results:** HAEMPWP showed a significant dose-dependent increase in muscle relaxant activity as compared to control. **Conclusion:** HAEMPWP possesses significant muscle relaxant activity and could be an effective treatment option for various spasmodic conditions.

KEY WORDS: *Mimosa pudica*; Muscle Relaxant; Rotarod; Chimney Test; Soxhlet Apparatus

INTRODUCTION

Skeletal muscle relaxants are drugs that reduce the muscle tone.[1] They act either peripherally at the neuromuscular junction (neuromuscular blockers) such as d-tubocurarine and succinylcholine or centrally in the cerebrospinal axis such as diazepam or directly on the contractile mechanism of the muscle as with dantrolene. The main clinical use of skeletal muscle relaxants (neuromuscular blockers) is as an adjuvant in surgical anesthesia, to obtain relaxation of skeletal muscles, particularly of abdominal wall and lower limbs so that operative manipulations become easier. Centrally acting drugs are useful in musculoskeletal disorders and spastic neurological disorders such as cerebral palsy. However, these drugs cause several adverse effects.

Today, phytopharmaceuticals are gaining more importance. Herbal drugs can be cost-effective alternatives for the costly Western drugs. It is, therefore, necessary to acquire and preserve the traditional system of medicine by proper documentation and identification of herbal remedies. In this study, an attempt has been made to evaluate the muscle relaxant activity of hydroalcoholic extract of *Mimosa pudica* whole plant (HAEMPWP).

The scientific name of this sensitive plant, *M. 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).[2-4] The name of the plant in most regional languages of India describes practically one of the properties of the plant, namely its response to touch. The plant grows wildly as a rapidly growing shrub. It is grown for its curiosity

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value - the fern-like leaves close up and droop when touched, usually reopening within minutes. Phytochemical studies had revealed the presence of alkaloids such as mimosine, crocetin, tubulin, turgorines, flavonoids, tannin, and sitisine.[5,6]

The medicinal use of the plant dates back to Charaka and Sushruta.[7,8] The plant is traditionally used for bleeding disorders such as menorrhagia, dysentery with blood and mucus, piles, and fistula. It is effective in relieving the symptoms of rheumatoid arthritis, spasmodic conditions, and muscular pain.[9] A decoction of the root of the plant is considered on the Malabar coast and elsewhere to be useful in gravel and other urinary complaints.[10,11] 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 such as antihistaminic,[12] antidepressant, hyperglycemic,[13] hemostatic, antifertility,[14] antibacterial,[15] anticonvulsant, anti-snake venom, antifungal, antimalarial, anticancer activities, and is also an immunomodulator. Hence, we decided to evaluate the muscle relaxant activity of HAEMPWP in albino mice.

MATERIALS AND METHODS

Plant Material and Extract

The whole plant of *M. pudica* was collected locally and authenticated by the Central Pharmacognosy Unit, Ayurveda Research Institute, Poojappura, Thiruvananthapuram. The fresh whole plants of *M. 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.[16] The solvent used was 50% water and 50% alcohol. About 2.4 kg of the plant yielded about 32.3 g of a sticky semisolid mass which was dark green in color with a pungent odor. The extract was stored in a refrigerator.

Experimental Animals

Swiss albino mice (20-25 g) 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

Valium tablet (diazepam - 5 mg) of Piramal Healthcare; 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[17] and Study on Gross Behavioral Changes

Albino mice of either sex weighing 15-20 g were used for the study. They were weighed and marked separately. They were divided into 5 groups of 2 mice each. The animals were fasted overnight with free access to water. Group 1 (control group) received distilled water orally. Groups 2-5 received the extract in a dose of 50, 70, 100, and 125 mg/kg. The mice were observed for effects on central nervous system (CNS) and autonomic changes continuously for 2 h and then occasionally for further 4 h and finally overnight mortality recorded. As there was no mortality, toxicity studies were repeated using 200, 400, 800, 1600, 2400, and 3200 mg/kg.

Assessment of Muscle Relaxant Activity

Muscle relaxant action was evaluated by Chimney test and Rotarod method.[18]

Chimney Test

The test "de la cheminee" has been introduced by Boissier et al. (1960)[19] as a simple test for tranquilizing and muscle relaxant activity. Male mice weighing 16-22 g were used. They were divided into 5 groups of 10 animals. The first group served as the control group and received distilled water (negative control). The second group was given the standard drug diazepam orally in the dose of 4 mg/kg (positive control). The remaining three groups were given the extract of *M. pudica* orally in the doses of 200, 400, and 800 mg/kg. Pyrex glass cylinders 30 cm long are required. The internal diameter varies with the animal weight: For mice weighing 16-18 g, the diameter is 22 mm; for mice weighing 18-20 g, 25 mm; and for mice weighing 20-22 g, 28 mm. Each tube has a mark 20 cm from its base.

Initially, the tube is held in a horizontal position. At the end of the tube near the mark, a mouse is introduced with the head forward. When the mouse reaches the other end of the tube, toward which it is pushed if necessary with a rod, the tube is moved to a vertical position. Immediately, the mouse tries to climb backward and performs coordinated movements similar to an alpinist to pass a chimney in the mountains (this gave the name for the test). The time required by the mouse to climb backward out at the top of the cylinder is noted.

Rotarod test was described by Dunham and Miya in 1956.[20,21] They suggested that the skeletal muscle relaxation induced by a test compound could be evaluated by testing the ability of mice to remain on a revolving rod. Male mice with a weight between 20 and 30 g undergo a pre-test on the apparatus. Only those animals which have demonstrated their

ability to remain on the rod for at least 3 min are used for the test. 50 male mice weighing between 20 and 30 g are used. They are divided into 5 groups of 10 animals. First group is the control group which receives distilled water 0.5 ml (negative control). The second group serves as the standard and receives diazepam orally in a dose of 4 mg/kg (positive control). Third, fourth, and fifth groups receive the test compound orally in a dose of 200, 400, and 800 mg/kg. The speed of the rotating rod is adjusted to 25 rotations per minute. The mice are placed on the rotating rod. The Rotarod is turned on. 1 h after drug administration, the fall off time is noted. Fall off time is the period of time for which the animal remains on the Rotarod before falling off. The difference in the fall off time from rotating rod before and after drug administration was taken as an index of muscle relaxation. Percentage decrease in fall off time was calculated using the formula ([Time b–Time a)/Time b]×100 where Time b and Time a are fall off time before and after drug administration, respectively.

Statistical analysis was done using one-way ANOVA, paired *t*-test, and Chi-square test to compare the muscle relaxant activity. The effective dose 50 (ED50) value; the dose for which 50% of animals fail to climb backward out of the tube within 30 s was calculated by log-probit analysis.

RESULTS

Acute Toxicity Studies

Administration of HAEMPWP did not show any sign of toxicity, except for decreased motor activity and muscle relaxation which were evident from 1 h 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 text groups even after 24 h.

Rotarod Test

The extract in doses of 200, 400, and 800mg/kg produced a significant (*t*-test, $P < 0.001$) decrease in fall off time in comparison with control after oral administration (Table 1). The percentage decrease in fall off time of the different doses of the extract as compared showed that extract produced a good muscle relaxation in a dose-dependent manner as shown in Figure 1. The results when analyzed using ANOVA showed $F = 116.31$, $P < 0.001$. *Post hoc* analysis with Dunnett's test revealed there was a significant difference in the muscle relaxation produced by extract 200 and extract 800 mg/kg ($P = 0.03$). There was, however, no statistically significant difference between muscle relaxant effect of extract 400 mg/kg with extract 200 mg/kg ($P = 0.17$) and with extract 800 mg/kg ($P = 0.99$). The percentage decrease in fall off time was greatest with a dose of 800 mg/kg. There was no change in fall of time with the use of distilled water.

Chimney Test

In chimney test, the inability of the mice to climb backward out of the tube within 30 s was considered as the end point to assess muscle relaxant action. At a dose of 200 mg/kg, two mice failed to climb out of the tube. Five mice failed to climb out of the tube at a dose of 400 mg/kg. At a dose of 800 mg/kg, 8 mice failed to climb. None of the mice were able to climb with diazepam. All the mice given distilled water could climb out of the tube. Statistical analysis done using Chi-square test showed that dose of 400 and 800 mg/kg body weight showed a significant muscle relaxation. Figure 2 clearly indicates the dose-dependent muscle relaxation produced by the extract.

As shown in Table 2, Chi-square test showed that there is a significant difference in fall off time value, with diazepam and extract at doses of 200 and 400 mg/kg. The extract at doses of 200 and 400 mg/kg has muscle relaxant activity but less than that of diazepam. At a dose of 800 mg/kg, the muscle relaxant action of the extract is comparable to diazepam.

ED50 value (Figure 3) observed was 482.266, confidence interval (86.077-766.100). The ED50 value is dependable because the goodness of fitness of Chi-square value is not significant ($P = 0.25$) which means the fitting is good.

DISCUSSION

Our study proves that *M. 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 et al., animals were subjected to different doses (5, 50, 300, and 2000 mg/kg). At the dose of 2000 mg/kg (P.O.), the extract showed certain changes in activity and was devoid of any toxicity.[22]

Table 1: Effect of hydroalcoholic extract of *Mimosa pudica* on muscle relaxation using Rotarod method

	Time b (s)	Time a (s)	% decrease	*t*-value	*P*-value
Distilled water	283±11.2		0		
Diazepam 4 mg/kg	263±22.1	46±6.7	82.74±2.13	29.7	<0.001
Extract 200 mg/kg	257±18.8	156±26.3	39.21±9.11	9.8	<0.001
Extract 400 mg/kg	272±19.9	121±34.9	55.17±13.71	15.21	<0.001
Extract 800 mg/kg	284±17.1	112±16.7	60.46±6.12	16.78	<0.001

Time b: Fall off time before drug administration, Time a: Fall off time before after drug administration, All values are mean±standard deviation, n=10, P<0.001 when compared with the control

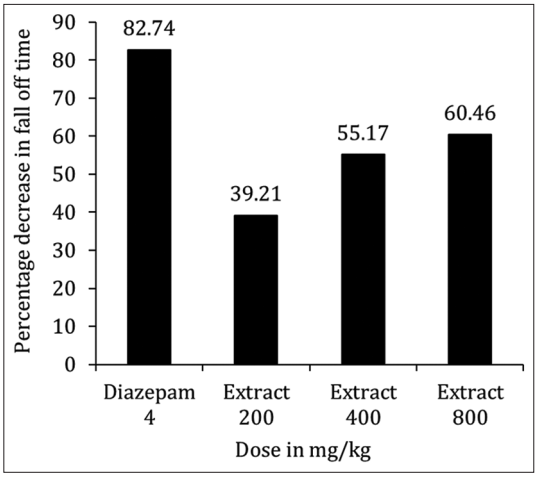

Figure 1: Percentage decrease in fall off time with hydroalcoholic extract of ***Mimosa pudica***

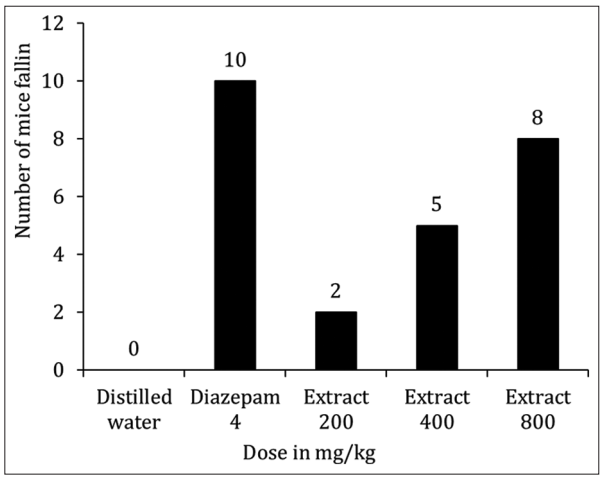

Figure 2: Comparison of muscle relaxant property of hydroalcoholic extract of Mimosa pudica

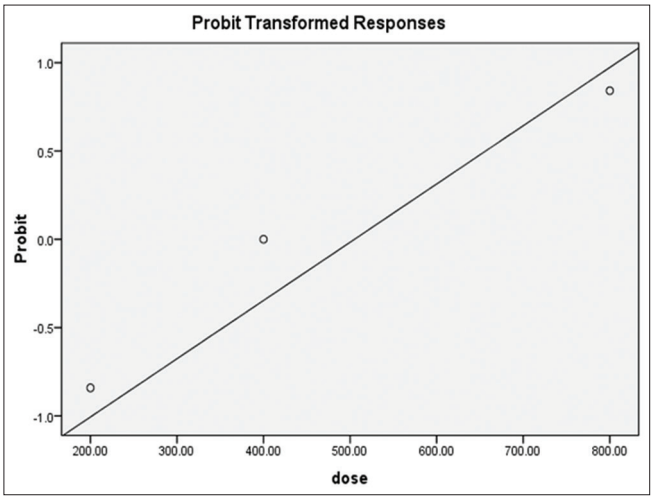

Figure 3: Probit transformed responses

Extensive literature search in PubMed and Google Scholar shows that so far no studies are published regarding the muscle relaxant effect of the plant till date. The objective of the present study was to investigate the effect of HAEMPWP

Table 2: Effect of hydroalcoholic extract of *Mimosa pudica* on muscle relaxation using chimney test

Groups	Number of mice failed to climb	Chi-square value	*P*-value
Distilled water	0		
Diazepam	10	20	<0.001
Extract 200 mg/kg	2	2.222	0.136
Extract 400 mg/kg	5	6.667	0.010
Extract 800 mg/kg	8	13.333	<0.001

on muscle relaxant activity in albino mice. Chimney test and Rotarod test are widely used screening methods for assessing muscle relaxant activity. Our study showed a dose-dependent increase in muscle relaxation with different doses of hydroalcoholic extract of *M. pudica* which was significant. In the present study, HAEMPWP showed muscle relaxation activity at doses of 200, 400, and 800 mg/kg. At a dose of 800 mg, the muscle relaxant activity was comparable to diazepam. Diazepam at a dose of 4 mg/kg body weight showed a significant lack in motor coordination, sedation, and muscle relaxant activity. Several herbal medicines have shown muscle relaxant activity. Muhammed et al. proved that methanolic extract of whole plant of *Viola betonicifolia* at doses of 400 and 500 mg/kg produced dose-dependent muscle relaxant activity with Chimney test.[23] Muscle relaxant property of aqueous leaf extract of *Prosopis cineraria* was studied using Rotarod apparatus, and total fall off time for standard and control group was recorded. Results showed that it produced a significant decrease in fall off time in a dose of 200 mg/kg and efficacy was found to be comparable with diazepam (1 mg/kg).[24] Intraperitoneal administration of 4.2 mg/kg aqueous extract of *Tridax procumbens* has shown significant skeletal muscle relaxant effect with Rotarod apparatus.[25] Aqueous extract of pericarp of *Sapindus trifoliatus* in doses of 50, 100, and 200 mg/kg has demonstrated significant skeletal muscle relaxant effect.[26]

M. 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 diuretic, anti-inflammatory, analgesic, and other effects of the plant.[27] Studies done on *M. pudica* justify the therapeutic application of this plant in indigenous system of medicine augmenting its therapeutic value.

Diazepam is a centrally acting skeletal muscle relaxant which acts by enhancing the effects of GABA. GABA is the most potent inhibitory neurotransmitter in the CNS. Different anxiolytic, muscle relaxant, sedative-hypnotic drugs mediate their action through GABA.[28] Therefore, it is possible that HAEMPWP may act by potentiating GABAergic inhibition in the CNS through membrane hyperpolarization causing a decrease in the firing rate of critical neurons in the brain.

Alternatively, it may be due to direct activation of GABA receptor by the extracts. Ayissi Mbomo et al. investigated the effect of aqueous extract of *M. pudica* on GABAergic regulation of serotonin neuronal activity in mice and suggested that *M. pudica* contains a positive modulator of GABA(A) receptor function.[29] The muscle relaxant effect of *Viola betonicifolia* and *Sapindus trifoliatus* is due to similar effect on the GABA(A) receptor. The muscle relaxant activity observed with HAEMPWP may be due to the presence of flavonoids, alkaloids, and terpenoids in the plant extract.

The standard reference drug diazepam acts as an anxiolytic at low doses and a muscle relaxant at higher doses. Diazepam has been used in several studies as a positive control for testing the skeletal muscle relaxation.[23-26] In this study, diazepam produced statistically significant muscle relaxation in comparison with control as well as extract. Diazepam has low muscle relaxant: Sedation ratio and sedation limit the dose used for muscle relaxation. The drug has long elimination t½ due to an active metabolite, and it is better avoided in elderly patients and in patients with hepatic impairment.[30] Diazepam can also cause respiratory depression. At this point, it is very interesting to note that the HAEMPWP produced muscle relaxation without causing sedation and respiratory depression. However, till date, there has been no published literature proving the skeletal muscle relaxant effect of this traditional medicine. This study proves that *M. pudica* is a skeletal muscle relaxant with two different tests. The major limitation of the study is that phytochemical analysis was not done to identify the exact constituents. Further extensive phytochemical analysis and research are necessary to identify the exact constituents and understanding of the possible mechanism of action of muscle relaxation of HEMPWP.

CONCLUSIONS

The present study showed that the extract of *M. pudica* is safe and possessed muscle relaxant activity in experimental models. Toxicity studies confirm the safety of the drug. These findings substantiate the medicinal values of the plant. The results of this study emphasize 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. This will help to reinforce the importance of the ethnobotanical approach as a potential source of bioactive substances.

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