

**Diuretic property of aqueous extract of leaves of *Mimosa pudica*
Linn. on experimental albino rats**

**Tultul Koksi Sangma^{1*}, Usham Dharmaraj Meitei², Rita Sanjenbam³,
Sharatchandra Khumbongmayum^{3*}**

¹Senior demonstrator, Pharmacology Department, 5th floor, College Building, Uni. College of Medical Sciences and Guru Teg Bahadur Hospital, Dilshad Garden, New Delhi- 110095.

²Senior demonstrator, Pharmacology Department, R.I.M.S., Imphal, Manipur, India-795004

³Professor, Department of Pharmacology, R.I.M.S., Imphal, Manipur, India-795004

* Corresponding Author

(Received 26 May 2010; Revised 07 June-10 July 2010; Accepted 05 July 2010)

ABSTRACT

In the present work, diuretic test of aqueous extract of *Mimosa pudica* Linn. leaves were evaluated using Lipschitz test in normally fed albino rats. The control group was given 0.9% NaCl, the 3 test groups were treated with aqueous extract of leaves of *M. pudica* in the doses of 100, 200 and 400 mg/kg respectively, and the standard group received furosemide. Rats were kept in metabolic cages, and overnight urine was collected. Urine biochemical analysis was done by colorimetry. The aqueous extract of *M. pudica* leaves at 100 mg/kg p. o. showed significant diuretic activity with increased electrolytes excretion ($P < 0.01$ for urine output, $P < 0.01$ for Cl^- , $P < 0.05$ for K^+ and $P < 0.01$ for Na^+). Increasing the dose of the test drug, however, does not bring about increase in diuretic property.

Keywords: *Mimosa pudica*; Diuretic; Coprophagia; Natriuresis.

INTRODUCTION

In various parts of the world, *Mimosa pudica* commonly known as touch-me-not plant was used in inflammations, burning sensations, bilious fever, piles, hydrocele, as diuretic etc. (Kiritikar and Basu, 1987). In Manipur, a state in India, it is reported that the consumption of the decoction of leaves boiled in water causes diuresis, and is used in urinary tract infection. The seeds of the plant was also said to have diuretic property (Krishnaraju, et al., 2006). *M. pudica* contain mimosine, a toxic compound that causes hair loss in animals and women (Crouse, et al., 1962). The methanolic extract of leaves of *M. pudica* showed the presence of bioactive components like terpenoids, flavonoids, glycosides, alkaloids, quinines, phenols, tannins, saponins and coumarin (Gandhiraja, et al., 2009). Roots contain tannin, ash, calcium oxalate crystals and alkaloid mimosine (Oudhia, 2006). This rare nonprotein amino acid, mimosine, has been investigated for the cancer therapy. It was found to inhibit both the initiation and the elongation step of DNA synthesis (Tsvetkov, et al., 1997). Teratogenic effects have been demonstrated in pigs and rats but no species of

Mimosa has been shown to have any such effect in humans (Evans, 2002). The chemical structure of mimosine (Fig. 1) has been worked out (Crounse, et al., 1962, Evans, 2002). Reports regarding scientific studies on diuretic action of *M. pudica* are not available. Taking all these reports and claims into consideration, the present study was designed to evaluate diuretic property of *M. pudica* Linn.

Increasing the urine output, though not the medical practice to correct scanty urine associated with urinary tract infection, diuretics, however, are normally required to remove edema fluid composed of water and solutes, of which sodium (Na^+) is the most important (Bennett and Brown, 2003).

MATERIALS AND METHODS

Plant collection and preparation of extract: Fresh leaves of *M. pudica* were collected from the campus of R.I.M.S. (Regional Institute of Medical Sciences), Manipur, India, in the months of March and April, 2007 and was authenticated (Col. No. 000202) by Professor P. Kumar Singh of Manipur University. The voucher specimen was deposited in the Life Sciences University Herbarium. The leaves were shade-dried at room temperature for 5 days and separated from the stalk. The leaves were then powdered in a mixture-grinder. The powdered plant material was extracted with distilled water using soxhlet apparatus at 100°C for about 5 h/day. The cycle was repeated in next 2 days till colorless solvent in the siphon exit appeared, which was taken as the end point of extraction. The aqueous extract was filtered and evaporated at a maintained temperature of 40°C till dark brown paste was obtained. The final dark brown residue was stored in a refrigerator at 8°C till used. The yield was 30.22%.

Animals: Adult albino rats of Wistar strain were brought from central animal house and acclimatized in departmental animal room for 4–5 days. They were kept (maximum of 4 animals each) in polypropylene cages measuring 24 × 40 × 15 cm³ with husk as bedding. Care was taken to prevent coprophagia. The animals were housed at controlled temperature (23 ± 2°C). Light:dark cycle of 12:12 was followed. The rats had free access to standard pelleted feed and water *ad libitum*. Approval for experimental procedures was taken from Institutional Animal Ethics Committee (IAEC) and the experiments were performed according to the CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) guidelines, 1998. The animals of both sexes were used and were randomly allocated. The rats were divided into 5 groups of 6 animals each.

Preliminary phytochemical analysis: The extract was treated for the presence of alkaloids by Wagner's reagent test, tannins by Ferric Chloride test and saponins by Foam test.

Test for diuretic effect: Lipschitz test as described by Lipschitz et al., (1943) was followed. Albino rats weighing 130–160 gm were used. In all the groups, rats of approximately equal weight were allocated. Six hour prior to the experiment, food was withdrawn with water *ad libitum* till the start of the experiment. Since numbers of metabolic cages were limited, only one rat per group was used per day. The drugs were dissolved in 0.9% NaCl (normal saline). It has been stated by Haravey (1966) that the indigenous drugs when tested on water-loaded animals, there was no appreciable difference between control and test groups; perhaps due to the fact that the water itself acts as a mild diuretic. It has also been mentioned by Dollery (1999) that the administration of normal saline maintains the extracellular fluid volume and corrects any pre-existing losses. The drug solutions were given by gavage at a volume of 25 ml/kg at approximately body temperature. The drugs given for the various groups were as follows: Control group- normal saline (25 ml/kg), Test 1- aqueous

extract of *M. pudica* (100 mg/kg), Test 2- aqueous extract of *M. pudica* (200 mg/kg), test 3- aqueous extract of *M. pudica* (400 mg/kg) and Standard group- furosemide (25 mg/kg). Soon after feeding, the animals were placed separately in metabolic cages provided with a mesh wire bottom and a funnel to collect the urine. No water or food was given during the time of the experiment. Both in control and test drug treated groups, often 5 h is not sufficient for collection of urine as the volume excreted was minimal or not at all. Thus, overnight (18 h) urine was collected for all the groups tested even though the urine output in furosemide treated group was enough. At the end of the experiment, residual urine from the bladder was expelled by gentle pressure on the abdomen and simultaneously pulling the base of the tail. The volume was measured and urine sample kept in refrigerator until Na^+ , K^+ and Cl^- levels were estimated. The urine samples were kept without adding any preservatives (Young and Bernes, 2001).

Quantitative estimation of Na^+ , K^+ and Cl^- : The electrolytes (Na^+ , K^+ and Cl^-) were determined by colorimetry. Quantitative estimation of Na^+ , K^+ and Cl^- were done by following the instructions as directed in the kit used (Medsorce Ozone Biomedicals Pvt Ltd). The kit was meant for human serum electrolyte estimation. The electrolyte concentration in urine was higher than in serum (Varley, 2005) and it was even higher in rats than in humans as observed by previous researchers (Durairaj, et al., 2007; Johnson, 2007). For determination of rat urine, trial and error method was followed in dilution of urine. Chloride kit was stored at room temperature and sodium-potassium kit was stored at 2–8°C. All glassware's and cuvette were washed with deionised water and dried in hot air oven before use.

Sodium assay

Step 1- Precipitation of Na^+ and protein: 1 ml of precipitating reagent (for sodium) was pipetted into clean, dry centrifuge tubes: one for the standard reagent labeled as S and the other 5 test tubes labeled as T, one each for all the experimental groups namely, T1 (Control), T2 (Test-100 mg/kg), T3 (Test-200 mg/kg), T4 (Test-400 mg/kg) and T5 (Standard drug-furosemide). Then 10 μl of standard reagent was added into test tube S and 10 μl of urine to all the T series test tubes. The solution was shaken vigorously and incubated at room temperature for 5 min, then centrifuged at 2000–3000 r.p.m. for 2 min to obtain a clear supernatant.

Step 2- Colour development: 1 ml of colour reagent (for sodium) was pipette into each of the clean, dry test tubes labeled B for Blank, S for Standard reagent and 5 test tubes labeled T1 to T5 as above. 20 μl of the appropriate supernatant from step1 was then added into the corresponding test tube S and tubes of the series T. Into test tube B, 20 μl of the precipitating reagent (for sodium) was added. Pipetting of sodium precipitating reagent (in step 1) and quick transfer of supernatant (in step 2) was followed. The solution was mixed well and allowed to stand at room temperature for 5 min, then the absorbance of Blank (Abs B), Standard (Abs S) and Test series (Abs T1, Abs T2, Abs T3, Abs T4 and Abs T5) against distilled water on a photocolorimeter with 530 nm within 10 min was measured.

Potassium assay: 1 ml of boron reagent for potassium was pipetted into dry clean centrifuge tubes labeled Standard (S) and Test (T)- T1, T2, T3, T4 and T5 as described for step 1 in Na^+ assay. 50 μl of standard reagent was added into the test tube S and the same amount of corresponding urine sample was added to the T series of test tubes. The solution was mixed well; incubated at room temperature for 5 min, then the absorbance of Standard (Abs S) and that of Test series (Abs T1, Abs T2.....Abs5) against distilled water on a photo-colorimeter with 620 nm within 10 min was measured. The urine Na^+ and K^+ for each group/test series was then

measured by the following formula: Na^+ in mMol/L = [(Abs of B - Abs of T) / (Abs of B - Abs of S)] \times 150, K^+ in mMol/L = (Abs of T / Abs of S) \times 5. This procedure is linear up to 200 m Mol/L for Na^+ and up to 7 m Mol/L for K^+ . If values exceed these limits, urine samples were diluted with deionised water and assay repeated, and the final value was calculated using appropriate dilution factor.

Quantitative estimation of Cl^- : Urine specimen was diluted 1+1 with distilled water. 1 ml of colour reagent was pipette into test tubes labeled Blank (B), Standard (S) and Test (T) - T1, T2, T3, T4 and T5. 10 μl of chloride standard was added to the test tube S while 10 μl of the appropriate urine sample was added to the corresponding T series test tubes. The solution was mixed well and the absorbance of standard (Abs S) and Test (Abs T) against Blank (Abs B) were read at 530 nm. The Cl^- content was calculated by using the given formula: Cl^- in mMol/L = (Abs of T / Abs of S) \times 100. Linearity for this procedure is 140–150 mMol/L. The final value was calculated by multiplying the dilution factor 2.

Preliminary toxicity testing: Two dose levels of test drug higher than the intended use was selected. Each dose was tested in a group of three female albino Swiss mice which has been fasted for 4 h. The animals were observed for a period of 14 days for any mortality. At first, 800 mg/kg of aqueous extract of *M. pudica* Linn. suspended in gum acacia was given orally at a volume of 1 ml/kg. Since no mortality occurred, the dose was escalated to 1000 mg/kg. The study was done following OECD 423 (Organisation for Economic Cooperation and Development) guidelines.

Statistical method: All data were expressed as mean \pm SD. One way Analysis of variance (ANOVA) followed by Dunnett's 't' test, was applied to find out statistical significance of the experimental results. Level of 5% was considered statistically significant.

RESULTS

Preliminary phytochemical analysis: The aqueous extract of leaves of *M. pudica* Linn. subjected to preliminary phytochemical analysis tested positive for alkaloids (Wagner's reagent test), tannins (Ferric Chloride test) and saponins (Foam test).

Effect of the plant extract on urinary output: For selection of test dose levels, the animals were first tried and tested with 50 mg/kg per orally. In this trial, no difference from the control group was seen, so this dose level was not included in the data. The drug doses were escalated in geometric progression. Oral administration of a single dose of aqueous extract of leaves of *M. pudica* significantly increased the urine output at the doses of 100 and 200 mg/kg (Table 1). The effect was dose dependent with more pronounced outflow at 100 mg/kg ($P < 0.01$ vs. control) and the significant effect was not seen anymore at 400 mg/kg. The effect of the reference drug, furosemide ($P < 0.001$ vs. control) was, however, more rapid and prominent than the test drug. As mentioned earlier, furosemide not only increased the urine output in the 5 h period but also the cumulative urinary excretion in the 18 h period was higher than any of the other groups tested.

Effect of the plant extract on urinary electrolytes: The effect of aqueous extract of leaves *M. pudica* Linn. on the excretion of urinary electrolytes is dose dependent (Table 2). The dose of 100 mg/kg significantly increased the excretion of all the electrolytes ($\text{Na}^+ = 400.00 \pm 35.77$ m Mol/L, $\text{K}^+ = 114.19 \pm 11.54$ m Mol/L and $\text{Cl}^- = 266.58 \pm 21.58$ m Mol/L) estimated in this study. At higher doses of 200 and 400 mg/kg, the test drug did not induce any significant increase in the Cl^- excretion. The test drug at 200 mg/kg still cause significant saluresis ($P < 0.01$) and kaliuresis ($P < 0.05$) when compared to control. However, the test drug at dose of 400 mg/kg did

not bring about any significant increase in the electrolytes excretion. The standard drug furosemide, as expected, increased the excretion of all the 3 electrolytes ($\text{Na}^+ = 486.67 \pm 46.76$ mMol/L, $\text{K}^+ = 133.74 \pm 21.62$ mMol/L and $\text{Cl}^- = 428.28 \pm 29.35$ mMol/L). The urinary level of these 3 electrolytes was higher than the test groups.

Preliminary toxicity testing: In the toxicity study, no mortality was observed in a 14 day test period in albino mice with the doses of 800 and 1000 mg/kg of the aqueous extract of *M. pudica*.

DISCUSSION

The current study evaluated the diuretic potential of *M. pudica* leaves in Wistar albino rats. The purpose of the present study was to establish the scientific basis for the traditional and the reported folk use of *M. pudica* Linn. for diuresis. The aqueous extract was given orally in the same way local people practice traditionally. The chemical analysis for the presence of alkaloids, tannins and saponins was also done. The earlier study has reported the presence of these chemicals in the plant (Gandhiraja, et al., 2009). The diuretic assay was done using Lipschitz test which is one of the commonly used standard models. This is probably the first study of the aqueous extract of leaves of *M. pudica* Linn. on the diuretic property. The study indicated that the diuretic activity of the plant extract was apparent in a narrow dose range. The effect was not seen at 50 mg/kg (not shown in the data), and also at a dose of 400 mg/kg the effect has declined. The dose of 100 mg/kg showed significant increase in urine output and excretion of electrolytes. This observation is in agreement with the findings of Krishnaraju et al., (2006) which showed the diuretic property of the seeds. The current study when compared with the previous studies, some differences were observed. In the study conducted by Durairaj et al., (2007) using the same control and the same standard drug intraperitoneally, the value of Cl^- excretion was more than that of Na^+ . In the present study, Na^+ excretion was more than that of Cl^- . Johnson (2007) has reported that the normal renal physiological values in a rat are a urine volume of 15 to 30 ml/24 h with Na^+ excretion of 200 mMol/L/24 h and K^+ excretion of 150 mMol/L/24 h. However, the author has further mentioned that the values will vary based on the strain of animal, supplier, feed and housing conditions.

The diuretic activity of the plant extract is relatively modest and slow in onset as compared to the reference drug, furosemide. The loop diuretic furosemide acts by inhibiting three-ion co-transporter system i.e. the $\text{Na}^+/\text{K}^+/2\text{Cl}^-$ (Sadki, et al., 2010). The plant extract must have acted in the same way like furosemide as it also cause increase excretion of all these 3 electrolytes. Li et al., (2007) has reported that L-mimosine indirectly cause pressure natriuresis. So, whether the diuretic activity of *M. pudica* can be attributed to the presence of the alkaloid mimosine still remains a question. The possibilities in the delayed diuretic activity seen with test drug may have resulted from the slow absorption of the active principle in the extract or due to biotransformation to its active metabolite or in vivo stimulation of endogenous diuretic compound. The decreasing urine output as well as decreasing urine electrolytes excretion at higher doses could be due to its toxic effect or may be due to different facets of drug action manifested at different doses. These possibilities meanwhile is purely hypothetical, and needs further studies in different animal models by various methods using wider range of doses and with different high-quality standardized extracts. In the toxicity study, though no mortality was observed during the course of the study, the drug cannot be considered safe without the thorough evaluation, as the late adverse effect like teratogenicity has been reported in experimental animals (Evans, 2002).

CONCLUSION

The present study revealed that the leaves of the aqueous extract of *M. pudica* possess significant diuretic activity at 100 and 200 mg/kg but the effect declined at higher dose. The findings partly supported the traditional claims of the plant as diuretic.

Acknowledgements: This study was supported by R.I.M.S. (PG/2006/RIMS-12) Manipur, India.

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Table-1: Effect of orally administered aqueous extract of leaves of *M. pudica* Linn. on 18 h urinary output in rats.

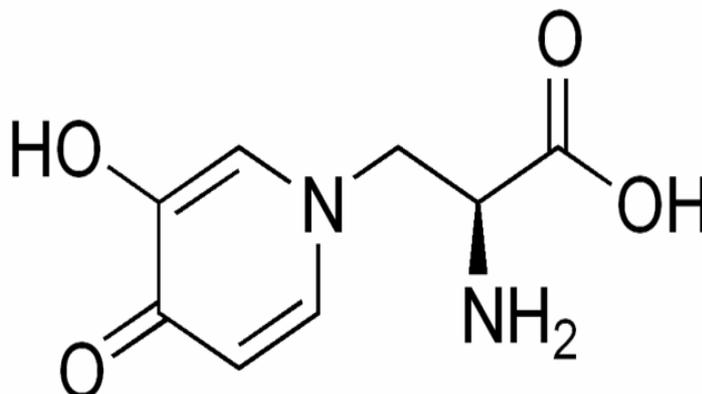
Treatment	Dose (oral)	Urine output in ml (mean \pm SD)
Normal saline	25 ml/kg	1.71 \pm 0.59
<i>M. pudica</i>	100 mg/kg	2.92 \pm 0.43 ^a
	200 mg/kg	2.42 \pm 0.95 ^b
	400 mg/kg	1.82 \pm 0.92
Furosemide	25 mg/kg	3.33 \pm 0.48 ^c

- n = 6 rats per group.
- ^aP < 0.01, ^bP < 0.05, ^cP < 0.001 compared to control (normal saline) group using Dunnett's 't' test.

Table-2: Effect of orally administered aqueous extract of leaves of *M. pudica* Linn. on 18 h urinary electrolytes in rats.

Treatment	Dose (oral)	Urine electrolytes (mMol/L/18 h)		
		Na ⁺	K ⁺	Cl ⁻
Normal saline	25 ml/kg	313.33 \pm 54.65	94.64 \pm 10.81	230.26 \pm 39.76
<i>M. pudica</i>	100 mg/kg	400.00 \pm 35.77 ^b	114.19 \pm 11.54 ^a	266.58 \pm 21.58 ^b
	200 mg/kg	386.67 \pm 54.65 ^b	114.19 \pm 11.54 ^a	250.42 \pm 33.01
	400 mg/kg	353.33 \pm 58.87	95.67 \pm 32.83	238.33 \pm 28.25
Furosemide	25 mg/kg	486.67 \pm 46.76 ^c	133.74 \pm 21.62 ^b	428.28 \pm 29.35 ^c

- Data are expressed as mean \pm SD, n = 6 in each group.
- ^aP < 0.05, ^bP < 0.01, ^cP < 0.001 compared to control (normal saline) group using Dunnett's 't' test.

**Figure-1: Chemical structure of mimosine**