DOI: 10.7860/JCDR/2015/14662.6877

Experimental Research

Pharmacology Section

Diuretic Activity of Ethanolic Root Extract of *Mimosa Pudica* in Albino Rats

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ABSTRACT

Introducation: Diuretics are the drugs which increase the urine output. This property is useful in various pathological conditions of fluid overload. The presently available diuretics have lot of adverse effects. Our study has evaluated the diuretic activity of ethanolic root extract of *Mimosa pudica* as an alternative/new drug which may induce diuresis.

Aim: To evaluate the diuretic activity of ethanolic root extract of *Mimosa pudicaa* in albino rats.

Materials and Methods: Ethanolic root extract of *Mimosa pudica* (EEMP) was prepared using soxhlet's apparatus. Albino rats were divided into 5 groups of 6 rats each. Group-I (Control) received distilled water 25ml/kg orally. Group-II (Standard) received Furosemide 20mg/kg orally. Group-III received EEMP 100 mg/kg, Group-IV received EEMP 200 mg/kg and Group-V received EEMP 400 mg/kg. The urine samples were collected for all the groups upto 5 hours after dosing and urine volume was measured. Urine was analysed for electrolytes (Na+, K+ and Cl-). ANOVA, Dunnet's test and p-values were measured and data was analysed.

Results: EEMP exhibited significant diuretic activity by increasing urine volume and also by enhancing elimination of Sodium (Na+), Potassium (K+) and Chloride (Cl-) at doses of 100 and 200mg/kg.

Conclusion: EEMP possesses significant diuretic activity and has a beneficial role in volume overload conditions.

Keywords: Soxhlet's apparatus, Furosemide

INTRODUCTION

Cardiac failure is a syndrome resulting from impaired pumping capacity of the heart which occurs because of genetic or acquired abnormality in cardiac structure and/ or function. This results in symptoms and signs of cardiac failure such as dyspnea, fatigue, oedema, rales leading to frequent hospitalization, a poor quality of life and a shortened life expectancy [1]. Inspite of high rates of morbidity and mortality, the pathophysiologic mechanisms and treatment of heart failure is poorly understood.

Body hydration status is of remarkable importance. Diuretics elevate the rate of urine flow and sodium excretion and are used to adjust the volume and/ or composition of the body fluids and this includes forced diuresis. Diuretics can adequately control fluid retention and restore and maintain normal volume status in patients with congestive symptoms (dyspnoea, orthopnoea, oedema) or signs of elevated filling pressures (rales, jugular venous distension, or peripheral oedema). Diuretics adjust the volume and composition of body fluids in a variety of clinical situations including hypertension, heart failure, renal failure, nephrotic syndrome, and cirrhosis [2]. The currently used diuretics are usually associated with many adverse reactions. Various plant extracts used in traditional medicine have shown significant diuretic activity when tested in animal models. In this current study an attempt has been made to evaluate the diuretic activity of ethanolic root extract of *Mimosa pudica*.

Mimosa pudica commonly known as "touch me-not" plant, belonging to the family "Fabaceae" is traditionally being used to cure various diseases such as leucoderma, vaginopathy, metropathy, ulcers, dysentery, inflammations, burning sensations, haemorrhoids, jaundice, asthma, fistula, strangury and fevers [3].

Mimosa pudica is reported to have antidepressant, anticonvulsant, hyperglycaemic and anti-implantation activities and also has effect on estrous cycle and ovulation [4-6]. The roots and leaves of *Mimosa pudica* are commonly used as astringent, acrid, cooling vulnerary, alexipharmic, resolvent, antispasmodic, emetic, constipating and as a febrifuge. In recent studies, ethanolic extract was shown to have analgesic and anti-inflammatory property [7] and aphrodisiac activity [3].

The various biomolecules isolated from *Mimosa pudica* include tubulin, C-glycosylflavones, phenolic ketone, a novel buffadienolide, mimosine, terpenoids, flavonoids, glycosides, alkaloids, etc [8]. Roots are especially rich in tannins and alkaloid mimosine [9].

AIM

To evaluate the diuretic activity of ethanolic root extract of *Mimosa pudica* in albino rats.

MATERIALS AND METHODS

Animals

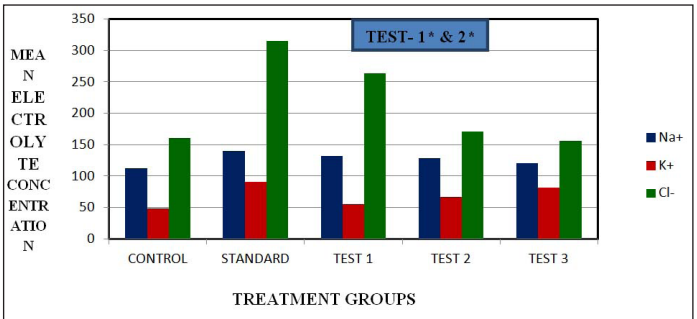
Albino rats (150-200g) of either sex were randomly selected from central animal facility, JSS Medical College, Mysore. Animals were housed into groups of six per cage at a controlled temperature (23 ± 2ºC). Light: dark cycle of 12:12 was followed. The rats had free access to standard pelleted feed and water ad libitum. The Institutional Animal Ethical Committee approved the protocol of this study (CPSEA approval number from IAEC of: JSSMC/PR/IAEC/11/26191/2013-14).

Preparation of the extract and isolation of active principle

The *Mimosa pudica* along with its roots found in the vicinity of Mysore were taken to JSS Ayurvedic College for authentication of the plant and the species. The roots were shade-dried and coarsely powdered. A weighed quantity (200g) of the powder was then subjected to continuous hot extraction in Soxhlet apparatus with Ethanol. The extract was filtered through a cotton plug, followed by Whartman filter paper (No.1) and dried at 40-50°C to get a blackish green semisolid mass, which was taken for final use.

Treatment group	Dose (Oral)	Urine volume(ml)	Na+ (mEq/L)	K + (mEq/L)	Cl – (mEq/L)
Control	25 ml/kg	2.93+0.20	112.00+2.52	48.75+1.20	160.50+4.32
Standard	20 mg/kg	8.43+0.89	140.33+1.86	90.70+1.44	314.50+12.48
Test- 1	100 mg/kg	5.80+0.35*	131.50+1.64*	55.61+3.15*	263.00+4.14*
Test- 2	200 mg/kg	5.23+0.40*	128.16+4.95*	66.31+3.41*	170.33+7.89*
Test- 3	400 mg/kg	5.16+0.56*	120.16+3.31	81.93+3.60*	155.50+3.21

[Table/Fig-1]: Effects of oral administration of Ethanolic root extract of *Mimosa pudica* on urine volume and electrolyte excretion
Values are in Mean+SD and the data was analysed by one-way ANOVA followed by Dunnett's test. *p-value < 0.05 when compared with control and treated groups.



[Table/Fig-2]: Diuretic activity (electrolyte concentration) of Ethanolic root extract of *Mimosa pudica*.
*p-value < 0.05 when compared with control.

Drugs and chemicals

Furosemide 20 mg/kg body weight (Sanofi India Limited), Ethanolic root extract of *Mimosa pudica*100, 200 and 400 mg/kg body weight and distilled water.

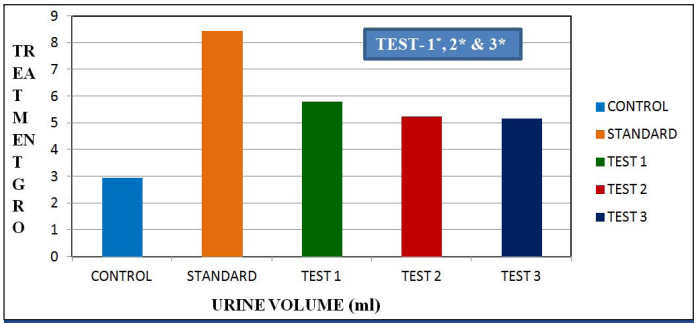
Animals were divided into five groups (with six rats each). Animals were deprived of food and water for 18 hours prior to the experiment. Group I received 25ml/kg of distilled water and served as the control, Group II received Furosemide 20mg/kg as standard [10], Groups III, IV and V received ethanolic root extract of *Mimosa pudica* at doses of 100, 200 and 400mg/kg respectively, all of which were administered orally immediately prior to the test in this acute study. The ethanolic extracts of the test drug were suspended in distilled water for oral administration.

ASSESSMENT OF DIURETIC ACTIVITY

Albino rats weighing 150–200 g were used. The rats were fed with standard pellet diet and provided water ad libitum. The same was withheld 18 hours prior to the experiment. They were hydrated with 5ml/kg of distilled water prior to drug/extract administration. Immediately after dosing the animals were placed in metabolic cages provided with a wire mesh bottom and a funnel to collect the urine. Stainless-steel sieves were placed in the funnel to retain feces and to allow the urine to pass. The urine was collected in measuring cylinder up to five hours after dosing. During this period, animals were deprived of food and water [11]. 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. The concentrations of urine Na+ and K+ were determined by flame photometry and concentration of Cl- was estimated titrimetrically using 0.02N $AgNO_3$ with 5% potassium chromate as indicator.

STATISTICAL ANALYSIS

The effects of EEMP were calculated by taking the Mean values and Standard Deviation of the outcome parameters. ANOVA (Analysis of Variance) was applied to compare the effects of drugs under study. The data were analysed using ANOVA followed by Dunnett's test.



[Table/Fig-3]: Diuretic activity (urine volume) of Ethanolic root extract of *Mimosa pudica*.
*p-value < 0.05 when compared with control

The *p-value < 0.05 was considered significant. All the statistical analysis was done by using IBM SPSS 21 software.

RESULTS

Oral administration of single dose of ethanolic root extract of *Mimosa pudica* significantly increased the urine output at the doses of 100 and 200 mg/kg. The effect was found to be dose dependent with more pronounced outflow at 100 mg/kg ($p<0.01$ vs control) and the results were not significant when the dose was increased to 400 mg/kg as indicated in [Table/Fig-1].

The effect of EEMP on the excretion of urinary electrolytes is dose dependent. The test doses of 100 mg/kg and 200 mg/kg significantly increased the excretion of all the electrolytes Na+, K + and Cl-estimated in the study. However, the test drug at higher dose of 400 mg/kg did not induce any significant increase in the Na+ and Cl- excretion except for significant kaliuresis as indicated in [Table/Fig-2].

DISCUSSION

Fluid overload is usually observed in various pathological states such as cardiac failure and secondary oliguric states. Heart failure is the leading cause of hospitalization in people older than 65. More than 20 million people have heart failure worldwide [12]. The prevalence and incidence of heart failure in India has been estimated to range from 1.3 to 4.6 million, with an annual incidence of 0.5- 1.8 million [13]. Inspite of high rates of morbidity and mortality, the pathophysiologic mechanisms and treatment of heart failure is poorly understood. Even with the advent of newer and selective agents, their side effect profile is a setback and also few cases show refractoriness to conventional treatment. The current study was aimed at evaluating the diuretic activity of ethanolic root extract of *Mimosa pudica* in albino rats.

Diuretics used in the treatment of heart failure act by enhancing urine outflow, decreasing plasma volume and venous return to the heart, and thereby subsequently decrease cardiac workload, oxygen demand and blood pressure. The major site of action of the loop diuretic, furosemide is the thick ascending limb of loop of Henle where it acts by inhibiting the Na+/K+/2Cl- co-transport carrier in the luminal membrane. It increases the urine output along with increased urinary excretion of Na+, K+ and Cl-.

The present study showed that the diuretic activity of the ethanolic root extract of *Mimosa pudica* is relatively modest and slow in onset as compared to the reference drug, furosemide. The plant extract also caused increased urine volume [Table/Fig-3] and increased urinary excretion of Na+, K+ and Cl- like that of furosemide [Table/Fig-2].

Therefore the probable diuretic action of ethanolic root extract of *Mimosa pudica* could be due to its interference with the Na+/K+/2Cl- co-transport carrier in the luminal membrane of the thick ascending limb of loop of Henle, similar to the mechanism of action of furosemide. And, this effect of the extract may be related mainly to the alkaloid L- mimosine that has also been reported to indirectly

cause pressure natriuresis. L-Mimosine is known to effectively induce Hypoxia inducible factor 1α (HIF-1α) in the kidneys in vivo and it significantly decreases prolyl-4-hydroxylase domain-containing proteins (PHDs) enzyme activity and, thereby, upregulates HIF-1α levels in the kidneys, especially in medullary areas. In addition, the binding of HIF-1α and the transcription of heme oxygenase-1 (HO-1), a prototype HIF-1α target gene, in the renal medulla is enhanced by L- mimosine. Thus the pressure natriuresis is increased by the inhibition of PHD activity which in turn increases the expression of HIF-1α and transcriptional activity [14].

Thus *Mimosa pudica* was observed to have diuretic activity in experimentally induced diuresis in albino rats. The study explores the complementary nature of *Mimosa pudica* with conventional treatment making it comparatively safer, economical, easily available and well tolerated therapy.

CONCLUSION

We conclude from the study that ethanolic root extract of *Mimosa pudica* has a beneficial role as a diuretic and thereby support the claim of traditional use of the plant as a diuretic. Further studies are indicated to identify the adverse effects, optimal treatment routes and dosage.

ACKNOWLEDGEMENT

We would like to thank our institution for permitting and providing the necessary assistance for the study.

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FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Apr 27, 2015**
Date of Peer Review: **Aug 09, 2015**
Date of Acceptance: **Oct 11, 2015**
Date of Publishing: **Dec 01, 2015**