Original Article

Evaluation of anti-diarrhoeal potential of ethanolic extract of *Mimosa pudica* leaves

Md. Saifuddin Khalid, Shah Jinesh Kumar, D. K. Suresh, Rajnish Kumar Singh, I. V. Narasimha Reddy, Sunil Kumar[1]
Department of Pharmacology, Luqman College of Pharmacy, Gulbarga, [1]Department of Pharmaceutical Chemistry, NET, College of Pharmacy, Raichur, Karnataka, India.

Diarrhoea is a major public health problem in developing countries and is said to be endemic in many regions of Asia. It is a leading cause of high degree of morbidity and mortality. The anti-diarrhoeal potential of the ethanolic extract of leaves of *Mimosa pudica* Linn (Mimosaceae) has been evaluated using several experimental models in Wistar albino rats. The ethanolic extract inhibited castor oil induced diarrhoea and PGE_2 induced enteropooling in rats and has also reduced gastrointestinal motility after charcoal meal administration. The ethanolic extract at 200 and 400 mg/kg was showed significantly inhibited diarrhoea. There was a significant (P<0.001) dose-dependent decrease in the diarrhoea produced by all the three models in rats as compared to the standard drug. The anti-diarrhoeal property may be related to the tannin and flavonoids present in the extract. These results clearly indicated that ethanolic extract of the leaves of *Mimosa pudica* is effective against diarrhoeal disease

Key words: Anti-diarrhoeal activity, castrol oil-PGE_2 induced diarrhoea, gastrointestinal motility, intestinal secretion, leaves ethanolic extract, *Mimosa pudica* linn

INTRODUCTION

Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them. Diarrhoea is a major public health problem in developing countries and is said to be endemic in many regions of Asia and is the leading cause of high degree of morbidity and mortality which contributes to the death of 3.3–6 million children annually.[1] Diarrhoea is characterised by the passage of abnormally soft or liquid feaces leading to excess loss of fluid, salts and nutrients.[2] In developing countries, a majority of people living in rural areas almost exclusively use traditional medicine in treating all sorts of diseases including diarrhoea. A medicinal plant provides an important source of new chemical substances with potential therapeutic effects. These have been used in traditional medicine for the treatment of several diseases.[3] Several herbs and shrubs are useful as medicines as reported by many scientists.[4] Many such herbs, shrubs and plants are known to protect the organs from the environmental, chemical and occupational challenges. *Mimosa pudica* Linn is one such green leaf shrubs.[5] The plant *Mimosa pudica* used in indigenous medicine for the treatment of hydrocele, scrofula, conjunctivitis, cuts, wounds, haemorrhages, bleeding disorders such as menorrhagia, dysentery with blood and mucus, piles, in herbal formulations.[6] The present study is aimed at investigating the anti-diarrhoeal activity of the plant extract.

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MATERIALS AND METHODS

The fresh leaves of *Mimosa pudica* Linn were collected from Luqman college of Pharmacy, Gulbarga, Karnataka. The plant herbarium specimen was identified and authenticated by Mr. P. G. Diwakar, Joint Director, Botanical Survey of India, Western circle,7, Koregaon Road, Pune -1. Voucher No. JINSHMI1.

Extraction

The authenticated leaves of *Mimosa pudica* Linn were dried in shade and powdered coarsely. Extraction was done according to standard procedure using analytical grade solvents. The coarse powder of the leaves was Soxhlet extracted with the solvents with increasing order of polarity, i.e. petroleum ether (60–80°C), chloroform (59.5–61.5°C), ethanol (64.5–65.5°C) and distilled water. The extracts obtained were concentrated under reduced pressure.[7] The dried extract was weighed (the yield of the extract was 12.38%) and stored in airtight containers in refrigerator below 10°C.

Address for correspondence: Mr. Md. Saifuddin Khalid, Department of Pharmacology, Luqman College of Pharmacy, Old Jewargi Road, Behind P and T Colony, P.O. Box No: 86, Gulbarga 585 102, Karnataka, India. E-mail: khalid2568@yahoo.com
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Phytochemical Screenin

Preliminary phytochemical screening of the ethanolic extract of leaves was performed for the presence of alkaloids, phenolics, flavonoids, saponins, carotenoids, carbohydrates and glycosides.[8]

Animals Used

Albino wistar rats of either sex weighing between 150 and 200 gm and albino mice of either sex weighing between 20 and 25g were procured from registered breeders (149/1999/CPCSEA, Mahavir Enterprises, Hyderabad.) used for studying anti-diarrhoeal activity and acute toxicity respectively. The animals were housed under standard conditions of temperature (25±2°C) and relative humidity (30%–70%) with a 12:12 light-dark cycle. The animals were fed with standard pellet diet (VRK Nutrition, Pune) and water *ad libitum*. Approval at the Institutional Animal Ethics Committee (IAEC) of Luqman College of Pharmacy, Gulbarga was taken for conducting anti-diarrhoeal activity.

Acute Toxicity Study

The acute toxicity of ethanolic extracts of *Mimosa pudica* leaves was determined in female albino mice. Animal were fasted overnight prior to the experiment. Fixed dose (Annexure-2d) method of CPCSEA, OECD guideline No. 420; was adopted for the study.[9] One fifth and one tenth of LD_{50} cut off (2000 mg/kg) values taken as screening dose.

Anti-diarrhoeal Activity

Castor oil induced diarrhoea

In the present study animals were divided into four groups of six rats each. Group I was administered vehicle orally and served as control. Group II served as standard and received loperamide (1 mg/kg), orally. Group III and IV were given orally ethanolic extract (200 and 400 mg/kg; orally) of *Mimosa pudica* leaves respectively. They were fasted overnight before the test with free access to water.

After 30 min of administration of above dose all the rats were given with 1 ml of castor oil orally. The numbers of wet fecal dropping were measured for six hours.[10]

Prostaglandin-E_2 induced enteropooling

In this test the animal were divided into five groups of six rats each. The animals were deprived of food and water for 18 h prior to the experiment. Group I received only 1 ml of 5% (v/v) ethanol in normal saline and then treated with 2% of (w/v) aqueous gum acacia suspension orally and served as vehicle control, Group II treated with PGE_2 (100 mg/kg, orally) and served as PGE_2 control, Group III served as standard and received loperamide (5 mg/kg) orally, Group IV and V were administered orally ethanolic extract (200 and 400 mg/kg), respectively. Immediately after the extract treatment each rat was administered PGE_2 (100 mg/kg in 5% (v/v) ethanol in normal saline, orally) in the group III, IV and V. All the rats were killed after 30 min and the whole length of the intestine from the pylorus to caecum was dissected out and its contents were collected in a test tube and volume was measured.[11]

Gastrointestinal motility test

In this study, the animals were divided into four groups of six rats each. They were fasted for 24 h before the test with free access to water. The Group I served as control (vehicle) while the Group II was administered standard drug atropine sulphate (5 mg/kg) intraperitoneally, Group III and IV was treated with ethanolic (200 and 400 mg/kg, orally) extracts. After 30 min they were orally administered 1 ml of charcoal meal (3% of charcoal in 2% aqueous tragacanth) after half an hour the rats were sacrificed and intestinal distance moved by the charcoal meal from pylorus to caecum was measured.[12]

Statistical Analysis

Results were expressed as mean±SEM, (n=6). Statistical analysis were performed with one way analysis of variance (ANOVA) followed by Dennett's t-test. P value less than <0.05 was considered to be statistically significant. *P<0.05, **P<0.01 and ***P<0.001, when compared with control and toxicant group as applicable.

RESULTS

Phytochemical Screening

The preliminary phytochemical investigation has revealed that the ethanolic extract of leaves of *Mimosa pudica* Linn said to contain alkaloids, carbohydrate, steroids, tannins, flavonoids, glycoside and saponins.

Selection of dose of the Extract

LD_{50} was done as per OECD guidelines for fixing the dose for biological evaluation. In LD_{50} studies, it was found that the animals were safe up to a maximum dose of 2000 mg/kg body weight. There were no changes in normal behaviour pattern and no signs and symptoms of toxicity and mortality were observed. The biological evaluation was carried out at doses (1/5th and 1/10th of LD_{50} cut off values) of 200 and 400 mg/kg body weight.

In castor oil induced diarrhoea, ethanolic extracts of *Mimosa pudica* leaves significantly reduced the mean weight of feces, Mean frequency of diarrhoea and mean number of fecal drops when compared to the control group [Table 1 and Figure 1].

In PGE_2 induced enteropooling, both doses have inhibited intestinal fluid accumulation in rats in dose dependant manner, when compared to PGE_2 control group. PGE_2 induced significant increase in fluid volume of the rat intestine when compared with vehicle control animals that received ethanol in normal saline [Table 2 and Figure 2].

In the third model the extracts has significantly decreased the propulsion of charcoal meal through the GIT and also in the Mean distance travelled by charcoal when compared with control group. However, the effect of the extract was found to be lesser than that of atropine in charcoal meal test [Table 3 and Figure 3].

Table 1: Effect of ethanolic extract of *Mimosa pudica* leaves on mean frequency of diarrhoea and their per cent protection in castor-oil induced diarrhoea in rats

Groups	Treatment	Dose (mg/kg)	Mean frequency of diarrhoea±SEM	Mean no. of fecal drops±SEM	Mean wt. of feaces±SEM after 4 hrs (gm)	Percent of protection
I.	Control	–	6.000±0.5774	8.000±1.5	3.335±0.14	–
II.	Standard (loperamide)	1	1.000±0.5164***	1.833±0.6***	0.6483±0.03***	80.99
III.	EEMP	200	3.000±0.5774**	3.833±0.6*	2.113±0.04**	36.13
IV.	EEMP	400	1.667±0.3333***	2.333±0.33**	1.075±0.03***	68.80

EEM – Ethanolic extract of *Mimosa pudica*. The values are Mean±SEM, n=6 *P<0.05, **P<0.01 and *** P<0.001 vs control

Table 2: Effect of ethanolic extracts of *Mimosa pudica* leaves on mean volume of intestinal fluid (ml) and their per cent protection in prostaglandin-E_2 induced diarrhoea in rats

Groups	Treatment	Dose	Mean volume of intestinal fluid (ml)±SEM	Per cent of inhibition
I.	Vehicle control	1 ml of 5% (v/v) ethanol and normal saline p.o.	1.567±0.08819	–
II.	PGE_2 control	PGE_2 100 µg/kg	3.367±0.09189	–
III.	Standard (loperamide)	5 mg/kg	1.690±0.03890***	79.21
IV.	EEMP	200 mg/kg	2.400±0.08760*	43.85
V.	EEMP	400 mg/kg	1.952±0.05850**	71.49

EEMP – Ethanolic extract of *Mimosa pudica*. The values are Mean±SEM, n=6 *P<0.05, **P<0.01 and *** P<0.001 vs control

Table 3: Effect of ethanolic extracts of *Mimosa pudica* leaves on mean movement of charcoal and their % inhibition in gastro-intestinal motility test in rats

Group	Treatment	Dose (mg/kg)	Mean length of intestine±SEM (cm)	Mean distance travelled by charcoal meal±SEM (cm)	Mean % movement of charcoal±SEM (cm)	Per cent of inhibition
I.	Control	–	106±0.7303	82.33±1.764	75.69±0.4357	–
II.	Standard (atropine sulphate)	5	102.7±1.022	53.33±0.8819	50.79±0.8274***	50
III.	EEMP	200	97.17±1.014	73.67±1.282	71.08±0.2288*	28.57
IV.	EEMP	400	104.2±0.7491	62.50±0.7638	63.40±0.1110**	36.27

EEMP – Ethanolic extract of *Mimosa pudica;* The values are Mean±SEM, n=6; *P<0.05. ** P<0.01 and *** P<0.001 vs control

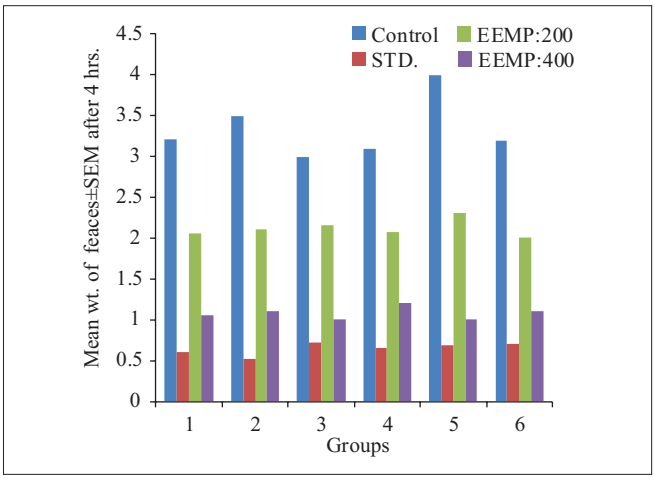

Figure 1: Effect of ethanolic extracts of *Mimosa pudica* leaves on mean weight of feaces in castor-oil induced diarrhoea in rats

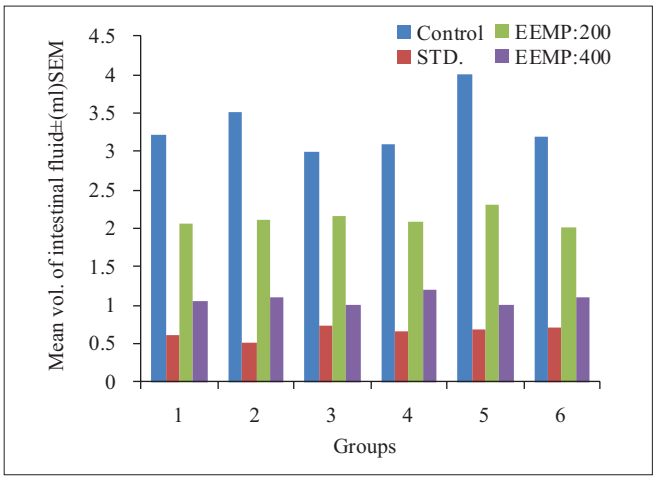

Figure 2: Effect of ethanolic extracts of *Mimosa pudica* leaves on mean volume of intestinal fluid in prostaglandin-E2 induced enteropooling model

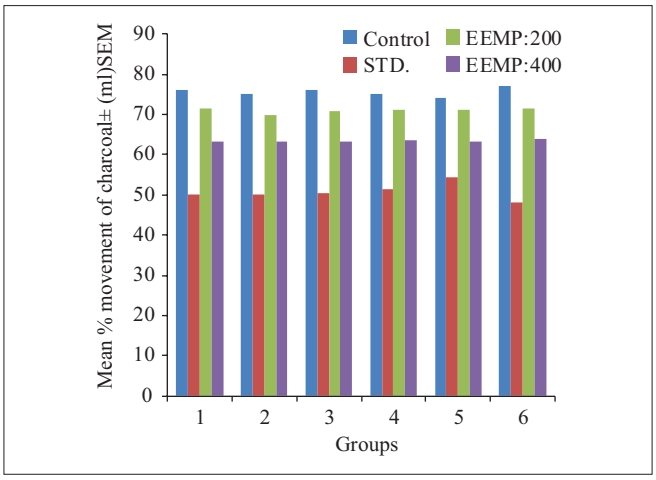

Figure 3: Effect of ethanolic extracts of *Mimosa pudica* leaves on mean per cent movement of charcoal meal in gastro-intestinal motility test

DISCUSSION

The *Mimosa pudica* Linn is used for the various gastrointestinal diseases in the folk medicine. Previous reports have demonstrated anti-diarrhoeal activity of tannins[13] and flavonoids[14] containing plant extracts. Tannins can evoke an anti-diarrhoeal effect since these substances may precipitate proteins of the electrolytes and reduce peristaltic movement and intestinal secretions.[15,16] The anti-diarrhoeal activity of flavonoids has been ascribed to their ability to inhibit intestinal motility and hydro electrolytic secretion[17,18] which are known to be altered in this intestinal condition. The preliminary phytochemical investigation of the Leaves of *Mimosa pudica* Linn showed the presence of tannins and flavonoids. Therefore, in our study the possible role for the significant anti-diarrhoeal property of ethanolic extract of leaves of *Mimosa pudica* may due to the presence of these phytochemicals.

CONCLUSION

On the basis of the present results and available reports, it can be concluded that the anti-diarrhoeal activity elucidated by *Mimosa pudica* leaves extract could be mainly due to its inhibitory effect both on gastrointestinal propulsion and fluid secretion. The inhibitory effect of the extracts justifies the use of the plant as a non-specific anti-diarrhoeal agent in folk medicine.

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