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Anti-ulcer activity of *Mimosa pudica* leaves against gastric ulcer in rats

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ABSTRACT

The effect of Methanolic, chloroform and diethyl ether extracts of *Mimosa pudica* was investigated in rats to evaluate the anti-ulcer activity by using three models, i.e. Aspirin, Alcohol and pyloric ligation models experimentally induced gastric ulcer. The parameters taken to assess anti-ulcer activity were volume of gastric secretion, PH, free acidity, total acidity and ulcer index. The results indicate that the alcoholic extract significantly ($P < 0.001$) decreases the volume of gastric acid secretion, PH, free acidity, total acidity and ulcer index with respect to control.

Keywords: *Mimosa pudica*, anti-ulcer, anti-secretory

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INTRODUCTION

Peptic ulcer is the most common gastrointestinal disorder in clinical practice. Considering the several side effects (arrhythmia's, impotence, funaecomastia and haematopoeitic changes) of modern edicine [1], indigenous drugs possessing fewer side effects should be looked for as a better alternative for the treatment of peptic ulcer. There is evidence concerning the participation of reactive oxygen species in the etiology and pathophysiology of human diseases, such as neurodegenerative disorders, inflammation, viral infections, autoimmune pathologies and digestive system disorders such as gastrointestinal inflammation and gastric ulcer [2]. The study assumes significance in the context that prolonged use of synthetic anti-ulcer drugs leads to adverse drug reactions and a search for new anti-ulcer agents that retain therapeutic efficacy and are devoid of adverse drug reaction is warranted. A study of the efficacy of an extracts of *Mimosa pudica* in gastric ulcer with pylorus ligation, alcohol and aspirin-induced ulcer was undertaken in a rat model.

Mimosa pudica [3,4]. (Fabaceae) known as Chue Mue, is a stout stragling prostrate shrubby plant with the compound leaves which gets sensitive on touching, spinous stipules and globose pinkish flower heads, grows as weed in almost all parts of the country. Leaves and stems of the plant have been reported to contain an alkaloid mimosine, leaves also contain mucilage and root contains tannins. *Mimosa pudica* is used for its anti-hyperglycemic, antidiarrhoeal, anti-convulsant and cytotoxic properties.

The plant also contains turgorins, leaves and roots are used in treatment of piles and fistula. Paste of leaves is applied to hydrocele. Cotton impregnated with juice of leaves is used for dressing sinus. Plant is also used in the treatment of sore gum and is used as a blood purifier. In ayurvedic and unani system of medicine, this plant has been used in diseases arising from corrupted blood and bile, billious fever, piles, jaundice, leprosy, ulcers, small pox. These plants are found to posses polyphenolic constituents like flavonoids, Quercetin, Naringin, Saponins, glycosides, tannins, gums and mucilages. Hence in the present study, the *Mimosa pudica* plant has been selected for investigate the anti-ulcer study.

MATERIALS AND METHODS

Plant material

The plant material was collected from Thaniparai hills, near watrap, tamilnadu. It was authenticated by Dr. Stephan, Dept of Botany, The American college, Madurai. The plant was collected in the month of May 2008 and shade dried at room temperature.

Extract Preparation [5]

The leaves were shade-dried and made into a coarse powder which was passed through a 40-mesh sieve to get a uniform particle size and then used for extraction. A weighed quantity

(500 g) of the powder was then subjected to continuous hot extraction in Soxhlet apparatus with methanol, chloroform and Diethyl ether and the residual marc was collected. The extract was filtered through a cotton plug, followed by whatman filter paper (no.1). The extract was evaporated under reduced pressure using a rotovac evaporator at a low temperature (40-60°C) until all the solvent had been removed to give an extract sample with a yield of 18% w/w, 16 %w/w and 13% w/w in relation to the dried starting material. Preliminary Phytochemical analysis was carried out to identify presence of Phytoconstituents in the crude extract.

Preliminary phytochemical analysis

The various extracts of *Mimosa pudica* were then subjected to preliminary phytochemical [6] analysis to assess the presence of various phytoconstituents, it revealed that the presence of alkaloids, steroids, polyphenolic constituents like flavonoids, quercetin, naringin, saponins, glycosides, tannins, gums and mucilages. Preliminary Thin layer chromatography studies also confirmed these constituents [7].

Animals

Wistar albino rats weighing 150-200g of either sex maintained under standard husbandary conditions (temp 23±2°C, relative humidity 55±10% and 12 hours light dark cycle) were used for the screening. Animals were fed with standard laboratory food and ad libitum during the study period. The experimental protocol has been approved by institutional animal ethics committee, Arulmigu Kalasalingam College of Pharmacy, Krishnankoil. (Regd No.509/02/C/CPCSEA/2002.) India.

Toxicity studies

Acute toxicity study was performed for various extracts of *Mimosa pudica* according to the acute toxic classic method as per OECD guidelines [8]. (Ecobichon, 1997). Female albino rats were used for acute toxicity study. The animals were kept fasting for overnight providing only water, after which the various extracts were administered orally at the dose of 300mg/kg and observed for 14 days. If mortality was observed in two animals out of three animals, then the dose administered was assigned as toxic dose. If the mortality was observed in one animal, then the same dose was repeated to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher doses such 400,500 & 2000mg/kg body weight. The animals were observed for toxic symptoms for 72 h.

Aspirin-induced gastric ulcer

In the aspirin-induced ulcer experiments [9], three groups of albino rats (150–200 g), with each group consisting of six animals were used. The first group served as a control group, the second group served as positive control and the third group served as the test group. The second and third groups were treated respectively with ranitidine (20 mg/kg) and methanolic,

chloroform and diethyl ether extracts of *Mimosa pudica* (100 & 200 mg/kg), orally for 8 days. Control animals received normal saline (2 ml/kg) for 8 days. After 8 days of treatment, animals were fasted for 24 h. Ulcer was produced by administration of aqueous suspension of aspirin (a dose of 200 mg/kg orally) on the day of sacrifice. The animals were sacrificed 4 h later and stomach was opened to calculate the ulcer index by Kunchandy method [10].

Alcohol-induced gastric ulcer

The male rats were randomly divided into three groups and fasted for 24 h with free access to water. Animals were given vehicle or Methanolic, chloroform and diethyl ether extracts of the *Mimosa pudica* at a dose of 100 and 200 mg/kg or Ranitidine (20 mg/kg) orally. One hour later, 1 ml of 80% ethanol was administered orally to each animal [11]. Animals were sacrificed by cervical dislocation, one hour after ethanol administration, stomachs were isolated and cut open along the greater curvature and pinned on a soft board. The length of each gastric lesion was measured and the lesion index was expressed as sum of the length of the entire lesion in mm.

Pylorus- ligation induced gastric ulcer

Male albino rats weighing 150-200g were selected for pyloric ligation ulcer model [12]. Rats were divided into three groups, each group consisting of six animals. Animals were fasted for 24 h. One group received normal saline 2 ml/kg (negative control), the second group received Ranitidine 20 mg/kg by oral route (positive control) and the third group received Methanolic, chloroform and diethyl ether extracts *Mimosa pudica* (100 & 200 mg/kg) by oral route, 30 min prior to pyloric ligation. Animals were sacrificed 4 h later and the stomach was opened to collect the gastric contents. The total volume of gastric content was measured. The gastric contents were centrifuged at 1000 rpm for 10 min. One ml of the supernatant liquid was pipetted out and diluted to 10 ml with distilled water. The solution was titrated against 0.01N NaOH using Topfer's reagent as indicator, to the endpoint when the solution turned to orange colour. The volume of NaOH needed was taken as corresponding to the free acidity. Titration was further continued till the solution regained pink colour. The volume of NaOH required was noted and was taken as corresponding to the total acidity. Acidity was expressed as:

$$\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Normality} \times 100}{0.1} \text{ mEq/l.}$$

Statistical analysis

The values Mean \pm SEM are calculated for each parameter. For determining the significant inter group difference each parameter was analysed separately and one-way analysis of variance [13] (Gennaro, 1995) was carried out and the individual comparisons of the group mean values were done using Dunnet's test [14] (Dunnet, 1964).

RESULTS

Preliminary phytochemical screening revealed the presence of Alkaloids, Steroids, polyphenolic constituents like flavonoids, Quercetin, Naringin, Saponins, glycosides, tannins, gums and mucilages. Acute toxicity studies of the various extracts of the *Mimosa pudica* did not exhibit any signs of toxicity up to 2 g/kg body weight. Since there was no mortality of the animals found at high dose. Hence 100 and 200 mg/kg dose of the extract selected for evaluation of anti-ulcer activity.

Aspirin induced ulcer

Table 1 summarizes the results obtained in the experimental model of aspirin-induced gastric ulceration in rats. The Methanolic extract was found to possess remarkable ulcer-protective properties at 100 and 200 mg/kg when compare to other two extracts. The maximum effect of ulcer protection (70.46%),(57.84%)&(46.46%) were produced at 200 mg/kg for methanolic, chloroform and diethyl ether extracts, and the standard drug (Ranitidine 20 mg/kg) gave 81.53% of ulcer protection (Table 1).

Alcohol induced ulcer

Pretreatment of rats with *Mimosa pudica* extracts produced a dose dependent protection in the ethanol induced ulceration model as compared to control group. However the protection was statistically significant reduced the severity of ulcer and caused a significant reduction of ulcer index in this model. Ranitidine produced significant gastric ulcer protection as compared to control group (Table 1).

Pylorus ligation induced ulcer

The Methanolic, chloroform and diethyl ether extracts of the *Mimosa pudica* in the doses of 100 and 200 mg/kg produced a reduction in the ulcer index, gastric volume, free acidity, total acidity and raised gastric pH significantly in comparison with control group. Ranitidine reference drug produced significant reduction gastric ulcer and total acid output as compared to control group (Table 2). The results of the present study indicate that the methanolic extract of *Mimosa pudica* significantly reduces the total volume of gastric juice, free and total acidity of gastric secretion and also has activity against gastric ulcers in rats when compare to other two extracts. (Figure 1) The control animals had ulcers and haemorrhagic streaks, whereas in animals administered with the extracts of *Mimosa pudica* there was significant reduction in ulcer index ($P < 0.001$) (Figure 2).

Table 1. Effect of various extracts of *Mimosa pudica* against Aspirin and Alcohol induced gastric ulcer in rats

Treatment	Dose (mg/kg) p.o	Aspirin		Alcohol	
		Ulcer Index	% of ulcer protection	Ulcer Index	% of ulcer protection
Control (Normal saline)	2ml/kg	6.5± 0.50	–	6.5± 0.50	–
Standard (Ranitidine)	20mg/kg	1.20± 0.24	81.53***	1.20± 0.24	81.53***
Methanolic Extract	100mg/kg	4.14± 0.24	36.30*	4.10± 0.22	36.92*
	200mg/kg	1.92± 0.32	70.46***	1.98± 0.38	66.53***
Chloroform Extract	100mg/kg	4.68± 0.28	28.00*	4.62± 0.25	28.92*
	200mg/kg	2.74± 0.36	57.84**	2.68± 0.33	58.76**
Diethyl Ether Extract	100mg/kg	4.86± 0.29	25.23*	4.82± 0.26	25.84*
	200mg/kg	3.48± 0.33	46.46**	3.42± 0.29	47.38**

Results are mean ± S.E.M.(n = 6). Statistical comparison was performed by using ANOVA coupled with student 't' test.* P<0.05, ** P<0.01, *** P<0.001 were consider statistically significant when compared to control group.

Table 2. Effect of various extracts of *Mimosa pudica* against Pylorus ligation induced gastric ulcer in rats

Treatment	Dose (mg/kg) p.o	Volume of gastric juice(ml/4h)	PH	Free Acidity (mEq/L)	Total Acidity (mEq/L)	Ulcer Index	%Inhibition of ulcer
Control (Normal saline)	2ml/kg	4.02± 0.11	1.84± 0.14	26.84± 0.08	70.16± 0.30	3.68± 0.56	–
Standard (Ranitidine)	20mg/kg	1.94± 0.06	4.96± 0.18	10.42± 0.02	22.24± 0.18	0.71± 0.14	80.70***
Methanolic Extract	100mg/kg	3.66± 0.16	3.12± 0.14	21.18± 0.05	52.14± 0.38	2.34± 0.24	36.41*
	200mg/kg	2.40± 0.14	4.56± 0.18	11.76± 0.06	30.62± 0.26	1.10± 0.29	70.10***
Chloroform Extract	100mg/kg	3.79± 0.16	3.20± 0.14	22.96± 0.08	60.48± 0.24	2.62± 0.36	28.80*
	200mg/kg	3.28± 0.21	3.88± 0.16	13.68± 0.02	35.45± 0.33	1.52± 0.44	58.69**
Diethyl Ether Extract	100mg/kg	3.86± 0.14	2.86± 0.14	25.54± 0.04	64.16± 0.19	2.76± 0.49	25.00*
	200mg/kg	3.68± 0.12	3.24± 0.16	16.62± 0.06	39.52± 0.32	1.96± 0.56	46.73**

Results are mean ± S.E.M.(n = 6). Statistical comparison was performed by using ANOVA coupled with student 't' test.* P<0.05, ** P<0.01, *** P<0.001 were consider statistically significant when compared to control group.

Fig. 1 Effect of various extracts (200mg/kg) of *Mimosa pudica* against Aspirin, Pylorus ligation and Alcohol induced gastric ulcer in rats

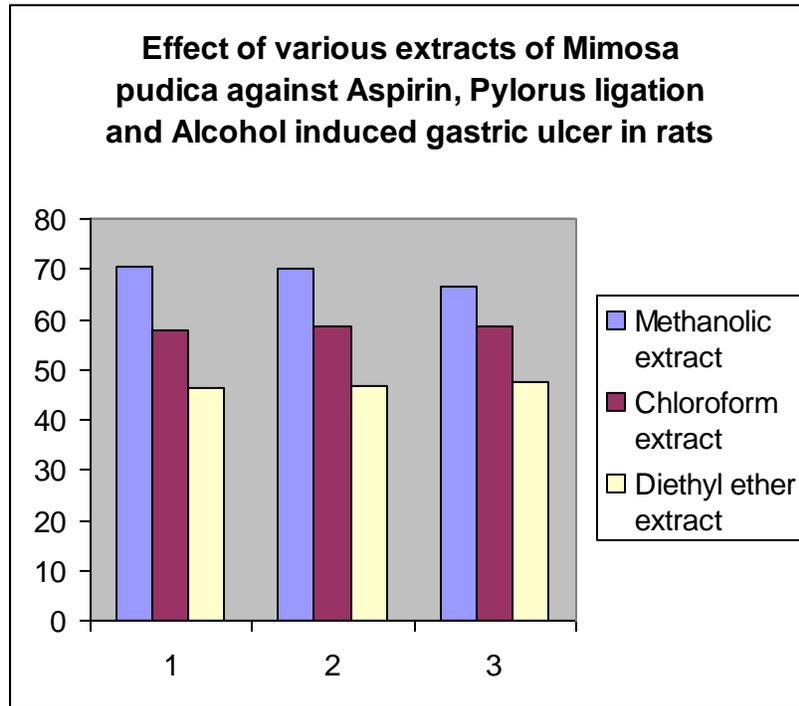
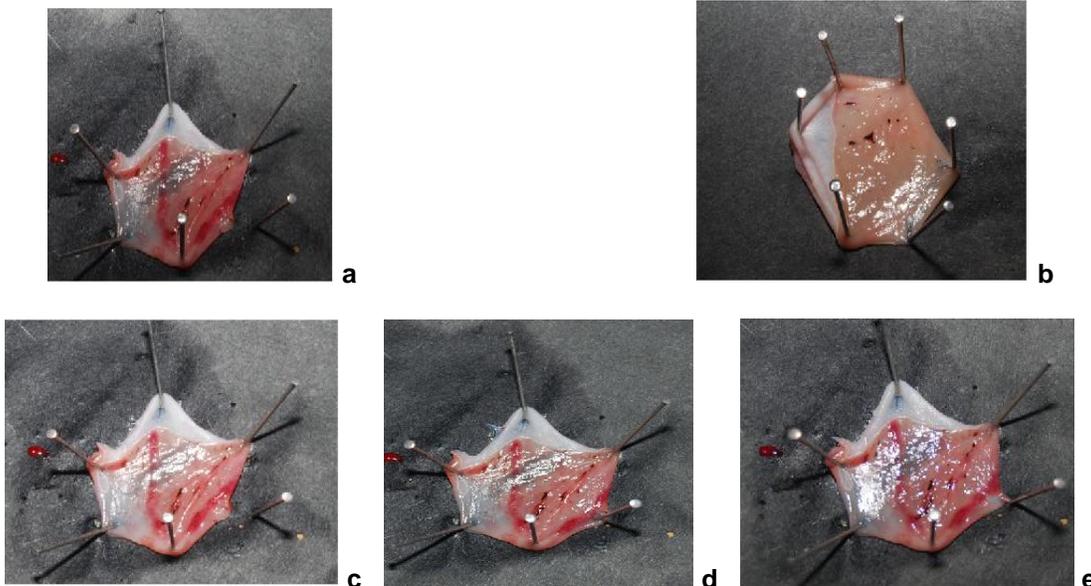


Fig. 2 : a, Stomach of control rat; b, standard drug treated; c, Effect of methanolic extract on aspirin induced gastric ulcer in rat; d, Effect of methanolic extract on alcohol induced gastric ulcer in rat; e, Effect of methanolic extract on pyloric ligation induced gastric ulcer in rat



DISCUSSION

The anti-ulcer activity of the plant of *Mimosa pudica* was evaluated by employing aspirin, alcohol and pylorus ligation ulcer models. These models represent some of the most common causes of gastric ulcer in humans. Many factors and mechanisms are implicated in the ulcerogenesis and gastric mucosal damage induced by different models employed in the present study involving, depletion of gastric wall, mucin mucosal damage induced by non-steroidal anti-inflammatory drugs and free radical production [15]. NSAID's like aspirin causes gastric mucosal damage by decreasing prostaglandin levels through inhibition of PG synthesis [16]. Methanol extract of the plant of *Mimosa pudica* was significantly effective in protecting gastric mucosa against aspirin induced ulcers at all the dose level studied. Ethanol induced gastric injury is associated with significant production of oxygen free radicals leading to increased lipid peroxidation, which causes damage to cell and cell membrane [17]. The extracts of the *Mimosa pudica* has significantly protected the gastric mucosa against ethanol challenge as shown by reduced values of lesion index as compared to control group suggesting its potent cytoprotective effect. It has been proposed that in pyloric ligation, the digestive effect of accumulated gastric juice and interference of gastric blood circulation are responsible for induction of ulceration [18]. The antiulcer activity of *Mimosa pudica* extracts in pylorus ligation model is evident from its significant reduction in gastric volume, total acidity, free acidity, ulcer index and increase in pH of gastric juice. Because of animals treated with *Mimosa pudica* extracts significantly inhibited the formation of pylorus ulcer in the stomach and also decreased both acid concentration, gastric volume and increased the pH values. It is suggested that *Mimosa pudica* extracts can suppress gastric damage induced by aggressive factors.

It is generally accepted that gastric ulcers result from an imbalance between aggressive factors and the maintenance of the mucosal integrity through endogenous defence mechanisms [19]. The excess gastric acid formation by prostaglandin (PG) includes both increase in mucosal resistance as well as a decrease in aggressive factors, mainly acid and pepsin[20]. Inhibitions of PG synthesis by aspirin coincide with the earlier stages of damage to the cell membrane of mucosal, parietal and endothelial cells[21]. The preliminary phytochemical studies revealed the presence of flavonoids in methanolic extract of *Mimosa pudica*; various flavonoids have been reported for its anti-ulcerogenic activity with good level of gastric protection [22,23]. So the possible mechanism of antiulcer action of *Mimosa pudica* may be due to its flavonoid content. In this study we observed that *Mimosa pudica* provides significant anti-ulcer activity against gastric ulcers in rats.

CONCLUSION

On the basis of the present results and available reports, it can be concluded that the anti-ulcer activity elucidated by *Mimosa pudica* could be mainly due to the modulation of defensive factors through an improvement of gastric cytoprotection and partly due to acid inhibition.

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