



Hygeia
Journal for drugs and medicines
April 2012-September2012
OPEN ACCESS

rid/C-3366-2012

www.hygeiajournal.com

A half yearly scientific, international, open access journal for drugs and medicines

Research article section: Pharmacognosy and Phytochemistry

Antiulcer activity of the Leaf ethanolic extract of *Mimosa pudica* in Rats

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Article history: Received: 19 January, 2012, revised: 25 Jan.2012, accepted: 15 February 2012, Available online: 5April 2012

Abstract:

Plan: The present study was designed to investigate the antiulcer potential of ethanolic extract of *Mimosa pudica* leaves.

Methodology: Antiulcer activity was evaluated by pylorus ligation, aspirin and ethanol induced ulcer models. The ethanolic extract of the leaves of *Mimosa pudica* was given by oral route at a dose of 100 mg/kg b.w.

Outcome: Ethanolic extract of *Mimosa pudica*, dose dependently reduce, the total acidity, ulcer index, and an increase in pH of gastric juice in pylorus ligated ulcer model. Extract of *Mimosa pudica* may be useful as a natural antioxidant in the treatment of ulcer.

Keywords: Antiulcer Activity, *Mimosa pudica*, leaf ethanolic extract

1. Introduction

Herbal drugs constitute a major part of therapeutics in all the traditional systems of medicine. Herbal medicine is a triumph of popular therapeutic diversity. There are evidences for the participation of reactive oxygen species in the etiology and pathophysiology of human disease, such as neurodegenerative disorders, inflammation, viral infections, autoimmune, gastrointestinal inflammation and gastric ulcer.

“Peptic ulcer disease” refers to breaks in the mucosa at the stomach and small intestine, principally the proximal duodenum, which are produced by the action of gastric secretion. Peptic ulcer is one of the major gastro intestinal disorders, which occurs due to an imbalance between the offensive (gastric acid secretion) and defensive (gastric mucosal integrity) factors. Consequently, reduction of gastric acid production as well as re-improvement of gastric mucosal production has been the major approaches for therapy of peptic ulcer disease. As a result drugs, of both herbal and synthetic origin are coming up offering newer and better options for treatment of peptic ulcer. The type of drugs varies from being proton-pump inhibitors to H₂ antagonist or a cytoprotective agent.

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<http://www.researcherid.com/rid/C-3366-2012>

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At the same time, each of these drugs confers simpler to several side effects like arrhythmias, impotence, gynaecomastia, hyperplasia and haemopoetic changes. This has been the major stimulus for the development of new antiulcer drugs and anti-inflammatory for novel molecules has been extended to herbal drugs that offer better protection and decreased relapse. Medicinal plants provide an important source of new chemical substances with potential therapeutic effects. These have been used in traditional medicine for the treatment of several diseases ^[1].

In view of the reported active constituents present in the various parts of the plant *Mimosa pudica* like flavonoides, alkaloids, sesquiterpenes, diterpenes, saponins etc. and its use in folk lore medicine.

2. Material and Methods

2.1. Preparation of the leaf extract

The authenticated *Mimosa pudica* leaves were shade dried and powdered coarsely. Extraction was done according to standard procedures using analytical grade solvents. The powdered drug was defatted by extracting with pet-ether (60-80°C). The coarse powder of the leaves was Soxhlet extracted with 90% ethanol. The aqueous extract was prepared by the processes of maceration ^[2].

2.2 Animals

The healthy Wistar albino rats of either sex weighing between 150-200 g were taken for the study. They were housed under controlled conditions of temperature (23±2°C), humidity (55±5%) and 12 h light and 12 h dark cycles. The animals were fed with standard pellet diet and water *ad libitum*.

2.3. Acute toxicity study

Acute toxicity studies for ethanolic extracts as *mimosa pudica* were conducted as per OECD guidelines 423 using albino wistar rats. Each animal was administered ethanolic solution of the extract by oral route. The animals were observed for any changes continuously for the first 2 h and upto 24 h for mortality ^[3].

2.4. Antiulcer activity:

Animals were randomly divided into six groups of six animals each. Group I served as control, Group II to VI were the drug treated groups. *Mimosa pudica* ethanolic extract 100 mg/kg body weight and ranitidine 20 mg/kg body weight respectively by oral route for a period of 5 days in both aspirin as well as ethanol induced ulcer models.

2.4.1. Ethanol induced ulcers:

On day 5, one hour after the administration of *Mimosa pudica* extracts / Ranitidine all the animals received absolute ethanol (1ml/rat p.o.). One hour after administration of ethanol, the animals were sacrificed and the stomach was then excised and cut along the greater curvature, washed carefully with 5.0 ml of 0.9% NaCl and ulcers were scored by a person unaware of the experimental protocol in the glandular portion of stomach. ^[4].

2.4.2. Pylorus ligation:

Ranitidine 20mg/kg was used as standard drug. After keeping the animals on fasting overnight, one hour after the administration of extracts/Ranitidine, pylorus ligation was done under ether anesthesia. Four hours after pylorus ligation, rats were sacrificed, stomach were isolated and opened along the greater curvature. Gastric fluid was collected for measurement of total gastric volume and estimation of free and total acidity. ^[5].

2.4.3. Aspirin induced ulcer:

On the 5, Aspirin at dose of 200 mg/kg was administered to the animals of all the groups (I to VI) one hour after the administration of last dose of the extract/Ranitidine. Four hours after the administration of Aspirin, the animals were sacrificed and the stomach was then excised and cut along the greater curvature, washed carefully with 5.0 ml of 0.9% NaCl and ulcers were scored by a person unaware of the experimental protocol in the glandular portion of stomach. Ulcer index has been calculated by adding the total number of ulcers per stomach and the total severity of ulcers per stomach. The total severity of the ulcers was determined by recording the severity of each ulcer. ^[6].

The numbers of ulcers is noted and the severity recorded with the following scores:

Normal coloration: 0, Red coloration; 0.5, Spot ulcer: 1.0, Hemorrhagic stress: 1.5

Ulcer ≥ 3 but ≤ 5 : 2, Ulcer > 5 : 3, Ulcer index (UI) was calculated using the formula ^[7], $UI = U_S + U_N + U_P \times 10^{-1}$,

Where, U_S = Mean severity of ulcer score., U_N = Average numbers of ulcer per animal

U_P = Percentage of animals with ulcer incidence,

$$\% \text{ protection from ulcer} = \frac{C_{UI} - T_{UI}}{UI}$$

Where, C_{UI} = Ulcer index of control groups T_{UI} = Ulcer index of treated groups

2.4.4. Statistical analysis:

The interpretation of the results was done after subjecting the data obtained from various studies to statistical analysis which included one way ANOVA followed by test like Dunnett and Tukey. $P < 0.05$ is considered as statistically significant.

3. Results

Acute toxicity studies for ethanolic extract of *mimosa pudica* were conducted as per OECD guidelines were 423 using albino wistar rats. The animals were observed for any changes continuously for the first 2 h and upto 24 h for mortality. There was no mortality and noticeable behavioral changes in all the groups tested. The extracts were found to be safe upto 2000mg/kg body weight.

At the end of the study, the stomach was isolated and washed with saline, it was then observed for ulceration and ulcers were scored, Ulcer index and percentage protection against ulcers was calculated.

Mimosa pudica extract at the dose of 100mg produced significant ($P<0.01$) reduction in the ulcer score when compared to the control. The percentage protection against ulcers by ranitidine, MPE 100 was found to be 66.96 (Table 1)

The control animals showed ulceration, redness, and hemorrhagic streaks, after pylorus ligation. There was also an increase in gastric volume, free acidity, total acidity and pH. MP extract at the 100 mg dose and ranitidine produced significant ($P<0.01$) reduction in ulcer score when compared to the control. The percentage protection against ulcers by ranitidine, MPE 100mg was found to be 49.22. *Mimosa pudica* ethanolic extract at 100mg dose and ranitidine significantly ($P<0.01$) reduced the gastric volume when compared to control. Extracts produced better ($P<0.01$) reduction in gastric volume, when compared to ethanolic extracts and ranitidine. Ethanolic extract of *mimosa pudica* at the doses significantly ($P<0.01$) reduced free acidity when compared to control.

Significant ($P<0.01$) reduction in ulcer score was production by ranitidine, ethanolic extract at the does 100mg body weight, when compared to the control.

4. Discussion:

It is generally accepted that gastric ulcers results from an imbalance between aggressive factors and the maintenance of the mucosal integrity through the endogenous defense mechanism^[8]. The role of free radicals is also reported in the indication of ulcers. Prostaglandins (PG) offer protection to duodenum through both increases in mucosal resistance as well as decrease in aggressive factors, mainly acid and pepsin^[9]. Ethanol induced gastric ulcers have been widely used for the evaluation of gastro protective activity. Ethanol is metabolized in the body and releases superoxide anion and hydroperoxy free radicals.

The incidence of ethanol induced ulcers is predominant in the glandular part of stomach. It was reported to stimulate the formation of leukotriene C4 (LTC₄), mast cell secretory products and reactive oxygen species resulting in the damage of rat gastric mucosa^[10]. It has been found that oxygen derived free radicals are implicated in the mechanism of acute and chronic ulceration in the gastric mucosa and scavenging these free radicals can play an appreciable role in healing these ulcer^[11].

When aspirin is in the lipid soluble undissociated form it can damage the gastric mucosa. Aspirin causes a dose dependent reduction in mucosal prostaglandins – PGE₂ and PGI₂ bio-synthesis accompanied by an increase in the mean area of gastric ulcerations.

Aspirin is known to inactivate irreversibly the PG synthetase system, which mediates synthesis of prostaglandin in the mucosa^[12, 13]. An increase in acid secretion and back diffusion at H⁺ ions is also noticed. It is reasonable to assume that the observed gastric mucosal lesions induced by aspirin are due to a deficiency of mucosal prostaglandin^[14]. Aspirin induced ulcer is mediated through tissue damaging free radicals, which are produced from the conversion of hydroperoxyl to hydroxyl fatty acids, which leads to cell destruction. The hydroperoxyl fatty acids are generated from the degeneration of mast cells and generalized lipid per oxidation accompanying cell damage^[11].

Pylorus ligation induced ulcers are due to auto digestion at the gastric mucosa and breakdown of the gastric mucosal barrier. In case of pyloric ligation, ulcer formation is mainly due to the stasis at the gastric juice and stress^[4, 15].

Mimosa pudica produced antiulcer activity in all the three models taken up for the study. Ethanolic extract at the doses, reduces ulcer incidence significantly ($P < 0.01$) when compared to the control as evident by decrease in ulcer score in all the three models. Protection against ulcerations in aspirin and ethanol induced ulcer models indicate cytoprotective action by extracts of *mimosa pudica*.

Anti-secretory activity of the extracts was noticed in pylorus ligation induced ulcer model. There was decrease in gastric volume and reduction in free and total acidity in the animals treated with ethanolic extract. *Mimosa pudica* is reported to contain quercetin^[16] apart from other flavonoids, tannins, alkaloids and terpenoids. From the phytochemical test done on the extracts of *mimosa pudica*, it was confirmed that the same classes of active constituents were present.

Quercetin is reported to prevent gastric mucosal lesions induced by various models (pylorus ligation, ethanol induced, cold restraint stress). Quercetin may increase the amount of natural glycoproteins, the most important proteins in the gastric mucosa, which may in turn facilitate the defence against an aggressive action. Quercetin also stimulates the synthesis of cyclooxygenase and of local prostaglandins.

Other mechanism proposed includes inhibition of the gastric proton pump, lipoxygenase pathway, or inhibition of lipid peroxidation^[17]. *Mimosa pudica* may owe its antiulcer activity to its active constituents like flavonoids and especially quercetin. From the phytochemical tests done on the extracts of *mimosa pudica*, it was confirmed that the same classes of active constituents were present.

5. Conclusion

Ethanollic extract of *Mimosa pudica* (MP) reduced ulcer incidence, when compared to the control as evident by decrease in ulcer score in all the three models. Anti-secretory activity of the extracts was noticed in pylorus ligation induced ulcer model. There was decrease in gastric volume and reduction in free and total acidity in the treated with ethanolic extract. This indicates that the leaf extracts of *Mimosa pudica* has antiulcer activity.

Table 1: Effects of ethanolic extract of mimosa pudica on ethanol induced ulcer model

Treatment	Ulcer Score	Ulcer Index	Percentage protection
Control	5.66±0.51	11.03	-----
Ranitidine	0.16±0 ^{**a}	0.01	95.32
MP 100	1.66±0.98 ^{**a}	3.64	66.96

n=6, mean ± SD, *P<0.05, **P<0.01, a=indicates comparison with control groups, MP: *Mimosa pudica* leaf Ethanolic extract

Table 2: Effects of ethanolic extract of mimosa pudica on pylorus ligation model

Treatment	Volume of gastric juice	Free acidity	Total acidity	pH
Control	8.36±0.36	36.13±1.27	51.81±0.60	2.59±0.33
Ranitidine	5.12±0.22 ^{**a}	9.5±0.20 ^{**a}	20.49±0.51 ^{**a}	5.64±0.13 ^{**a}
MP 100	5.92±0.51 ^{**a,*b}	9.68±1.66 ^{**a,*b}	3.98±2.53 ^{**a,*b}	3±0.68

n=6, mean ± SD, *P<0.05, **P<0.01,***P<0.001,

Table 3: Effects of ethanolic extract of mimosa pudica on aspirin induced ulcer model

Treatment	Ulcer Score	Ulcer Index	Percentage protection
Control	5.83±0.40	11.06	-----
Ranitidine	0.83±0.57 ^{**a}	0.083	99.24
MP 100	1.33±1.16 ^{**a,*b}	1.865	83.13

n=6, mean ± SD, *P<0.05, **P<0.01

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