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Research Article

Preliminary Investigation of *Mimosa pudica* Linn Seeds Extracts for Antiulcer Potential on Experimental Albino Rats

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ABSTRACT

Ulcer is a common disorder in oral mucosa and gastrointestinal. Number of synthetic drugs are available to treat ulcers but these drugs have undesirable side effects. The present study aimed to investigate Preliminary screening of *Mimosa pudica* Linn seeds for oral and stomach ulcers. Plant material of both plants were extracted with different organic solvents and yield was calculated. Preliminary phytochemical tests were applied for the detection of presence of different chemical constituents in petroleum ether, chloroform, ethyl acetate, ethanol and aqueous extracts. All these extracts were also screened for anti-ulcer effect on ethanol induced stomach ulcer and glacial acetic acid oral ulcer. The effect was observed by the measurement of ulcer index, percentage inhibition, pH Total acidity, and gastric volume. Biochemical assessment of antioxidant substances and pro-inflammatory mediators (TNF-α, IL-6 and PGE-2) were done in the mucosal tissues. Phytochemical screening revealed the presence of steroids, terpenoids, and fatty acid in petroleum ether and alkaloids, tannins and phenolic compounds in chloroform extract of *M. pudica* Linn seeds the ethyl acetate and ethanol extract contain flavonoids, tannins and phenolic compounds, glycosides. Results of Preliminary pharmacological screening was showed that ethanolic extract of *M. pudica* Linn seeds with 200 and 400 mg/kg b.w. dose have significant anti-ulcer potential and is comparable to the standard (Omeprazole and ORASORE Gel). The significant restoration of antioxidant substance and inflammatory mediators indicated supporting mechanism of ulcer healing of ethanolic extract. In conclusion, the anti-ulcer effects were showed in dose-dependent manner. The potential anti-ulcer effect may be due to presence of flavonoid components i.e., quercetin, luteolin, etc. in the ethanolic extract of *M. pudica* Linn seeds.

Introduction

Ulcer is most common disease in India in any part of the body. It ruptures the mucous membrane or soft tissues normally in the mouth. Oral ulcer generally seems to be a occur in the mucous membrane and typically has white or yellow color and very painful. An ulcer in the tissue susceptible to infection leads to inflammation and tissue necrosis. Our country's prevalence rate of oral ulcer is more than 10%.[1] Oral ulcer is superficial lesion which vary in size and shape from round to oval. They often occur on the lip, checks and tongue edges, usually accompanied by congestion and local pain.it is known as oral mucosal pain.

Peptic ulcer disease (stomach and duodenal ulcers) is one of the most painful diseases. Stomach ulcers, a communal investigational gastro-intestinal demonstration accompanied by augmented oxidative pressure and inflammation and disruption of the mucosal fence of gastric coating. Vulnerability to ulcers is amplified by destructive issues. Endogenic antagonistic reasons can cause stomach lesions such as smoking, non-steroidal anti-inflammatory treatment (NSAID) medicines, ethanol, *Helicobacter pylori* contagion. excessive production of HCl and pepsin, leukotriene, refluxed bile, and stress oxygen classes.[2]

Number of synthetic drugs are available to treat ulcers, including proton pump inhibitors and H-2 receptor

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antagonists are available for the treatment of peptic ulcers, but clinical evaluation of these drugs has shown an incidence of relapses and various side effects may cause nausea, abdominal pain, constipation, and diarrhea and are expensive in comparison to Indigenous medicinal plants. However, Most of the remedies ware taken from plants and proved to be useful in the indigenous system of medicine.[3] Nature always has been a valuable source of drugs; plant is the largest reservoir of the bioactive principals that have a long history of use in modern medicine and in certain system of traditional medicine.

Mimosa pudica Linn is a creeping annual or perennial herb commonly known as a sensitive plant (touch-me-not) and identified as lajjalu in Ayurveda the plant is a native of tropical America and naturalized nearly all through the tropical and subtropical parts of India. It is commonly distributed in open spaces, such as waste area, roadside, agricultural land, and natural forests. Phyto medicines potentially benefit to the community thus they are still being practiced in all traditional systems of therapies, including Greco-Arab (Unani-Tibb), Ayurveda, and Chinese medicine.[4]

Secondary metabolites such as flavonoids, alkaloids, tannins, and saponins are responsible for anti-ulcer pharmacological activity.[5] The Preliminary "phytochemical" screening of *M. Pudica Linn* seeds ethanolic extract showed the presence of bioactive components like terpenoids, flavonoids, alkaloids, phenols, tannins and saponins.[6] Moreover, *M. Pudica Linn* seeds extract acting as an antioxidant.[7] Thus, can reduce mucosal damage by oxygen free radicals. The present research was carried out to investigate the therapeutic efficacy of *M. pudica Linn* seeds extracts against oral and stomach ulcers.

Materials And Methods

Plant Material Collection and Authentication

The fresh dried seeds *M. pudica Linn* seeds were purchased from Kartik foods product west Delhi, identified & authenticated by the Department of Botany, APS University, Rewa M.P. India. Herbarium specimens of each were prepared and deposited with voucher specimen No /B/PAN/484.

Preparation of Plant Extracts

The fresh dried seeds of *M. pudica Linn* shattered and screened with 40 mesh. shade dried coarsely powdered seeds (250 g) were loaded in soxhlet apparatus and was extracted with petroleum ether (60–62°C), chloroform, ethyl acetate, ethyl alcohol and aqueous until the extraction ware done. After completion of extraction, the solvent was removed by distillation. The extracts were dried using rotator evaporator. The residue was then stored in a desiccator and percentage yield was determined.[8,9]

Phytochemical Screening

Phytochemical screening carried out to get the presence of various phytocompounds *viz.* flavonoids, saponins, glycosides, alkaloids, carbohydrate, tannins, phenols in different extracts of *M. pudica Linn* seeds using standard method.[10]

Experimental Animals

Whole experiments were performed accordance with the guidelines issued by the Institutional Animal Ethics Committee (IAEC), Vedica College of B. Pharmacy, RKDF University, Bhopal MP. The animals were used with permission number IAEC/VCP/2019/001/7. As per Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. Adult albino rats both sex weighed 150 to 180 g were used for the *in-vivo* anti-ulcer study. The animals were housed in clean polypropylene cages and maintained in well-ventilated, temperature-controlled animal house with a constant 12 hours' light/dark schedule. The animals were fed with a standard rat pelleted diet and clean drinking water was made available ad libitum.

Toxicity Study

The acute oral toxicity studies were carried out as per the guidelines of Organization for Economic Co-operation and Development (OECD) 423. The acute toxicity of all extracts ware determinutesed in albino rats. The animals were fasted overnight prior to the experiment. Animals were divided into 8 groups and the extracts were administered orally to various groups of albino rats in doses ranging from 500, 1000, 1500, 2000, 2500, 3000, 3500 and 4000 mg/kg for the acute toxicity study.[11] Observation of all animals for any lethality in any of the groups after 7 days of treatment was noted.

Experimental Protocol

Albino rats 150–180 g of both sex were used for the study. All the animals were divided into 12 groups with 6 animals ware kept each group. Group I indicated as disease control Saline 5 mL/kg p. o.; group II-treated with Omeprazole 20 mg/kg p. o. for ethanol induced ulcer model and ORASORE Gel (Wings Biotech, Delhi) for glacial acetic acid induced oral ulcer model; group III treated with petroleum ether extract of *M. pudica* Linn seeds (PEEMP) 200 mg/kg p. o.; group IV treated with petroleum ether extract of *M. pudica Linn* seeds (PEEMP) 400 mg/kg p. o.; group V treated with chloroform extract of *M. pudica* Linn seeds (CEMP) 200 mg/kg p. o.; group VI treated with chloroform extract of *M. pudica* Linn seeds (CEMP) 400 mg/kg p. o.; group VII treated with ethyl acetate extract of *M. pudica* Linn seeds (EAEMP) 200 mg/kg p. o.; group VIII treated with ethyl acetate extract of *M. pudica* Linn seeds (EAEMP) 400 mg/kg p. o.; group IX treated with ethanol extract of *M. pudica* Linn seeds (EEMP) 200 mg/kg p. o.; group X treated with

ethanol extract of *M. pudica* Linn seeds (EEMP) 400 mg/kg p. o.; group XI treated with aqueous extract of *M. pudica* Linn seeds (AEMP) 200 mg/kg p. o. and group XII treated with aqueous extract of *M. pudica* Linn seeds (AEMP) 400 mg/kg p. o.

Ethanol Induced Ulcer

M. pudica Linn seeds extract 200 mg/kg, 400 mg/kg, and omeprazole 20 mg/kg was administered orally to 48 hours fasted albino rats. The ulcer was induced using 1-mL of 80 % ethanol was administered orally to each animal.[12] After 1-hour the albino rats were anesthetized using diethyl ether and then euthanized by cervical dislocation. The stomach was removed and opened along the greater curvature. All the stomachs were gently rinsed with water to remove the gastric contents and blood clots. Stomach along the greater curvature pinned on a soft board for evaluating gastric ulcers. Each stomach was examinutesed for lesions in the four-stomach portion and indexed according to severity. The ulcer scoring was done and the percentage protection was calculated.[13,14]

Glacial Acetic Acid Induced Oral Ulcer Model

The animals were provided and kept under controlled environmental conditions (24°C relative humidity 40–60%) at 12 hours alternate light-dark cycles. Animals were received food and water *ad libitum*) in the animal house of RKDF University, Bhopal for whole study. After one week of acclimatization, the animals were randomLy distributed into 12 groups.

The albino rats were anesthetized with 3% of sodium pentobarbital at an injection dose of 30 mg/kg. then, 40% glacial acetic acid was administered into the buccal membrane for 60 second using a glass rod of diameter of 7 mm and length 10 cm. then, the cauterization parts ware rinsed for 1-minutes to form a white lesion. Congestion and red swelling were observed in the area of buccal. Yellow or white pseudo membrane covered the treated parts, indicating that the oral ulcer model was successfully established.[15] All groups were observed macroscopically and were calculated ulcer index and percentage inhibition. After a week of duration oral mucosa tissue sample was collected from each group and analyses antioxidants and cytokines assay.

Macroscopic Evaluation

The stomachs were opened along the wider curvature, rinsed with saline to eated animals extract stomach contents and blood clots, and inspected for ulcer formation by a 10-lens magnifier.[16]

In case of oral ulcer model, oral mucosal tissue was collected at the junction of ulcer and normal mucosa from different treatment groups for morphological features of oral ulcer e.g. hyperemia, color of oral mucosa. They counted the number of ulcers.

Scoring of ulcers was observed as in following manner:

Normal color ..... (0)
Red coloration..... (0.5)
Spot ulcer..... (1)
Hemorrhagic streak... (1.5)
Deep ulcers..... (2)
Perforation..... (3)

The mean ulcer score is expressed as the ulcer index for each experimental animal. The percentage of protection against ulcer has been as follows. The ulcer index (UI) was calculated using formula:

$$UI = UN + US + UP \times 10^{-1}$$

Where-

UI= Ulcer index;
UN = Average number of ulcers per animal;
US = Average number of severity score;
UP = Percentage of animals with ulcers

Percentage inhibition of ulceration

$$\% \text{ Inhibition of Ulcer} = \frac{\text{Ulcer index of Control group} - \text{Ulcer index of Test group}}{\text{Ulcer index of Control group}} \times 100$$

Determinutesation of Total Acidity in Stomach Ulcer Model

The stomachs were removed and the content was measured before drained into a centrifuge tube and subjected to centrifugation at 2000 rpm for 10 minutes pH of the gastric secretion was recorded with a pH meter. The total acidity of the gastric secretion was determined by titration with 0.01N NaOH and phenolphthalein as indicator. The total acidity is expressed as mEquiv/l using the following formula:

$n \times 0.01 \times 40 \times 1000$

where, *n* is volume of NaOH quantified, 40 is the molecular weight of NaOH, 0.01 is normality of NaOH and 1000 is the factor represented in liter.

Determination of pH in Stomach Ulcer Model

An aliquot of 1-mL gastric juice was diluted with 1-mL of distilled water and pH of the solution was measured using pH meter.[16]

Measurement of Gastric Content in Stomach Ulcer

The stomachs were removed and the volume of the gastric juice was collected and measured.

Assessment of Antioxidant Parameters

Assessment of stomach tissue and oral mucosal tissues were collected from all experimental animal groups for antioxidant assay. Catalase was estimated following the breakdown of hydrogen peroxide accordingto the method.[17] Superoxide dismutase (SOD) was assayed.[18] Based on the inhibition of epinephrine autoxidation by the enzyme. Reduced glutathione (GSH) content was determined in tissue by the method.[19]

The SOD activity was measured as a degree of inhibition of auto-oxidation of epinephrine at an alkaline pH by the method.[18] A 0.1 mL of tissue homogenate was added

to the tubes containing 0.75 mL ethanol and 0.15 mL chloroform (chilled in ice) and centrifuged. To 0.5 mL of supernatant, 0.5 mL of 0.6 mM EDTA solution and 1-mL of 0.1 M carbonate–bicarbonate (pH 10.2) buffer were added. The reaction was initiated by the addition of 0.5 mL of 1.8 mM epinephrine (freshly prepared) and the increase in absorbance at 480 nm was measured by using Shimadzu UV-vis spectrophotometer.

Beers and Seizer method was used to determinutese the activity of the enzyme CAT. A total 3 mL of reaction mixture containing 1.9 mL of phosphate buffer (0.05 M) pH 7.0, 1-mL of hydrogen peroxide (5.0 mM) and 0.1 mL of diluted enzyme (skin homogenate) was used in this assay. The activity was measured by reading absorbance at 240 nm at 30 second and interval for 3 minutes using UV-vis spectrophotometer.

Reduced glutathione (GSH) level was determinutesed by method of Moron.[19] Skin homogenates were immediately precipitated with 0.1 mL of 25% TCA and the precipitate was removed after centrifugation. Free-SH groups were assayed in a total 3 mL volume by the addition of 2 mL of 0.6 mM DTNB and 0.9 mL 0.2 mM sodium phosphate buffer (pH 8.0) to 0.1 mL of the supernatant and the absorbance was read at 412 nm using a UV-vis spectrophotometer.

Cytokines Assays

Cytokines parameters such as tumor necrosis factor-α (TNF-α), prostaglandin E2 (PGE2), and interlukin-6 (IL-6) were assayed in the tissue samples by ELISA reader (Erba Lisa Scan EM, Mumbai). The assays were performed according to protocol recommended by the manufacturer's (TRANSASIA, Mumbai, India).[20]

Statistical Analysis

Statistical analysis of the results was done by one way analysis of variance (ANOVA) using GraphPad Prism 5 software followed by Bonferroni comparison test for significance. Significance has been set at ($p < 0.05$) and compared to the inducer. Results ware presented as mean ± S.D.

Results

Phytochemical Screening

The plant material *M. pudica* Linn seeds were collected and identified. The powdered materials plants were successively extracted with petroleum ether, chloroform, ethyl acetate, ethyl alcohol and aqueous. The percent yields of each extract were calculated. The percent yields of all extracts of *M. pudica* Linn. were found 14.2 % w/w in petroleum ether, 13.2% w/w in chloroform, 5.4 % w/w in ethyl acetate, 26.4 % w/w in ethanol and 4.5 % w/w in aqueous. The phytochemical analyses of all extracts were performed qualitatively for different phytoconstituents. The *M. pudica* Linn seeds give positive test of steroids, terpenoids, and fatty acid in petroleum ether and alkaloids, Tannins and Phenolic compounds in chloroform extract. The ethyl acetate and ethanol extract contain flavonoids, tannins and phenolic compounds, glycosides. aqueous extract was found presence of, saponins, glycosides, Phenolic compounds and flavonoids.

Acute Toxicity Study

All extracts were found safe in the dose used and there was no mortality up to a dose of 4000 mg/kg body weight. In the first to fourth step of the acute toxicity studies, there were no remarkable signs of toxicity observed at 2000 and 4000 mg/kg dosage. There is zero mortality 0/3 and total mortality 0/3 *i.e.,* between 500 and 4000 mg/kg. On the basis of above study, the dose 200 and 400 mg/kg P.O. was taken for the anti-ulcer activity.

Table 1: Effect of different extracts of *M. pudica* Linn seeds on Ulcer Index and percent inhibition in ethanol induced ulcer model in albino rats

S. No.	*Treatment Groups*	*Ulcer Index*	*% Inhibition*
1.	Disease Control (saline 5 mL/kg)	37.51 ± 2.10	-
2.	Omeprazole (20 mg/kg)	14.63 ± 0.84*	60.99*
3.	PEEMP (200 mg/kg)	28.42 ± 2.61	24.23
4.	PEEMP (400 mg/kg)	27.64 ± 2.08	26.31
5.	CEMP (200 mg/kg)	28.55 ± 2.33	23.88
6.	CEMP (400 mg/kg)	26.41 ± 3.01	29.59
7.	EAEMP (200 mg/kg)	25.77 ± 2.98	33.06
8.	EAEMP (400 mg/kg)	24.11 ± 1.88	35.72
9.	EEMP (200 mg/kg)	13.94 ± 0.45*	62.83*
10.	EEMP (400 mg/kg)	12.55 ± 1.67*	66.54*
11.	AEMP (200 mg/kg)	31.22 ± 2.52	16.76
12.	AEMP (400 mg/kg)	29.66 ± 2.11	20.92

n = 6, value represents Mean ± S.D. *p< 0.05

Table 2: Effect of different extracts of *M. pudica* Linn seeds on Ulcer Index and percent inhibition in glacial acetic acid induced oral ulcer model in albino rats

S. No.	*Treatment groups*	*Ulcer index*	*%Inhibition*
1.	Disease Control (saline 5 mL/kg)	33.51 ± 2.17	-
2.	ORASORE Gel (once a day)	10.28 ± 0.85*	69.32*
3.	PEEMP (200 mg/kg)	29.22 ± 1.85	12.80
4.	PEEMP (400 mg/kg)	27.31 ± 1.32	18.50
5.	CEMP (200 mg/kg)	30.96 ± 2.10	07.60
6.	CEMP (400 mg/kg)	28.42 ± 1.96	15.18
7.	EAEMP (200 mg/kg)	25.33 ± 1.83	24.41
8.	EAEMP (400 mg/kg)	23.17 ± 1.36	30.85
9.	EEMP (200 mg/kg)	12.05 ± 0.76*	64.04*
10.	EEMP (400 mg/kg)	11.62 ± 0.64*	65.32*
11.	AEMP (200 mg/kg)	24.61 ± 1.52	26.55
12.	AEMP (400 mg/kg)	22.82 ± 1.38	31.90

n = 6, value represents Mean ± S.D. *p < 0.05

Determination of Ulcer Index

Ethanol administration resulted in the production of gastric mucosal damage. The ulcer index of animals in ethanol induced ulcer model, in case of disease control group of animals was 37.51 ± 2.10. Ethanol extract of *M. pudica* Linn seeds at the dose 200 mg/kg body weight and 400 mg/kg body weight treated animals were showed 13.94 ± 0.45 and 12.55 ± 1.67, respectively that is significantly reduced the ulcer index at level ($p < 0.05$) as compared to control group (Table 1). However, the reduction in ulcer index by other extracts ware not found significantly. Glacial acetic acid induced oral ulcer shows the ulcer index of animals in glacial acetic acid induced oral ulcer model, in case of control group of animals was 33.51 ± 2.17. Ethanol extract of *M. pudica* Linn seeds 200 and 400 mg/kg treated animals were showed 12.05 ± 0.76 and 11.62 ± 0.64, respectively that is significantly reduced the ulcer index ($p < 0.05$) as compared to control group (Table 2). The reduction in ulcer index by other extracts was not found to be significantly.

pH of Gastric Contents

In control animals, without any drug treatment the average pH was 2.1, the ethanol extract of *M. pudica* Linn seeds 200 and 400 mg/kg were showed significant rise in pH 4.0 and 4.9, respectively as compared to control (Table 3). The rise in pH shown by omeprazole, a standard drug was 4.8, which is statistically significant ($p < 0.05$). This is revealed the extract i.e., EEMP at dose 400 mg/kg body weight is more potent than omeprazole.

Table 3: Effect of different extracts of *M. pudica* Linn seeds on various ulcer parameters in Ethanol induced ulcer model in albino rats

S. No.	*Groups*	*Gastric volume (mL)*	*pH*	*Total acidity (mEq/l)*
1.	Disease Control (saline 5 mL/kg)	8.31 ± 0.07	2.1 ± 0.02	598.42 ± 23.51
2.	Omeprazole 20 mg/kg	4.28 ± 0.06*	4.8 ± 0.04*	264.35 ± 11.24*
3.	PEEMP (200 mg/kg)	7.66 ± 0.01	3.7 ± 0.02	485.22 ± 17.25
4.	PEEMP (400 mg/kg)	6.98 ± 0.07	3.4 ± 0.04	432.75 ± 18.32
5.	CEMP (200 mg/kg)	6.34 ± 0.04	3.9 ± 0.03	410.75 ± 18.66
6.	CEMP (400 mg/kg)	6.12 ± 0.03	3.5 ± 0.01	389.55 ± 17.64
7.	EAEMP (200mg/kg)	5.88 ± 0.02	3.7 ± 0.03	401.44 ± 20.10
8.	EAEMP (400mg/kg)	5.41 ± 0.04	3.8 ± 0.04	395.21 ± 19.85
9.	EEMP (200 mg)	3.61 ± 0.06*	4.0 ± 0.06*	274.20 ± 17.45*
10.	EEMP (400 mg)	2.85 ± 0.04*	4.9 ± 0.03*	241.33 ± 16.55*
11.	AEMP (200 mg/kg)	7.10 ± 0.07	3.0 ± 0.01	405.22 ± 22.48
12.	AEMP (400 mg/kg)	6.58 ± 0.05	3.5 ± 0.02	388.27 ± 21.30

n = 6, value represents Mean ± S.D. *$p < 0.05$

Table 4: Effect of different extracts of *M. pudica* Linn seeds on antioxidants level of stomach tissues of albino rats in Ethanol induced ulcer model

S. No	*Groups*	*Antioxidants level*		
		SOD (µg/50 mg tissue)	*CAT (µmol/50 mg tissue)*	*GSH (µmol/50 mg tissue)*
1.	Disease Control (saline 5 mL/kg)	18.22 ± 2.32	16.24 ± 1.25	21.38 ± 1.18
2.	Omeprazole (20 mg/kg)	52.64 ± 3.02*	38.62 ± 3.74*	48.77 ± 2.52*
3.	PEEMP (200 mg/kg)	23.28 ± 1.23	19.88 ± 1.14	23.25 ± 1.24
4.	PEEMP (400 mg/kg)	25.22 ± 2.98	21.25 ± 2.51	25.36 ± 1.61
5.	CEMP (200 mg/kg)	26.61 ± 1.54	20.42 ± 1.37	19.64 ± 1.36
6.	CEMP (400 mg/kg)	24.28 ± 2.25	22.66 ± 2.36	20.84 ± 1.56
7.	EAEMP (200 mg/kg)	26.66 ± 3.58	24.43 ± 2.12	23.45 ± 1.81
8.	EAEMP (400 mg/kg)	28.26 ± 2.86	25.50 ± 2.41	25.29 ± 1.53
9.	EEMP (200 mg)	49.17 ± 2.21*	35.20 ± 2.05*	42.30 ± 2.78*
10.	EEMP (400 mg)	50.35 ± 3.27*	37.42 ± 2.98*	47.23 ± 2.05*
11.	AEMP (200 mg/kg)	21.54 ± 2.65	20.94 ± 1.36	22.29 ± 1.86
12	AEMP (400 mg/kg)	32.56 ± 2.81	21.67 ± 1.84	22.10 ± 1.73

n = 6, value represents Mean ± S.D. *$p < 0.05$

Table 5: Effect of different extracts of *M. pudica* seeds on antioxidants level of oral mucosal tissue of albino rats in glacial acetic acid induced oral ulcer model

S. No	*Groups*	*Antioxidants level*		
		SOD (µg/50 mg tissue)	*CAT (µmol/50 mg tissue)*	*GSH (µmol/50 mg tissue)*
1.	Disease Control (saline 5 mL/kg)	15.27 ± 1.58	17.34 ± 1.37	26.47 ± 2.45
2.	ORASORE Gel (once a day)	58.47 ± 4.23*	37.25 ± 2.35*	48.33 ± 3.51*
3.	PEEMP (200 mg/kg)	21.75 ± 1.47	19.81 ± 1.14	27.54 ± 1.65
4.	PEEMP (400 mg/kg)	22.72 ± 1.46	20.42 ± 1.54	28.54 ± 1.15
5.	CEMP (200 mg/kg)	24.56 ± 1.54	21.23 ± 1.48	26.42 ± 1.18
6.	CEMP (400 mg/kg)	25.43 ± 1.73	24.52 ± 1.42	28.74 ± 1.40
7.	EAEMP (200 mg/kg)	23.49 ± 1.25	20.56 ± 1.13	28.52 ± 1.78
8.	EAEMP (400 mg/kg)	24.59 ± 1.64	21.45 ± 1.43	28.72 ± 1.16
9.	EEMP (200 mg)	55.36 ± 2.56*	35.53 ± 2.42*	46.52 ± 2.29*
10.	EEMP (400 mg)	57.73 ± 2.37*	36.42 ± 2.17*	47.85 ± 2.17*
11.	AEMP (200 mg/kg)	27.67 ± 2.16	19.74 ± 1.41	27.54 ± 1.18
12.	AEMP (400 mg/kg)	33.23 ± 2.87	21.51 ± 1.96	27.51 ± 1.73

n = 6, value represents Mean ± S.D. *p < 0.05

Total Acidity of Gastric Contents

The disease control group was showed gastric total acidity 598.42 ± 23.51 mEq/litre. After treatment with ethanol extract 200 and 400 mg/kg of *M. pudica* Linn seeds was showed significant decrease in total acidity 274.20 ± 17.45 and 241.33 ± 16.55 mEq/litre, respectively and compared to control group of animals. other extracts, i.e., petroleum ether, chloroform, ethyl acetate and aqueous, do not show significant reduction in total acidity (Table 3).

Effect of Different Extracts on Oxidative Status in Ethanol Induced Ulcer Model and Glacial Acetic Acid Induced Oral Ulcer Model in Albino Rats

The level of antioxidants in stomach tissues were observed significant decrease in control group, may be due to increasing free radicles generation. This decreasing level of SOD, CAT and GSH was slightly improved in treatment group with ethanol extract at 200 mg/kg dose of *M. pudica* Linn seeds. But a significant improvement in level of SOD, CAT and GSH were found in treatment group of 400 mg/kg dose of ethanol extract of *M. pudica* Linn seeds as well as standard drug treated group, when compared to disease control group (Table 4).

In glacial acetic acid induced oral ulcer model significant improvement in level of SOD, CAT and GSH were found in treatment group of 400 mg/kg dose of ethanol extract of *Mimosa pudica* Linn seeds as well as standard drug treated group when compared to disease control group (Table 5).

Effect of Different Extracts on Pro-inflammatory Mediators in Ethanol Induced Ulcer Model and Glacial Acetic Acid Induced Oral Ulcer Model in Albino Rats

Inhibitory effect of ethanol extract on the tissue level of pro-inflammatory mediators (TNF-α, IL-6 and PGE2) were measured in stomach tissue of albino rats by ELISA (Table 6 and 7). Results confirmed that *M. pudica* Linn seeds extracts treatment groups showed a significant decrease in the tissue level of pro-inflammatory mediators i.e., TNF-α, IL-6, and PGE2 when compared to the control group of animals.

Ethanolic extract of *M. pudica* Linn seeds at both doses 200 and 400 mg/kg showed significant reduction of tissue cytokines level such as TNF-α 18.67 ± 1.78 and 16.25 ± 1.23, respectively, IL-6 15.20 ± 0.95 and 12.89 ± 0.64, respectively and prostatglandin E2 23.41 ± 1.96 and 21.05 ± 1.07 pg/L. This significant reduction was comparable to standard group of animals also. All other extracts did not show significant reduction in the cytokine levels of treated animals (Table 6).

Glacial acetic acid induced oral ulcer model also characterized by the marked expression of some pro-inflammatory cytokines. Inhibitory effect of ethanol extract on the tissue level of pro-inflammatory mediators (TNF-α, IL-6 and PGE2) were measured in oral mucosa of albino rats by ELISA (Table 7).

Ethanolic extract of *M. pudica* Linn seeds at both doses 200 and 400 mg/kg showed significant reduction of tissue cytokines level such as TNF-α 19.26 ± 1.08 and 16.32 ± 0.87, respectively, IL-6 15.24 ± 0.54 and 13.27 ± 0.73, respectively and prostaglandin E2 22.32 ± 1.08 and 21.32 ± 1.05 pg/L. This significant reduction was comparable to standard group of animals also. All other extracts did not show significant reduction in the cytokine levels of treated animals (Table 7).

Discussion

Ulcer, considered one of the modern age epidemics, has been affecting approximately 10% of the world population. Earlier study suggested that peptic ulcer

Table 6: Effect of different extracts of *M. pudica* Linn seeds on pro-inflammatory mediators in albino rats stomach tissue of ethanol induce ulcer model

S. No	*Animal groups*	*TNF-α(pg/L)*	*IL-6 (pg/L)*	*PGE2 (pg/L)*
1.	Disease Control (saline 5 mL/kg)	37.65 ± 2.17	28.29 ± 1.95	47.15 ± 2.04
2.	Omeprazole (20 mg/kg)	15.34 ± 0.75*	13.85 ± 0.68*	20.64 ± 1.08*
3.	PEEMP (200 mg/kg)	35.20 ± 2.03	22.24 ± 2.31	38.52 ± 2.02
4.	PEEMP (400 mg/kg)	31.05 ± 2.13	20.43 ± 2.34	37.41 ± 2.33
5.	CEMP (200 mg/kg)	29.82 ± 2.56	26.86 ± 1.90	34.87 ± 1.92
6.	CEMP (400 mg/kg)	26.34 ± 1.86	24.75 ± 1.99	32.85 ± 2.24
7.	EAEMP (200 mg/kg)	26.82 ± 1.36	21.82 ± 1.45	34.24 ± 1.97
8.	EAEMP (400 mg/kg)	24.61 ± 2.12	21.10 ± 2.23	31.55 ± 2.12
9.	EEMP (200 mg)	18.67 ± 1.78*	15.20 ± 0.95*	23.41 ± 1.96*
10.	EEMP (400 mg)	16.25 ± 1.23*	12.89 ± 0.64*	21.05 ± 1.07*
11.	AEMP (200 mg/kg)	23.84 ± 2.32	25.52 ± 2.11	30.82 ± 2.21
12.	AEMP (400 mg/kg)	22.46 ± 2.12	24.32 ± 1.89	29.53 ± 1.89

n = 6, value represents Mean ± S.D. *p < 0.05

Table 7: Effect of different extracts of *Mimosa pudica* Linn seeds on pro-inflammatory mediators in albino rats oral mucosal tissue of glacial acetic acid induce oral ulcer model

S. No	*Animal groups*	*TNF-α(pg/L)*	*IL-6 (pg/L)*	*PGE2 (pg/L)*
1.	Disease Control (Saline 5 mL/kg)	43.52 ± 2.05	31.30 ± 1.05	53.56 ± 2.01
2.	ORASORE Gel (Once a day)	18.73 ± 0.85*	13.25 ± 0.97*	23.24 ± 1.85*
3.	PEEMP (200mg/kg)	39.75 ± 1.06	24.32 ± 1.13	46.53 ± 2.15
4.	PEEMP (400mg/kg)	37.56 ± 2.12	21.50 ± 2.06	44.12 ± 1.98
5.	CEMP (200 mg/kg)	28.54 ± 1.08	23.52 ± 1.34	39.54 ± 1.56
6.	CEMP (400 mg/kg)	26.35 ± 1.07	26.32 ± 1.04	36.32 ± 1.54
7.	EAEMP (200mg/kg)	25.14 ± 2.04	21.56 ± 1.05	33.54 ± 1.08
8.	EAEMP (400mg/kg)	21.45 ± 2.21	20.36 ± 2.04	31.24 ± 1.65
9.	EEMP (200 mg)	19.26 ± 1.08*	15.24 ± 0.54*	22.32 ± 1.08*
10.	EEMP (400 mg)	16.32 ± 0.87*	13.27 ± 0.73*	21.32 ± 1.05*
11.	AEMP (200 mg/kg)	30.25 ± 1.28	22.21 ± 1.24	37.08 ± 1.12
12.	AEMP (400 mg/kg)	29.45 ± 1.22	21.05 ± 2.12	30.27 ± 1.23

n = 6, value represents Mean ± S.D. *p < 0.05

is due to an imbalance between acid and pepsin along with the weakness of the mucosal barrier. Due to these, it is commonly associated with damage of the stomach's mucosal layer and oral mucosa, which is simply generated via excess generation of exogenous and endogenous active oxygen and free radicals. Some of the main causes of gastric ulcers include chronic use of alcoholic beverages and anti-inflammatory drugs, stress, and Helicobacter pylori infection.

The *M. pudica* Linn seeds extracts were investigated for two different models i.e., Ethanol induced stomach ulcer and Glacial acetic acid induced oral ulcer model. The *M. pudica* Linn seeds give positive test of flavonoids, tannins and Phenolic compounds, and glycosides in aqueous extract and flavonoids. By producing natural antioxidants (SOD, CAT, GSH), the cell reacts to increased concentrations of free radicals, which can reduce or remove free-radical harm to cellular structures. In particular, glutathione peroxidase catalyzes the conversion of hydroxide ions to water. SOD able to converts ions from superoxide to hydrogen peroxide and is then converted by catalase to oxygen and water. Superoxide dismutase occurs in a variety of different isoforms, each specializing in particular areas of the cell.[18] The cell increases the expression of antioxidant enzymes when subjected to rising ionizing radiation levels. However, if these cellular defenses are overcome by the amount of ROS, the cell will experience damage (dose-dependent) that can lead to carcinogenesis, teratogenesis, necrosis or apoptosis. Anti-inflammatory and antioxidant compounds can control free radical generation, eliminutesate free radicals and induce natural development of antioxidant (such as SOD, GSH and CAT). They improve DNA repair, suppress many inflammatory reactions, or stay cell

division, allowing cells to undergo apoptosis for longer.[21] The results revealed that a significant ($p < 0.05$) enhancement after treatment with *M. pudica* Linn seeds extract 200 and 400 mg/kg doses. in dose dependent manner as indicating antioxidant effect of ethanol extracts. The tissue antioxidant level is very close to normal (Table 4 and 5). Most of the cell and organ damage caused by free radicles comes through induced free radicals. The GSH detoxification system is important in cellular defense against a large group of injurious agents. GSH can protect against free radicals and cell death after various inflammation reactions.[22] The endogenous protection mechanism, like the GSH and antioxidant enzymes, defends against oxidative damage under normal conditions. GSH is a flexible protector and performs its protective function by free radical scavenging, restoring the damaged molecules by contributing hydrogen, reducing peroxides and retaining protein thiols in a reduced state.[23] Flavonoids such as quercetin are believed to act as health-promoting substances as they have antioxidant and anti-inflammatory properties.[24] In the inflammation phase, macrophages and neutrophils are attracted to the injured tissues that release inflammatory mediators, such as TNF-a and IL-1. Neutrophils contain high levels of destructive proteases and oxygen free radicals that are released into the wound area when cells die. This can cause extensive tissue damage and prolong the inflammatory phase. These free radicals are produced during oxidative stress, which causes lipid peroxidation, DNA breakage and scavenging enzymes inactivation. In case of hemorrhoid, one of the major causes of delayed healing is the persistence of inflammation or an inadequate angiogenic response.[25] It has been postulated that an anti-inflammatory response after cutaneous wound induction is a prerequisite for healing.[26] Potent antioxidant, anti-inflammatory agents such as luteolin can play an important role in restoring physiological conditions, significantly improving ulcer healing. Ethanol induced stomach ulcer also characterized by the marked expression of some pro-inflammatory cytokines. Glacial acetic acid induced oral ulcer model also characterized by the marked expression of some pro-inflammatory cytokines. Inhibitory effect of ethanol extract on the tissue level of pro-inflammatory mediators (TNF-α, IL-6 and PGE2) were measured in oral mucosa of albino rats by ELISA (Table 7).[27]

In conclusion, plant extracts i.e., ethanol extract of M. pudica Linn seeds showed significant anti-ulcer effect in both type of ulcer, i.e., mouth and stomach ulcer, through possible antioxidant mechanisms. Additionally, some reported flavonoids e.g., quercetin, may be responsible for the protective effect.

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