Potential of *Mimosa pudica* leaf in the treatment of ulcerative colitis in rat
Introduction

Ulcerative colitis and Crohn’s disease are the two major types of inflammatory bowel diseases. Still today the etiology and pathogenesis of both are not confirmed (Fiocchi, 1998). The literature studies suggest that increased production of reactive oxygen species plays a very important role in the destruction of mucosal epithelial layer in inflammatory bowel diseases patient (Hyam et al., 2013; Pullman et al., 1992). The pathophysiology of ulcerative colitis has highlighted the importance of cytokines like tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1β), IL-6 and IL-8, which are responsible for the production of reactive oxygen species which activates the oxidative stress-responsive gene that plays an important role in development and maintenance of inflammatory bowel diseases (Oz et al., 2005).

If the disease is long-term, the risk of developing colon cancer increases (McIlmurray and Langman, 1975). Corticosteroid, 5-aminosalicylate, sulfasalazine, methotrexate, prednisolone and immunosuppressive agents (mercaptopurine and azathioprine) are the recent treatment regimens for inflammatory bowel diseases. However, these agents provide partial relief in the patients and these also have several adverse effects and high relapse rate limit their utility in the treatment of inflammatory bowel diseases (Joshi et al., 2005). Therefore, there is a need to develop new medication to treat the inflammatory bowel diseases.

Many herbal formulations conations polyphenolics and flavonoid derivatives show good potential for the treatment of inflammatory bowel diseases (Rahimi et al., 2009). Aloe vera, wheat grass, licorice, slippery elm and curcumin can be used in the treatment of ulcerative colitis (Fei Ke et al., 2012).

Mimosa pudica L. belongs to the family of Fabaceae. The plant, having various polyphenolics and flavonoid derivatives, has been traditionally used for its anti-diarrheal,
Materials and Methods

Plant material

The leaves of *M. pudica* were collected from the rural areas of Ahmednagar district, Maharashtra in November 2014. Authentication of the plant was carried out at the Botanical Survey of India, Pune (Voucher specimen number: BBZ01).

Preparation of extract

A matured leaves of *M. pudica* were collected, shade dried and made into a coarse powder and then used for extraction. A weighed quantity (1 kg) of the powder was then subjected to continuous hot extraction in soxhlet apparatus with organic solvents like petroleum ether, chloroform, ethyl acetate, ethanol and water. The extract was concentrated under reused pressure using a rotary evaporator (BUCHI, Rotavapor R-215) at 40-60°C until all the solvent had been removed to give an extract sample.

Phytochemical analysis

The phytochemical study of all the extracts of *M. pudica* was carried out according to the standard methods (Khandelwal, 2007; Kokate, 2005).

Animals

Female Sprague Dawley female rats (230-250 g) were obtained from the National Institute of Bioscience, Pune, India. Six rats per cage were housed together in the autoclaved polypropylene cages. Cage changing was done once weekly. They were maintained at 24 ± 1°C, with a relative humidity of 45 to 65% and 12:12 hours dark/light cycle. The rats were allowed to acclimatize for five days prior to dosing. During this period, animals were observed daily for clinical signs. The animals were fed with standard rodent diet and filtered water *ad libitum* throughout the experimental protocol, with the exception of overnight fasting before induction of experimental colitis. During this period, the animals had access to filtered water only.

Drugs and chemicals

Acetic acid, ether, ethanol, ethyl acetate, chloroform, formalin, ether, carbon tetrachloride, ethylene glycol, hydrochloric acid and concentrated sulfuric acid were purchased from Poona Chemical Lab, India. Prednisolone was obtained as a gift sample from Wyeth Pharma Ltd. India.

Toxicity studies

The acute toxicity study of *M. pudica* on rats was carried out as per OECD guidelines (Rajendran et al., 2009). The selected doses were 50, 100, 200, 400 and 2,000 mg/kg body weight. The animals were observed for toxic symptoms for 72 hours. The methanol extract of *M. pudica* did not produce any toxic symptoms or mortality up to the dose level of 2,000 mg/kg body weight in rats, hence the extract was considered to be safe and non-toxic for further pharmacological screening.

Experimental design

The rats were divided into nine groups with 6 animals in each group as follows: Group I received saline water (2 mL/kg/day); Group II received 4% acetic acid (1 mL) intrarectally on day 8; Group III received prednisolone (2 mg/kg, p.o for 3 days). Prednisolone and acetic acid treatment were started on the same day; Group IV, VI, VIII received 7 days pretreatment with 200 mg/kg of ethanol, ethyl acetate, chloroform extract of *M. pudica* respectively, p.o. and 1 mL of 4% acetic acid solution, intrarectally on day 8. Drug treatment was continued till day 11; Group V, VII and IX received 7 days pretreatment with 400 mg/kg of ethanol, ethyl acetate, chloroform extract of *M. pudica* respectively, p.o. and 1 mL of 4% acetic acid solution, intrarectally on day 8. Drug treatment was continued till day 11.

Colonic inflammation was induced in overnight fasted rats. After 48 hours of colitis induction blood was withdraw by retro-orbital puncture and animals were sacrificed by cervical dislocation to remove the colon. Five centimeter long piece of the colon was flushed gently with saline, scored for inflammation based on the macroscopic features. Portions of colonic specimens were kept in 10% formalin for histopathological studies (Millar et al., 1996; Mascoto et al., 1995).

Evaluation of the disease

The intrarectal instillation of 1 mL of 4% acetic acid produced disease in experimental animals, these disease-induced experimental animals were evaluated based on its macroscopic characteristics. In this evaluation, pieces of the rat colon (10 cm long each) were scored for macroscopic features using a scoring pattern (Morris et al., 1989).

Determination of ulcer index

The whole alimentary canal was isolated, opened longitudinally, and rinsed with phosphate buffer saline. The ulceration of the opened colon was measured with help of a microscope and the ulcer index was calculated.
using the following formula (Zaware et al., 2011):

\[
\text{Ulcer index} = \frac{\text{Grade of ulcer in positive control} - \text{Grade of ulcer in test}}{\text{Grade of ulcer in test} - \text{Grade of ulcer in normal control}} \times 100
\]

**Biochemical analysis of colon for myeloperoxidase and malondialdehyde levels**

Sample preparation: The proximal 5 cm of the dissected colon specimen was used for biochemical analysis of myeloperoxidase and malondialdehyde levels. The colonic samples were minced and homogenized using a polytron homogenizer. The supernatant was obtained by centrifuging at 3,000 rpm for 20 min (Murat et al., 2004).

Determination of colonic myeloperoxidase activity: Supernatant sample mixed with citric phosphate buffer having pH 5.0 containing 0.4 mg/mL O-phenylene diamine and 0.015% hydrogen peroxide. The change in absorbance measured spectrophotometrically at 492 nm. Test absorbance compared with the standard dilution with horseradish peroxidase. Myeloperoxidase was expressed in U/g of wet scrapings (Dighe et al., 2015; Evans et al., 2000).

Determination of malondialdehyde level: The reaction mixture containing 0.1 mL tissue sample, 0.2 mL 8.1% sodium dodecyl sulfate LR, 1.5 mL 2% acetic acid, and 1.5 mL 0.8% aqueous solution of thiobarbituric acid LR. The mixture pH was adjusted to 3.5 and the volume was finally made up to 4 mL with distilled water and 5 mL of the mixture of n-butanol and pyridine (15%) was added. The mixture was shaken vigorously. After centrifugation at 4,000 rpm for 10 min, the absorbance of the organic layer was measured spectrophotometrically at 532 nm. Malondialdehyde was expressed as unit per gram of protein (Ganjare et al., 2011).

**Histopathological studies**

A representative sample of the colon from each group was washed with saline and the tissue was fixed with 10% formalin for histopathological studies. It was processed for 24-36 hours and then trimmed at the suitable site and washed under running tap water for 2 hours then the tissue is dehydrated with help alcohol. Then, the tissue was cleaned with xylene and embedded with paraffin wax. 5 mm thick tissue was deparafinated and immersed in the xylene for 3 min. Sections were rehydrated with alcohol, kept in water for 5 min and in hematoxylin for 10 min. Dip in 1% ammonia water was done and instantly washed under running tap water. Add 2 or 3 drops of alcoholic eosin and dehydrated with alcohol. Again slides were cleaned with xylene, stained with Hematoxylin-Eosin and finally examined under a microscope.

**Statistical analysis**

The values mean ± SEM were calculated for each parameter. Data analysis was performed using GraphPad Prism 5.0 software. All data on biochemical parameters were analyzed using one-way ANOVA; Dunnett’s multiple range tests were applied for post hoc analysis. A value of p<0.05 was considered to be statistically significant.

**Results**

**Phytochemical analysis**

The preliminary phytochemical analysis of *M. pudica* extracts revealed the presence of phytoconstituents like alkaloids, carbohydrates, phenols, saponins, flavonoids, glycosides and terpenoids (Table I).

<table>
<thead>
<tr>
<th>Phytochemical test for extract of <em>M. pudica</em> leaf</th>
<th>Chloroform</th>
<th>Ethyl acetate</th>
<th>Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Phenols</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Proteins</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: (+) positive result, (-) negative result

**Acetic acid-induced colitis**

As observed from this study, intrarectal instillation of 1 mL of 4% acetic acid to the experimental control group caused colonic inflammation when compared to the normal control group. Ethanol extract-treated group showed significant suppressed of inflammatory reaction.

**Effect of extract on the macroscopic score**

After intrarectal instillation of 1 mL of 4% acetic acid, the colons of the rats were examined macroscopically for signs of hemorrhage, ulceration and inflammations by an independent observer, in a blinded fashion. The mean macroscopical score in control group rats (acetic acid control group) was found to be significantly increased (p<0.01) as compared to normal group rats. Ethanol extract of *M. pudica* (200 and 400 mg/kg) decreased the macroscopical lesions of colon showed better effect comparable with the standard drug (Table II).

**Effect of extract on ulcer protection**
Acetic acid control group showed a less protective effect in ulcer index. The seven days pretreatment of ethanol extract of *M. pudica* (200 and 400 mg/kg) showed a better protective effect in ulcer index (Table II).

### Effect of extract on colonic myeloperoxidase and malondialdehyde concentrations

The acetic acid-induced colonic inflammation results in increase in myeloperoxidase and malondialdehyde concentrations. Ethanol extract of *M. pudica* (200 and 400 mg/kg) found best in reducing myeloperoxidase and malondialdehyde activities in tissues which were raised by the acetic acid (Table III).

### Histopathological study

Histopathological observation ulcerated parts of the colon of the rat treated with 4% acetic acid showed transmural necrosis, edema, ulceration, hemorrhages, hyperemia, and cellular infiltration in the colon of rat (Figure 1). The 7 days pretreatment of ethanol extract of *M. pudica* (400 mg/kg) found to be preventive progression of colitis. The preventive effect observed by morphologically as well as histopathological studies (Table IV).

### Discussion

Acetic acid-induced colitis in laboratory rats model is

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**Table II**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Macroscopical score</th>
<th>Microscopic ulcer index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0.0 ± 0.0</td>
<td>100</td>
</tr>
<tr>
<td>Control (4% acetic acid)</td>
<td>8.1 ± 0.4*</td>
<td>00</td>
</tr>
<tr>
<td>Prednisolone (2 mg/kg)</td>
<td>3.0 ± 0.4</td>
<td>91</td>
</tr>
<tr>
<td>Ethanol (200)</td>
<td>4.6 ± 0.6b</td>
<td>72</td>
</tr>
<tr>
<td>Ethanol (400)</td>
<td>3.6 ± 0.4</td>
<td>75</td>
</tr>
<tr>
<td>Ethyl acetate (200)</td>
<td>5.8 ± 0.5</td>
<td>50</td>
</tr>
<tr>
<td>Ethyl acetate (400)</td>
<td>5.1 ± 0.4</td>
<td>50</td>
</tr>
<tr>
<td>Chloroform (200)</td>
<td>6.8 ± 0.5</td>
<td>40</td>
</tr>
<tr>
<td>Chloroform (400)</td>
<td>6.5 ± 0.5</td>
<td>31</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SEM (n = 6) and analyze by ANOVA followed by Dunnett’s test. *p < 0.05, p < 0.01, p < 0.001 as compared to acetic acid control group; *p < 0.001 as compared to normal group.

**Table III**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Myeloperoxidase (U/g)</th>
<th>Malondialdehyde (U/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>6.2 ± 0.5</td>
<td>2.3 ± 0.3</td>
</tr>
<tr>
<td>Control (4% acetic acid)</td>
<td>15.9 ± 0.4*</td>
<td>9.5 ± 0.4*</td>
</tr>
<tr>
<td>Prednisolone (2 mg/kg)</td>
<td>12.4 ± 0.4</td>
<td>4.8 ± 0.4</td>
</tr>
<tr>
<td>Ethanol (200)</td>
<td>13.7 ± 0.5b</td>
<td>7.0 ± 0.5b</td>
</tr>
<tr>
<td>Ethanol (400)</td>
<td>13.0 ± 0.3*</td>
<td>6.1 ± 0.45*</td>
</tr>
<tr>
<td>Ethyl acetate (200)</td>
<td>14.3 ± 0.4*</td>
<td>7.6 ± 0.5*</td>
</tr>
<tr>
<td>Ethyl acetate (400)</td>
<td>14.0 ± 0.3*</td>
<td>7.5 ± 0.4*</td>
</tr>
<tr>
<td>Chloroform (200)</td>
<td>14.8 ± 0.3</td>
<td>8.5 ± 0.4</td>
</tr>
<tr>
<td>Chloroform (400)</td>
<td>15.0 ± 0.3</td>
<td>8.4 ± 0.4</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SEM (n = 6) and analyze by ANOVA followed by Dunnett’s test. *p < 0.05, p < 0.01, p < 0.001 as compared to acetic acid control group; *p < 0.001 as compared to normal group.

**Table IV**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ulceration</th>
<th>Hyperemia</th>
<th>Necrosis</th>
<th>Edema</th>
<th>Cellular infiltration</th>
<th>Goblet-cell hyperplasia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>(4% acetic acid)</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Prednisolone (2 mg/kg)</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ethanol (200)</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Ethanol (400)</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ethyl acetate (200)</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Ethyl acetate (400)</td>
<td>++</td>
<td>++</td>
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<td>Chloroform (200)</td>
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<tr>
<td>Chloroform (400)</td>
<td>++</td>
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<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

(0): no abnormality detected; (+): damage/active changes up to less than 25%; (++): damage/active changes up to less than 50%; (+++): damage/active changes up to less than 75%; (++++): damage/active changes up to more than 75%
one of the most commonly used experimental models while screening drugs active against ulcerative colitis. The intrarectal administration of acetic acid produces inflammation appears to involve the entry of the protonated form of the acid within the intracellular space and causes intracellular acidification resulting in epithelial damage and localized erosion of the colonic mucosa leading to hemorrhages and severe localized inflammation. The inflammatory response by acetic acid activates the cyclooxygenase enzymes and lipoxygenase enzymes pathways which results generation of inflammatory mediators like prostaglandin and leukotrienes (Jagtap et al., 2004; Nakhai et al., 2007; Macpherson and Pfeiffer, 1978).

Mucosal immune system is the main effectors of intestinal inflammation and injury, with cytokines playing a central role in modulating inflammation (Ardizzone and Bianchi, 2005; Nakamura et al., 2006).

Increased levels of both TNF-α and PGE₂, in this work caused epithelial cell necrosis, edema and neutrophil infiltration, as proved by the histopathological study.

Ethanolic extract of *M. pudica* has possessed a significant protective activity against experimental colitis in rats, as indicated by macroscopic, microscopic observations ulcer index and biochemical evaluations.

Ulcer index was quantitatively determined. Pre-treatment of ethanol extract showed a better protective effect in ulcer index than other extracts comparable to standard drug.

Myeloperoxidase is a peroxidase enzyme mostly found in neutrophil granulocytes. It is a good marker of tissue injury, inflammation and neutrophil infiltration. Acetic acid raised the levels of colonic myeloperoxidase, indicating infiltration of neutrophils and perturbation of the inflammatory system, it indicates that neutrophil
accumulation contributes to the colitis induce oxidative injury. (Krawisz et al., 1984). Pretreatment with ethanol extract of M. pudica leaves ameliorated neutrophil infiltration as evidenced by inhibition of colon myeloperoxidase level and development of histological features (Joshi et al., 2011; Shiratora et al., 1989).

Malondialdehyde is a good indicator of lipid peroxidation (Zama et al., 2007), which is found to be increased in colonic tissue of the rats treated with acetic acid. Elevated lipid peroxidation can initiate vicious cycles that produce reactive metabolites, which weaken cellular anti-oxidants and help the development of more inflammation. Pretreatment of ethanol extract of M. pudica increase colonic oxidative balance in rats on colitis since it was able to reduce the malondialdehyde level significantly (Ohkawa et al., 1979). The M. pudica contains phytochemicals like flavonoid, alkaloids, glycosides and phenolic compounds. The present results may lead pharmacological support to folkloric, etnomedical uses of the plant in the management of inflammatory gastrointestinal tract disorders. Flavonoid and phenolic substances found in green plants which possess anti-oxidant and anti-inflammatory property (Sharma et al., 2011).

Conclusion

M. pudica leaves ethanol extract possesses potent activity against various pathological changes caused by administration of acetic acid. The flavonoid derivatives present in M. pudica may possess anti-oxidant as well as anti-inflammatory potential against acetic acid-induced experimental colitis by inhibition of release of oxidative-inflammatory mediators like myeloperoxidase and malondialdehyde.

Financial Support

Self-funded

Ethical Issue

All procedures of the proposed study was in accordance with the standard operating procedures of PRADO (Preclinical Research and Development Organization laboratory, Pune where the activity performed) and the guidelines provided by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) as published in The Gazette of India, December 15, 1998. Prior approval of the Institutional Animal Ethics Committee (IAEC) was obtained before initiation of the study (IAEC-15-010).

Conflict of Interest

Authors declare no conflict of interest

References


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