Evaluation of Antiulcer Activity of Whole Plant Extract of *Malvastrum tricuspidatum* in Experimental Animals

NEELAM BALEKAR, DINESH KUMAR JAIN, PANKAJ V. DIXIT, and SANDEEP SINGH BHADORIYA

ABSTRACT

*Malvastrum tricuspidatum* is recommended in Ayurveda and Folklore Medicine for the management of gastric ulcers. Therefore, the purpose of the study was to investigate the antiulcer effect of whole plant extract of *Malvastrum tricuspidatum* (MTE) on ethanol (EtOH)-induced, aspirin (ASP)-induced, cold-restraint-stress (CRU) and pylorus-ligation (PL)-induced gastric ulcer models in rats. Aqueous extract (MTAE 250, 500 mg/kg) and ethanolic extract (MTEE 250, 500 and 1000 mg/kg) were tested orally in ethanol-induced ulcer model. The ethanolic extract (MTEE 500 mg/kg) showed better ulcer protection than aqueous extract in ethanol induced ulcer model. Hence, effective dose of ethanolic extract (500 mg/kg) was further investigated in remaining models. The ethanolic extract (MTEE at the dose of 500 mg/kg) significantly inhibited the gastric lesions induced by EtOH (82.35 %), ASP (83.10 %), CRU (84.61%) and PL (75.78%), respectively. In addition MTEE showed concomitant attenuation of gastric secretory volume, free acidity, total acidity and peptic activity in ulcerated rats. Also the phytochemical tests revealed presence of antiulcer phytochemical constituents like flavonoids, tannins, terpenes and glycinebetaine in ethanolic extract. These results suggest that ethanolic extract (MTEE) of whole plant of *Malvastrum tricuspidatum* is effective against all the four experimentally induced acute gastric ulcers.

Keywords: *Malvastrum tricuspidatum*, Antiulcer, Antisecretory, Ulcer index comma
inflammatory, analgesic, antipyretic [10,11].

Preparation of ethanolic extract

The dried coarsely-powdered whole plant was extracted with petroleum ether for 48 h to remove fatty matter. The defatted marc was then subjected to soxhlet extraction with 95 % ethanol for 8 h. The total ethanolic extract was concentrated using rotary evaporator. The dried extract was weighed and then kept in refrigerator until ready for use. The yield of extract was 10.5 % (w/w) of powdered drug [14,15]. In each experiment, the ethanolic and aqueous extracts were suspended in sodium carboxymethyl cellulose (0.5%) before use.

Phytochemical screening

The chemical constituents of aqueous and ethanolic extracts were identified by qualitative phytochemical analysis [16-19] and quantitative phytochemical analysis [20,21].

Experimental Animals

Adult male albino rats (150-200 g) of Wistar strain and albino mice (20-30 g) were used in the study. The animals were procured from Veterinary College, Mhow (Indore, India). The animals were acclimatized for 10 days’ under standard, husbandry conditions, room temperature (27 ± 3°C), relative humidity (65 ± 10 %) and 12h light/dark cycle. They were allowed free access to standard dry pelleted diet (M/s Godrej Pvt Ltd., Mumbai, India) and water ad libitum under hygienic conditions. Five rats were used for each group in antiulcer study. The study was approved by the institutional animal ethics Committee, which follows the guidelines of CPSCEA (Committee for the Purpose of Control and Supervision of Experiments on Animals, which complies with international norms of INSA.

Toxicity study

Acute oral toxicity study of aqueous and ethanolic extract of the M. tricuspidatum was carried out for determination of LD₅₀ by adapting dosing schedule as per OECD guideline no. 425. The female albino mice weighing 20-30 g were used for the study. The animals were continuously observed for 12 h to detect changes in autonomic or behavioral responses. Mortality was observed for 24h. The doses of 250, 500 and 1000 g/Kg, Pune. A voucher specimen (DANVIMAL5) has been assigned by Dept. of Botany, Botanical Survey of India. Toxicity testing [22].

Treatment Schedule

For ethanol induced ulcer model rats were divided into seven groups. Each groups containing five rats.

Preparation of aqueous extract

The dried coarsely powdered whole plant (5 kg) was extracted with petroleum ether for 48 h to remove fatty matter. The defatted marc was then subjected to soxhlet extraction with 95 % ethanol for 8 h. The total aqueous extract was concentrated using rotary evaporator. The dried extract was weighed and kept in refrigerator until ready for use. The yield of extract was 5.2 % (w/w) of powdered drug [9].

Preparation of ethanolic extract

The dried coarsely-powdered whole plant was extracted with petroleum ether for 48 h to remove fatty matter. The defatted marc was then subjected to soxhlet extraction with 95 % ethanol for 8 h. The total ethanolic extract was concentrated using rotary evaporator. The dried extract was weighed and kept in refrigerator until ready for use. The yield of extract was 10.5 % (w/w) of powdered drug [14,15]. In each experiment, the ethanolic and aqueous extracts were suspended in sodium carboxymethyl cellulose (0.5%) before use.

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Treatment Schedule

For ethanol induced ulcer model rats were divided into seven groups. Each groups containing five rats.
Aspirin-induced ulcers

For aspirin-induced ulcer model rats were divided into three groups. Each group contained five rats. Group I was control and given sodium carboxymethyl cellulose (0.5 %) p.o. Group II was standard and given ranitidine (50 mg/kg) p.o. Group III was given ethanolic extract of Malvastrum tricuspidatum (500 mg/kg) p.o. After 1 h of pretreatment with ethanolic extract (500 mg/kg) and ranitidine (50 mg/kg), ASP (1000 mg/kg) suspended in 0.5% sodium carboxymethyl cellulose was given p.o. to induce gastric ulcers. After 5 h, the animals were killed and ulcer scoring was done [25].

Cold-restraint-stress-induced ulcers

For cold-restraint-stress-induced ulcer model rats were divided into three groups. Each group contained five rats. Group I was negative control (restraint-stress-controlled) and given sodium carboxymethyl cellulose (0.5 %) p.o. Group II was positive control (cold- and restraint-stress-controlled) and given sodium carboxymethyl cellulose (0.5 %) p.o. Group III was standard and given Omeprazole (20 mg/kg) p.o. Group IV was given ethanolic extract of Malvastrum tricuspidatum (500 mg/kg) p.o. In this method, male albino rats were fasted in individual cages for 24 h and care was taken to avoid coprophagy. Pylorus ligation was applied by ligating the pyloric end of the stomach of rats under ether anaesthesia for 6 h after 1 h of ethanolic extract (500 mg/kg) or omeprazole (20 mg/kg) treatment. Animals were were allowed to recover and stabilize in individual cage and were deprived of water during postoperative period. After 6 h of surgery, rats were sacrificed with over dose of chloroform and the stomach was dissected out. The glandular portion was then exposed and examined for ulceration as described earlier [28]. Gastric juice was collected and its volume [26], pH [2], free acidity and total acidity [2], mucus content [26], protein content [21] and peptic activity [29,30] were determined.

Pylorus-licigation-induced ulcer

For pylorus-ligated ulcer model, rats were divided into three groups. Each group contained five rats. Group I was control and given sodium carboxymethyl cellulose (0.5 %) p.o. Group II was standard and given omeprazole (20 mg/kg) p.o. Group III was given ethanolic extract of Malvastrum tricuspidatum (500 mg/kg) p.o.

Ethanol-induced ulcers

The male rats were randomly divided into seven groups and fasted for 24 h with free access to water. Animals were given sodium carboxymethyl cellulose (0.5%), ethanolic extract of the M. tricuspidatum at dose screening were shown Table 2.

Table 1. Qualitative phytochemical analysis of aqueous and ethanolic extract of Malvastrum tricuspidatum

<table>
<thead>
<tr>
<th>Sr.no.</th>
<th>Phytochemical tests</th>
<th>Aqueous extract</th>
<th>Ethanolic extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Saponins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Tannins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Phytosterols</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Carbohydrates</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Proteins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Terpenoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Volatile oil</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+ indicates present
- indicates absent

RESULT

Phytochemical screening

Preliminary phytochemical screening revealed the presence of flavonoids, triterpenes, saponins, tannins, alkaloids, glycosides and carbohydrates.
Acute oral toxicity study of aqueous and ethanolic extracts of *Malvastrum tricuspidatum* revealed that it 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, doses of 250, 500 and 1000 mg/kg of the extracts were selected for evaluation of anti-ulcer activity.

### Effect of MTAE and MTEE on gastric ulcer studies

Effect of MTAE and MTEE on various types of gastric ulcer models was shown in Tables 3 and 4 and Fig 1. In ulcerogen-treated animals, extensive gastric ulcers in the stomach of all the experimental models were shown. Both ethanol and cold restraint stress provoked haemorrhagic form of ulcers in the stomach with adequate evidence with intraluminal bleeding whereas aspirin caused mostly petechial ulcers and erosions. MTAE (250 and 500 mg/kg) and MTEE (250, 500 and 1000 mg/kg) given orally showed dose-dependent protective effect against gastric ulcer induced by ethanol and was comparable with omeprazole. MTEE at a dose of 500 mg/kg significantly \((p < 0.05)\) reduced gastric ulcers in pylorus ligated ulcer model.

In 6 h pylorus-ligated rats, MTEE (500 mg/kg) decreased the gastric juice volume and reversed the increased output of acid and peptic secretion (Table 3). Omeprazole showed significant \((p < 0.05)\) reduction in protein content and output of acid and peptic activity in pylorus ligation.

<p>| Table 3. Effect of MTAE and MTEE on EtOH-, ASP-, CRU- and PL-induced ulcers in rats |</p>
<table>
<thead>
<tr>
<th>Treatment dose (mg/kg)</th>
<th>Ulcer index</th>
<th>Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>EtOH-induced ulcer control (EtOH)</td>
<td>22.1 ± 0.33</td>
<td>--</td>
</tr>
<tr>
<td>OMP (20) + EtOH</td>
<td>2.5 ± 0.50*</td>
<td>88.68</td>
</tr>
<tr>
<td>MTAE (250) + EtOH</td>
<td>13.9 ± 0.18*</td>
<td>37.10</td>
</tr>
<tr>
<td>MTAE (500) + EtOH</td>
<td>4.2 ± 0.84*</td>
<td>80.90</td>
</tr>
<tr>
<td>MTEE (250) + EtOH</td>
<td>9.7 ± 0.58*</td>
<td>56.10</td>
</tr>
<tr>
<td>MTEE (500) + EtOH</td>
<td>3.9 ± 0.10*</td>
<td>82.35</td>
</tr>
<tr>
<td>MTEE (1000) + EtOH</td>
<td>3.7 ± 0.12*</td>
<td>83.25</td>
</tr>
<tr>
<td>ASP induced ulcers control (ASP)</td>
<td>14.80 ± 0.560</td>
<td>--</td>
</tr>
<tr>
<td>Ranitidine (50)</td>
<td>1.50 ± 0.223*</td>
<td>89.86</td>
</tr>
<tr>
<td>MTEE (500) + ASP</td>
<td>2.5 ± 0.220*</td>
<td>83.10</td>
</tr>
<tr>
<td>Negative control (CRU)</td>
<td>0.5 ± 0.223</td>
<td>--</td>
</tr>
<tr>
<td>Positive control (CRU)</td>
<td>6.5 ± 0.353b</td>
<td>--</td>
</tr>
<tr>
<td>OMZ (20) + CRU</td>
<td>0.9 ± 0.187*</td>
<td>85.93</td>
</tr>
<tr>
<td>MTEE (500) + CRU</td>
<td>1.0 ± 0.220*</td>
<td>84.61</td>
</tr>
<tr>
<td>PL-induced ulcers control (PL)</td>
<td>9.5 ± 0.50</td>
<td>--</td>
</tr>
<tr>
<td>OMZ (20) + PL</td>
<td>1.4 ± 0.33*</td>
<td>85.26</td>
</tr>
<tr>
<td>MTEE (500) + PL</td>
<td>2.3 ± 0.25*</td>
<td>75.78</td>
</tr>
</tbody>
</table>

EtOH: Ethanol; MTAE: Malvastrum tricuspidatum Aqueous extract; MTEE: Malvastrum tricuspidatum Ethanolic extract; OMP: omeprazole; ASP: aspirin; CRU: Restraint controlled ulcer; PL: pylorus-ligation.

Results are expressed as mean ± SEM; \(n=5\) in each group comparison made with control and with standard group. Data were analyzed by one way ANOVA followed by Tukey’s multiple comparison test.

\(*p < 0.05 = \text{compared to control group}\)

\(b p < 0.05 = \text{compared to standard group}\)
The anti-ulcer activity of the whole plant extract of *Malvastrum tricuspidatum* as evaluated by employing aspirin, ethanol, cold restraint 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 aggressive factors and correct the imbalance between protective and defensive factors indicating its cytoprotective, antioxidant, neutralizing, and anti-secretory properties. In the pylorus ligation ulcer model, the fo rmal extract of *Malvastrum tricuspidatum* has significantly protected the gastric mucosa against ethanol challenge as shown by reduced values of lesion index and increased mucus content as compared to control group.

**Table 4.** Gastroprotective activity of ethanolic extract of whole plant of *Malvastrum tricuspidatum* on various parameters in pylorus ligated ulcer

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Volume of gastric juice (ml)</th>
<th>pH</th>
<th>Free acidity (mEq/l/100g)</th>
<th>Total acidity (mEq/l/100g)</th>
<th>Gastric mucus content (µg of alcian blue/g of stomach)</th>
<th>Total protein (µg/ml)</th>
<th>Pepsin activity (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>--</td>
<td>4.32 ± 0.25</td>
<td>2.4 ± 0.31</td>
<td>27.2 ± 2.45</td>
<td>47.4 ± 2.13</td>
<td>4.82 ± 0.11</td>
<td>286.38 ± 15.68</td>
<td>45.75 ± 1.39</td>
</tr>
<tr>
<td>OMZ</td>
<td>20</td>
<td>2.24 ± 0.19*</td>
<td>3.94 ± 0.20*</td>
<td>11.0 ± 0.70*</td>
<td>26.2 ± 1.53*</td>
<td>7.44 ± 0.49*</td>
<td>165.3 ± 8.53*</td>
<td>22.04 ± 0.84*</td>
</tr>
<tr>
<td>MTEE</td>
<td>500</td>
<td>1.68 ± 0.18*</td>
<td>4.52 ± 0.18*</td>
<td>11.48 ± 0.54*</td>
<td>21.8 ± 1.49*</td>
<td>5.83 ± 0.16*</td>
<td>191.7 ± 12.84*</td>
<td>31.85 ± 0.59*</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM; n=5 in each group comparison made with control and with standard group. Data were analyzed by one way ANOVA followed by Tukey’s multiple comparison test.

*ap < 0.05= compared to control group

*bp < 0.05= compared to standard group
histamine secretion. On the other hand, tannins and polyphenols may prevent ulcer development due to their protein precipitating and vasoconstricting effects. Their astringent action can help precipitating microproteins on ulcer site thereby forming an impervious layer over the lining that hinders gut secretions and protects underlying mucosa from toxins and other irritants and stimulate PGE2 formation. Terpenes are known to possess antulcer activity and their action has been suggested to be due to the activation of cellular protection, reduction of mucosal prostaglandins metabolism-cytoprotective action and reduction of gastric vascular permeability. Betaine also known as glycinebetaine closely related to amino acid, glycine.

Earlier experimental studies indicated that betaine could preserve cellular and subcellular membranes from free radical mediated oxidative damage by its antioxidant activity. The ability of betaine to maintain the mucosal antioxidant status at higher rate demonstrates its possible preventive efficacy in inhibiting free radical mediated ulcerogenesis. The antulcer activity of betaine is probably related to its ability to neutralize the hydrochloric acid secreted in to stomach and/or its antioxidant nature by which it maintain the level of GSH and the activities of the mucosal antioxidant enzymes near normal status. Thus it protects the gastric mucosa against oxidative damage by decreasing lipid peroxidation and strengthening the mucosal barrier.

In conclusion, On the basis of the present results and available reports, it can be concluded that the anti-ulcer activity elucidated by Malvastrum tricuspidatum could be mainly due to the modulation of defensive factors through an improvement of gastric cytoprotection and partly due to decreased acid secretion. The results also supported the presence of flavonoids, tannins, and terpenes in ethanolic extract of Malvastrum tricuspidatum that are reported to possess antulcer activity by various mechanisms like free radical scavenging, increased mucosal PGE2, increased mucosal blood flow, decreased histamine secretion, astringent action, neutralizing HCl secreted and antioxidant nature. Hence, it is suggested that Malvastrum tricuspidatum ethanolic extract show antulcer activity by suppressing gastric damage induced by aggressive factors as well as by regulating the defensive factors.

REFERENCES


Antiulcer Activity of Malvastrum tricuspidatum


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