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-division (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], antibacterial [9] and antinociceptive activity [12]. Chronic toxicity study of *Malvastrum tricuspidatum* showed that extract of whole plant given orally to Wistar rats at the dose of 0.2-20 g/kg for 60 days did not produce toxicity in the animals [13]. Our research interest in this plant arose because of its potential medicinal value against peptic ulcer, as used in folk medicine and presence of antitumor phytochemical constituents like flavonoids, tannins, and glycinine. Experimental study to determine antitumor potential of *M. tricuspidatum* and possible mechanisms for inhibition of gastric ulcer is not reported earlier so it was worthwhile to undertake such investigation using aqueous and ethanolic extract of whole plant of *M. tricuspidatum*. The present study incorporates the evaluation of antitumor effect of aqueous and ethanolic extract of whole plant of *M. tricuspidatum* in Ethanol-induced (CRU), aspirin-induced (ASP), cold restraint stress (IND), and pylorus ligation (PL)-induced ulcer models. In addition possible mechanisms for gastroprotection by major antitumor phytochemical constituents of *M. tricuspidatum* in all the four acute gastric ulcer models were suggested in the present study. This study thus provides an insight on the mechanism of the antitumor effect of *M. tricuspidatum*.

**MATERIALS AND METHODS**

**Drugs and chemicals**

Aspirin (bulk drug) was obtained as gift sample from Cyno Pharma, Indore, India and omeprazole and ranitidine was obtained from Alpa Lab. Indore, I & M, and ranitidine was obtained from Alpa Lab. Indore, India. Ethanol (Merck Pvt. Ltd., Mumbai) and diethyl ether (Sisco Research Lab. Pvt. Ltd., Mumbai). All the other chemicals and reagents used were prepared immediately before use and were of analytical grade.

**Plant material**

* *M. tricuspidatum* whole plant was collected from the local garden of College of IPS academy, Indore. The plant was identified and authenticated by T. Chakraborty, Jeiffntist ‘D’ Botanical Survey of India, Pune. A voucher specimen (DANVIMALT5) has been assigned by Dept. of Botany, Botanical Survey of India.

**Preparation of extracts**

1. 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 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.

**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 antitumor 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 24 h. The doses of 250, 500 and 1000 g/Kg, p.o. were selected based on the results of preliminary toxicity testing [22].

**Treatment Schedule**

Ethanol-induced ulcers

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 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.

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Pylorus-ligation-induced gastritis

In this method, male albino rats were fasted in individual cages for 24 h and were deprived of water during the 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.

Antilulcer study

Ethanol-induced ulcers

The male rats were randomly divided into seven 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) or mucus content was determined [26]. Group IV was standard and given omeprazole (20 mg/kg) p.o. Group III was given anethic extract of *Malvastrum tricuspidatum* (500 mg/kg) p.o.

Pylorus-ligation-induced ulcers

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 anethic extract of *Malvastrum tricuspidatum* (500 mg/kg) p.o.

Cold-restraint-stress-induced gastric ulcer

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.

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) 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 ulcers scoring was done [25]. Group III was given anethic extract of *Malvastrum tricuspidatum* (500 mg/kg) p.o.

Cold-stress-induced gastric ulcer

After 1 h of restraint stress, the rats were subjected to cold stress in a refrigerator for 2 h. The animals were sacrificed 2 h later and ulcer index was determined following previously-described method [26] and mucus content was determined [27].

Preliminary phytochemical screening revealed the presence of flavonoids, triterpenes, saponins, tannins, carbohydrates, glycosides and carbohydrates. Antilulcer activity of *M. tricuspidatum* extract at dose of 250, 500 and 1000 mg/kg and aqueous extract 250, 500 mg/kg or Omeprazole (20 mg/kg) orally. After pretreatment of extract and omeprazole, EtOH (1 ml/200 gm of absolute ethanol) was administered orally to each group [23]. Animals were sacrificed after 1 h by cervical dislocation. Stomachs were isolated, opened along the greater curvature and were gently rinsed with saline to remove the gastric content and blood clot. The ulcer scoring was done and the percentage protection was calculated [24]. Percent ulcer inhibition = Mean ulcer index of control - Mean ulcer index of test / Mean ulcer index of control × 100

Result

Phytochemical screening

The results of quantitative phytochemical study (Table 1). The presence of flavonoids, triterpenes, saponins, tannins, carbohydrates, glycosides and carbohydrates. Antilulcer activity of *M. tricuspidatum* extract at dose of 250, 500 and 1000 mg/kg and aqueous extract 250, 500 mg/kg or Omeprazole (20 mg/kg) orally. After pretreatment of extract and omeprazole, EtOH (1 ml/200 gm of absolute ethanol) was administered orally to each group [23]. Animals were sacrificed after 1 h by cervical dislocation. Stomachs were isolated, opened along the greater curvature and were gently rinsed with saline to remove the gastric content and blood clot. The ulcer scoring was done and the percentage protection was calculated [24]. Percent ulcer inhibition = Mean ulcer index of control - Mean ulcer index of test / Mean ulcer index of control × 100.
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.

### Table 2. Quantitative phytochemical analysis of aqueous and ethanolic extract of *Malvastrum tricuspidatum*

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>Quantity in aqueous extract</th>
<th>Quantity in ethanolic extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids (%)</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>Flavonoids (%)</td>
<td>12.50</td>
<td>20.50</td>
</tr>
<tr>
<td>Carbohydrates (mg/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>4.7</td>
<td>4.3</td>
</tr>
<tr>
<td>Fructose</td>
<td>5.4</td>
<td>4.56</td>
</tr>
<tr>
<td>Lactose</td>
<td>6.5</td>
<td>5.93</td>
</tr>
<tr>
<td>Maltose</td>
<td>7.47</td>
<td>6.37</td>
</tr>
<tr>
<td>Lipids (mg/ml)</td>
<td>0.208</td>
<td>0.28</td>
</tr>
</tbody>
</table>

### Table 3. Effect of MTAE and MTEE on EtOH-, ASP-, CRU- and PL-induced ulcers in rats

<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&lt;sup&gt;a&lt;/sup&gt;</td>
<td>88.68</td>
</tr>
<tr>
<td>MTAE (250) + EtOH</td>
<td>13.9 ± 0.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>37.10</td>
</tr>
<tr>
<td>MTAE (500) + EtOH</td>
<td>4.2 ± 0.84&lt;sup&gt;a&lt;/sup&gt;</td>
<td>80.90</td>
</tr>
<tr>
<td>MTEE (250) + EtOH</td>
<td>9.7 ± 0.58&lt;sup&gt;b&lt;/sup&gt;</td>
<td>56.10</td>
</tr>
<tr>
<td>MTEE (500) + EtOH</td>
<td>3.9 ± 0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>82.35</td>
</tr>
<tr>
<td>MTEE (1000) + EtOH</td>
<td>3.7 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</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&lt;sup&gt;a&lt;/sup&gt;</td>
<td>89.86</td>
</tr>
<tr>
<td>MTEE (500) + ASP</td>
<td>2.5 ± 0.220&lt;sup&gt;a&lt;/sup&gt;</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 ± 0.353&lt;sup&gt;b&lt;/sup&gt;</td>
<td>--</td>
</tr>
<tr>
<td>OMZ (20) + CRU</td>
<td>0.9 ± 0.187&lt;sup&gt;a&lt;/sup&gt;</td>
<td>85.93</td>
</tr>
<tr>
<td>MTEE (500) + CRU</td>
<td>1.0 ± 0.220&lt;sup&gt;a&lt;/sup&gt;</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&lt;sup&gt;a&lt;/sup&gt;</td>
<td>85.26</td>
</tr>
<tr>
<td>MTEE (500) + PL</td>
<td>2.3 ± 0.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>75.78</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.

<sup>a</sup>*p* < 0.05= compared to control group

<sup>b</sup>*p* < 0.05= compared to standard group
The anti-ulcer activity of the whole plant extract of *Malvastrum tricuspidatum* as evaluated by employing various models [30]. Ethanolic extract of *Malvastrum tricuspidatum* significantly protected the gastric mucosa against ethanol-induced mucosal damage by decreasing proinflammatory cytokines and aggressive factors indicating its potent cytoprotective, antioxidant, neutralizing and antisecretory properties. NSAIDs like aspirin cause gastric mucosal damage by decreasing prostaglandin levels and through inhibition of prostaglandin synthesis [33,35] by several mechanisms in the present study.

Ethanolic extract of *Malvastrum tricuspidatum* was significantly effective in protecting gastric mucosa against aspirin-induced stress ulcers at the dose of 500 mg/kg [36]. In pylorus ligation models, the anti-ulcer activity of the whole plant extract of *Malvastrum tricuspidatum* was significantly effective in protecting gastric mucosa against cold restraint stress ulcers at the dose of 500 mg/kg as shown by reduced values of lesion index and increased mucus content as compared to control group, suggesting its potent cytoprotective and antisecretory 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 [35]. The anti-ulcer activity of ethanolic extract of *Malvastrum tricuspidatum* at the dose of 500 mg/kg 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. In animals treated with ethanolic extract of *Malvastrum tricuspidatum*, the formation was mainly due to gastric hypermotility, which could lead to mucosal over friction and generation of free radical during stress ulcer [34].

### DISCUSSION

The anti-ulcer activity of the whole plant extract of *Malvastrum tricuspidatum* was significantly effective in protecting gastric mucosa against cold restraint stress ulcers at the dose of 500 mg/kg as shown by reduced values of lesion index and increased mucus content as compared to control group, suggesting its potent cytoprotective and antisecretory 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 [35]. The anti-ulcer activity of ethanolic extract of *Malvastrum tricuspidatum* at the dose of 500 mg/kg 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. In animals treated with ethanolic extract of *Malvastrum tricuspidatum*, the formation was mainly due to gastric hypermotility, which could lead to mucosal over friction and generation of free radical during stress ulcer [34].

### Table 4. Gastrophotoprotective 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/l100g)</th>
<th>Total acidity (mEq/l100g)</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>8.74 ± 0.44*</td>
<td>165.3 ± 8.53*</td>
<td>18.04 ± 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 ± 12.85*</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.

* p < 0.05* compared to control group

* p < 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 antiulcer activity and their action has been suggested to be due to the activation of cellular protection, reduction of mucosal prostaglandins metabolism-cytotoxic 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 antiulcer 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 maintains the level of GSH and the activities of the mucosal antioxidant enzymes to 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 antiulcer 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 antiulcer activity by suppressing gastric damage induced by aggressive factors as well as by regulating the defensive factors.

### REFERENCES


Antiulcer Activity of Malvastrum tricuspidatum


