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) at the dose of 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

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inflammatory, analgesic, antipyretic [10,11]. 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 antulcer phytochemical constituents like flavonoids, tannins, and glycinebetaine. Experimental study to determine antulcer 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 antulcer effect of aqueous and ethanolic extract of whole plant of M. tricuspidatum in Ethanol-induced (EtOH), aspirin-induced (ASP), cold restraint stress(CRU)- and pylorus ligation (PL)-induced ulcer models. In addition possible mechanisms for gastroprotection by major antulcer 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 antulcer 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, India. Ethanol (Merck Pvt. Ltd., Mumbai) and diethyl ether (Sisco Research Lab. Pvt. Ltd., Mumbai). All the other chemicals and reagent 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, Scientist ‘D’ Botanical Survey of India, Indore, India. 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 antulcer 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, India) 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 LD<sub>50</sub> 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, 19 were suspended in 1% carboxymethyl cellulose (0.5%) before use.

**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.

**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.

**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 antulcer 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, India) which complies with international norms of INSA.

**Treatment Schedule**

**Ethanol-induced ulcers**

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

**Preparation of extracts**

**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.

**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.
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.

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.

Pylorus-lation-induced gastric 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.

Result

Phytochemical screening

Preliminary phytochemical screening revealed the presence of flavonoids, triterpenes, saponins, tannins, phytosterol, alkaloids, glycosides and carbohydrates. Animals were given sodium carboxymethyl cellulose (0.5%), ethanolic extract of the M. tricuspidatum at dose screening were shown Table 1.
Toxicity study

Acute oral toxicity study of aqueous and ethanolic extracts of the *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.

**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>4.7</td>
<td>4.3</td>
</tr>
<tr>
<td>Glucose</td>
<td>5.4</td>
<td>4.56</td>
</tr>
<tr>
<td>Fructose</td>
<td>6.5</td>
<td>5.93</td>
</tr>
<tr>
<td>Lactose</td>
<td>7.47</td>
<td>6.37</td>
</tr>
<tr>
<td>Maltose</td>
<td></td>
<td></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 ± 0.50¹</td>
<td>88.68</td>
</tr>
<tr>
<td>MTAE (250) + EtOH</td>
<td>13.9 ± 0.18ᵃ</td>
<td>37.10</td>
</tr>
<tr>
<td>MTAE (500) + EtOH</td>
<td>4.2 ± 0.84ᵃ</td>
<td>80.90</td>
</tr>
<tr>
<td>MTEE (250) + EtOH</td>
<td>9.7 ± 0.58ᵃ</td>
<td>56.10</td>
</tr>
<tr>
<td>MTEE (500) + EtOH</td>
<td>3.9 ± 0.10ᵃ</td>
<td>82.35</td>
</tr>
<tr>
<td>MTEE (1000) + EtOH</td>
<td>3.7 ± 0.12ᵃ</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 ± 0.22</td>
<td>--</td>
</tr>
<tr>
<td>Positive control (CRU)</td>
<td>6.5 ± 0.35³</td>
<td>--</td>
</tr>
<tr>
<td>OMZ (20) + CRU</td>
<td>0.9 ± 0.18⁰</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 ± 0.33⁰</td>
<td>85.26</td>
</tr>
<tr>
<td>MTEE (500) + PL</td>
<td>2.3 ± 0.25⁰</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.

¹p < 0.05= compared to control group
³p < 0.05= compared to standard group
Antiulcer Activity of Malvastrum tricuspidatum

**DISCUSSION**

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 defensive factors and mechanisms are implicated in the ulcerogenesis and gastric mucosal damage induced by aggressive factors and correct the imbalance between defensive factors. Inhibiting, depletion of gastric wall, mucosal damage, cytoprotective, antioxidant, neutralizing and induced by non-steroidal anti-inflammatory drugs and antispasmodic properties.

Flavonoids have antiulcer and gastroprotective activities. Several gastroprotective mechanisms have

<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 20</td>
<td>2.24 ± 0.19^a</td>
<td>3.94 ± 0.20^a</td>
<td>11.0 ± 0.70^a</td>
<td>26.2 ± 1.53^a</td>
<td>8.74 ± 0.44^a</td>
<td>165.3 ± 8.53^a</td>
<td>18.04 ± 0.84^a</td>
<td></td>
</tr>
<tr>
<td>MTEE 500</td>
<td>1.68 ± 0.18^b</td>
<td>4.52 ± 0.18^b</td>
<td>11.48 ± 0.54^b</td>
<td>21.8 ± 1.49^b</td>
<td>5.83 ± 0.16^b</td>
<td>191.7 ± 12.85^b</td>
<td>31.85 ± 0.59^b</td>
<td></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-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 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 maintain the level of reduced 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


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