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

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Received January 9, 2012; Revised April 14, 2012; Accepted May 23, 2012

**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].
50 Antibacterial [9] and antinociceptive activity [12].
51 Chronic toxicity study of Malvastrum tricuspidatum
52 showed that extract of whole plant given orally to
53 Wistar rats at the dose of 0.2-20 g/kg for 60 days did
54 not produce toxicity in the animals [13]. Our research
55 interest in this plant arose because of its potential
56 medicinal value against peptic ulcer, as used in folk
57 medicine and presence of antitumor phytochemical
58 constituents like flavonoids, tannins, and glycosides.
59 Experimental study to determine antitumor potential of
60 M. tricuspidatum and possible mechanisms for
61 inhibition of gastric ulcer is not reported earlier, so it
62 was worthwhile to undertake such investigation using
63 aqueous and ethanolic extract of whole plant of M.
64 tricuspidatum.

65 MATERIALS AND METHODS

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

77 Phytochemical screening
78 The chemical constituents of aqueous and ethanolic
79 extracts were identified by qualitative phytochemical
80 analysis [16-19] and qualitative phytochemical
81 analysis [20,21].

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

98 Toxicity study
99 Acute oral toxicity study of aqueous and ethanolic
100 extract of the M. tricuspidatum was carried out for
101 determination of LD₅₀ by adapting dosing schedule as
102 per OECD guideline no. 425. The female albino mice
103 weighing 20-30 g were used for the study. The animals
104 were continuously observed for 12 h to detect changes
105 in autonomic or behavioral responses. Mortality was
106 observed for 24h. The doses of 250, 500 and 1000 g/Kg,
107 were selected based on the results of preliminary
108 toxicity testing [22].

109 Treatment Schedule
110 For ethanol induced ulcer model rats were divided
111 into seven groups. Each groups containing five rats.
112 Preparation of extracts
113 The dried coarsely powdered whole plant (5 kg) was
114 extracted with petroleum ether for 48 h to remove fatty
115 matter. The defatted marc was then subjected to
116 decocation for 1 h. Then it was filtered through muslin
117 (20) cloth. The total aqueous extract was concentrated
118 using rotary evaporator. The dried extract was weighed and
119 then kept in refrigerator until ready for use. The yield of
120 extract was 5.2 % (w/w) of powdered drug [9].

121 Malvastrum tricuspidatum (250, 500 mg/kg) p.o.
122 Malvastrum tricuspidatum (250, 500, 1000 mg/kg) p.o.

123 Malvastrum tricuspidatum (250, 500 mg/kg) p.o.
Table 1. Qualitative phytochemical analysis of aqueous and ethanolic extract of Malvastrum tricuspidatum

<table>
<thead>
<tr>
<th>Sr.no.</th>
<th>Phytochemicals tested</th>
<th>Aqueous extract</th>
<th>Ethanol 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

**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-restraint-stress-controlled) and given sodium carboxymethyl cellulose (0.5%) p.o.
- **Group III** was standard and given omeprazole (20 mg/kg) p.o.

**Pylorus-formation-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.

**Antiulcer 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) p.o.
- **Group III** was given ethanolic extract of Malvastrum tricuspidatum (500 mg/kg) p.o.

**Pylorus-formation induced gastric ulcer**

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 gastric ulcer**

After 1 h of pretreatment with ethanolic extract (500 mg/kg), rats were subjected to cold stress in restraint cages that were placed at 2 - 4°C 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].

**Result**

Preliminary phytochemical screening revealed the presence of flavonoids, triterpenes, saponins, tannins, phytosterol, alkaloids, glycosides and carbohydrates.(0.5%), ethanolic extract of the M. tricuspidatun at dose 258 screening were showed Table 2.
Toxicity study

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

**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 ± 0.50a</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 ± 0.223a</td>
<td>89.86</td>
</tr>
<tr>
<td>MTEE (500) + ASP</td>
<td>2.5 ± 0.220a</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 ± 0.187a</td>
<td>85.93</td>
</tr>
<tr>
<td>MTEE (500) + CRU</td>
<td>1.0 ± 0.220a</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 ± 0.33a</td>
<td>85.26</td>
</tr>
<tr>
<td>MTEE (500) + PL</td>
<td>2.3 ± 0.25a</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.

*a* *p* < 0.05 = compared to control group  
*b* *p* < 0.05 = compared to standard group
These phytochemicals have been proposed to explain the biological effects of Malvastrum tricuspidatum. Ethanol 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 regeneration of free radical during stress ulcer [34].

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>24.3 ± 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</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>
<td></td>
</tr>
<tr>
<td>MTEE 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>
<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

*b* = 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 astrigent 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 PGE$_2$ 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 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 Malvus 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 Malvus tricuspidatum that are reported to possess antiulcer activity by various mechanisms like free radical scavenging, increased mucosal PGE$_2$, increased mucosal blood flow, decreased histamine secretion, astringent action, neutralizing HCl secreted and antioxidant nature. Hence, it is suggested that Malvus tricuspidatum ethanolic extract show antiulcer activity by suppressing gastric damage induced by aggressive factors as well as by regulating the defensive factors.

REFERENCES

Antilulcer Activity of Malvastrum tricuspidatum


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