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

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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 antiulcer phytochemical constituents like flavonoids, tannins, and glycinebetaine.

Experimental study to determine antiulcer 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 antiulcer effect of aqueous and ethanolic extract of whole plant of *M. tricuspidatum* in Ethanol-induced (ASP), aspirin-induced (ASP), cold restraint stress (CRU)- and pylorus ligation (PL)-induced ulcer models. In addition possible mechanisms for gastrotection by major antiulcer phytochemicals 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 antiulcer 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 Dr. Chakraborty, Seientist ‘D’ Botanical Survey of India, observed for 24h. The doses of 250, 500 and 1000 g/Kg, Pune. A voucher specimen (DANVIMALT5) has been assigned by Dept. of Botany, Botanical Survey of India. Toxicity testing [22].

The whole plant was collected in the month of July, 2009 and shade dried at room temperature.

**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-powerd 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 recorded on day 14 as a measure of lethality. The data were analyzed by the method of Litchfield and Wilcoxon.

**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 5.2 % (w/w) of powdered drug [9].

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Antiulcer Activity of *Malvastrum tricuspidatun*

### 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>Phenylterpenoids</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

#### 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 (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-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 ethanolic extract of *Malvastrum tricuspidatum* (500 mg/kg) p.o.

### 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 negative control (restraint stress-controlled) and given sodium carboxymethyl cellulose (0.5 %) p.o.

**Group III** was given ethanolic extract of *Malvastrum tricuspidatum* (250, 500 and 1000 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].

**Group IV** was positive control (cold- and restraint-stress-controlled) and given sodium carboxymethyl cellulose (0.5 %) p.o.

**Group V** was standard and given *Omeprazole* 20 mg/kg p.o.

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

**Group VII** was standard and given *Omeprazole* 20 mg/kg p.o.

In this method, male albino rats were fasted in individual cages for 24 h 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.

### phytochemical screening

Preliminary phytochemical screening revealed the presence of flavonoids, triterpenes, saponins, tannins, alkaloids, glycosides and carbohydrates. Animals were given sodium carboxymethyl cellulose (Table 1). The results of quantitative phytochemical study showed 16% of 250, 500 and 1000 mg/kg and aqueous extract 250, 500 mg/kg or *Omeprazole* (20 mg/kg) orally. After 6 h of surgery, rats were sacrificed with overdose 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.
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 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>
<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>Glucose</td>
<td>4.7</td>
</tr>
<tr>
<td></td>
<td>Fructose</td>
<td>5.4</td>
</tr>
<tr>
<td></td>
<td>Lactose</td>
<td>6.5</td>
</tr>
<tr>
<td></td>
<td>Maltose</td>
<td>7.47</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*</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 ± 0.353#</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= compared to control group

*p* < 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 ulcerogenesis and gastric mucosal damage induced by aggressive factors and correct the imbalance between defensive factors indicating its protective, antioxidant, neutralizing and cytoprotective effect. It is suggested that *Malvastrum tricuspidatum* ethanolic extract can suppress gastric damage induced by free radical production. Ethanol-induced gastric ulcer in the stomach was significantly inhibited, both as shown by reduced values of lesion index as compared to control group. 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. 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*, gastric ulcer formation was mainly due to gastric hypermotility, which could lead to mucosal over friction and regeneration of free radical during stress ulcer. 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. Ethanol extract of *Malvastrum tricuspidatum* has been proposed to explain the biologic effects of flavonoids, alkaloids, tannins, saponins, terpenes, amino acids, gums and mucilages are reported to possess oxygen free radicals leading to increased lipid peroxidation, which causes damage to cell and cell membrane. In phytomedicine, various phytoconstituents like flavonoids, tannins, terpenes, amino acids, gums and mucilages are reported to possess scavenging effect. NSAIDs like aspirin cause gastric ulcer by several mechanisms employed in the present study. Antioxidant and anti-inflammatory drugs and anti-secretory properties.

Ethanol extract of *Malvastrum tricuspidatum* has been proposed that in pyloric ligation, the oxidative stress ulcers at the dose of 500 mg/kg been proposed to explain the biological effects of flavonoids including free radical scavenging during stress ulcer which could lead to mucosal over friction and regeneration of free radical during stress ulcer have been proposed to explain the biological effects of flavonoids, alkaloids, tannins, saponins, terpenes, amino acids, gums and mucilages are reported to possess scavenging effect. NSAIDs like aspirin cause gastric ulcer by several mechanisms employed in the present study. Antioxidant and anti-inflammatory drugs and anti-secretory properties.

### 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 20</td>
<td>2.24 ± 0.19a</td>
<td>3.94 ± 0.20a</td>
<td>11.0 ± 0.70</td>
<td>26.2 ± 1.53a</td>
<td>8.74 ± 0.44a</td>
<td>165.3 ± 8.53</td>
<td>18.04 ± 0.84</td>
<td></td>
</tr>
<tr>
<td>MTEE 500</td>
<td>1.68 ± 0.18a</td>
<td>4.52 ± 0.18a</td>
<td>11.48 ± 0.54</td>
<td>21.8 ± 1.49a</td>
<td>5.83 ± 0.16b</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.

*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 PGE\textsubscript{2} 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-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 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 may elevate 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 antulcer 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 PGE\textsubscript{2}, 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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