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

For author affiliations, see end of text.

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

This paper is available online at http://ijpt.iums.ac.ir

**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 glycinin 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

Peptic ulcer mostly refers to Amlapitta or gastrointestinal inflammations. Amlapitta, in Ayurveda, Amlapitta is a disease of the gastrointestinal tract, especially the stomach [1]. Peptic ulcer is one of the major ailments affecting about 60% of human adults and nearly 80% of child population in tropical countries [2]. Peptic ulcer is the most common gastrointestinal disorder in clinical practice. Considering the several side effects (arrhythmia's, impotence, Indian subcontinent [4]. The leaves are applied to the treatment of peptic ulcer. There is evidence concerning the participation of reactive oxygen species [5]. This plant is used in the etiology and pathophysiology of human diseases, such as neurodegenerative disorders, inflammation, viral infections, autoimmune pathologies and digestive system disorders such as gastrointestinal inflammations. It is traditionally used as antipyretic, smooth muscle relaxant and system disorders such as gastrointestinal inflammations. It is traditionally used as antipyretic, smooth muscle relaxant and...
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 12 h 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 ant ulcer study. The study was approved by the institutional animal ethics Committee, which follows the guidelines of CPCSEA (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 extracts

Preparation of aqueous extract

The dried coarsely powdered whole plant (5 kg) was extracted with petroleum ether for 48 h to remove fatty carboxymethyl cellulose (0.5 %) p.o.

Preparation of alcoholic extract

The total aqueous 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].

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 12 h 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 ant ulcer study. The study was approved by the institutional animal ethics Committee, which follows the guidelines of CPCSEA (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.
ARTICLE IN PRESS

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>Phytoesters</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- 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.

Result

Ethanol-induced ulcers

The male rats were randomly divided into seven groups and fasted for 24h 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.
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</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;a&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;a&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</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;a&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>

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.

<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 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* at the dose of 500 mg/kg in pylorus ligation model is evident from reduced values of lesion index and 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 of pylorus ulcer in the stomach was significantly inhibited, both by several mechanisms in the present study. The anti-ulcerogenic and gastric mucosal damage induced by aggressive factors and correct the imbalance between different protective and defensive factors indicating its involving, depletion of gastric wall, mucosal damage, cytoprotective, antioxidant, neutralizing and induced by non-steroidal anti-inflammatory drugs and antispetic properties.

**DISCUSSION**

The anti-ulcer activity of the whole plant extract of *Malvastrum tricuspidatum* as evaluated by employing pylorus ligation model is evident from reduced values of lesion index and 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 reduced values of lesion index and 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 reduced values of lesion index and 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 reduced values of lesion index and 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 reduced values of lesion index and 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].

![Fig 1. Comparison of mean ulcer index among ethanol-induced ulcer, aspirin-induced ulcer, cold restraint ulcer and pylorus ligation models](image)

**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>2.41 ± 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 ± 0.14</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

\( ^* p < 0.05 \) 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 microparticles on ulcer site thereby forming an impervious layer over the lining that hinders gut secretions and protects underlying mucus from toxins and other irritants and stimulate PGE₂ formation. Terpenes are known to posses 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.

487

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 GSH and the activities of the mucosal antioxidant enzymes to near normal status. Thus it protects the gastric mucus against oxidative damage by decreasing lipid peroxidation and strengthening the mucosal barrier.

488

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.

489

REFERENCES

490


Antiulcer Activity of Malvastrum tricuspidatum


Current Author Addresses
Neelam Balekar, College of Pharmacy, IPS Academy, Rajendra Nagar, A.B. Road, Indore- 452012, India. E-mail: neelambalekar@gmail.com (Corresponding author)
Dinesh Kumar Jain, College of Pharmacy, IPS Academy, Rajendra Nagar, A.B. Road, Indore- 452012, India.
Pankaj V. Dixit, College of Pharmacy, IPS Academy, Rajendra Nagar, A.B. Road, Indore- 452012, India.
Sandeep Singh Bhadoriya, College of Pharmacy, IPS Academy, Rajendra Nagar, A.B. Road, Indore- 452012, India.