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-igation (PL)-induced gastric ulcer models in rats. Aqueous extract (MTEA 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

Peptic ulcer mostly refers to Amlapitta or system disorders such as gastrointestinal inflammations Parinamasula; in Ayurveda, Amlapitta is a disease of the and gastric ulcer [3].

gastrointestinal tract, especially the stomach [1]. Peptic is one of the major ailments affecting about 60% one plant possessing anti-ulcer activity is *M.*

of human adults and nearly 80% of child population in *Malvastrum tricuspidatum*. *M. tricuspidatum* (Malvaceae), also tropical countries [2]. Peptic ulcer is the most common known as Kharenti or Bala, is an erect under shrub or gastrointestinal disorder in clinical practice. Considering herb, found as a weed distributed world wide, also in the the several side effects (arrhythmia’s, impotence, Indian subcontinent [4]. The leaves are applied to fumeomastia and haematopoietic changes) of modern inflamed sores and wound. The flowers are given as a antulcer medicine, indigenous drugs possessing fewer pectoral and diaphoretic [5]. This plant is used side effects should be looked for as a better alternative ethnomedicinally in cough, chest and lung disease. The for the treatment of peptic ulcer. There is evidence decoction of leaf is given in dysentery and smelling of concerning the participation of reactive oxygen species root helps to prevent vomiting [6]. It is traditionally in the etiology and pathophysiology of human diseases, such as neurodegenerative disorders, inflammation, ulceroprotective [7-9]. *M. tricuspidatum*, crude water viral infections, autoimmune pathologies and digestive extract (Whole plant) was reported to possess anti-
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 (EtOH), aspirin-induced (ASP), cold restraint stress (CRU)- and pylorus ligation (PL)-induced ulcer models. In addition possible mechanisms for gastroprotection by major antiulcer 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 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**

Malvastrum tricuspidatum whole plant was collected from the local garden of College of IPS academy, Indore. The plant was identified and authenticated by T. Chakraborty, Sejentist ‘D’ Botanical Survey of India, Pune. A voucher specimen (DANVIMALT5) has been assigned by Dept. of Botany, Botanical Survey of India. 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, 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). 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 dose 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, p.o. were selected based on the results of preliminary toxicity testing [22].

**Treatment Schedule**

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

**Ethanol-induced Ulcers**

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-IV were given aqueous extract of Malvastrum tricuspidatum (250, 500 mg/kg) p.o.

Groups V-VII were given ethanolic extract of Malvastrum tricuspidatum (250, 500, 1000 mg/kg) p.o.
Antifungal Activity of Malvastrum tricuspidatum

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.

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 p.o. screening were shown Table 2.

result

Preliminary phytochemical screening revealed the presence of flavonoids, triterpenes, saponins, tannins, carbohydrates, glycosides and alkaloids.

Table 2. 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>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
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.

Effect of MTAE and MTEE on gastric ulcer studies

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*</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
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 represents 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 indicating its antiulcer effect. The ethanolic extract of *Malvastrum tricuspidatum* significantly protected the gastric mucosa against gastric acid concentration and gastric volume were decreased in pylorus ligation models. The ethanolic extract of *Malvastrum tricuspidatum* has been prepared and phytochemical analysis revealed flavonoids, alkaloids, tannins, saponins, terpenes, amino acids, gums and mucilages are reported to possess free radical scavenging effect. NSAIDs like aspirin cause gastric mucosal damage by decreasing prostaglandin levels and the pH values, mucus content were increased as shown by reduced values of lesion index as compared to control group. The ethanolic 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 antisecretary 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. 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 free radical during stress ulceration is associated with significant production of flavonoids and tannins as a major factor and the pH values, mucus content were increased. Many phytochemical constituents like flavonoids, alkaloids, tannins, terpenes and glycinomembrane of ethanolic extract of *Malvastrum tricuspidatum* have been proposed that in pyloric ligation, the interference of gastric blood circulation are responsible for induction of ulceration.

**Table 4.** Gastrophrotective 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>Peptic 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 the underlying mucosa from toxins and other irritants and stimulates PGE\textsubscript{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 protective 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 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 cytoprotective 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 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 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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