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

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**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

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

Peptic ulcer is one of the major ailments affecting about 60% one plant possessing anti-ulcer activity is *M. 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 fumeacomastia and haematopoetic changes) of modern inflamed sores and wound. The flowers are given as a antiulcer 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 concerning the participation of reactive oxygen species root helps to prevent vomiting [6]. It is traditionally in the etiology and pathophysiology of human diseases, used as antipyretic, smooth muscle relaxant and 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-
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 a rotary evaporator. The dried extract was weighed and then kept in a 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 a 12-h light/dark cycle. They were allowed free access to standard dry pellet 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 INSAR.

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, 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 group 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 a rotary evaporator. The dried extract was weighed and then kept in a 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 a rotary evaporator. The dried extract was weighed and then kept in a 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.

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Antilulcer Activity of Malvastrum tricuspidatum

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>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- 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.

Aspirin-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].

Pylorus-ligation-induced gastric ulcer

In this method, male albino rats were fasted in individual cages for 24 h and care was taken to avoid coprophagy. Pylorus ligation was applied by ligating the pyloric end of the stomach of rats under ether anaesthesia for 6 h after 1 h of ethanolic extract (500 mg/kg) or omeprazole (20 mg/kg) treatment. Animals were allowed to recover and stabilize in individual cage 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.

Antilulcer study

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%) and omeprazole (50 mg/kg) p.o. (Table 1) . The results of quantitative phytochemical screening were shown 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 (250and 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 ± 0.50/*</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>OMR (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>OMR (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 ethanol, aspirin, 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 these aggressive and defensive factors indicating its involvement, depletion of gastric wall, mucosal damage cytoprotective, antioxidant, neutralizing and induced by non-steroidal anti-inflammatory drugs and antisecretory properties.

Free radical production [31]. Ethanol-induced gastric injury is associated with significant production of flavonoids, alkaloids, tannins, saponins, terpenes, amino acids, gums and mucilages are reported to possess antioxidant and anti-inflammatory activities. Several gastroprotective mechanisms like increased mucus content as compared to control group, suggesting its potent cytoprotective activity and also present in scavenging effect. NSAIDs like aspirin cause gastric mucosal damage by decreasing prostaglandin levels and been proposed to explain their gastroprotective effects through inhibition of prostaglandin synthesis [33, 35] by several mechanisms in the present study.

Flavonoids have antiulcer and gastroprotective properties. Several gastroprotective mechanism have been proposed to explain the biological effects of flavonoids including free radical scavenging during stress ulcer, which could lead to mucosal over friction and ulcer formation was mainly due to gastric hypermotility. Many phytochemical constituents like betaine that are suggested to possess antiulcer activity are also present in *Malvastrum tricuspidatum*. These phytochemicals have been reported to suppress gastric damage induced by stress ulcer [34].

The anti-ulcer activity of the whole plant extract of *Malvastrum tricuspidatum*, the formation of pylorus ulcer in the stomach was significantly inhibited, both acid concentration and gastric volume were decreased. The ethanolic extract of *Malvastrum tricuspidatum* has been proposed to explain the biological effects of flavonoids and tannins as a major mucosa against ethanol challenge as shown by reduced protein constituent. Many phytochemical constituents like flavonoids, tannins, terpenes and glycinebetaine that are suggesting its potent cytoprotective and free radical reported to possess antiulcer activity are also present in scavenging effect. NSAIDs like aspirin cause gastric mucosal damage by decreasing prostaglandin levels and been proposed to explain their gastroprotective effects through inhibition of prostaglandin synthesis [33, 35] by several mechanisms in the present study.

**DISCUSSION**

**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</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
36 histamine secretion. On the other hand, tannins and 37 polyphenols may prevent ulcer development due to their 38 protein precipitating and vasoconstricting effects. Their 39 astrigent action can help precipitating microparticles on 40 ulcer site thereby forming an impervious layer over the 41 lining that hinders gut secretions and protects the 42 underlying mucosa from toxins and other irritants and 43 stimulate PGE₂ formation. Terpenes are known to 44 possess antulcer activity and their action has been 45 suggested to be due to the activation of cellular 46 protection, reduction of mucosal prostaglandins 47 metabolismo-cytprotective action and reduction of 48 gastric vascular permeability. Betaine also known as 49 glycinebetaine closely related to amino acid, glycine. 50 Earlier experimental studies indicated that betaine could 51 preserve cellular and subcellular membranes from free 52 radical mediated oxidative damage by its antioxidant 53 activity. The ability of betaine to maintain the mucosal 54 antioxidant status at higher rate demonstrates its 55 possible preventive efficacy in inhibiting free radical 56 mediated ulcerogenesis. The antulcer activity of betaine 57 is probably related to its ability to neutralize the 58 hydrochloric acid secreted in to stomach and/or its 59 antioxidant nature by which it maintain the level of 60 GSH and the activities of the mucosal antioxidant 61 enzymes to near normal status. Thus it protects the 62 gastric mucosa against oxidative damage by decreasing 63 lipid peroxidation and strengthening the mucosal barrier 64 [37-39].

In conclusion, On the basis of the present results and 65 available reports, it can be concluded that the anti-ulcer 66 activity elucidated by Malvastrum tricuspidatum could 67 be mainly due to the modulation of defensive factors 68 through an improvement of gastric cytoprotection and 69 partly due to decreased acid secretion. The results also 70 supported the presence of flavonoids, tannins, and 71 terpenes in ethanolic extract of Malvastrum 72 tricuspidatum that are reported to possess antulcer 73 activity by various mechanisms like free radical 74 scavenging, increased mucosal PGE₂, increased 75 mucosal blood flow, decreased histamine secretion, 76 astrigent action, neutralizing HCl secreted and 77 antioxidant nature. Hence, it is suggested that 78 Malvastrum tricuspidatum ethanolic extract show 79 antulcer activity by suppressing gastric damage induced 80 by aggressive factors as well as by regulating the 81 defensive factors.

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Antiulcer Activity of Malvastrum tricuspidatum


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