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

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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
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 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 antulcer 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 INS A).

Toxicity study

Acute oral toxicity study of aqueous and ethanolic extract of the M. tricuspidatum was carried out for determination of LD₅₀ by adapting dosage 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, 50 g/Kg were taken to observe the effect of ethanolic extract on ulcer. A voucher specimen (DANVIMALT5) has been assigned by Dept. of Botany, Botanical Survey of India.

Treatment Schedule

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 matter. The defatted marc was then subjected to soxhlet extraction with 95 % ethanol for 8 h. 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.

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Antiulcer 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>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>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 (500 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-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-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.

Pylorus-formation-induced ulcer

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 [23, 26], pH [2], free acidity and total acidity [2], mucus content [26], protein content [21] and peptic activity [29, 30] were determined.

Antiulcer study

Ethanol-induced ulcers

The male rats were randomly divided into seven groups and fasted for 24 h with free access to water. Animals were given sodium carboxymethyl cellulose (0.5%) p.o.

Phytochemical screening

Preliminary phytochemical screening revealed the presence of flavonoids, triterpenes, saponins, tannins, glycosides and carbohydrates. Animals were given sodium carboxymethyl cellulose (0.5%), ethanolic extract of the M. tricuspidatum at dose screening were shown 25%.
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$^a$</td>
<td>88.68</td>
</tr>
<tr>
<td>MTAE (250) + EtOH</td>
<td>13.9 ± 0.18$^a$</td>
<td>37.10</td>
</tr>
<tr>
<td>MTAE (500) + EtOH</td>
<td>4.2 ± 0.84$^a$</td>
<td>80.90</td>
</tr>
<tr>
<td>MTEE (250) + EtOH</td>
<td>9.7 ± 0.58$^a$</td>
<td>56.10</td>
</tr>
<tr>
<td>MTEE (500) + EtOH</td>
<td>3.9 ± 0.10$^a$</td>
<td>82.35</td>
</tr>
<tr>
<td>MTEE (1000) + EtOH</td>
<td>3.7 ± 0.12$^a$</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$^a$</td>
<td>89.86</td>
</tr>
<tr>
<td>MTEE (500) + ASP</td>
<td>2.5 ± 0.220$^a$</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$^a$</td>
<td>--</td>
</tr>
<tr>
<td>OMZ (20) + CRU</td>
<td>0.9 ± 0.187$^a$</td>
<td>85.93</td>
</tr>
<tr>
<td>MTEE (500) + CRU</td>
<td>1.0 ± 0.220$^a$</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$^a$</td>
<td>85.26</td>
</tr>
<tr>
<td>MTEE (500) + PL</td>
<td>2.3 ± 0.25$^a$</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 *in vivo* models represents some of the most common causes of gastric ulcer in humans. Many factors and mechanisms are implicated in the formation of *in vivo* ulceration. NSAIDs like aspirin cause gastric mucosal damage by increasing proinflammatory factors and mechanisms in the present study. The anti-ulcer activity of the whole plant 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 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 pylorus ulcer in the stomach was significantly inhibited, both as shown by reduced values of lesion index and the pH values, mucus content were increased. It is suggested that *Malvastrum tricuspidatum* ethanolic extract can suppress gastric damage induced by ulcerogenesis and gastric mucosal damage induced by aggressive factors and correct the balance between different models employed in the present study. Aggressive and defensive factors indicating its antiulcer activity are also present in several mechanisms in the present study. Ethanol-induced gastric ulcer. In phytomedicine, various phytoconstituents like oxygen free radicals leading to increased lipid peroxidation, which causes damage to cell and cell antulcer effect. Aqueous and ethanolic extract of *Malvastrum tricuspidatum* has significantly protected the gastric mucosa against ethanol challenge as shown by reduced presence of flavonoids and tannins as a major constituent. Many phytochemical constituents like values of lesion index as compared to control group, flavonoids, tannins, terpenes and glycinogen containing tannins and are suggesting its potent cytoprotective and free radical reported to possess antihypertensive effect. NSAIDs like aspirin cause gastric damage induced by *Malvastrum tricuspidatum*. These phytochemicals have been proposed to explain their gastroprotective effects through inhibition of prostaglandin synthesis by several mechanisms in the present study. 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 including free radical scavenging during reduction in gastric volume, total acidity, free acidity, ulcer index and increase in pH of gastric juice. Flavonoids have antiulcer and gastroprotective effect. In the cold-restraint stress model, gastric ulcer is increased mucus blood flow, decreases

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

**DISCUSSION**

The anti-ulcer activity of the whole plant 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 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 pylorus ulcer in the stomach was significantly inhibited, both as shown by reduced values of lesion index and the pH values, mucus content were increased. It is suggested that *Malvastrum tricuspidatum* ethanolic extract can suppress gastric damage induced by ulcerogenesis and gastric mucosal damage induced by aggressive factors and correct the balance between different models employed in the present study. Aggressive and defensive factors indicating its antiulcer activity are also present in several mechanisms in the present study. Ethanol-induced gastric ulcer. In phytomedicine, various phytoconstituents like oxygen free radicals leading to increased lipid peroxidation, which causes damage to cell and cell antulcer effect. Aqueous and ethanolic extract of *Malvastrum tricuspidatum* has significantly protected the gastric mucosa against ethanol challenge as shown by reduced presence of flavonoids and tannins as a major constituent. Many phytochemical constituents like values of lesion index as compared to control group, flavonoids, tannins, terpenes and glycinogen containing tannins and are suggesting its potent cytoprotective and free radical reported to possess antihypertensive effect. NSAIDs like aspirin cause gastric damage induced by *Malvastrum tricuspidatum*. These phytochemicals have been proposed to explain their gastroprotective effects through inhibition of prostaglandin synthesis by several mechanisms in the present study. 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 including free radical scavenging during reduction in gastric volume, total acidity, free acidity, ulcer index and increase in pH of gastric juice. Flavonoids have antiulcer and gastroprotective effect. In the cold-restraint stress model, gastric ulcer is increased mucus blood flow, decreases

<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 ± 0.70a</td>
<td>26.2 ± 1.53a</td>
<td>8.74 ± 0.44a</td>
<td>165.3 ± 8.53a</td>
<td>18.04 ± 0.84a</td>
<td></td>
</tr>
<tr>
<td>MTEE 500</td>
<td>1.68 ± 0.18ab</td>
<td>4.52 ± 0.18ab</td>
<td>11.48 ± 0.54ab</td>
<td>21.8 ± 1.49ab</td>
<td>5.83 ± 0.16b</td>
<td>191.7 ± 12.85b</td>
<td>31.85 ± 0.59ab</td>
<td></td>
</tr>
</tbody>
</table>

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*However, compared to control group

*However, 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 PGE2 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 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 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 PGE2, increased mucosal blood flow, decreased histamine secretion, astrigent 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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