

ORIGINAL ARTICLE

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

Peptic ulcer mostly refers to Amlapitta or Parinamasula, 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, funaecomastia and haematopoeitic changes) of modern antiulcer medicine, indigenous drugs possessing fewer side effects should be looked for as a better alternative for the treatment of peptic ulcer. There is evidence concerning the participation of reactive oxygen species 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 and gastric ulcer [3].

According to traditional and ethnomedicinal claims, one plant possessing anti-ulcer activity is *M. tricuspidatum*. *M. tricuspidatum* (Malvaceae), also known as Kharenti or Bala, is an erect under shrub or herb, found as a weed distributed world wide, also in the Indian subcontinent [4]. The leaves are applied to inflamed sores and wound. The flowers are given as a pectoral and diaphoretic [5]. This plant is used ethnomedicinally in cough, chest and lung disease. The decoction of leaf is given in dysentery and smelling of root helps to prevent vomiting [6]. It is traditionally used as antipyretic, smooth muscle relaxant and ulceroprotective [7-9]. *M. tricuspidatum*, crude water extract (Whole plant) was reported to possess anti-

57 inflammatory, analgesic, antipyretic [10,11],
58 antibacterial [9] and antinociceptive activity [12].
59 Chronic toxicity study of *Malvastrum tricuspidatum*
60 showed that extract of whole plant given orally to
61 Wistar rats at the dose of 0.2-20 g/ kg for 60 days did
62 not produce toxicity in the animals [13]. Our research
63 interest in this plant arose because of its potential
64 medicinal value against peptic ulcer, as used in folk
65 medicine and presence of antiulcer phytochemical
66 constituents like flavonoids, tannins, and glycinebetaine.
67 Experimental study to determine antiulcer potential of
68 *M. tricuspidatum* and possible mechanisms for
69 inhibition of gastric ulcer is not reported earlier, so it
70 was worthwhile to undertake such investigation using
71 aqueous and ethanolic extract of whole plant of *M.*
72 *tricuspidatum*.
73 The present study incorporates the evaluation of
74 antiulcer effect of aqueous and ethanolic extract of
75 whole plant of *M. tricuspidatum* in Ethanol-induced
76 (EtOH), aspirin-induced (ASP), cold restraint stress
77 (CRU)- and pylorus ligation (PL)-induced ulcer models.
78 In addition possible mechanisms for gastroprotection by
79 major antiulcer phytochemical constituents of *M.*
80 *tricuspidatum* in all the four acute gastric ulcer models
81 were suggested in the present study. This study thus
82 provides an insight on the mechanism of the antiulcer
83 effect of *M. tricuspidatum*.

84 MATERIALS AND METHODS

85 *Drugs and chemicals*

86 Aspirin (bulk drug) was obtained as gift sample
87 from Cyno Pharma, Indore, India and omeprazole and
88 ranitidine was obtained from Alpa Lab. Indore, India.
89 Ethanol (Merck Pvt. Ltd., Mumbai) and diethyl ether
90 (Sisco Research Lab. Pvt. Ltd., Mumbai). All the other
91 chemicals and reagent used were prepared immediately
92 before use and were of analytical grade.

93 *Plant material*

94 *M. tricuspidatum* whole plant was collected from the
95 local garden of College of IPS academy, Indore. The
96 plant was identified and authenticated by T.
97 Chakraborty, Scientist 'D' Botanical Survey of India,
98 Pune. A voucher specimen (DANVIMALT5) has been
99 assigned by Dept. of Botany, Botanical Survey of India.
100 The whole plant was collected in the month of July
101 2009 and shade dried at room temperature.

102 *Preparation of extracts*

103 *Preparation of aqueous extract*

104 The dried coarsely powdered whole plant (5 kg) was
105 extracted with petroleum ether for 48 h to remove fatty
106 matter. The defatted marc was then subjected to
107 decoction for 1 h. Then it was filtered through muslin
108 cloth. The total aqueous extract was concentrated using
109 rotary evaporator. The dried extract was weighed and
110 then kept in refrigerator until ready for use. The yield of
111 extract was 5.2 % (w/w) of powdered drug [9].

112 *Preparation of ethanolic extract*

113 The dried coarsely-powdered whole plant was
114 extracted with petroleum ether for 48 h to remove fatty
115 matter. The defatted marc was then subjected to soxhlet
116 extraction with 95 % ethanol for 8 h. The total ethanolic
117 extract was concentrated using rotary evaporator. The
118 dried extract was weighed and then kept in refrigerator
119 until ready for use. The yield of extract was 10.5 %
120 (w/w) of powdered drug [14,15]. In each experiment,
121 the ethanolic and aqueous extracts were suspended in
122 sodium carboxymethyl cellulose (0.5%) before use.

123 *Phytochemical screening*

124 The chemical constituents of aqueous and ethanolic
125 extracts were identified by qualitative phytochemical
126 analysis [16-19] and quantitative phytochemical
127 analysis [20,21].

128 *Experimental Animals*

129 Adult male albino rats (150-200 g) of Wistar strain
130 and albino mice (20-30 g) were used in the study. The
131 animals were procured from Veterinary College, Mhow
132 (Indore), India. The animals were acclimatized for 10
133 day's under standard husbandry conditions, room
134 temperature ($27 \pm 3^\circ\text{C}$), relative humidity ($65 \pm 10\%$)
135 and 12h light/dark cycle. They were allowed free access
136 to standard dry pelleted diet (M/s Godrej Pvt Ltd.,
137 Mumbai, India) and water ad libitum under hygienic
138 conditions. Five rats were used for each group in
139 antiulcer study. The study was approved by the
140 institutional animal ethics Committee, which follows
141 the guidelines of CPSCEA (Committee for the Purpose
142 of Control and Supervision of Experiments on Animals,
143 which complies with international norms of INSA.

144 *Toxicity study*

145 Acute oral toxicity study of aqueous and ethanolic
146 extract of the *M. tricuspidatum* was carried out for
147 determination of LD₅₀ by adapting dosing schedule as
148 per OECD guideline no. 425. The female albino mice
149 weighing 20-30 g were used for the study. The animals
150 were continuously observed for 12 h to detect changes
151 in autonomic or behavioral responses. Mortality was
152 observed for 24h. The doses of 250, 500 and 1000 g/Kg,
153 p.o. were selected based on the results of preliminary
154 toxicity testing [22].

155 *Treatment Schedule*

156 *Ethanol-induced ulcers*

157 For ethanol induced ulcer model rats were divided
158 into seven groups. Each groups containing five rats.
159 Group I was control and given sodium
160 carboxymethyl cellulose (0.5 %) p.o.
161 Group II was standard and given omeprazole (20
162 mg/kg) p.o.
163 Groups III-IV were given aqueous extract of
164 *Malvastrum tricuspidatum* (250, 500 mg/kg) p.o
165 Groups V-VII were given ethanolic extract of
166 *Malvastrum tricuspidatum* (250, 500, 1000 mg/kg) p.o.

Table 1. Qualitative phytochemical analysis of aqueous and ethanolic extract of *Malvastrum tricuspidatum*

Sr.no.	Phytochemical tests	Inference	
		Aqueous extract	Ethanolic extract
1	Alkaloids	+	+
2	Saponins	+	+
3	Tannins	+	+
4	Flavonoids	+	+
5	Phytosterols	+	+
6	Carbohydrates	+	+
7	Proteins	+	+
8	Terpenoids	+	+
9	Volatile oil	-	-

+ 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.

Antiulcer 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%), ethanolic extract of the *M. tricuspidatum* at dose

of 250, 500 and 1000 mg/kg and aqueous extract 250, 500 mg/kg or Omeprazole (20 mg/kg) orally. After pretreatment of extract and omeprazole, EtOH (1 ml/200 gm of absolute ethanol) was administered orally to each group [23]. Animals were sacrificed after 1 h by cervical dislocation. Stomachs were isolated, opened along the greater curvature and were gently rinsed with saline to remove the gastric content and blood clot. The ulcer scoring was done and the percentage protection was calculated [24].

0.5 Red colouration

1 Spot ulcer

1.5 Haemorrhagic streak

2 Ulcers

3 Perforation

Percentage of ulcer inhibition = Mean ulcer index of control - Mean ulcer index of test / Mean ulcer index of control × 100

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.

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RESULT**Phytochemical screening**

Preliminary phytochemical screening revealed the presence of flavonoids, triterpenes, saponins, tannins, phytosterol, alkaloids, glycosides and carbohydrates (Table 1). The results of quantitative phytochemical screening were shown Table 2.

Table 2. Quantitative phytochemical analysis of aqueous and ethanolic extract of *Malvastrum tricuspidatum*

Phytoconstituents	Quantity in aqueous extract	Quantity in ethanolic extract
Alkaloids (%)	10	12
Flavonoids (%)	12.50	20.50
Carbohydrates (mg/ml)		
Glucose	4.7	4.3
Fructose	5.4	4.56
Lactose	6.5	5.93
Maltose	7.47	6.37
Lipids (mg/ml)	0.208	0.28

259 Toxicity study

260 Acute oral toxicity study of aqueous and ethanolic
261 extracts of the *M. tricuspidatum* revealed that it did not
262 exhibit any signs of toxicity up to 2 g /kg body weight.
263 Since there was no mortality of the animals found at
264 high dose, doses of 250, 500 and 1000 mg/kg of the
265 extracts were selected for evaluation of anti-ulcer
266 activity.

267 Effect of MTAE and MTEE on gastric ulcer studies

268 Effect of MTAE and MTEE on various types of
269 gastric ulcer models was shown in Tables 3 and 4 and
270 Fig 1. In ulcerogen-treated animals, extensive gastric
271 ulcers in the stomach of all the experimental models
272 were shown. Both ethanol and cold restraint stress
273 provoked haemorrhagic form of ulcers in the stomach
274 with adequate evidence with intraluminal bleeding
275 whereas aspirin caused mostly petechial ulcers and
276 erosions. MTAE (250 and 500 mg/kg) and MTEE (250,
277 500 and 1000 mg/kg) given orally showed dose-
278 dependent protective effect against gastric ulcer induced
279 by ethanol and was comparable with omeprazole.
280 MTEE at a dose of 500 mg/kg significantly ($p < 0.05$)
281 reduced gastric ulcers in pylorus ligated ulcer model.

282 Effect of MTAE and MTEE on gastric ulcer studies

283 In 6 h pylorus-ligated rats, MTEE (500 mg/kg)
284 decreased the gastric juice volume and reversed the
285 increased output of acid and peptic secretion (Table 3).
286 Omeprazole showed significant ($p < 0.05$) reduction in
287 protein content and output of acid and peptic activity in
288 pylorus ligation.

Table 3. Effect of MTAE and MTEE on EtOH-, ASP-, CRU- and PL-induced ulcers in rats

Treatment dose (mg/kg)	Ulcer index	Protection
EtOH-induced ulcer control (EtOH)	22.1 ± 0.33	--
OMP (20) + EtOH	2.5 ± 0.50 ^a	88.68
MTAE (250) + EtOH	13.9 ± 0.18 ^{ab}	37.10
MTAE (500) + EtOH	4.2 ± 0.84 ^a	80.90
MTEE (250) + EtOH	9.7 ± 0.58 ^{ab}	56.10
MTEE (500) + EtOH	3.9 ± 0.10 ^a	82.35
MTEE (1000) + EtOH	3.7 ± 0.12 ^a	83.25
ASP induced ulcers control (ASP)	14.80 ± 0.560	--
Ranitidine (50)	1.50 ± 0.223 ^a	89.86
MTEE (500) + ASP	2.5 ± 0.220 ^a	83.10
Negative control (CRU)	0.5 ± 0.223	--
Positive control (CRU)	6.5 ± 0.353 ^b	--
OMZ (20) + CRU	0.9 ± 0.187 ^a	85.93
MTEE (500) + CRU	1.0 ± 0.220 ^a	84.61
PL-induced ulcers control (PL)	9.5 ± 0.50	--
OMZ (20) + PL	1.4 ± 0.33 ^a	85.26
MTEE (500) + PL	2.3 ± 0.25 ^a	75.78

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.

^a $p < 0.05$ = compared to control group

^b $p < 0.05$ = compared to standard group

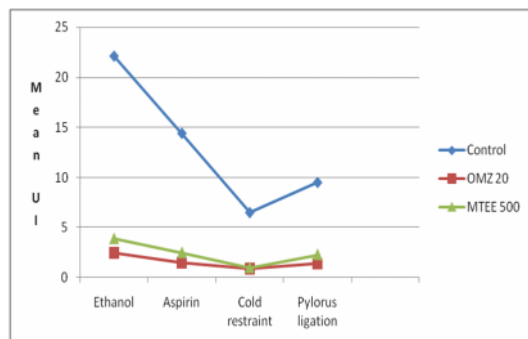


Fig 1. Comparison of mean ulcer index among ethanol-induced ulcer, aspirin-induced ulcer, cold restraint ulcer and pylorus ligation models

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DISCUSSION

The anti-ulcer activity of the whole plant extract of *Malvastrum tricuspidatum*, the formation of pylorus *Malvastrum tricuspidatum* as evaluated by employing ulcer in the stomach was significantly inhibited, both aspirin, ethanol, cold restraint and pylorus ligation ulcer acid concentration and gastric volume were decreased models. These models represent some of the most common and the pH values, mucus content were increased. It is common causes of gastric ulcer in humans. Many suggested that *Malvastrum tricuspidatum* ethanolic factors and mechanisms are implicated in the extract can suppress gastric damage induced by ulcerogenesis and gastric mucosal damage induced by aggressive factors and correct the imbalance between different models employed in the present study aggressive and defensive factors indicating its involving, depletion of gastric wall, mucosal damage cytoprotective, antioxidant, neutralizing and induced by non-steroidal anti-inflammatory drugs and antisecretory properties.

In phytomedicine, various phytoconstituents like flavonoids, alkaloids, tannins, saponins, terpenes, amino acids, gums and mucilages are reported to possess antioxidant, which causes damage to cell and cell membrane [32]. The ethanolic extract of *Malvastrum tricuspidatum* has significantly protected the gastric mucosa against ethanol challenge as shown by reduced values of lesion index as compared to control group, suggesting its potent cytoprotective and free radical scavenging effect. NSAIDs like aspirin cause gastric mucosal damage by decreasing prostaglandin levels through inhibition of prostaglandin synthesis [33].

Ethanolic extract of *Malvastrum tricuspidatum* was significantly effective in protecting gastric mucosa against aspirin-induced ulcers at the dose of 500 mg/kg as shown by reduced values of lesion index as compared to control group, suggesting its potent cytoprotective effect. In the cold-restraint stress model, gastric ulcer PGE₂, increases mucosal blood flow, decreases

Table 4. Gastroprotective activity of ethanolic extract of whole plant of *Malvastrum tricuspidatum* on various parameters in pylorus ligated ulcer

Treatment	Dose (mg/kg)	Volume of gastric juice (ml)	pH	Free acidity (mEq/l/100g)	Total acidity (mEq/l/100g)	Gastric mucus content (µg of alcian blue/g of stomach)	Total protein (µg/ml)	Pepsin activity (µg/ml)
Control	--	4.32 ± 0.25	2.4 ± 0.31	27.2 ± 2.45	47.4 ± 2.13	4.82 ± 0.11	286.38 ± 15.68	45.75 ± 1.39
OMZ	20	2.24 ± 0.19 ^a	3.94 ± 0.20 ^a	11.0 ± 0.70 ^a	26.2 ± 1.53 ^a	8.74 ± 0.44 ^a	165.3 ± 8.53 ^a	18.04 ± 0.84 ^a
MTEE	500	1.68 ± 0.18 ^{ab}	4.52 ± 0.18 ^a	11.48 ± 0.54 ^a	21.8 ± 1.49 ^a	5.83 ± 0.16 ^b	191.7 ± 12.85 ^a	31.85 ± 0.59 ^{ab}

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

364 histamine secretion. On the other hand, tannins and
 365 polyphenols may prevent ulcer development due to their
 366 protein precipitating and vasoconstricting effects. Their
 367 astringent action can help precipitating microproteins on
 368 ulcer site thereby forming an impervious layer over the
 369 lining that hinders gut secretions and protects
 370 underlying mucosa from toxins and other irritants and
 371 stimulate PGE₂ formation. Terpenes are known to
 372 possess antiulcer activity and their action has been
 373 suggested to be due to the activation of cellular
 374 protection, reduction of mucosal prostaglandins
 375 metabolism-cytoprotective action and reduction of
 376 gastric permeability. Betaine also known as
 377 glycinebetaine closely related to amino acid, glycine.
 378 Earlier experimental studies indicated that betaine could
 379 preserve cellular and subcellular membranes from free
 380 radical mediated oxidative damage by its antioxidant
 381 activity. The ability of betaine to maintain the mucosal
 382 antioxidant status at higher rate demonstrates its
 383 possible preventive efficacy in inhibiting free radical
 384 mediated ulcerogenesis. The antiulcer activity of betaine
 385 is probably related to its ability to neutralize the
 386 hydrochloric acid secreted in to stomach and/or its
 387 antioxidant nature by which it maintain the level of
 388 GSH and the activities of the mucosal antioxidant
 389 enzymes to near normal status. Thus it protects the
 390 gastric mucosa against oxidative damage by decreasing
 391 lipid peroxidation and strengthening the mucosal barrier
 392 [37-39].

393 In conclusion, On the basis of the present results and
 394 available reports, it can be concluded that the anti-ulcer
 395 activity elucidated by *Malvastrum tricuspidatum* could
 396 be mainly due to the modulation of defensive factors
 397 through an improvement of gastric cytoprotection and
 398 partly due to decreased acid secretion. The results also
 399 supported the presence of flavonoids, tannins, and
 400 terpenes in ethanolic extract of *Malvastrum*
 401 *tricuspidatum* that are reported to possess antiulcer
 402 activity by various mechanisms like free radical
 403 scavenging, increased mucosal PGE₂, increased
 404 mucosal blood flow, decreased histamine secretion,
 405 astringent action, neutralizing HCl secreted and
 406 antioxidant nature. Hence, it is suggested that
 407 *Malvastrum tricuspidatum* ethanolic extract show
 408 antiulcer activity by suppressing gastric damage induced
 409 by aggressive factors as well as by regulating the
 410 defensive factors.

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