IJPT
1735-2657/12/112-53-59
IRANIAN JOURNAL OF PHARMACOLOGY & THERAPEUTICS

IJPT 11: 53-59, 2012

ORIGINAL ARTICLE

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

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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 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 errect 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-

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

M. tricuspidatum whole plant was collected from the local garden of College of IPS academy, Indore. The plant was identified and authenticated by T. Chakraborty, Scientist '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 decoction for 1 h. Then it was filtered through muslin cloth. 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 day's 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 LD_{50} 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 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

Ethanol-induced ulcers

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

Groups 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.

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. tricuspidatun* 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 - 4ºC 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.

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

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.

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 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

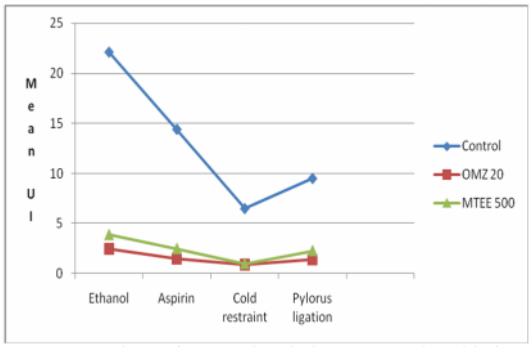

Fig 1. Comparison of mean ulcer index among ethanol-induced ulcer, aspirin-induced ulcer, cold restraint ulcer and pylorus ligation models

DISSCUSION

The anti-ulcer activity of the whole plant extract of *Malvastrum tricuspidatum* as evaluated by employing asprin, ethanol, 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 different models employed in the present study involving, depletion of gastric wall, mucosal damage induced by non-steroidal anti-inflammatory drugs and free radical production [31]. Ethanol-induced gastric injury is associated with significant production of oxygen free radicals leading to increased lipid peroxidation, 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 formation was mainly due to gastric hypermotility, which could lead to mucosal over friction and generation of free radical during stress ulcer [34]. 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 [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 acid concentration and gastric volume were decreased and the pH values, mucus content were increased. It is suggested that *Malvastrum tricuspidatum* ethanolic extract can suppress gastric damage induced by aggressive factors and correct the imbalance between aggressive and defensive factors indicating its cytoprotective, antioxidant, neutralizing and antisecretary properties.

In phytomedicine, various phytoconstituents like flavonoids, alkaloids, tannins, saponins, terpenes, amino acids, gums and mucilages are reported to possess antiulcer effect [36]. Aqueous and ethanolic extract were prepared and phytochemical analysis revealed presence of flavonoids and tannins as a major constituent. Many phytochemical constituents like flavonoids, tannins, terpenes and glycinebetaine that are reported to possess antiulcer activity are also present in *Malvastrum tricuspidatum*. These phytochemicals have been proposed to explain their gastroprotective effects by several mechanisms in the present study.

Flavonoids have antiulcer and gastroprotective activities. Several gastroprotective mechanism have been proposed to explain the biological effects of flavonoids including free radical scavenging during hyperoxidation of lipid memebrane, increases mucosal PGE_2 , 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.

[a] $p < 0.05$= compared to control group

[b] $p < 0.05$= 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 linning that hinders gut secretions and protects underlying mucosa from toxins and other irritants and stimulate PGE_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 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 mucosa against oxidative damage by decreasing lipid peroxidation and strengthening the mucosal barrier [37-39].

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 PGE_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.

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