



Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage: www.elsevier.com/locate/apjtd



Document heading doi:10.1016/S2222-1808(12)60024-3

Evaluation of gastric anti-ulcer activity of methanolic extract of *Cayratia trifolia* in experimental animals

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ARTICLE INFO

Article history:

Received 15 October 2011

Received in revised form 27 December 2011

Accepted 28 February 2012

Available online 28 April 2012

Keywords:

Cayratia trifolia

Pylorus ligation

Ethanol induced ulcer model

Ulcer index

ABSTRACT

Objective: To evaluate the gastric anti-ulcer activity of methanolic extract of *Cayratia trifolia* L. (MECT) (Vitaceae) leaves in experimental animals. **Methods:** MECT was investigated in pylorus ligation and ethanol induced ulcer models in Wistar rats. In both models, the common parameter determined was ulcer index. MECT at doses of 250, 500 mg/kg (p.o.) was used to determine whether it could produce significant inhibition of the gastric lesions induced by pylorus ligation and ethanol. **Results:** The extract (250 and 500 mg/kg) showed significant ($P < 0.05$) reduction in gastric volume and ulcer index as compared to the control in both of the two models. **Conclusions:** It can be concluded that MECT possesses antiulcerogenic as well as ulcer healing properties, which might be due to its antisecretory activity.

1. Introduction

Peptic ulcer disease is a serious gastrointestinal disorder[1]. The formation of peptic ulcers depends on the presence of acid and peptic activity in gastric juice plus a breakdown in mucosal defenses. There are two major factors that can disrupt the mucosal resistance to injury: non-steroidal anti-inflammatory drugs (NSAIDs) e.g. aspirin and *Helicobacter pylori* (*H. pylori*) infection[2]. As a matter of fact, many drugs were used to treat this disease but many of them cause adverse effects and recurrent infections frequently occur within a few weeks because of difficulty in eradication of *H. pylori*[3]. This has been rationale for the development of new antiulcer drugs and search for novel molecule. Drugs of plants origin are gaining popularity and investigating for the various disorders including peptic ulcer. The objective of the present study was to evaluate the effectiveness of leaves extract in preventing the formation of gastric ulcer

experimentally by ethanol-induced gastric damage in rats. Drug treatment of peptic ulcers is targeted at either counteracting aggressive factors [acid, pepsin, active oxidants, platelet aggravating factor (PAF), leukotrienes, endothelins, bile or exogenous factors including NSAIDs] or stimulating the mucosal defences [(mucus, bicarbonate, normal blood flow, prostaglandins (PG), nitric oxide)[4]. The goals of treating peptic ulcer disease are to relieve pain, heal the ulcer and prevent ulcer recurrence. Currently there is no cost-effective treatment that meets all these goals. Hence, efforts are on to find a suitable treatment from natural product sources. *Cayratia trifolia* (*C. trifolia*) Linn. Domin Syn. *Vitis trifolia* (Family: Vitaceae) is commonly known as Fox grape in English; Amlabel, Ramchana in Hindi and Amlavetash in Sanskrit. It is native to India, Asia and Australia. Flowers are small greenish white and brown in colour[5]. Whole plant of *C. trifolia* has been reported to contain yellow waxy oil, steroids/ terpenoids, flavonoids and tannins upon preliminary phytochemical screening. Leaves contain stilbenes (piceid, reveratrol, viniferin, ampelopsin). Stem, leaves, roots are reported to possess hydrocyanic acid and delphinidin. Several flavonoids such as cyanidins are reported in the leaves. This plant also contains kaempferol, myricetin, quercetin, triterpenes and epifriedelanol[6].

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Foundation Project: This work was financially supported by University Grants Committee (UGC), New Delhi (F.No. 39-955/2010).

Leaves are also used as antiulcer agents[7]. Root paste is mixed with coconut oil and applied as decoction for three days. Roots are grounded with black peeper and applied as poultice on boils[8]. Infusion of seeds along with extract of tubers is traditionally given orally to diabetic patients to check sugar level of blood. Paste of tubers is applied on the affected part in the treatment of snake bite. Whole plant is used as diuretic, in tumors, neuralgia and splenopathy[9]. Its climbers are wrapped around the neck of frantic bullock whereas poultice of leaves is used to yoke sores of bullock[10]. The methanolic extract of bark extract shows the antiviral, antibacterial, antiprotozoal, hypoglycemic, anticancer and diuretic activities[8].

2. Materials and methods

2.1. Plant material

The leaves of *C. trifolia* Linn. were collected from the campus Kurukshetra University, Kurukshetra in the month of October 2010. The plant was authenticated by Dr. HB Singh, Scientist–F and Head, Raw Herbarium and Museum NISCAIR, New Delhi, India. A voucher specimen of the plant was preserved in the herbarium (NISCAIR/RHMD/Consult/–2010–11/1548/146) for further reference.

2.2. Preparation of the extract

Leaves of *C. trifolia* Linn. were washed under running tap water and dried in shade for two weeks. Dried leaves were powdered, sieved and stored in an air tight container at room temperature. Dried powder (400 g) was extracted sequentially with petroleum ether and hydro–alcohol (30:70) by using soxhlation method. The extracts were concentrated to dryness using rotary evaporator (Heidolph, model number–4011, USA). The extracts were preserved in refrigerator at 4 °C.

2.3. Acute toxicity study of the extract

Adult albino mice (25–30 g) were divided into five groups each containing 10 mice. The mice were fasted for 6 h with only access to water *ad libitum* before experimental study. Group I, II, III and IV animals were administered various doses of methanolic extract of *C. trifolia* (MECT) *i.e.* 500, 1000, 2000, 3000 and 4000 mg/kg. Group V received Tween 80 only. All the doses and vehicle were administered by oral route. The animals were observed for 72 h for mortality[11].

2.4. Pyloric ligation in rats

Animals were divided into four groups, each consisting of six rats. Rats in group I, served as control group, received distilled water (1 mL) orally. Rats in group II received omeprazole (20 mg/kg) which was used as a reference drug for ulcer protective studies. Rats in group III and IV received MECT at doses of 250 and 500 mg/kg, respectively. After 45

min of MECT and omeprazole treatment, pyloric ligation was done by ligating the pyloric end of stomach of rats of respective groups under ether anaesthesia at a dose of 35 mg/kg bw. Ligation was done without causing any damage to the blood supply of the stomach. Animals were allowed to recover and stabilize in individual cages and were deprived of water during postoperative period. After 4 h of surgery, rats were sacrificed and ulcer scoring was done. Gastric juice was collected and gastric secretion studies were performed[12,13].

2.5. Ethanol induced ulcer model

The ulcer was induced by administering ethanol. All the animals were fasted for 36 h before administration of ethanol. The animals were divided into four groups, each consisting of six rats. Rats in group I, served as control group, received distilled water (1 mL) orally. Rats in group II were administered with omeprazole (20 mg/kg) as a standard reference drug. Rats in group III and IV received MECT at doses of 250 and 500 mg/kg, respectively. The gastric ulcers were induced in rats by administering absolute ethanol (90%) (1 mL/200 g) orally, after 45 min of MECT and omeprazole treatment. They were kept in specially constructed cages to prevent coprophagia during and after the experiment. The animals were anaesthetized 1 h later with anaesthetic ether and stomach was incised along the greater curvature and ulceration will be scored. A score for the ulcer was study similar to pyloric ligation induced ulcer model[14].

2.6. Scoring of ulcer will be made as follows

Normal stomach.....	(0)
Red coloration.....	(0.5)
Spot ulcer.....	(1)
Hemorrhagic streak..	(1.5)
Ulcers.....	(2)
Perforation.....	(3)

Mean ulcer score for each animal will be expressed as ulcer index.

2.7. Percentage protection

Percentage protection=(Control mean ulcer index – Test mean ulcer index) / Control mean ulcer index × 100

2.8. Calculation of ulcer index[15]

$$U1 = (UN + US + UP) \times 10^{-1}$$

U1 = Ulcer index

UN = Average of number of ulcer per animal

US = Average of severity score

UP = Percentage of animal with ulcer

2.9. Statistical analysis

The Dunnett's test was employed for statistical comparison.

In all the cases, values of $P < 0.05$ were considered significant. All values were presented as mean \pm SE.

3. Results

3.1. Pyloric ligation induced gastric ulcer

In pyloric ligation induced ulcer model, oral administration of MECT in two different doses showed significant reduction in ulcer index, gastric volume, as compared to the control group. It was showing protection index of 74% and 82% at the doses of 250 and 500 mg/kg, respectively in comparison to control whereas omeprazole as reference standard drug showed reduction of ulcer 86% (Table 1 and Figure 1).

Table 1
Effect of *C. trifolia* leaves extract on various parameters in pyloric ligation induced gastric ulcer.

Groups	Treatments	Doses (mg/kg)	Ulcer index	% Protection	pH of gastric juice	Gastric juice (mL)
I	Control	1 mL/animal	15.60 \pm 1.50	–	2.40 \pm 0.20	9.20 \pm 0.20
II	Omeprazole	20	2.20 \pm 0.50*	86	4.70 \pm 0.15*	2.20 \pm 0.18*
III	MECT	250	3.50 \pm 0.50	74	3.40 \pm 0.20	4.60 \pm 0.12
IV	MECT	500	2.50 \pm 0.60*	82	4.20 \pm 0.18*	3.90 \pm 0.15*

*: $P < 0.05$ as compared to control group.

Table 2
Effect of *C. trifolia* leaves extract on various parameters in ethanol induced gastric ulcer.

Groups	Treatments	Dose (mg/kg)	Ulcer index	% Protection	pH of gastric juice
I	Control	1 mL/animal	8.60 \pm 0.08	–	3.10 \pm 0.20
II	Omeprazole	20	3.02 \pm 0.26*	72	5.20 \pm 0.90*
III	MECT	250	4.20 \pm 0.04*	54	3.60 \pm 0.15
IV	MECT	500	3.70 \pm 0.60*	68	4.80 \pm 0.17*

*: $P < 0.05$ as compared to control group.

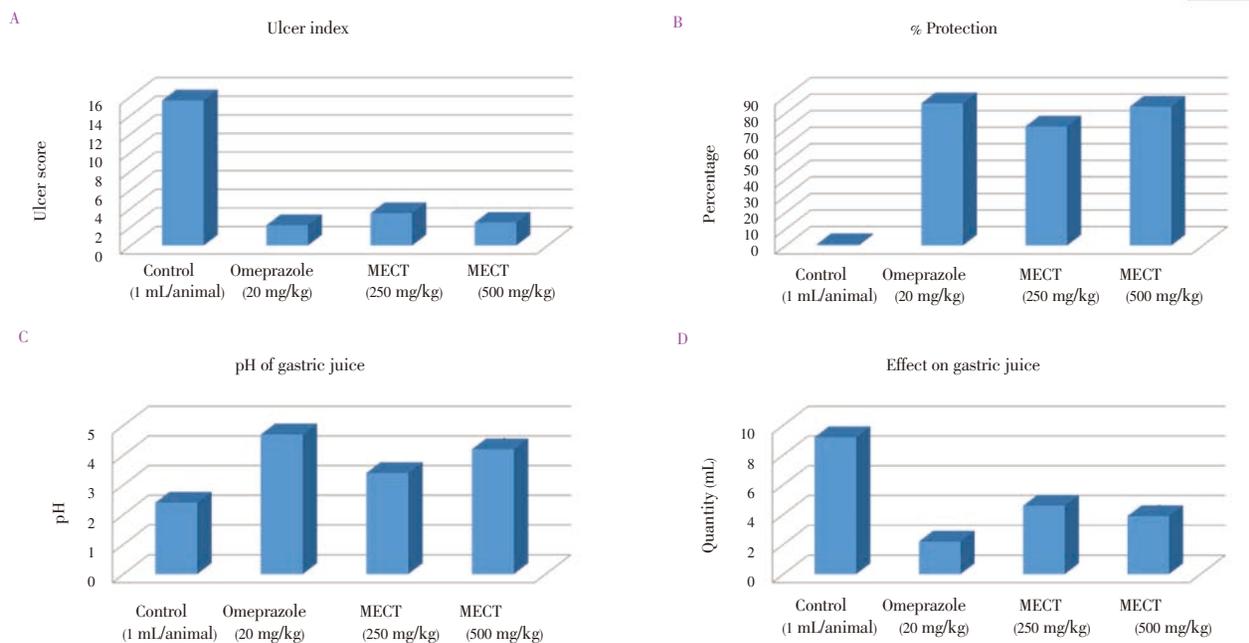


Figure 1. Effect of *C. trifolia* leaves extract on various parameters in pyloric ligation induced gastric ulcer. A: Ulcer index in pyloric ligation induced gastric ulcer; B: Percentage of protection in pyloric ligation induced gastric ulcer; C: Effect on pH of gastric juice in pyloric ligation induced gastric ulcer; D: Effect on gastric juice in pyloric ligation induced gastric ulcer.

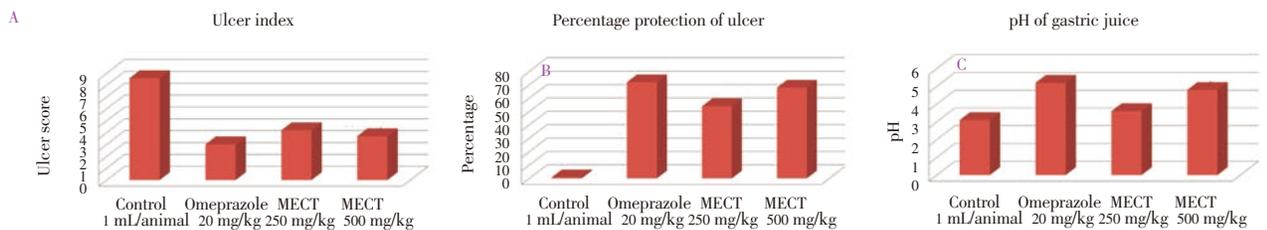


Figure 2. Effect of *C. trifolia* leaves extract on various parameters in ethanol induced gastric ulcer. A: Ulcer index in ethanol induced gastric ulcer; B: Effect on percentage protection of ulcer in ethanol induced gastric ulcer; C: Effect on pH of gastric juice in ethanol induced gastric ulcer.

3.2. Ethanol-induced gastric ulcer

In control animal, oral administration of absolute ethanol produced characteristic lesions in the glandular portion of rat stomach which appeared as elongated bands of thick, black and dark red lesions. MECT showed significant protection index of 54% and 68% with the doses of 250 and 500 mg/kg, respectively in comparison to control. Omeprazole as reference standard drug showed reduction of ulcer 72% (Table 2 and Figure 2).

4. Discussion

The etiology of peptic ulcer is unknown in most of the cases, yet it is generally accepted that it results from an imbalance between aggressive factors and the maintenance of mucosal integrity through the endogenous defence mechanism^[16].

Pylorus ligation induced ulcer was used to study the effect of leaves extracts on gastric acid secretion and mucus secretion. The ligation of the pyloric end of the stomach causes accumulation of gastric acid in the stomach. This increase in the gastric acid secretion causes ulcers in the stomach. The original Shay rat model involves fasting of rats for 36 h followed by ligation of pyloric end of the stomach. The ulcer index is determined 5 h after pylorus ligation. The lesions produced by this method are located in the lumen region of the stomach. Many authors have modified the original model. In the present study, the Shay rat model described by Kulkarni was followed. Ethanol induced gastric ulcer was employed to study the cytoprotective effect of the extracts. Ethanol induced gastric lesion formation may be due to stasis in gastric blood flow which contributes to the development of the haemorrhage and necrotic aspects of tissue injury. Alcohol rapidly penetrates the gastric mucosa apparently causing cell and plasma membrane damage leading to increased intracellular membrane permeability to sodium and water. The massive intracellular accumulation of calcium represents a major step in the pathogenesis of gastric mucosal injury. This leads to cell death and exfoliation in the surface epithelium^[17,18].

In conclusion, MECT possesses anti-ulcer activity which might be due to its antisecretory and cytoprotective activities.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The authors are thankful to UGC, New Delhi for financially supporting (F.No. 39–955/2010).

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