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Antiulcer Activity of Aqueous Extract of *Citrus medica* Linn. Fruit Against Ethanol-Induced Ulcer in Rats

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Abstract: *Citrus medica* Linn. (Rutaceae) known as Gajanimbe and is used as a folk medicine for the treatment of gastric ulcers. The present study was planned to evaluate the antiulcer activity of aqueous extract of the fruits against ethanol-induced ulcers in rats. The extract was subjected to phytochemical screening and found to contain carbohydrates, proteins, amino acids and flavonoids. The rats were pretreated with the extract at two doses (250 and 500 mg kg^{-1} p.o.) and the antiulcer effect was compared with that of ranitidine (20 mg kg^{-1} p.o.). The extract of both doses showed a significant reduction in ulcer formation. Histopathological sections showed significant decrease in mucosal ulceration, inflammatory mucosal changes and submucosal edema compared to ethanol treated group and the ranitidine group. It is concluded that, the fruits of *C. medica* possesses significant antiulcer activity against ethanol-induced ulcers in rats and the antiulcer activity could be due to the presence of flavonoids as these compounds have well documented antiulcer activity.

Key words: Citron % Ulcer % Flavonoids % Ranitidine % Histopathology % Gajanimbe

INTRODUTION

Citrus medica Linn. (Rutacae) commonly known as Citron, Baranimbu, Matulunga, Gajanimbe is an evergreen shrub/small tree about 3.6 m high with short, thick and thorny branches cultivated sparsely throughout the warm moist regions in India [1]. Various parts of this plant are widely used in the Indian traditional system of medicine. Ripe fruits are used in sore throat, cough, asthma, thirst, hiccough, earache, nausea, vomiting, potent anti scorbutic, stomachic, tonic, stimulant, expellant of poison, correct fetid breath; distilled water of the fruit is sedative; fruits and seeds are cardiac tonic and useful in palpitation and fruit decoction is analgesic [2-6]. Roots, flowers, seeds, peels and leaves are also used in many ailments. Fruit extracts have also shown good antioxidant activity [7]. The fruit wrapped in cloth is used to protect clothes from moths indicating its insect-repellent activity [8,9]. In ancient literature, citron was mentioned as an antidote for various kinds of poison [10]. Both the leaves and juice of the citron are used by the populations of South Eastern Nigeria for febrile illness [11].

Citrus fruits and juices have long been known to contain secondary metabolites including ascorbic acid, flavonoids, phenolics and pectin that are important to human nutrition. These metabolites especially ascorbic acid not only possess excellent antioxidant properties, but also promote healing of mucosal lining by stimulating procollagen formation and thereby enhancing collagen synthesis [12]. The flavonoids reported from the fruits are hesperidin:3,5,6-trihydroxyl-4,7-dimethoxyflavone;3,5,6-trihydroxy-3',4',7-trimethoxy flavones [3, 13]. The peel is reported to contain coumarins, limettin, scoparone, scopoletin and umbelliferone, while seeds contain limonin, limonol and nomilinic acid [14,15]. In view of the above, the present study was planned to evaluate the antiulcer activity of the aqueous extract of the fruit against ethanol-induced gastric ulcers in rats.

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MATERIALS AND METHODS

Chemicals and Drugs: Ranitidine powder (gift sample) was obtained from Cipla Pharmaceuticals Ltd. Bangalore. All the other chemicals and reagents used in the study were of analytical grade.

Plant Material and Preparation of the Extract: The fruits were collected from farms of Amrutha Nursing Home, Yelahanka, Bangalore, authenticated by the Taxonomist of Bangalore University and a herbarium specimen was deposited in the Department of pharmacology, Nargund College of Pharmacy, Bangalore for future reference. Aqueous exrtract was prepared by macerating the fruits with water (1:8 w/v) and extraction was further continued on a mechanical shaker at 70°C for 24 h. The extract was then filtered and freeze dried to obtain waxy residue which was further subjected for phytochemical evaluation [16-18].

Animals: Healthy male Albino rats of wistar strain weighing 200-250 g were procured from Sri Venkateswara Enterprises, Bangalore and divided into 5 groups (n = 6) as following.

- Group I: Control group, received distilled water (1 mL kg^{-1}, p.o)
- Group II: Untreated group, received distilled water (1 mL kg^{-1}, p.o) for 9 days followed by ethanol (5 ml kg^{-1}, p.o) on 11th day.
- Group III: Ranitidine group (20 mg kg^{-1}, p.o) for 9 days followed by ethanol (5 mL kg^{-1}, p.o) on 11th day.
- Group IV: CM1 group (250 mg kg^{-1}, p.o) for 9 days followed by ethanol (5 mL kg^{-1}, p.o) on 11th day.
- Group V: CM2 group (500 mg kg^{-1}, p.o) for 9 days by ethanol (5 mL kg^{-1}, p.o) on 11th day.

The rats were housed in polyacrylic cages and maintained at 27 ± 2°C, 45-60% RH and 12 h photo period. The rats were provided with a standard pellet diet (Hindustan Lever Ltd. Bangalore, India) and water *ad libitum.* All animal procedures have been approved by the Animal Ethical Committee (No: IAEC/NCP/35/10) in accordance with animal experimentation and care guidelines provided by IAEC/CPCSEA.

All the animals of ethanol treated group were fasted for 36 h before administration of ethanol. The animals in the standard drug group and aqueous extract (test drug) group, animals are pretreated with respective drugs for 9 days. Later, food and water were withdrawn for 36 hours and respective drugs were administered 1 hour before ethanol administration. Ethanol (90%) was administered to all animals at a dose of 1ml/200g and after 1 hour, the animals were sacrificed, stomach was removed slightly inflated by injecting15% formalin solution for 10 minutes. Subsequently, the stomachs were cut opened along the greater curvature and ulcer scoring was done using the dissecting microscope with a square grid eye piece [20-21]. Average No of ulcers, Ulcer scoring, Percentage of ulcers and Ulcer index (UI) is calculated by using formula.

$UI = U_N + U_S + U_P \times 10^{-1}$

U_N -Average No. of ulcers per animal.

U_S - Average of severity score

U_p - % of ulcers with ulcer.

The inhibition percentage was calculated by the following formula.

Inhibition % = [(UI control - UI treated) /UI control] x 100

The isolated stomachs were kept in formalin solution (15%) and then sent to the pathologist for histopathological observation and comments.

Statistical Analysis: The values were expressed as Mean ± SEM. Statistical Analysis was performed using one-way analysis of variance (ANOVA) fallowed by Dunnett's test. $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

The present study reports the antiulcer activity of the aqueous extract of *C. medica* fruits against ethanol-induced gastric ulcers in rats. Although, the etiology of ulcer is unknown, there are several factors that may induce ulcers such as stress, chronic alcoholism and frequent use of non stimulant anti-inflammatory drugs etc. In general, it can be perceived that, ulcer is the result of an imbalance between aggressive factors and defensive factor which maintains the mucosal integrity through the endogenous defense mechanism [22].

The phytochemical screening of aqueous extract of *C. medica* showed the presence of carbohydrates, proteins and flavonoids. Administration of ethanol resulted in the formation of significant number of ulcers compared to control where no ulcers were found. Pretreatment of the extract at 250 mg kg^{-1} resulted in

Table 1: Effect of Aqueous Extract of the Fruit of *Citrus medica* Linn. on stomach for the antiulcer activity through ethanol-induced mucosal damage

Groups	Avg no. of ulcers (Mean ± SEM)	Severity of ulcer score	% of ulcers	Ulcer index	% of inhibition
Group I	-	-	-	-	-
Group II	24.67±2.06***b	3.000±0.00***b	100%	12.77	-
Group III	8.500±1.025***a	2.000±0.00***a	100%	11.05	66%
Group IV	3.667±1.282***a	1.333±0.210***a	66.68%	7.03	85%
Group V	3.667±0.843***a	1.333±0.210***a	83.35%	8.81	85%

MEAN±SEM; n=6 animals in each group; Data was analyzed using one way ANOVA followed by Dunnett's *t* test; Symbol represent statistical significance:*P<0.05, **P<0.01, ***P<0.001; [a] when compared with stress control group, [b] when compared with Normal control group.

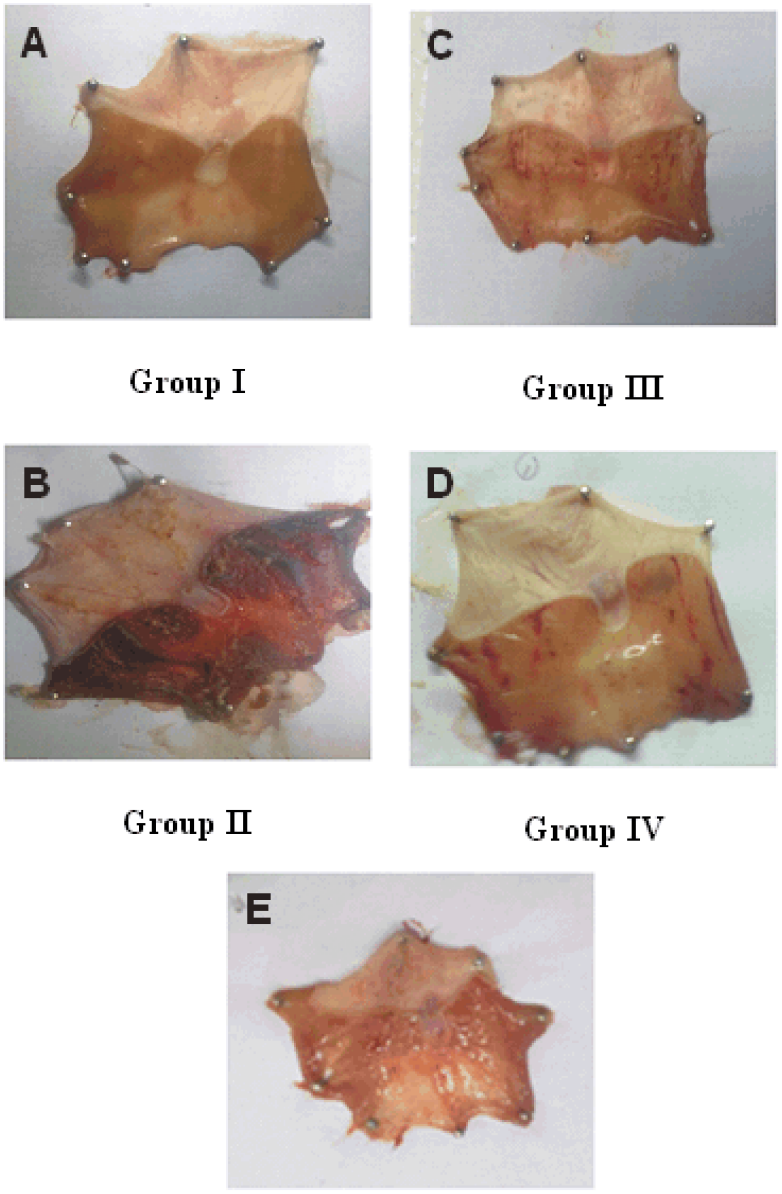

Fig 1: (A) Gastric mucosa of normal rats showing absence of ulcers. (B) Gastric mucosa of untreated rats showing severe ulceration. (C) Gastric mucosa of ranitidine group showing less intense ulceration. (D) Gastric mucosa of extract treated group (250 mg kg^{-1}) showing mild ulceration. (E) Gastric mucosa extract treated group (500 mg kg^{-1}) mild ulceration.

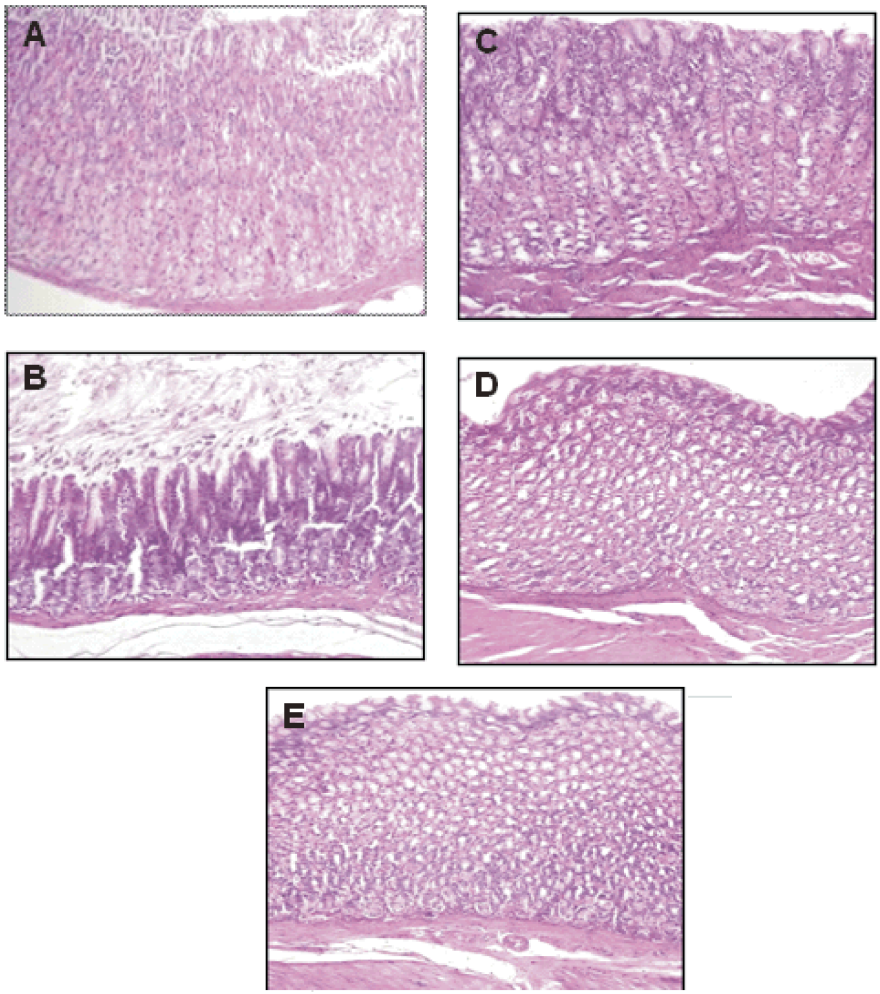

Fig 2: (A) Gastric section form normal rats showing normal mucosa and sub-mucosa. (B) Gastric section form untreated rats showing ulceration of the mucosal cells associated with inflammatory changes and necrosis. (C) Gastric section form ranitidine group showing gastric mucosa with intact epithelium, lamina propria and muscularis mucosa. Intervening the epithelial cells are seen scattered mononuclear inflammatory cells and few congested vascular spaces. (D) Gastric section form extract treated group (250 mg kg^{-1}) showing scattered neutrophils and mild submucosal edema. (E) Gastric section form extract treated group (500 mg kg^{-1}) showing gastric mucosa with intact epithelium, lamina propria and muscularis mucosa. Intervening the epithelial cells are seen scattered mononuclear inflammatory cells and few congested vascular spaces.

significant reduction in average number of ulcers, severity of ulcer score, percentage of ulcers and ulcer index compared to the untreated group (Table 1). It is known that gastric lesions produced by ethanol administration appear as multiple hemorrhagic red bands of different sizes along the glandular stomach. Ethanol is commonly used for inducing ulcers in experimental rats and leads to intense gastric mucosal damage. Studies suggested that the ethanol damage to the gastrointestinal mucosa starts with micro vascular injury, namely disruption of the vascular endothelium resulting in increased vascular permeability, edema formation and epithelial lifting. Ethanol produces necrotic lesions in the gastric mucosa by its direct toxic effect, reducing the secretion of bicarbonates and production of mucus [23].

The reduction in the ulcers was significantly higher in 500 mg kg^{-1} extract pre-treated group and the effect was comparable with that of ranitidine group (Figure 1A-1E). Ethanol-induced gastric lesion formation due to stasis

in gastric blood flow contributes to the development of hemorrhagic and necrotic changes of gastric tissue. Exposure to ethanol increases the extension of cellular damage in a dose-dependent way [24]. Cytoprotective action has been decreased by ethanol, which is due to the inhibition of prostaglandin synthesis which promotes the formation of ulcer. *C. medica* showed statistically significant decrease in the ulcer scores, % of ulcers and ulcer index. The antiulcer effect of *C. medica* could be due to the presence of flavonoids as one of its constituents, as polyphenolic compounds are known to exhibit gastro protective effect by virtue of their antioxidant property.

These observations were further substantiated by the histopathological findings wherein, decreased mucosal ulceration, inflammatory infiltration in mucosa and edema in sub mucosa were observed in the extract pre-treated groups compared untreated group (Figure 2A-2E).

CONCLUSION

It is concluded the *C. medica* fruit extract possesses antiulcer activity and also validated the traditional use of aqueous extract of *C. medica* an antiulcer remedy.

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