



Antiulcer Activity of Ethanolic Extract of *Terminalia catappa* Leaves against Gastric Ulcers by Pyrolic Ligation Induced Model in Rats

Bharath Kumar G, Divya Kumari, Sravanthi G, Rajeshwar G, Umadevi V,
Niranjan Goud Kotla*

Dhanvanthri College of Pharmaceutical Sciences, Tirumala Hills, Mahabubnagar-509001, Andhra Pradesh, India

ABSTRACT

Terminalia catappa family combretaceae moreover known as Indian almond was a large, spreading tree scattered throughout the tropics in coastal environments in India. Anti-ulcer activity of ethanolic extract of *T. catappa* (250 and 500 mg/kg b.w) was examined on pyrolic ligated induced ulcer model in rats. Omeprazole was used as standard. The anti-ulcer activity of *T. catappa* was evaluated with the help of ulcer index and histopathological examination. Preliminary phytochemical screening and acute toxicity studies of *T. catappa* also carried out. The extract showed significant ($p < 0.001$) reduction in pH, gastric volume, free acidity, total acidity and ulcer index in dose dependent manner as compared to control. The extract did not produce any toxic effects even at high doses. The anti-ulcer activity was probably due to the presence of flavonoids.

Keywords: *Terminalia catappa*, Omeprazole, pyrolic ligation, ethanolic extract, Ulcer index, flavonoids.

INTRODUCTION

Peptic ulcer disease (PUD) is an illness that affects a considerable number of people worldwide. It progresses when there is a disparity between the “aggressive” and “protective” factors at the luminal surface of the epithelial cells of stomach. Aggressive factors comprise *Helicobacter pylori*, pepsin, non-steroidal anti-inflammatory drugs (NSAIDs), bile acids, ischemia, hypoxia, smoking and alcohol. Whereas defensive factors include bicarbonate, mucus layer protection, mucosal blood flow, prostaglandins and growth factors.^[1]

The intervening causes of peptic ulcer infection with the bacterium called *Helicobacter pylori* (*H. pylori*) and the use of Non-Steroidal Anti Inflammatory Drugs (NSAIDs) such as aspirin and ibuprofen.^[2] The stomach guards itself from hydrochloric acid and pepsin by creating a mucus coating and producing bicarbonates. *Helicobacter pylori* infection and NSAIDs has been reported to damage these defending functions as the GI tract become susceptible to hydrochloric acid and pepsin and leads to the formation of ulcer. *Helicobacter pylori* infections alone are the major causative factor (95% of the duodenal ulcers and 80% of the gastric ulcers). And sometimes lifestyle factors such as smoking, alcohol, spicy foods and stress are also associated with peptic

ulcer formation.^[3] With this aspect to explore herbal drugs which would be effective in the management of ulcers.

Terminalia catappa family Combretaceae also known as Indian almond is a large, spreading tree distributed throughout the tropics in coastal environments in India. The dried leaves are used for fish pathogen treatment, as a substitute to antibiotics. The leaves have antioxidant as well as anti clastogenic properties.^[4] The leaves of the plant contain flavonoids in rich quantity, henceforward; these flavonoids are responsible for anti-ulcer activity. The various extracts of leaves and bark of *T. catappa* have been reported to be anticancer, anti-HIV reverse transcriptase^[5] and hepato-protective^[6] as well as anti-inflammatory^[7], hepatitis^[8] anti diabetic^[9] and aphrodisiac activities.^[10]

The moderate consumption of the seed kernel is useful in the treatment of men with sexual dysfunctions, primarily from premature ejaculation. The ethanol extract of the leaves of *Terminalia catappa* L. (Combretaceae) inhibits osmotically-induced hemolysis of human erythrocytes in a dose-dependent manner. Punicalagin and punicalin, from the leaves are used to treat dermatitis and hepatitis as both have strong anti-oxidative activity. The leaves had rich in flavonoids; these agents are responsible to treat gastric ulcers. In view of this, the present study was set up with the objective to assess the antiulcer activity of ethanolic extract of leaves of *Terminalia catappa*.

MATERIALS AND METHODS

Plant material

*Corresponding author: Mr. Niranjan Goud Kotla,
Dhanvanthri College of Pharmaceutical Sciences, Tirumala Hills, Mahabubnagar-509001, Andhra Pradesh, India;
Tel.: +91-9493112829; E-mail: niranjandqa@gmail.com

The leaves of *Terminalia catappa* was collected from Thirumala hills near Tirupathi, Chittoor district, Andhra Pradesh, India and the plant material was taxonomically identified and authenticated by the Dr. Madhava Shetty (Research Officer, Department of botany), Andhra Pradesh.

Ethanolic extract of *Terminalia paniculata* [ETP]

The leaves of *Terminalia catappa* was dried under shade and then powdered with a mechanical grinder to obtain coarse powder. Equal quantity of powder was passed through 40 mesh sieve and extracted with ethanol (70% v/v) in soxhlet apparatus at 60°C. The solvent was completely removed by rotary vacuum evaporator. The extract was freeze dried and stored in vacuum desiccators.^[11]

Phytochemical screening

The presence of phytochemical constituents in the ethanolic extract of leaves of *Terminalia catappa* was tested by using the standard methods. These standard methods revealed the presences of carbohydrates, glycosides, flavonoids, steroids, tannins, saponins, triterpenoids and alkaloids.^[12]

Animals

Studies were carried out using Wistar albino male rats (150-200 g), obtained from Raghavendra Enterprises, Bangalore India. The animals were grouped and housed in polyacrylic cages (38×23×10 cm) with not more than six animals per cage and maintained under standard laboratory conditions (temperature 25 ± 2°C) with dark and light cycle (12/12 h). The animals were fed with standard pellet diet and fresh water *ad libitum*. All the animals were acclimatized to laboratory condition for a week before commencement of experiment.^[13]

Acute toxicity studies

Male albino mice were used to study the acute toxicity of ethanolic extract of leaves of *T. catappa*. Animals were fasted overnight and administered with ethanolic extract. Changes in the animal behavior were noted before and after administration for 24 hours. Treated animals were further observed for up to 14 days for any toxic symptoms.^[14]

Pyloric ligation in rats

Animals were divided into four groups, each consisting of six rats. Group-1 treated as control group, received distilled water orally for 7 days. Group-2 treated as standard group was received Omeprazole 30 mg/kg b.w. for 7 days. Group 3 and 4 treated as treatment groups were received ethanolic extract of *Terminalia catappa* in a dose of 250 and 500 mg/kg b.w. for 7 days respectively. Animals in all groups were fasted for 18 hours after the respective assigned treatment and were anaesthetized with diethyl ether at the dose of 35 mg/kg b.w. 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 gastric contents were collected into the centrifuge tubes and centrifuged at 1000 rpm for 10 min and the pH, free acidity, total acidity of the gastric juice was determined. In addition the ulcer index was determined by opening the stomach on greater curvature and scores were given 0 to 3 depending upon the severity of ulcers.

Histopathology

After collecting the gastric contents and cut into small pieces from each group were fixed in 10% (v/v) formalin solution and subsequently embedded into the paraffin wax. Sections of 5µm thick were cut in a microtome and mounted on the

glass microscope slides using standard techniques. After staining with hematoxylin-eosin, the sections were examined under light microscope and photographed.^[15]

Statistical analysis

The results are expressed as mean ± S.D. values, statistical differences between means were determined by one way ANOVA followed by Tukey's test. P value <0.001 has been considered as statistically significant.

RESULTS

Effect of *Terminalia catappa* extracts on acute toxicity studies

The body weight of the mice's before and after administration of drugs was noted and the changes in the body weight were not so prominent. No changes in skin, fur, eyes, mucous membrane, respiratory, circulatory, autonomic, central nervous system, motor activity and behavior pattern are observed and also no sign of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma were noted. The onset and signs of toxicity was also observed. No mortality was observed. The aforementioned parameters reveal that the selected dose levels of 250 mg/kg and 500 mg/kg b.w. were appropriate.

Effect of *Terminalia catappa* extract on pyloric ligation induced gastric ulcers

In pyloric ligation induced ulcer model, oral administration of ethanolic extract of *T. catappa* in two different doses showed significant reduction in ulcer index, gastric volume, free acidity, total acidity and P^H as compared to the control group. It was showing protection index of 42 % and 77 % at the dose of 250 and 500 mg/kg respectively in comparison to control whereas Omeprazole as reference standard drug was reduction of ulcer 83%. Results are tabulated in Table I.

Macroscopical and histopathological evaluation:

Macroscopical changes of pylorus ligation model were shown in figure (1a, 1b, 1c, 1d). Histopathological changes on pylorus ligation model showed the degeneration, hemorrhage, edematous appearance of the gastric tissue, where as EETC (250 and 500 mg/kg) and Omeprazole (30 mg/kg) treated groups showed regeneration and prevents the formation of hemorrhage and edema and it was shown in figure (2a, 2b, 2c, 2d).

DISCUSSION

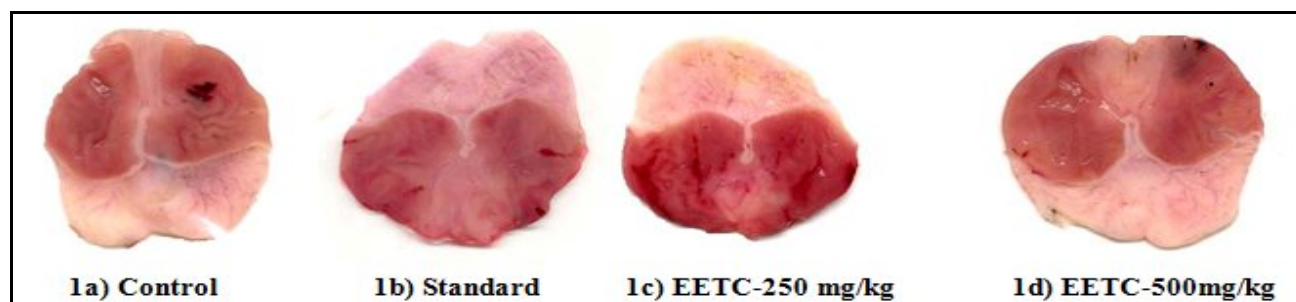
The etiology of peptic ulcer is unidentified in most of the cases, yet it is generally accepted that it results from an imbalance between aggressive factors and the maintenance of mucosal integrity through the endogenous defense mechanisms.^[16] To recuperate the balance, different therapeutic agents are used to obstruct the gastric acid secretion or to boost the mucosal defense mechanisms by increasing mucosal production, stabilizing the surface epithelial cells or interfering with the prostaglandin synthesis. The causes of gastric ulcer by pyloric ligation were believed to be due to stress induced increase in gastric hydrochloric acid secretion and stasis of acid and the volume of secretion is also an important factor in the formation of ulcer due to exposure of the unprotected lumen of the stomach to the accumulating acid.^[17] Pylorus ligation-induced ulcers are due to auto digestion of the gastric mucosa and break down of the gastric mucosal barrier.^[18]

Although the mechanism of ulcer prevention by this extract is not clear, phytochemicals like flavonoids present in the extract might play an important role.

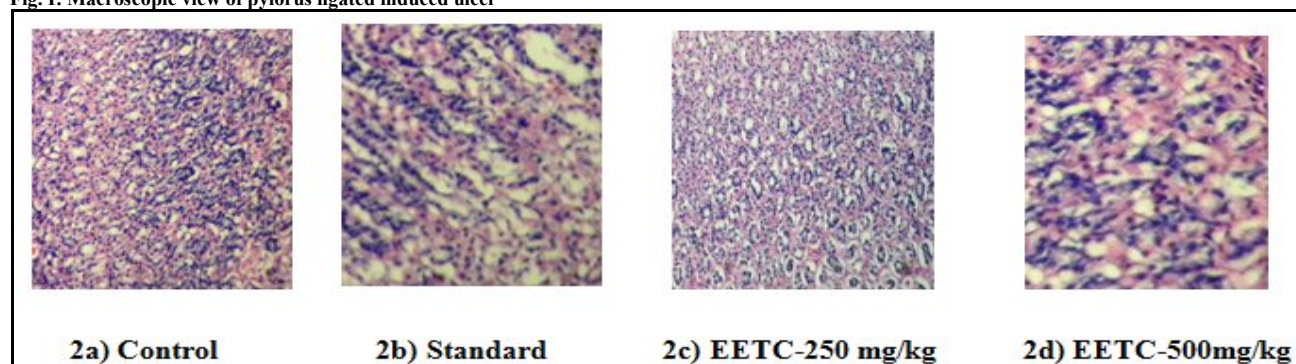
Table I: Effect of ethanolic extract of *T. catappa* on various parameters in pyloric ligated ulcer model

Group	Treatment	Gastric volume	pH	Free acidity	Total acidity	Ulcer index	% protection
Group-1	Control (pyloric ligated)	6.48 ± 0.35	1.9±0.68	11.6±1.08	33±2.19	3±0.54	--
Group-2	Standard (Omeprazole 30 mg/kg b w)	3.36 ± 0.36***	2.88±0.52	5.41±1.02***	10.58±1.11***	0.5±0.44	83
Group-3	EETC(250 mg/kg b w)	5.16 ± 0.72	2.13±0.77	8.6±1.18***	16.08±1.85***	1.75±0.27	42
Group-4	EETC(500 mg/kg b w)	4.91 ± 0.38***	2.1±0.63	5.66±0.87***	11±0.7	0.7±0.27	50

All values are mean ± S.D., ***P<0.001 Tukey's test with respect to control. (one way of ANOVA followed by Tukey's test)



1a) showed severe damage of mucosal layer, 1b) showed protective mucosal layer, 1c, 1d) also showed protective mucosal layers

Fig. I: Macroscopic view of pylorus ligated induced ulcer

2a) showed mucosal ulceration and inflammation, 2b, 2c, 2d) showed no significant change in histopathology

Fig. II: Histopathological view of pylorus ligated induced ulcer

The phytochemical screening confirms the presence of carbohydrates, proteins, tannins, flavonoids and phenolic compounds. Compounds like flavonoids are of particular interest, as they have been reported for their anti-ulcer activity and gastric protection. From this study, it is clear that *T. catappa* extract have significant anti-ulcer activity in animal models. The extract is not toxic even at relatively high concentrations. The antiulcer activity is probably due to the presence of flavonoids. The present investigation has also opened avenues for further research especially with reference to the development of potent phytomedicine for treatment of ulcer and its complication from the title plant.

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