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Evaluation of Anti-ulcer activity of methanolic extract of *Terminalia chebula* fruits in experimental rats

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ABSTRACT
The anti-ulcer activity of methanolic extract of *Terminalia chebula* (Combretaceae)fruits METC was investigated in pylorus ligation and ethanol induced ulcer models in wistar rats. In both models the common parameter determined was ulcer index. METC at doses of 250,500 mg/kg p.o produced significant inhibition of the gastric lesions induced by Pylorus ligation induced ulcer & Ethanol induced gastric ulcer .The extract (250 mg/kg & 500 mg/kg) showed significant ($P<0.01$) reduction in gastric volume, free acidity and ulcer index as compared to control. This present study indicates that *Terminalia chebula* fruit extract have potential anti ulcer activity in the both models. These results may further suggest that methanolic extract was found to possess antiulcerogenic as well as ulcer healing properties, which might be due to its antisecretory activity.
Keywords: *Terminalia chebula*, Pylorus ligation, Ethanol induced ulcer model, ulcer index.

INTRODUCTION

Gastric ulcer, one of the most widespread, is believed to be due to an imbalance between aggressive and protective factors [1]. The gastric mucosa is continuously exposed to potentially injurious agents such as acid, pepsin, bile acids, food ingredients, bacterial products (*Helicobacter pylori*) and drugs [2]. These agents have been implicated in the pathogenesis of gastric ulcer, including enhanced gastric acid and pepsin secretion, inhibition of prostaglandin synthesis and cell proliferation growth, diminished gastric blood flow and gastric motility [3]. 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.

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Terminalia chebula (Family: Combretaceae) was one of the traditional medicine used in many folkclaims and it is called as "King of medicine". It is an middle-sized tree leaves are ovate, or elliptic, flowers are yellowish white, fruits are yellowish brown in colour distributed through out in India[5,6].The plant has extensively used in ayurveda and sidda for Constipation, diarrhea, ulcers, gastroenteritis, asthma, cough, dyspnea, dyspepsia, hemorrhoids, candidiasis, parasites, malabsorption syndrome, hepatomegaly, vesicular and renal calculi, urinary discharges, tumors, skin diseases, leprosy, intermittent fever, rheumatism, arthritis, gout, neuropathy, paralysis, memory loss, epilepsy, depression, diabetes, cardiovascular diseases, anorexia, wounds[7,8]. *Terminalia chebula* contains tannin,chebulic acid, glycosides. sugar, triterpenoids ,steroids and small quantity of phosphoric acid.The pharmacological activities previously reported are Antibacterial, Antifungal, Antiviral, Anticarcinogenic, Antioxidant, Adaptogenic and Antianaphylactic, Hypolipidemic, Hepatoprotective , Cardio protective, Antidiabetic Wound healing, Immunomodulatory and Chemo preventive[9]. However there are no reports on the antiulcer activity of the plant hence the present study was designed to verify the claims of the native practioners.

MATERIAL AND METHODS

Plant Collection

The fruits of *Terminalia chebula* were collected from kolli hills,namakkal district,tamilnadu during the month of November. It was identified and authentificated by Prof. P.Jayaraman Ph.D., Director-Plant Anatomy Research Centre (PARC) Tambaram. The voucher specimen number is PARC/2008/212 and it was submitted to institute of SRM College of pharmacy, for further reference.

Preparation of extract

The fruits of *Terminalia chebula* were shade dried and reduced to coarse powder in a mechanical grinder. The powdered material obtained was then subjected to successive extraction by Hot Percolation Method using petroleum ether, chloroform, and methanol solvents in a soxhelet extractor. The different extracts obtained were evaporated at 45˚C to get a semisolid mass. The extracts thus obtained were subjected to phytochemical analysis. The percentage yield of Alcoholic extract was found to be 38.50%w/w and the methanolic extract was used for further studies.[10]

Preliminary phytochemical screening

The phytochemical examination of the METC was performed by the standard methods [11].

Animals used

Wistar albino rats of either sex weighing between 150-250 gm were used. Institutional Animal Ethics Committee approved the experimental protocol; animals were maintained under standard conditions in an animal house approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA). Albino rats were used in this thesis was obtained from the Bioneeds Animal House Dhavas Pet, Tumkur. The animals were housed in Poly propylene cages and maintained at 24˚C ± 2˚C under 12h light/ dark cycle and were feed *ad libitum* with standard pellet diet and had free access to water. The animals were given standard diet supplied by Pranav Agro Industries Ltd. Sangli. The composition of the diet are protein 10%, Arachis oil 4%, Fibers 1%, Calcium1%, Vitamin A 1000 IU/gm and Vitamin D 500 IU/gm.

Pyloric ligation in rats

Animals are divided into five groups,each consisting of six rats. Control group were received distilled water orally. Second group having pyloric ligated. Third & Fourth Groups received methanolic extract of *Terminalia chebula* in a dose of 250 and 500 mg/kg. Omeprazole, in the dose of 20 mg/kg was be administered orally for Group Fifth as a reference drug for ulcer protective studies. After 45 min of METC and Omeprazole treatment, pyloric ligation was be done by ligating the pyloric end of stomach of rats of respective groups under ether anaesthesia at a dose of 35 mg/kg of body weight. 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 post-operative 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]

Ethanol induced ulcer model

The ulcer was induced by administering ethanol. All the animals were fasted for 36 hours before administration of ethanol .The animals were divided into five groups, each consisting of six rats. One Group represented the control group, which received distilled water orally. Second group receive ethanol. Third & Fourth Groups received methanolic extract of *Terminalia chebula* 250 and 500 mg/kg and, Omeprazole, in the dose of 20 mg/kg were administered orally for Fifth group as reference standard drug. The gastric ulcers were induced in rats by administrating absolute ethanol (90%) (1ml/200g.) Orally, after 45 min of methanolic extract and Omeprazole treatment .They were kept in specially constructed cages to prevent coprophagia during and after the experiment. The animals were anaesthetized 1h latter 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, 15]

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. The percentage of ulcer protection was determined as follows:-

$$\% \text{ Protective} = \frac{\text{Control mean ulcer index - Test mean ulcer index}}{\text{Control mean ulcer index}} \times 100$$

Determination of acidity

$$\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Normality of NaOH} \times 100}{0.1} mEq/L$$

Statistical analysis

The values are represented as mean ± S.E.M, and statistical significance between treated and control groups was analyzed using of One way ANOVA, followed by Dunnett's test where $P<0.05$ was considered statistically significant.

Histopathological evaluation

The gastric tissue samples were fixed in neutral buffered formalin for 24 h. Sections of tissue from stomachs were examined histopathologically to study the ulcerogenic and/ or anti-ulcerogenic activity of *Terminalia chebula*. The tissues were fixed in 10% buffered formalin and were processed using a tissue processor. The processed tissues were embedded in paraffin blocks and about 5-μm thick sections were cut using a rotary microtome. These sections were stained with hematoxylin and eosin using routine procedures. The slides were examined microscopically for Pathomorphological changes such as congestion, haemorrhage, oedema and erosions using an arbitrary scale for the assessment of severity of these changes.[16]

RESULTS

Phytochemical screening

The results of preliminary phytochemical screening of the Methanolic extract of *Terminalia chebula* (METC) revealed that presence of alkaloids, flavonoids, carbohydrates, glycosides, tannins, terpenoids,
Phenols and absence of fixed oils and steroids.

Pyloric ligation induced gastric ulcer

In pyloric ligation induced ulcer model, Oral administration of METC in two different dose showed significant reduction in ulcer index, gastric volume, free acidity, total acidity as compared to the control group It was showing protection index of 76 % and 82 % at the dose of 250 and 500 mg/kg respectively in comparison to control whereas Omeprazole as reference standard drug was reduction of ulcer 84%. **(**Results are tabulated in Table-1).

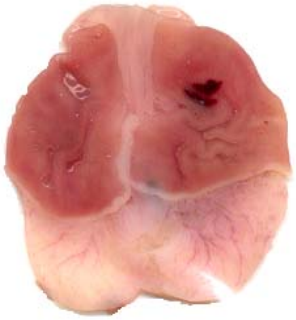
Ethanol-induced gastric ulcer

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 & dark red lesions. METC has shown significant protection index of 54% and 66% with the dose of 250 and 500 mg/kg respectively in comparison to control, Omeprazole as reference standard drug was reduction of ulcer 72%. (Results are tabulated in Table-2)

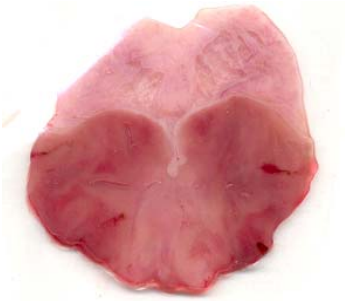
Macroscopical and Histopathological Evaluation

Macroscopical change of pylorus ligation and ethanol induced models were shown in figure (1a,1b,1c&2a,2b,2c).Histopathological changes on pylorus ligation model showed the degeneration, hemorrhage, edematous appearance of the gastric tissue, where as METC (500 mg/kg) and Omeprazole (20 mg/kg) treated groups showed regeneration and prevents the formation of hemorrhage and edema and it was shown in figure (3a,3b,3c).

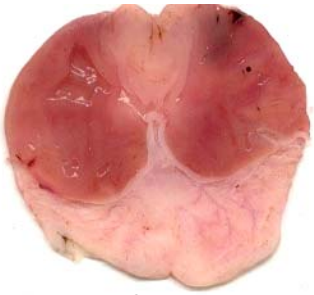
Macroscopical view of Pylorus Ligation induced Ulcer



1 a) Control (P.L.) shows severe damage of mucosal layer



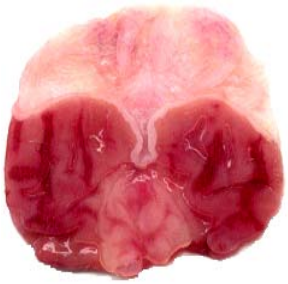
1 b) Omeprazole (20 mg/kg) shows protected mucosal layer



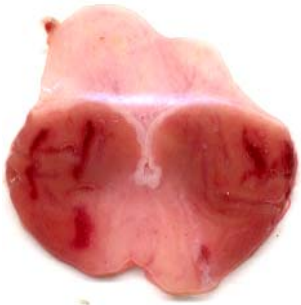
Methanol fruit extract (500 mg/kg)

1c) METC (500 mg/kg) Shows protected mucosal layer

Macroscopical view of Ethanol induced Ulcer



2 a) Ethanol Treated rats shows congestion, oedema, mucosal damage

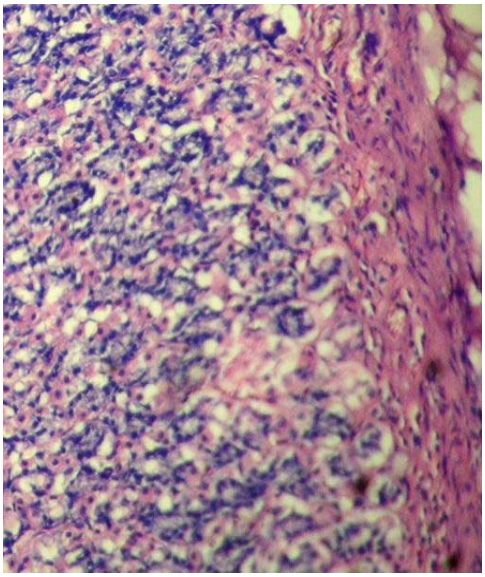


2 b) Omeprazole (20 mg/kg) shows protection of mucosal layer

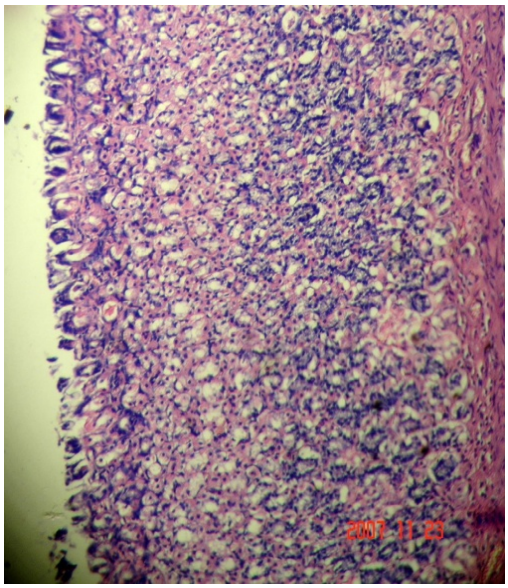
Methanol fruit extract (500 mg/kg)

2c) METC (500 mg/kg) shows protection of mucosal layer

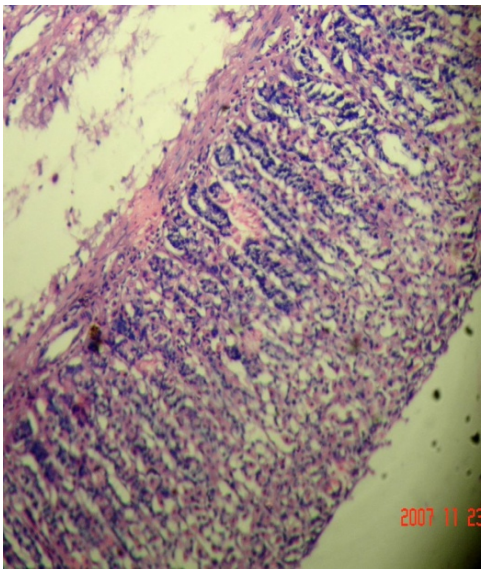
Histopathology of pyloric ligation induced ulcer model (Hematoxin&Eosinx100)



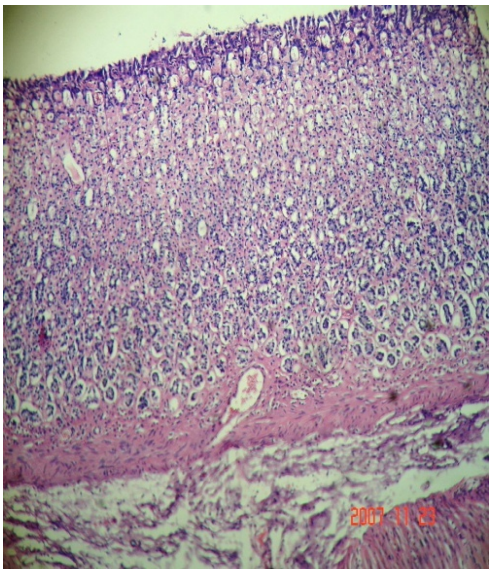
3a) Section of gastric mucosal layer Shows normal appearance Control



3b) pylorus ligation groups shows mucosal ulceration and inflammation



3c)Omeprazole (20 mg/kg) shows no significance change in histopathology almost normal appearance



3d) METC (500mg/kg) shows no significance change in histopathology almost normal appearance

Table:-1 Effect of *Terminalia chebula* fruit extracts on various parameters in pyloric ligation induced Gastric ulcers

Group	Treatment	Ulcer index	Protection (%)	P^H of gastric juice	Gastric juice (ml)	Free acidity meq/ltr	Total acidity meq/ltr
I	Control (Pyloric ligation)	15.8±1.4	-----	2.4±.20	9.4±.20	97.8±1.4	117.8±.24
II	Omeprazole (20 mg/kg)	2.4±.05*	84 %	4.9±.15*	2.4±.18*	32.8±2.4*	57.8±1.4*
III	METC (250 mg/kg)	3.7±.05	76 %	3.6±.20	4.4±.12	47.8±1.4	67.8±.38
IV	METC (500 mg/kg)	2.7±.06*	82 %	4.5±.18*	3.9±.15*	37.8±1.4*	62.8±1.4*

Table:-2 Effect of *Terminalia chebula* fruit extracts on various parameters in ethanol induced gastric ulcer

Group	Treatment	Ulcer index	% Protection	P^H of gastric juice
I	Control (1 ml/ animal)	12.6±.08	-----	3.1±.20
II	Omeprazole,(20 mg/kg)	3.5±.07*	72 %	5.4±.09*
III	METC(250 mg/kg)	5.7±.05	54 %	3.8±.15
IV	METC(500 mg/kg)	4.2±.04*	66 %	4.9±.17*

Values are express as mean ± SEM of 6 observations, Statistical comparisons as follows: Significant at * $p<0.05$ compared to control group.

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 mechanisms.[17] To regain the balance, different therapeutic agents are used to inhibit the gastric acid secretion or to boost the mucosal defence mechanisms by increasing mucosal production, stabilising the surface epithelial cells or interfering with the prostaglandin synthesis. The causes of gastric ulcer pyloric ligation are believed to be due to stress induced increase in gastric hydrochloric acid secretion and/or 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. [18]

Pylorus ligation induced ulcer was used to study the effect of fruit 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 hours followed by ligation of pyloric end of the stomach. The ulcer index is determined 5 hours 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. The METC and Omeprazole significantly decreased the total acidity and free acidity; this suggests that it having an antisecretory effect. Its antiulcer activity is further supported by histopathological study shows that protection of mucosal layer from ulceration and inflammation.

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 intra cellular 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 [19, 20].The extract shows protection against characteristic lesions produced by ethanol administration this antiulcer effect of METC may be due to both reductions in gastric acid secretion and gastric cytoprotection. Further studies are needed for their exact mechanism of action on gastric acid secretion and gastric cytoprotection.

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