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Evaluation of Gastroprotective Effect of *Terminalia chebula* Fruit Extract in Stress-Induced Peptic Ulcers in Wistar Rats

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ABSTRACT

Background: Ulcer can be developed inside the inner lining of the stomach (gastric ulcer) or the small intestine (duodenal ulcer). Both the ulcers are also referred as peptic ulcers. It affects nearly 10% of world population. From olden days, there is an evidence of plant sources like *Terminalia chebula* in the treatment of various diseases modalities. The aim of the study is to evaluate the gastroprotective and anti-peptic ulcer activity of ethanolic extract of *Terminalia chebula* fruits. Methods: The present study was carried by stress-induced ulcer models in Wistar rats. The antiulcer activity of *T. chebula* (0.5, 1 mg/kg p.o. for 7 days) was compared with standard drugs (Ranitidine). The studied parameters were mucin content, gastric volume, pH, total acidity, free acidity, ulcer index, size & number. Results: The low and high dose of *T. chebula* extract significantly reduced gastric mucosal lesion, mucin content, volume of gastric juice, gastric pH, free & total acidity when compared to positive control group. The high dose of *T. chebula* extract showed comparable results in parameters like effect on mucin content, gastric volume, pH, free acidity & total acidity with standard group. The statistical significant changes noted only in ulcer size, number & index. Conclusions: Although the high dose *T. chebula* (1mg/kg) group showed significant gastric protection against ulcer induced by cold restraint method. However, no clear inference can be drawn at this stage and hence we consider the work for further extensive research.

INTRODUCTION

Gastric ulcer diseases are a group of heterogeneous disorders, which arises as a break in the lining of gastrointestinal mucosa bathed by acid & pepsin. Among the gastrointestinal diseases, gastric ulcer is the most predominant with a worldwide prevalence of about 40% in the developed countries & 80% in the developing countries. The etiology of the gastric ulcer is lack of equilibrium between the gastric aggressive factors & the mucosal defensive factors. [1] The present treatment for a gastric ulcer is the use of histamine H2- receptor antagonists, antacids, anticholinergic drugs & PPI's. From olden days, there is an evidence of plant sources in the treatment of various diseases modalities, one such product is *Terminalia chebula* Retz. (Combretaceae), known as "chebulic myrobalan" (English), "abhaya" (Sanskrit), "harahra" (Hindi), and "allalekai (Kannada), fruits of *T. chebula* are a rich source of gallic acid-based secondary metabolites (20–36%). Major constituents are chebulagic acid, chebulinic acid, and chebulic acid; other constituents are tannic acid, gallic acid, ethyl gallate, ellagic acid, chebulanin, corilagin, and terflavin.

T. chebula is used to cure chronic ulcer, carious teeth pain, heart problems (Ishtiaq et al., 2007). *T. chebula* possesses laxative (Miglani *et al.,* 1971), hypolipidemic (Shaila & Udup, 1998), antioxidant (Lee *et al.,* 2005, 2007), hepatoprotectant (Tasduq *et al.,* 2006), antiviral (Jeong *et al.,* 2002), and antibacterial (Sato *et al.,* 1997) activities. One group of researchers found that it is effective in inhibiting the urease activity of *Helicobacter pylori* (Malckzadeh

et al., 2002) and the ubiquitous bacterium implicated in the development of gastritis, ulcers, and stomach cancers.

However, detailed investigations of the antiulcer activity of *T. chebula* have not been carried out so far. Hence this led us to the study the antiulcer activity of *T. chebula* in different ulcer-induced models.

METHODS

The study is conducted after the approval issued by Institutional Animal Ethics Committee - 1284/ac/16/CPCSEA.

CHEMICALS/ DRUGS/ INSTRUMENTS USED

Ranitidine, *T. chebula* fruit extract, ketamine, orcinol 1.6%, sulphuric acid 60%, phenolphthalein, topfer's reagent, 0.5% carboxymethyl Cellulose, distilled water, surgical kit, pH meter.

PLANT PREPARATION AND EXTRACTION

The fresh fruits were purchased from the local market & the fruits of *T. chebula* were shade dried and coarsely powdered. The powder is filled in to filter paper bag and placed in the soxhlet apparatus for extraction. The soxhlet apparatus is connected to round bottom flask which is filled with ethanol (90%) solvent & water bath to maintain temperature. The ethanol was boiled at 40°C for over a period of 24 hours. The extract obtained was 10 % & was stored in a desiccator at room temperature.

EXPERIMENTAL ANIMALS

The animals were taken from central animal house - male/female albino rats of Wistar strain, weight 200-250 gms. The animals were housed under standard condition, housed individually with normal water and food granules, 12:12 hours light dark cycle, 50% humidity and 28°C temperature and provided with standard food granules and water ad libitum.

ANIMAL GROUPING AND TREATMENT

The animals were divided into 5 groups 6 rats in each group.

Group no.	**Group name**	**Drugs to be administered**	**No. of rats**
I	Negative Control	0.9% Normal saline	6
II	Positive Control	0.9% Normal saline + ulcer induced rats	6
III	Standard	Ranitidine(30mg/kg)	6
IV	Test dose 1	*T. chebula* extract (0.5mg/kg) for 7 days	6
V	Test dose 2	*T. chebula* extract (1mg/kg) for 7 days	6
		Total rats	**30**

All drugs are given via oral route via a feeding tube and animals are dosed once a day, with 0.5% carboxymethyl Cellulose (vehicle).

ULCER INDUCING METHOD

The procedure for inducing ulcers is with the stress-induced cold restraint ulcer model, which include animals being fasted for a period of 18-24 hours prior to the experiment. Ulcers are then induced by placing animals individually in a restricted cage for 8 hours and in restraint cold ventilated refrigerator at a temperature of 2-3°C for 2–4 hours. [1]

DRUG DOSING

Group I - Negative control, 0.9% normal saline - no ulcers induced x 7 days

Group II - Positive control 0.9% saline + ulcer induced rats x 7 days

Group III - Standard - Ranitidine 30mg/kg x 7 days

Group IV - Test 1 - *T. chebula* extract 0.5mg/kg x 7 days

Group v - Test 2 - *T. chebula* extract 1mg/kg x 7 days

The first day of the experiment the ulcers were induced by the Stress-induced cold restraint ulcer model.

From day 3-9: Standard & test drugs are given via oral route via a feeding tube and animals are dosed once a day, with 0.5% carboxymethyl Cellulose (vehicle) for 7 days, with the dosage mentioned above. On day 10 - the animals are euthanized using ketamine, the abdomen is dissected then the stomach was removed for the assessment of gastric mucosal damage.

MEASUREMENT OF ULCER

The stomach was dissected along the greater curvature and fixing on a board or transparent glass examination can be carried out with hand lens macroscopically and by a tracing on the transparent paper and the paper on to graph sheets and size of ulcers and the ulcer index was calculated as per the method of Rao *et al.* 1990 *i.e.*

ULCER INDEX = ULCER SIZE X ULCER NUMBER

MUCIN CONTENT DETERMINATION: (WINZLER METHOD)

Diluted sample orcinol (1.6%) and sulphuric acid (60%) are added, vortexed and boiled for 10 min mixtures are cooled in ice-cooled water to stop reaction and absorbance studied at 425nm.

MEASUREMENT OF VOLUME OF GASTRIC JUICE

Gastric juice from the stomach will be drained into a centrifuge tube after the animals were sacrificed. The tube will be centrifuged at 3000rpm for 10 minutes and the Centrifuged sample will be decanted and analyzed for the volume of gastric juice.

MEASUREMENT OF pH OF GASTRIC JUICE: pH of the centrifuged sample of gastric juice is measured using a digital pH Meter, type DPH-100 (Dalal instruments)

MEASUREMENT OF FREE AND TOTAL ACIDITY

The free and total acidity is measured by titrating 0.1 ml of gastric juice with 0.01NaOH using topfers reagent and phenolphthalein as INDICATORS (HAWK, 1965). Orange yellow and point with topfers reagent Is for the free acid content and the pink endpoint with phenolphthalein gives a measure of total acid content. Acidity is expressed as mEq/L per 100 grams body weight.

STATISTICAL ANALYSIS

The results will be analyzed using one way ANOVA in SPSS 21 Software for Microsoft. The statistical significant value for any measure was set to $p<0.05$ at a confidence interval of 95%. The results expressed are in mean +/- standard error mean.

RESULTS

The high dose of *T. chebula* extracts [group-5] significantly reduced gastric mucosal lesion [table1], mucin content [table-2], volume of gastric juice [table-2], gastric pH [table-2], free and total acidity [group-3] when compared to positive control group.

The low dose test group [group-4] showed significantly reduced gastric mucosal lesion [table1], mucin content [table-2], volume of gastric juice [table-2], gastric pH [table-2], free and total acidity [group-3] when compared to positive control group. When compared with

the standard group it showed significant changes only in ulcer size, number & index but not in other laboratory parameters.

The high dose of T. chebula extract [group-5] showed comparable results in parameters like effect on mucin content, gastric volume, pH, free acidity & total acidity with the standard group [table-2, 3& 4]. The statistical significant changes noted only in ulcer size, number & index [table-1].

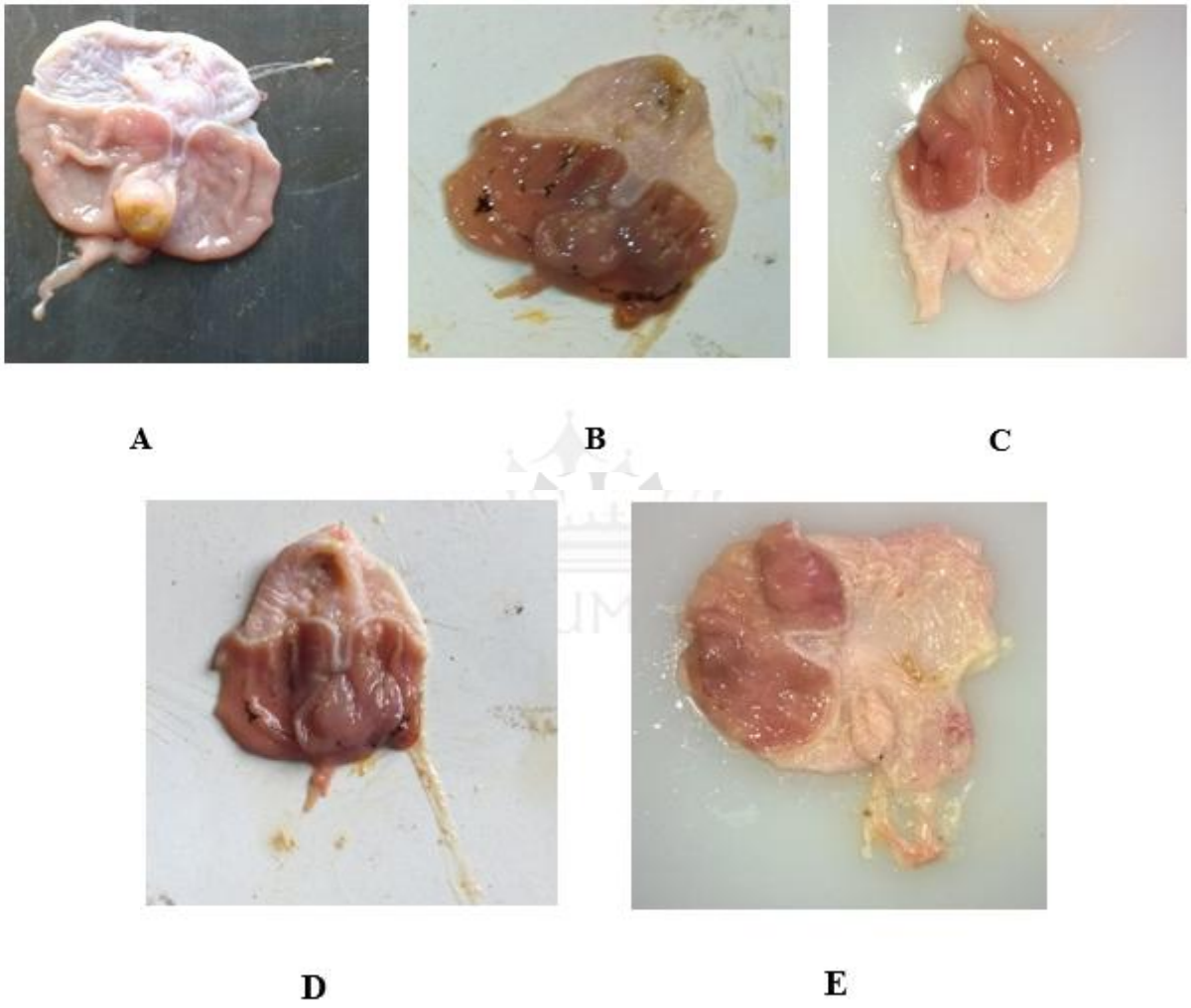

Figure 1. **A** - Negative control group, **B** -Positive control group, **C** - Standard group, **D** -Test 1 group, **E** -Test 2 group

Table: 1 Effect of *Terminalia chebula* on Gastric Ulcer

Group	Mean Ulcer Size(mm)	Mean Ulcer Number	Mean ulcer Index
Group I	-	-	-
Group II	26.8±0.47[c,d,e]	13.5±0.76 [c,d,e]	298±19.5 [c,d,e]
Group III	1.8±0.3[b,d]	3±0.4 [b,d]	4.15±0.6 [b,d]
Group IV	11.9±0.4[b,c,e]	10.5±0.4 [b,c,e]	101.3±0.7 [b,c,e]
Group V	2.4±0.3[b,d]	3.8±0.65 [b,d]	6.4±1.4 [b,d]

Values are mean ± SEM (n=6).Values are statistically significant at *P<0.05 using one way ANOVA followed by Tukey's test. **b:** *p*<0.05 vs positive control, **c:** *p*<0.005 vs standard, **d:** *p*< 0.05 vs test 1, **e:** p< 0.05 vs test 2

Table: 2 Effect of Terminalia chebula on Mucin Content, pH & Vol. of Gastric Juice

Group	Mucin	pH	Volume of Gastric Juice(ml)
Group I	358.33±3.8 [b,c,d,e]	4.86±.04 [b]	2.02±0.07 [b,c,d,e]
Group II	228.8±1.7 [c,d,e]	2.66±0.03 [c,d,e]	7.47±0.12 [a,c,d,e]
Group III	339.1±3.7 [b]	4.66±0.73 [b]	4.07±0.07 [a,b]
Group IV	346.5±2.1 [b]	5.02±0.06 [b]	4.7±0.10 [a,b]
Group V	319±8.8 [b]	5.01±0.16 [b]	4.2±0.29 [a,b]

Values are mean ± SEM (n=6).Values are statistically significant at *P<0.05 using one way ANOVA followed by Tukey's test. **a:** *p*<0.05 vs negative control **b:** *p*<0.05 vs positive control, **c:** *p*<0.005 vs standard, **d:** *p*< 0.05 vs test 1, **e:** p< 0.05 vs test 2

Table: 3 Effect of *Terminalia chebula* on Free Acidity & Total Acidity

Group	Free Acidity(mEq/L/Hr/100gm)	Total Acidity(mEq/L/Hr/100gm))
Group I	7.05±0.05 [b,c,d,e]	32.8±1.4 [b,c,d,e]
Group II	21.4±0.09 [c,d,e]	89.6±0.37 [c,d,e]
Group III	7.98±0.08 [a,b,d]	39.1±0.37 [a,b]
Group IV	7.24±0.13 [a,b,c,e]	32.7±0.54 [a,b]
Group V	7.66±0.08 [a,b,d]	32.2±0.51 [a,b]

Values are mean ± SEM (n=6).Values are statistically significant at *P<0.05 using one way ANOVA followed by Tukey's test. **a:** *p*<0.05 vs negative control **b:** *p*<0.05 vs positive control, **c:** *p*<0.005 vs standard, **d:** *p*< 0.05 vs test 1, **e:** p< 0.05 vs test 2

DISCUSSION

The present study revealed that *Terminalia chebula* fruit extract accelerated the gastric healing in stress-induced cold restraint ulcer after a period of 7 days dosing the rate of healing is comparable to the standard group ranitidine.

The evidence is based on the changes noticed in the gastric mucosa, pH, volume, mucin content, free acidity and total acidity.

The high dose *T. chebula* group (1mg/kg) showed the significant reduction in the gastric mucosal damage when compared with the control and standard ranitidine group (30mg/kg). All other parameters like mucin content, gastric pH, volume, free and total acidity were comparable.

Group 3 (ranitidine 30mg/kg) and group 4 (low dose *T chebula* extract 0.5mg/kg) showed the significant difference in all the parameters when compared with group I & II.

Following severe stress, the ulcer occurs. The possible factors may be Psychological stress, physiological stress which occurs in conditions like shock, severe trauma, extensive burn (Curling's ulcer), septicaemia, Cushing's ulcer. [11]

In our study, the ulcer induction was done using restraint cage and placing the rats in cold ventilated freezer for 2 hours. Edward et al. (1967) studied the Synergism between cold and restraint for rapid production of stress ulcers in rats and they observed that restraint and exposure to cold acted synergistically to produce gastric ulcers.

The effectiveness of extract of *T. chebula* protection against mucosal damage and increase in gastric mucus content caused by ethanol may be an indication of its effect on prostaglandin production (Hollannder *et al.*, 1984). The gastroprotective action of extract of *T. chebula* against stress-induced ulceration could be due to its antihistaminic, anticholinergic and/or antisecretory effects.

T. chebula fruit exhibited the antioxidant activity of different magnitudes of potency (Cheng *et al*., 2003; Lee *et al*., 2005). It has stronger antioxidant activity than α-tocopherols; HPLC analysis with diode array detection indicated the presence of hydroxybenzoic acid derivatives, hydroxycinnamic acid derivatives (chebulagic acid, chebulinic acid, and chebulic acid), flavonol aglycones and their glycosides, as main phenolic compounds (Saleem 1990) and chebulic acid compound were isolated from the plant (Lee *et al.*, 2007).

In the present study, it is observed that the extract of T. chebula fruit attenuated the gastric mucosal damage by its antioxidant and free radical scavenging property, so this model (cold restraint) can confirm the gastro-protective property.

CONCLUSION

Although the high dose *T. chebula* (1mg/kg) group showed significant gastric protection against ulcer induced by cold restraint method. However, no clear inference can be drawn at this stage and hence we consider the work for further extensive research.

Conflict of interest: The authors report no conflicts of interest.

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