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GASTROPROTECTIVE EFFECTS OF FRUITS OF *TRIBULUS TERRESTRIS* L. IN PYLORUS-LIGATED WISTAR RAT MODEL

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ABSTRACT

Tribulus terrestris L. (TT; Zygophyllaceae) is employed in the folk medicine against sexual impotence, oedemas, abdominal distention and cardiovascular diseases. Gastroprotective (i.e. antiulcer and anti-secretory) potential of methanolic extract of TT fruits was evaluated in pylorus-ligated rat model of Wistar rat. The methanolic extract of TT was tested orally at the doses of 150, 300 & 600 mg/kg, on gastric ulcerations experimentally induced by pylorus ligation. Preliminary phytochemical screening of the methanol extract of TT showed the presence of alkaloids, carbohydrates, cardiac glycosides, flavonoids, saponins, tannins and proteins. The methanolic extract at the doses of 300 & 600 mg/kg produced more significant inhibition when gastric ulcerations were induced by pylorus ligation respectively. The methanol extract of the fruits of *Tribulus terrestris* L. possess gastroprotective i.e. antiulcer and anti-secretory effect.

INTRODUCTION: *Tribulus terrestris* L. (TT; Zygophyllaceae) is an annual plant native of Mediterranean region, but now extensively distributed in the warm regions all over the world [1]. It is employed in the folk medicine of India, China, Bulgaria and South Africa against sexual impotence, oedemas, abdominal distention and cardiovascular diseases [2]. TT has reported to have antimicrobial, antihypertension, diuretic, antiacetylcholine, haemolytic activity, to stimulate spermatogenesis, libido and antitumor activity and effects on cardiovascular system [3, 4].

Despite the vast amount of research on ulcer, the cause of chronic peptic ulceration is still not clear. Although in most of the cases the causes of the ulcers is unknown. It is commonly accepted that they result from an imbalance between aggressive factors and the maintenance of mucosal integrity through endogenous defence mechanisms [5]. Drug management of peptic ulcers is targeted at either counteracting these aggressive factors or stimulating the mucosal defence [6]. Regardless of the progress in conventional chemistry and pharmacology in producing effective drugs, the plant kingdom might provide a useful source of new antiulcer compounds for development as pharmaceutical entities or, alternatively, as simple dietary adjuncts to existing therapies [7].

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There are no at present scientific basis or reports in the modern literature regarding its usefulness as antiulcer agent against acetaminophen-induced liver damage in Wistar rat. Therefore, the present study was conducted to evaluate the effect of the methanolic extract of the TT fruits against pylorus ligation-induced ulcer in Wistar rats.

MATERIAL AND METHODS:

Plant*:* Fruits of TT were collected from Chidambaram, Tamil Nadu, India. The plant was identified and authenticated by a Chief Botanist, Department of Botany, Annamalai University, Annamalai Nagar Chidambaram, T.N., India.

Drugs and Chemicals: Omeprazole was obtained from Zydus Research Centre, India. All other chemicals used in this study were obtained commercially and were of analytical grade.

Preparation of the Fruit Extract: The fruits of the TT were dried in shade, powdered and passed through a 40-mesh sieve. Dried powder (200 g) was taken and subjected to successive extraction with Petroleum ether, chloroform and methanol in soxhlet apparatus. The extracts were concentrated to dry residue by distillation (temperature 60 °C without vacuum) and dried completely in a desiccator and weighed. The yield of the methanol extract was found to be 20 g. Only the methanol extract was found to be effective as gastroprotective.

Phytochemical screening: The methanol extract was subjected to phytochemical and pharmacological screening. On preliminary phytochemical study, the methanol extract showed the presence of alkaloids, carbohydrates, flavonoids, saponins, and terpenes. The extract showed the absence of proteins, amino acids, phenols, glycosides, fixed oils, volatile oils steroids and tannins [8, 9]. For dosing, the methanol extract was uniformly suspended in 1% carboxymethyl cellulose (CMC) dissolved in water and administered orally (p.o.).

Animals: The study was conducted after obtaining institutional ethical committee clearance bearing the number 160/1999/CPCSEA. Albino rats of Wistar strain of either sex (100–150 g; 4–6 weeks old) were maintained under controlled conditions of light (12 h/12 h), temperature (26±2°C) and relative humidity (44–56%) for 1 week before and during the experiments. The animals had access to standard laboratory feed (Gold Mohur, Hindustan Lever Ltd., Mumbai, India) and water *ad libitum*. For experimental purposes, animals were kept fasting overnight but were allowed free access to water.

Experimental procedure:

Total acidity: An aliquot of 1ml gastric juice was taken into a 50 ml conical flask and two drops of phenolphthalein indicator was added to it and titrated with 0.01N NaOH until a permanent pink colour was observed. The volume of 0.01N NaOH consumed was recorded. The total acidity is dentaed as meq./l by the following formula: $n \times 0.01 \times 36.45 \times 1000$ where n is volume of NaOH consumed, 36.45 is molecular weight of NaOH, 0.01 is normality of NaOH, 1000 is the factor (to be represented in litre).

Free acidity: Instead of phenolphthalein indicator, the Topfer's reagent was employed. Aliquot of gastric juice was titrated with 0.01N NaOH until canary yellow colour was seen. The volume of 0.01N NaOH consumed was recorded. The free acidity was calculated by the formula for the determination of total acidity [8].

Ulcer score: The gastric mucosa was evaluated for ulcers by magnifying lens and the ulcer scored according to its severity in comparison with that of standard. Ulcer score was noted as 0, normal stomach/no ulcer; 0.5, red coloration 1, isolated haemorrhagic spot; 1.5, hemaorrhagic streaks 2, Ulcer > 3mm but <5mm; 3, ulcer >5mm [10].

The percentage protection was calculated using the formula.

$$\text{Percentage protection} = 100 - \left(\frac{ut}{uc} \times 100\right)$$

Where, *ut* = Ulcer index of treated group, *uc* = Ulcer index of control group.

Mean ulcer score for each animal is expressed as ulcer index.

Effect of TTon pylorus ligated-induced ulcers: The Wistar rats were divided into 5 groups of 6 animals each. Groups I served as negative controls and received suspension of 1% carboxymethyl cellulose in distilled water (10 ml/kg). Groups II served as positive controls and received Omeprazole (8 mg/kg) as standard. Groups III–V received the methanolic extracts at the doses of 150, 300 & 600 mg/kg. All treatments were administered orally at corresponding volume of 1ml/100 g body weight.

Pylorus ligation was made 1 h after treatment. Six hours after the ligation, the rats were sacrificed and the stomach removed. The gastric contents were collected, centrifuged and the supernatant measured. The ulcer formed in the gastric mucosa were measured and scored as described by Shay et al. (1945) [11]. The ulcer index, the percentage ulcerated surface and the percentage of inhibition were determined as described above. One millilitre of the total centrifuged gastric contents from each pylorus-ligated rat was analyzed for hydrogen ion concentration by titrating against a 0.01N solution of NaOH using a pH meter (Santex TS-2). The experiment was done in triplicate.

Statistical analysis: Statistical analysis was perfomed using ANOVA followed by Tukey's test and significance of difference between treatments was accepted at $p < 0.05$. Data are expressed as mean ± standard error of the mean.

RESULTS AND DISCUSSION: Administration of methanolic extracts of TT in different doses (125, 250 & 500 mg/kg) showed significant graded and dose dependent decrease in ulcer index. The TT also significantly reduced the gastric volume, total and free acidity, and increased the pH of the gastric fluid (**Table 1 & 2**).

TABLE 1: EFFECT OF *TRIBULUS TERRESTRIS* L. EXTRACT ON ACID SECRETARY PARAMETERS IN PYLORUS LIGATION-INDUCED GASTRIC SECRETION MODEL

Treatment	Volume of Gastric Juice (ml)	Free Acidity (m eq/l 100 gm)	Total Acidity (m eq/l 100 gm)	pH
Control (1% w/v CMC, 10 ml/kg)	3.6±4.176	35.66±7.42	76.66±14.06	2.6±0.356
Omeprazole (8 mg/kg)	1.433 ± 0.180***	3.66±0.49***	9.00±0.85**	6.36±0.448***
TT (125 mg/kg)	3.13±0.306	24.83±2.0	45.5±6.58	3.95±0.489
TT (250 mg/kg)	2.5±0.274	18.33±2.90	39.166±2.60	4.283±0.242
TT (500 mg/kg)	2.0±0.183	14.83±1.49	24.33±2.107	5.45±0.265

All values represents mean ± SEM, n=6 in each group. ** P<0.05, **P<0.01 and *** P< 0.001 when compared with control group (ANOVA, followed by Tukey's multiple range test).

TABLE 2: EFFECT OF *TRIBULUS TERRESTRIS* L. EXTRACT ON PYLORUS-LIGATED ULCER MODEL IN RATS

Treatment	Mean ulcer index
Control (10 ml/kg)	6.500±0.670
Omeprazole (8 mg/kg)	0.66±0.27***
TT (125 mg/kg)	3.66±0.848
TT (250 mg/kg)	2.92±0.83**
TT (500 mg/kg)	2.58±0.848

All values represents mean ± SEM, n=6 in each group. ** P<0.01 and ***P<0.001 when compared with control group (ANOVA, followed by Tukey's multiple range test).

Gastric ulcers caused by pyloric ligation are due to enhanced accumulation of gastric acid and pepsin, leading to the autodigesion of gastric mucosa [12] and break down of the gastric mucosal barrier [13]. The present study reveals that TT treated groups showed a significant ($P < 0.001$) increase in gastric juice pH, reduced gastric volume, free acidity and total acidity when compared to control. This effect was similar to omeprazole treated group. TT decreased the ulcer index more effectively in a dose dependent manner. These results showed that the antiulcer activity of TT might be due to its antisecretory activity.

The phytochemical profile carried out in the present study pointed out the presence of alkaloids, carbohydrates, cardiac glycosides, flavonoids, saponins, tannins and proteins in methanolic. Flavonoids have been reported to have a significant anti-ulcer activity, in various experimental models of gastric and duodenal ulceration.

Kaempferol which is a flavonol glycoside reportedly have anti-ulcer activity against pylorus-ligated and alcohol-induced ulceration models [14, 15].

The present results clearly indicate that oral administration of methanolic extract of TT fruits at different doses of 150, 300 & 600 mg/kg in pylorus ligated model produce a significant graded and dose dependent antiulcer as well as anti-secretary activity when compared to control (vehicle) group using Omeprazole 8 mg/kg as standard.

CONCLUSION: It can be conceived that methanolic extract of TT, exerts its anti-ulcer activity with the flavonoids. Results suggest that TT extract could be beneficial component of gastroprotection preventing ulcer formation as well as antisecretory activity. Thus the present study established a significant gastroprotective i.e. antiulcer, anti-secretory effect of methanolic extract of TT fruits. However, further studies are needed to establish its exact mode of action, isolation and characterization of constituents responsible for activity.

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