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Antiulcer Activity Using Ethanolic Extract of *Tectona grandis* Linn. Root

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ABSTRACT

The study was designed to study Anti-ulcer model in albino rats in which ability of root extracts of *Tectona grandis Linn.* was tested in at dose level of 100 & 200mg/kg body weight orally and compared with Omeprazole (10mg/kg) as standard. In the antiulcer model, various study like gastric volume, pH, total acidity, free acidity, ulcer index and inhibition of ulceration was determined. Phytochemicals like glycosides, alkaloids, amino acid, carbohydrates, tannins are present in the extract with some biological activities like antidiabetic, antihyperglycemic has been reported but antiulcer activity was not reported. From the results, it is concluded that root extracts of selected dose level, perform excellent antiulcer activity when compared to standard drug.

INTRODUCTION

Peptic ulcer is a very common worldwide problem today, which cause discomfort to the patient, disturbing their daily routine and cause mental distress [(1)]. It is caused due to imbalance between offensive HCl, pepsin, and *H. pylori* and defensive factors mucin, prostaglandin, bicarbonate ions and nitric oxide. Now days, there are two main parameters for treating gastric ulcer, the first due to reduced production of gastric acid and second with gastric mucus layer protection[(2)]. Today's many antiulcer drugs like H_2 antagonists, proton pump inhibitors and ulcer protective are available for the treatment, but they have many side effects and complications. Natural drugs like plants and plant extracts used in the treating of many diseases. These natural drugs are considered to be safe because they contain natural chemical constituents and has no any side effects [(3)].

Tectona grandis (TG), belonging to the family *Lamiaceae* is locally called as "sagwan" and commonly known as Indian teak. In traditional medicine, its root is extensively used for wound healing activity when administered orally; it contains mainly naphthoquinone, lapachol as the active constituent of the extract [(4)]. On literature review there are many activities like Gastric protection, Hyperglycemic, aldose inhibitory potential activity, Antidiabetic, Antihyperlipidemic, Antioxidant potential activity, Antibacterial, gastric secretion activity, Anti-hyperglycemic, Anthelmintic, Diuretic activity, Cytotoxic activity and Anemia of various parts of plant were reported [(5-13)] but there are no any activity was defined, related to the antiulcer activity and responsible for the gastroprotective effects of root. So, the present study was selected for the anti-ulcer property of dried root of TG.

MATERIALS AND METHODS

Plant materials

The roots are collected from the local area of Allahabad (U.P.) and identified by the BOTANICAL SURVEY OF INDIA, ALLAHABAD. (Accession No.-100355).

Experimental animals

Male albino rats, weight 170-200g were obtained from Central drug research institute (CDRI), Lucknow and kept in highly controlled rooms at 26±3 °C with 12 hours dark/light schedule. Animals were feed with pellets and water. Experiments on the rats were approved

by our Institutes Ethical Committee which follows guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals).

Reagents and Equipments

Ethanol (Changshu Yangyum chemical, China), Chloroform (S.D. fine chemical, Bangalore), Pet. Ether (Qualichem fine chem. Pvt. Ltd Vadodara), Carboxymethyl cellulose (CMC) (Central drug house P. Ltd, New Delhi), Omeprazole as a gift sample obtained from Cipla Laboratory, Rotatory evaporator, Centrifuge machine (Remi), Soxhlet apparatus (J-sil), pH meter (systronics).

Methods

Extraction and purification

Shed dried roots of TG and grind in fine powder with the help of grinder, and extracted using various solvents like ether, chloroform, ethanol and water at room temperature. All the extract was concentrated under vacuum then further dried using rotator evaporator for three hours. TLC Chromatography was performed of the dried extract, using Silica Gel 60 (Merck) using Chloroform: Methanol in 9:1 and 8:2 ratio, as mobile phase, sprayed the detecting agent Dragendroff's reagent and visualized under UV light (254–363 nm) for the detection of alkaloids[14] .

Phytochemical test [15]

The phytochemical tests like glycosides, alkaloids, carbohydrates, amino acid, tannins, and steroids test were performed using various respective detecting agents, Table 1.

Treatment schedule

Ethanolic extract and standard drug prepared separately in sodium carboxymethyl cellulose (CMC) dissolve in purified water which is act as vehicle. Prepared suspension and the dose were provided orally 45min before the exposure of ulcerogens to the animals at a volume of 1 ml/200 g according to their body weight. All animals were kept without food for 24 h before ulcerogens study and they divided into four groups, each group containing six animals. The Control group of animals experimented with vehicle similar to the other groups.

Anti-ulcer studies model

Pyloric ligation induced ulcer model (PL) [16]

Pyloric ligation model was done under diethyl ether anesthesia. After 45 min pretreatment with Ethanolic extract and standard drug (10 mg/kg, p.o.), the pyloric part of the stomach was ligated with the help of suture. After 4 hours of the pylorus ligation method, rats were sacrificed and the stomach was dissected and gastric juice was collected. Ulcers were also counted from the dissected stomach.

Macroscopic evaluation

The stomachs were opened along the greater curvature, washed with normal running water to remove gastric contents and blood clots. 10X microscope used for the detection of ulcers.

Mean ulcer score for each animal will be expressed as ulcer index. The percentage of ulcer protection was determined, reported in Table 2.

Ulcer index (UI) was measured by using following formula:

$$\text{Ulcer Index (UI)} = (\text{UN} + \text{US} + \text{UP}) \text{ X } (10\text{-}1)$$

Where,

UI= Ulcer Index; UN = Average number of ulcers per animal; US = Average number of severity score; UP = Percentage of animals with ulcers

Percentage inhibition of ulceration was calculated using following formula:

$$\% \text{ Inhibition of Ulceration} = \frac{(\text{Ulcer index controle} - \text{ulcer index test}) \text{ x } 100}{\text{ulcer index controle}}$$

Identification of pH

Gastric juice was collected and pH was determined by using a digital pH meter, reported in Table 3.

Identification of total acidity

1ml gastric juice diluted with 9ml of distilled water and taken into a conical flask and two drops of phenolphthalein indicator was added to it and titrated with 0.01N NaOH until a permanent pink color was observed. The volume of 0.01N NaOH consumed was noted.

The total acidity is expressed as mEq/L by the following formula:

$$\text{Acidity} = \frac{\text{Vol. of NaOH} \times \text{N} \times 100\ \text{mEq/L}}{0.1}$$

Identification of free acidity

1ml gastric juice diluted with 9ml of distilled water and taken into a conical flask, this Gastric juice was titrated with 0.01N NaOH until yellow color was observed Topfer's reagent was used as indicator. The volume of 0.01N NaOH consumed was noted. The free acidity was calculated by the same formula:

$$\text{Acidity} = \frac{\text{Vol. of NaOH} \times \text{N} \times 100\ \text{mEq/L}}{0.1}$$

Statistical Analysis [17-18]

Statistical analysis was performed as the mean ± SD for each group. The main objective of Analysis of variance (ANOVA) is, to compare two or more group means. The *t*-test is a special case of ANOVA in which only two means are compared. The result should be statistically significant to $P<0.001$.

RESULTS AND DISCUSSION

Thin layer chromatography was performed for the detection of the alkaloids, on the TLC plate yellow and orange red color was formed which shows the presence of alkaloid.

In present study, the preliminary phytochemical test shows the presence of Carbohydrates, Glycosides, amino acid, tannins, alkaloids reported in Table 1.

Table 1: Phytochemical Test results for root extract of *TG.*

Sr. No.	Name of Test	Pet. Ether extract	Chloroform extract	Ethanol extract	Aqueous extract
1.	Glycoside test				
	(a) Foam test	_	_	+	+
	(b) Keller-kelliani	_	_	+	_
2.	Alkaloid test				
	(a) Mayer's test	_	+	+	_
	(b) Dragendroff's test	_	_	+	_
	(c) Wagner's test	_	_	_	+
3.	Carbohydrate's test				
	(a) Molisch's test	+	_	+	+
	(b) Barfoed's test	_	_	+	+
4.	Amino acid test				
	(a) Ninhydrin's test	_	_	+	_
	(b) Biuret test	_	+	+	+
5.	Tannin's test				
	(a) Ferric chloride's test	_	_	+	_
	(b) Iodine's test	_	+	+	_
6.	Reducing sugar test	_	_	+	+
	(a) Fehling's test	_	_	_	+
	(b) Benedict's test				
7.	Steroid's test	_	_	_	_
	(a) Burchard's reaction				

Results of ulcer score reported in Table 2. Comparing the Ulcer control group both the test extract showed increasing in pH shows the reduction the acidity of the gastric juice. The extract of 200mg/kg indicates almost equivalent to the standard, Fig. 1 & 2 and Table 3.

Table 2: Results of ulcer score

Ulcer	**Normal colored stomach**	**Red coloration**	**Spot ulcer**	**Hemorrhagic streak**	**Deep Ulcers**	**Perforation**
Score	0	0.5	1	1.5	2	3

The extracts expressed the decrease in volume of gastric juice when compared to control group. But the extract (200mg/kg) showed significant effect on that of standard (10mg/kg) in reducing the gastric juice volume, Fig. 1 & 3 and results are reported in Table 3.

Due to pylorus ligation the free acidity increases in control group; the extracts at 200mg/kg decreased the gastric free acidity when compared to standard, Fig. 1 & 4 and Table 3.

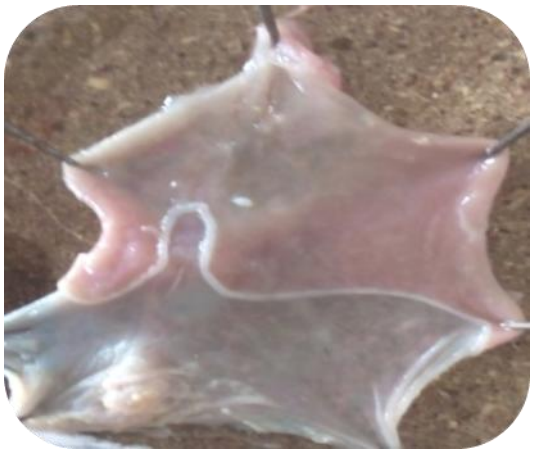
The extracts express decrease the total acidity when compared to control group, Fig 1 & 5 and Table 3.

Normal control

Ulcer control

Standard drug

EETG-100mg/kg



EETG-200mg/kg

Fig. 1. Effect of EETG on pylorus ligation induced ulcer model

The ulcer score decreases when compared to the ulcer control group and extract 200mg/kg was mostly equivalent to the standard, Fig. 1 & 6 and results are reported in Table 3.

Percentage protection of extract at 200mg/kg was almost similar to the standard, Fig. 1 & 7 and results are reported in Table 3.

Table 3: Effect of extract against pylorus ligation induced gastric ulcer

Group (n=6)	Dose (mg/kg)	Gastric pH ±SEM	Gastric Volume (mL) ±SEM	Free Acidity (mEq/L) ±SEM	Total Acidity (mEq/L) ±SEM	Ulcer index	% protection
Normal Control (Water)	-	2.42±0.076	2.56±0.026	33.26±0.0964	34.55±0.045	0.33±0.105	-
Ulcer Control (CMC)	-	2.36±0.021	4.28±0.021	78.19±0.016	97.11±0.048	6±0.634	-
Standard (Omeprazole)	10mg/kg	3.14±0.018	2.91±0.011	24.21±0.0136	21.83±0.082	0.916±0.201	84.73
EETG	100mg/kg	3.24±0.013	3.35±0.01	28.31±0.0101	37.33±0.015	3.083±0.508	36.16
EETG	200mg/kg	3.07±0.006	3.02±0.015	22.37±0.011	29.16±0.012	1.25±0.404	79.16

Results are expressed as mean±SEM; n=6 in each group, EETG= ethanolic extract of TG, CMC= carboxymethyl cellulose, P<0.001.

Table 4: ANOVA result for different mean value

Group (n=6)	Sum of square		d. f.	variance	F	P
Gastric pH	Between group	4.262	4	1.0657	129.94	0.0000
	Within group	0.205	25			
	Total	4.467	29	0.0082		
Gastric volume	Between group	10.246	4	2.561	1341.60	0.0000
	Within group	0.0477	25			
	Total	10.294	29	0.0019		
Free acidity	Between group	13275.78	4	3318.94	279533.05	0.0000
	Within group	0.2968	25			
	Total	13276.08	29	0.0119		
Total acidity	Between group	21998.78	4	5499.69	884115.45	0.0000
	Within group	0.1555	25			
	Total	21998.93	29	0.0062		
Ulcer index	Between group	127.13	4	31.78	30.06	0.0000
	Within group	26.42	25			
	Total	153.55	29	1.057		

From the one way of ANOVA, It was found that the P value close to <0.0001(P=0), which indicate the more significant.

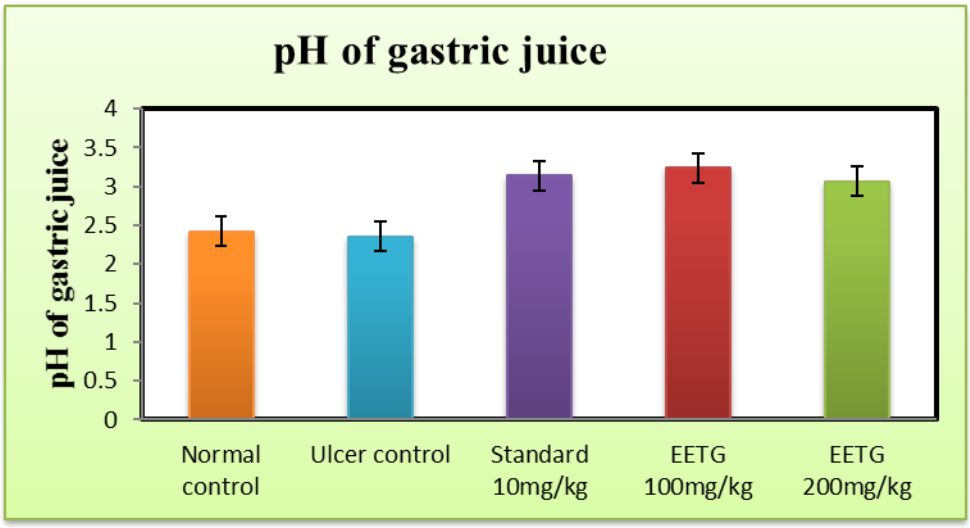


Fig. 2. Effect of EETG on pH of gastric juice in pylorus ligation model

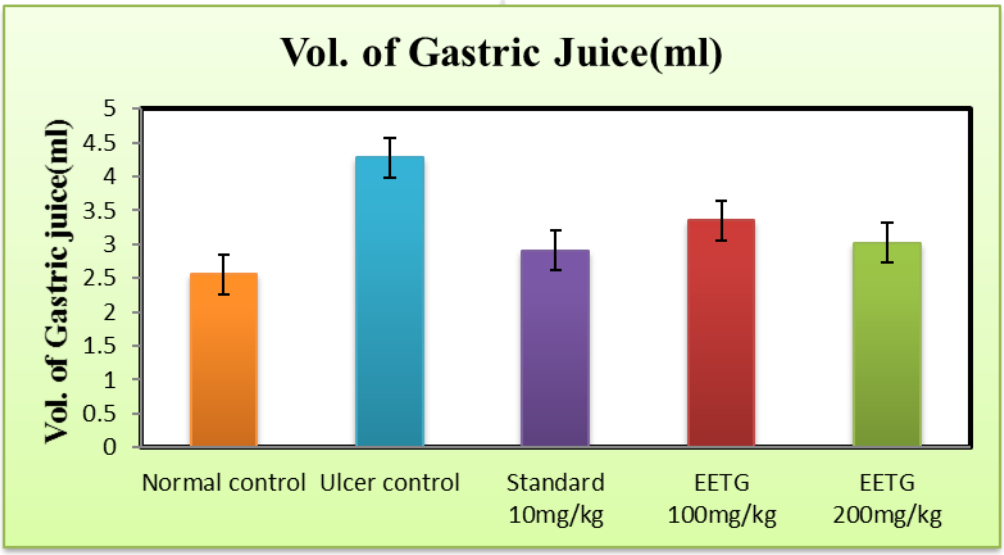


Fig 3. Effect of EETG on vol. of gastric juice in pylorus ligation model

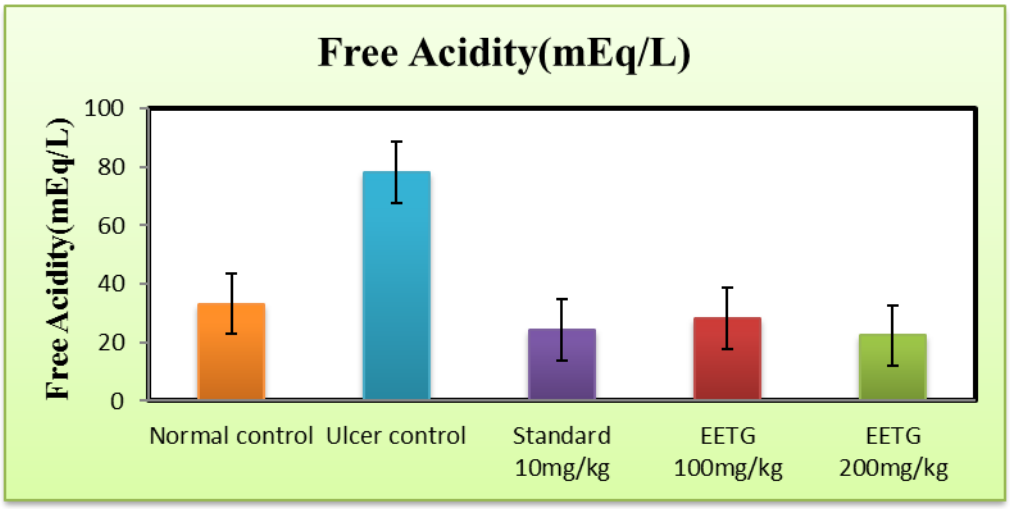


Fig 4. Effect of EETG on free acidity in pylorus ligation model

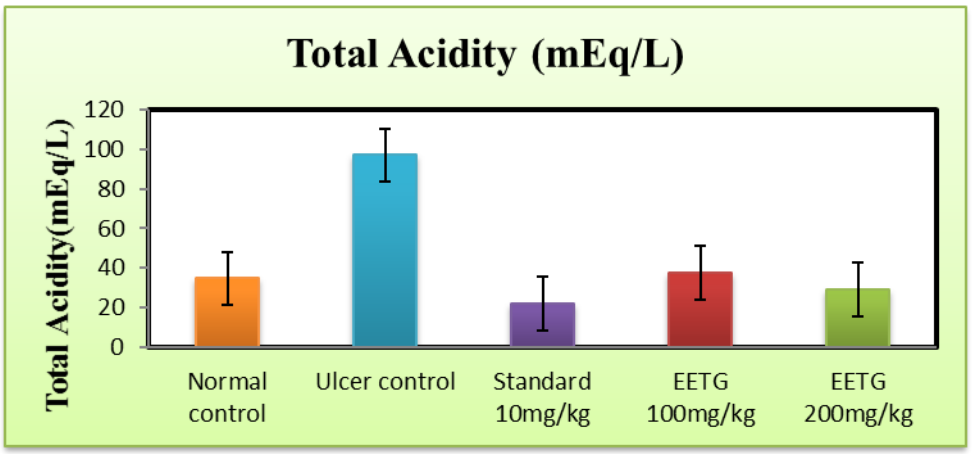


Fig 5. Effect of EETG on total acidity in pylorus ligation model

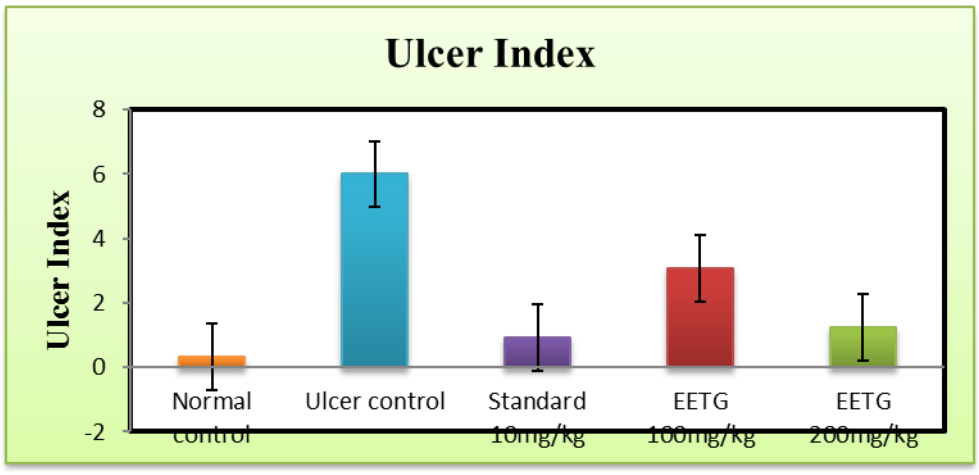


Fig 6. Effect of EETG on ulcer index in pylorus ligation model

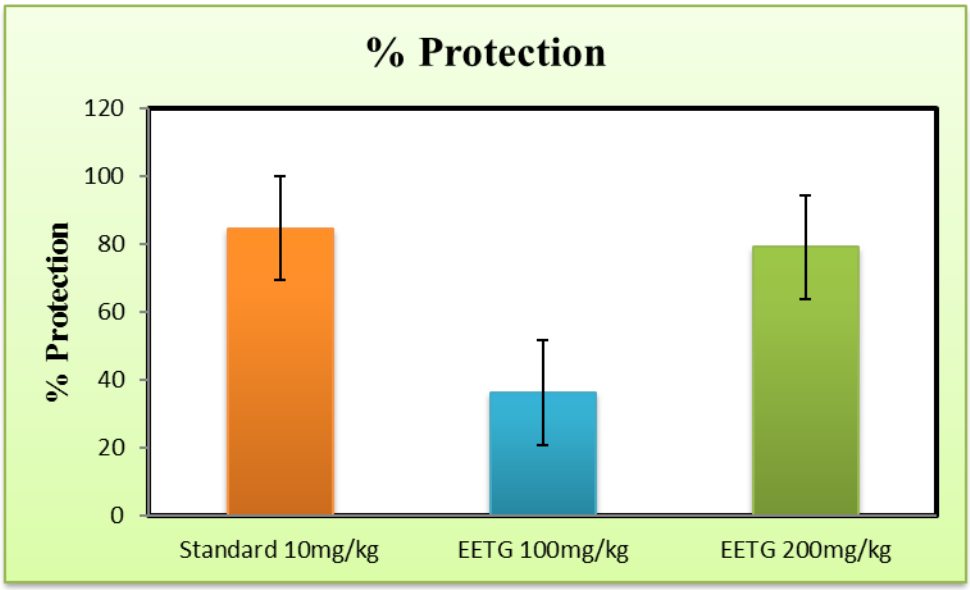


Fig 7. % Protection of EETG in pylorus ligation induced model

CONCLUSION

The antiulcer activity of *root* extracts in pylorus ligation model shows the decrease in gastric volume, free acidity, total acidity, ulcer score and elevated in pH when compared to the standard drug. The antiulcer activity of extract at 200mg/kg is more significant than the extract at 100mg/kg. Thus it has been proved that these extracts contain more antiulcer activity, which can used to control the acidity.

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