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Research Article

EVALUATION OF ANTIPYRETIC AND ANTIULCER ACTIVITY OF ETHANOLIC EXTRACT OF LEAVES OF *TREMA ORIENTALIS* L. IN ALBINO WISTAR RATS

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ABSTRACT

Objectives: The purpose of the present study was aimed at evaluating the antipyretic and antiulcer activity on leaves of *Trema orientalis* L. (family Cannabaceae) on albino Wistar rats. The antipyretic and antiulcer activity of the ethanolic extract of the *T. orientalis* L. leaves in three different concentrations (100, 200, and 300 mg/kg) was compared with standard paracetamol and pantoprazole, which was evaluated by employing Brewer's yeast-induced pyrexia and ethanol-induced ulcer model. The biochemical parameters such as the volume of gastric juice secretion, pH, total acidity, ulcer index, percentage protection, and lipid peroxidation were also studied.

Methods: *T. orientalis* leaves were extracted with ethanol by the Soxhlet extraction method. The dried extract was used for further phytochemical and pharmacological analysis. The antipyretic effect was studied using Brewer's yeast-induced pyrexia. The ethanol-induced ulcer model was used to study the antiulcer effect.

Results: The percentage yield of ethanolic extract of *T. orientalis* leaves was found to be 6.71% w/w, respectively. The extract showed significant antipyretic and antiulcer effect when compared with standard paracetamol and pantoprazole.

Conclusion: The ethanolic extract of *T. orientalis* has significant antipyretic and antiulcer action.

Keywords: *Trema orientalis* L., Antiulcer activity, Ethanol-induced ulcer, Ulcer index.

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INTRODUCTION

Peptic ulcer

The peptic ulcer is characterized by an area of the gastrointestinal tract (GIT) which has been damaged by gastric acid or pepsin. A peptic ulcer can refer to gastric ulcer, duodenal ulcer, or esophageal ulcer. The peptic ulcer is characterized by an area of the GIT which has been damaged by gastric acid or pepsin. A peptic ulcer can refer to gastric ulcer, duodenal ulcer, or esophageal ulcer [1]. Peptic ulcer is one of the major gastrointestinal disorders and it affecting about 10% of the world population. About 15000 deaths occur every year due to the consequences of peptic ulcer [2].

Trema orientalis is a species of flowering tree in the hemp family, Cannabaceae. It is known by many common names, including charcoal-tree, Indian charcoal-tree, pigeon wood, and oriental trema. *T. orientalis* is widely distributed all over the world in countries. Species in this family are significant in food. *T. orientalis* is used in the treatment of antidiabetic [3,4], antiplasmodial and antimicrobial [5-7], diuresis [8], anticonvulsant [9], anti-inflammatory and anti-arthritic [8,10], antibacterial [11,12], antihelmintic [12,13], antisickling [14], and antioxidant activity [15]. The leaves of T. orientalis contain tannins, saponins, flavonoids, and triterpenoid (simiarenol, simiarenone, and tramadol) [16]. T. orientalis plants are used in folk medicine for the treatment of trauma, blood stasis, hematuria, and bleeding of intestines and stomach [17]. Flavonoids are among the cytoprotective materials for which antiulcerogenic efficacy has been extensively confirmed [18-20], they protect the gastric mucosa against a variety of ulcerogenic agents through several mechanisms of action, mainly free-radical scavenging and antioxidant properties, increased mucus production, antisecretory action, and inhibition of the Helicobacter pylori growth (Fig. 1) [19].

METHODS

Animals

Male albino Wistar rats (180–220 g) were used for the study. The animals were obtained from Bharat Serums and Vaccines Limited, Thane West, Maharashtra 400604. The rats were housed in the standard polypropylene rat cages, which were covered by stainless steel coverlids. Wheat husk was used as bedding material. The animals were kept at the animal house of Oriental College of Pharmacy, Sanpada, Navi Mumbai. Standard environmental conditions such as photoperiod (12:12 h dark:light cycle) and temperature (22±2°C) were maintained. Rats were provided with commercial rat feed and water given *ad libitum*. The use of these animals and the study protocols was approved by CPCSEA recognized Institutional Animal Ethics Committee (IAEC) of Oriental College of Pharmacy, Sanpada, Navi Mumbai - 400 705 under protocol no. OCP/IAEC/2017-18/03.

Drugs and chemicals

Paracetamol and pantoprazole were obtained from Cipla Ltd., and ethanol was obtained from Thomas Baker Chemicals, Brewer's yeast powder (now foods). All other chemicals used in this study were obtained commercially and were of analytical grade.

Plant material

The fresh leaves of *T. orientalis* L. were collected from Ghodbunder Road, Thane West, Mumbai, in June 2017 and were submitted to Dr. Bindoo Gopal Krishnan Assistant Professor Department of Botany at Mithibai College of Arts, Chauhan Institute of Science and Amruthben Jivanlal College of Commerce and Economics. The leaves were washed with tap water and shade dried at normal room temperature with the aid of circulating airflow using the fan. Then, leaves were ground to make a coarse powder. The powder was stored in a suitable container. This powder was subjected to Soxhlet extraction.

The extracts obtained from the Soxhlet were evaporated to obtain the dry powder of extract. This crude dry extract was stored in suitable container and kept in refrigerator 4°C until use. The percentage yield of ethanol extract was 6.71% w/w. The ethanol extract of *T. orientalis* L. was used for the entire study.

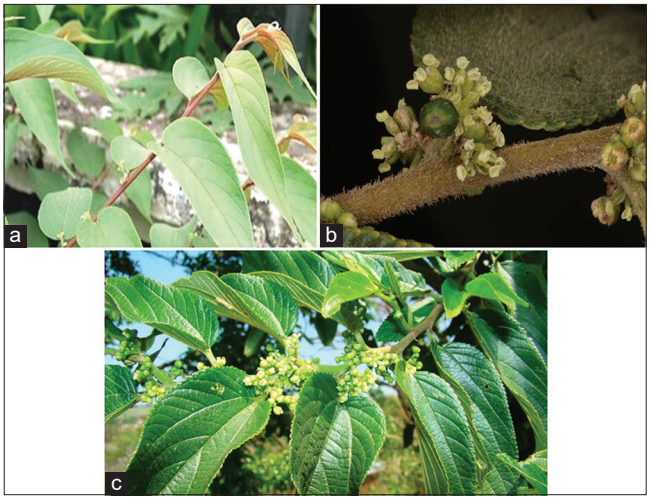

Fig. 1: (a-c) Leaves of *Trema orientalis* L

Qualitative phytochemical screening

Preliminary chemical tests were carried out on ethanolic extract of *T. orientalis* L. for determination of the presence of different phytoconstituents. All tests were performed by the procedures given in "practical pharmacognosy" by Dr. Khandelwal [21].

Preparation of Ethanolic extract of *T. orientalis* (EETO) stock suspension

To prepare the stock suspension of 10 mg/ml of EETO, the EETO powder was taken in mortar and pestle and crushed to get it into a fine consistency. This powder was then passed through the 80# sieve. About 0.2% carboxymethyl cellulose in 1000 ml saline water was mixed in parts with 10 g of the fine powder to get a 10 mg/ml suspension of EETO.

Selection of doses

Protocol no. OCP/IAEC/2018-19/02 conducted acute oral toxicity study prescribed by the OECD guideline 423 and the results suggested that there were no toxic signs observed in clinical parameters during acute toxicity study up to 2000 mg/kg. Hence, it indicates that the LD_{50} of the ethanolic extract of *T. orientalis* leaves formulation is >2000 mg kg. The doses for the groups were selected as 100 mg/kg, 200 mg/kg, and 300 mg/kg.

Antipyretic study

Brewer's yeast induced pyrexia model in one of the common models for induction of pyrexia. This model was used by various researchers such as Hossain *et al*. and Akapa *et al*. [16,22,23]. Yeast is able to produce the pyrogens in the body, causing generation of immune response, thus ultimately leading to the synthesis and release of prostaglandins, which is the final fever mediator in the brain, particularly in the preoptic area of the anterior hypothalamus.

The animals were divided into five groups (six animals in each group) for antipyretic studies:
Group I (negative): They received 20% yeast suspension
Group II (standard): They received paracetamol 150 mg/kg
Group III (test 1): They received EETO 100 mg/kg
Group IV (test 2): They received EETO 200 mg/kg
Group V (test 3): They received EETO 300 mg/kg.

Procedure

The normal body temperature of each rat was measured rectally and recorded. Pyrexia was induced by injecting 10 ml/kg body weight of 20% brewer's yeast suspension in saline solution into the subcutaneous route. Hick's digital thermometer coated with the lubricant was inserted 3–4 cm deep into the rectum and basal temperature was recorded. After 18 h of yeast injection, each group receives the respective oral dosage of control/standard/test drug [24-28].

The temperature was recorded at 30, 60, 90, 120, 150, and 300 min after yeast injection. Rectal temperature before and after treatment was compared and the percentage change in rectal temperature was calculated by the following formula:

$$\text{Percent inhibition of rectal temperature} = \frac{\text{Temperature at time "t"} - \text{Initial temperature}}{\text{Initial temperature}} \times 100 \quad (1)$$

Behavioral changes before and after injection of yeast suspension was also monitored.

Ethanol-induced gastric ulcer model

Ethanol-induced gastric ulcer model is used on rats and is one of the widely used models due to its principle involving the cytoprotective action. Ethanol causes damage to the superficial epithelial layers, as well as is known to inhibit the prostaglandin release. It also increases the concentration of blood neutrophils, causing microcirculatory abnormality and generation of reactive oxygen species, leading to activation of H^+/K^+ ATPase, i.e., proton-pump, which leads to acid hypersecretion, causing damage to GI mucosa [29].

Pantoprazole is widely used, one the most commonly used drugs to treat gastric ulcers. It belongs to the class of proton-pump inhibitors, which is the end step in secretion of gastric acid. Proton-pump inhibitors are prodrugs, which underwent sulfenamide cation form and irreversibly bind to the sulfhydryl group of H^+/K^+ ATPase, causing complete inhibition of the acid secretion [30]. It also provides this activity on nocturnal acid secretion [31]. Any chemical agent that prevents the ethanol-induced ulcers might have the cytoprotective activity and may exert its action by stimulating the release of endogenous prostaglandins, as well as mucin. Similarly, this model can be used to screen possible antisecretory activity effectively.

Procedure

The animals were divided into six groups (six animals in each group) for antiulcer studies [32-35].
Group I (normal): They received distilled water
Group II (negative): They received ethanol 5 ml
Group III (standard): They received pantoprazole 20 mg/kg
Group IV (test 1): They received EETO 100 mg/kg
Group V (test 2): They received EETO 200 mg/kg
Group VI (test 3): They received EETO 300 mg/kg.

Ethanol-induced gastric ulcer method was done by the mechanism given by Oates and Hakkinen [32]. The ulcer was induced by ethanol 95% (absolute ethanol). Male albino Wistar rats were divided into six groups. Six rats in each group, all groups were under a fasting state for 24 h; however, they were allowed to drink water. The purpose of fasting was to inhibit the cross-reaction of gastric content with administered treatments. All rats were orally given particular pre-treatment based on their groups and their weight. After 24 h, test groups 1, 2, and 3 were administered with ethanolic extract of *T. orientalis* L. leaves (100, 200, and 300 mg/kg, p.o.), the standard group received pantoprazole (20 mg/kg, p.o.). The control group received vehicle (distilled water). Two hours after drug treatment, the animals were sacrificed in the CO_2 chamber and dissected to extract their stomach. Stomachs are cut open along the greater curvature, and content of the stomachs was collected in test tubes for evaluation of gastric volume, total acidity, lipid peroxidation (LPO), and pH. Then, stomachs were gently rinsed with 0.9% saline solution and mounted on the wax plate.

Determination volume of gastric content, total acidity, and pH

Gastric contents of each rat were collected in the test tubes and their volume was determined. To determine the pH, 1 ml of distilled water is added to the 1 ml aliquot of gastric juice. With help of pH meter, the pH of the resultant solution was measured. To determine the total acidity, 1 ml gastric juice was diluted by addition of 1 ml distilled water. The resultant solution was transferred to the conical flask and

titrated against the 0.01 N NaOH with few drops of phenolphthalein solution as an indicator. The total acidity was determined by the following formula [36-39]:

$$\text{Total acidity} = \left(\frac{\text{Vol. of NaOH} \times 0.01}{0.1}\right) \times 100 \quad (2)$$

Evaluation of antiulcer activity

For determination of protection from the ulcer, ulcer index (UI) given by Ganguly and Bhatnagar was used, which is as follows [40,41]:

Normal colored stomach – (0), red coloration – (0.5), spot ulcer – (1), hemorrhagic streak – (1.5), deep ulcers – (2), perforation – (3)

UI was measured using following formula:

$$UI = (UN+US+UP) \times 10^{-1}$$

Where,
UI: Ulcer index
UN: Average number of ulcers per animal
US: Average number of severity score
UP: Percentage of animals with ulcers.

Similarly, from the data obtained after using the above equation, percent protection was determined using the following formula:

Table 1: Result of physicochemical analysis of powdered leaves of *Trema orientalis* L.

S. No.	Test	Result (%)
1.	Water-soluble extractive value	5
2.	Ethanol-soluble extractive value	6.71
3.	Chloroform-soluble extractive value	0.12
4.	Benzene-soluble extractive value	0.52
5.	Petroleum-ether soluble extractive value	2.72

Table 2: Result of quantitative phytochemical analysis of powdered leaves of *Trema orientalis* L.

S. No.	Phytoconstituents	Ethanolic extract of the leaves *Trema orientalis* L.
1.	Alkaloids	Present
2.	Flavonoids	Present
3.	Cardiac glycosides	Present
4.	Saponins	Absent
5.	Steroids	Absent
6.	Terpenoids	Present
7.	Tannins and phenolic compounds	Present
8.	Carbohydrates	Absent
9.	Protein	Present

$$\text{Percent protection} = \left(\frac{UIa - UIt}{UIa}\right) \times 100 \quad (3)$$

Where,
UIa: UI of ulcer control group
UIt: UI of test groups.

Furthermore, another method to determine the area of stomach affected by ulcer was suggested by Rathod *et al*. 2014, which requires the use of ImageJ software [42]. Using this software, the total area of the antrum and the total ulcerated area was determined, and from this, percent protection was calculated with the following formula:

$$\text{Percent protection} = \left(\frac{UAa - UAt}{UAa}\right) \times 100 \quad (4)$$

Where,
UAa: Ulcerated area of ulcer control group
UAt: Ulcerated area of test groups.

Biochemical estimation of LPO from post mitochondrial supernatant (PMS)

Procedure

The stomach was homogenized in chilled phosphate buffer (pH 7.4) using a homogenizer. The homogenates were centrifuged at 800 rpm for 5 min at 4°C to separate the molecular debris. The supernatant so obtained was centrifuged at 10,000 rpm for 20 min at 4°C to get the PMS [43,44].

About 0.5 ml of PMS was taken and to it was added 0.5 ml of tris hydrogen chloride buffer and incubated at 37°C for 2 h, and then 1 ml of ice-cold trichloroacetic acid was added, centrifuged at 1000 rpm for 10 min. From the above, 1 ml of supernatant was taken and added 1 ml of thiobarbituric acid and the tubes were kept in boiling water bath for 10 min. The tubes were removed and brought up to room temperature and 1 ml of distilled water was added. Absorbance was measured at 532 nm using a UV–visible spectrophotometer.

Blank

It was prepared without tissue homogenate.

$$\text{Calculation} = \frac{3 \times \text{Absorbance of sample}}{50.156 \times (\text{mg of tissue taken}} = \mu\text{m/mg tissue} \quad (5)$$

Statistical analysis

The data were analyzed with InStat Software by GraphPad (version 3.10). The results are expressed as the mean±standard error of mean for each group. Statistical differences were evaluated using a one-way analysis of variance followed by Dunnett's t-test.

RESULTS

Physicochemical analysis of powdered leaves

Physicochemical analysis of powdered leaves is mentioned in Table 1.

Table 3: Effect of ethanolic extract of *Trema orientalis* L. leaves on percent change in rectal temperature in yeast-induced pyrexia in albino Wistar rats

Group	0 min	30 min	60 min	90 min	120 min	150 min	300 min
Yeast control	104.58±0.315	104.83±0.041	105.03±0.374$^{\#\#}$	105.19±0.178$^{\#\#}$	105.25±0.108$^{\#\#}$	105.42±0.180$^{\#\#}$	105.67±0.170$^{\#\#}$
Standard	103.60±0.231	104.42±0.279	103.13±0.382**	102.41±0.350**	102.06±0.365**	101.68±0.375**	101.07±0.477**
Test 1 (EETO 100 mg/kg)	104.18±0.557	104.25±0.649	104.25±0.482	103.80±0.504*$^{\#}$	103.39±0.533**$^{\#}$	103.08±0.570**$^{\#}$	102.50±0.618**
Test 2 (EETO 200 mg/kg)	105.80±0.354$^{\#\#}$	105.23±0.160	104.85±0.171$^{\#}$	103.92±0.256$^{\#}$	103.15±0.162**	102.87±0.282**	102.37±0.325**
Test 3 (EETO 300 mg/kg)	104.12±0.293	103.48±0.427	103.30±0.414*	103.04±0.363**	102.71±0.296**	102.01±0.279**	101.38±0.246**

Values are the mean±standard error of mean of n=6 rats/treatment, significance **indicates p≤0.01, *indicates p≤0.05 when compared with negative control, when compared with standard, significance $^{\#\#}$p≤0.01, $^{\#}$p≤0.05

Table 4: Effect of ethanolic extract of *Trema orientalis* L. leaves on ulcer index and percent ulcer in albino Wistar rats

Groups	Ulcer index (Ganguly and Bhatnagar)	Percent protection	Percent ulcer (ImageJ Software)	Percent protection
Normal control	-	-	-	-
Ulcer control	14.50±0.67$^{\#\#}$	-	18.48±3.26$^{\#\#}$	-
Standard (pantoprazole)	4.83±0.60**	66.33**	0.69±0.32**	99.31**
Test 1 (100 mg/kg EETO)	9.53±0.32**$^{\#\#}$	34.00**$^{\#\#}$	3.20±1.16**	96.80**
Test 2 (200 mg/kg EETO)	7.13±0.19**$^{\#}$	50.33**$^{\#\#}$	1.36±0.42**	98.64**
Test 3 (300 mg/kg EETO)	4.93±0.549**	65.33**	0.98±0.814**	99.02**

Values are the mean±standard error of mean of n=6 rats/treatment. Significance **p≤0.01, *indicates p≤0.05 when compared with negative control, when compared with standard. Significance $^{\#\#}$p≤0.01, $^{\#}$p≤0.05

Table 5: Effect of ethanolic extract of *Trema orientalis* L. leaves on gastric volume, total acidity, and pH of gastric juice in albino Wistar rats

Group	Gastric volume (ml/100 g)	Total acidity (mEq/L)	pH
Normal control	2.12±0.070**	2.36±0.03$^{\#\#}$	14.39±0.54**$^{\#\#}$
Alcohol control	3.97±0.249$^{\#\#}$	1.01±0.01$^{\#\#}$	19.43±0.39$^{\#\#}$
Standard (pantoprazole)	2.172±0.200**	7.94±0.22**	7.35±0.97**
Test 1 (100 mg/kg EETO)	1.99±0.411**	13.23±0.59**$^{\#\#}$	3.18±0.28**$^{\#\#}$
Test 2 (150 mg/kg EETO)	2.26±0.095**	9.94±0.14**$^{\#\#}$	3.96±0.29**$^{\#\#}$
Test 3 (250 mg/kg EETO)	1.86±0.212**	7.53±0.90**	6.05±0.59**

Values are the mean±standard error of mean of n=6 rats/treatment. Significance **p≤0.01, *indicates p≤0.05 when compared with negative control, when compared with standard. Significance $^{\#\#}$p≤0.01, $^{\#}$p≤0.05

Table 6: Effect of ethanolic extract of *Trema orientalis* L. leaves on lipid peroxidation value in albino Wistar rats

S. No.	Treatment	Lipid peroxidation
1.	Normal control	1.09±0.58**
2.	Alcohol control	3.75±0.35$^{\#\#}$
3.	Standard (pantoprazole)	1.40±0.180**
4.	Test 1 (100 mg/kg EETO)	3.31±0.28$^{\#\#}$
5.	Test 2 (200 mg/kg EETO)	1.74±0.40**
6.	Test 3 (300 mg/kg EETO)	1.60±0.36**

Values are the mean±standard error of mean of n=6 rats/treatment. Significance **p≤0.01, *indicates p≤0.05 when compared with a negative control, when compared with standard. Significance $^{\#\#}$p≤0.01, $^{\#}$p≤0.05

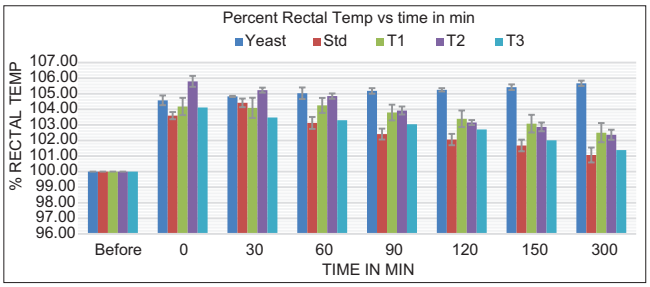

Fig. 2: Effect of ethanolic extract of *Trema orientalis L.* leaves on percent change in rectal temperature yeast-induced pyrexia in albino Wistar rat

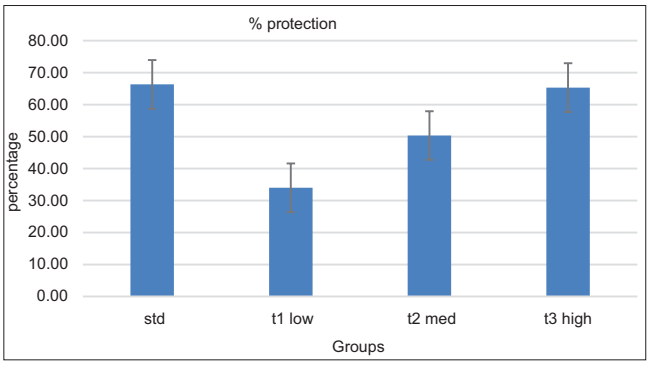

Fig. 3: Effect of ethanolic extract of *Trema orientalis L.* leaves on percent ulcer in albino Wistar rats calculated by Ganguly and Bhatnagar formula

Qualitative phytochemical screening

Qualitative phytochemical screening is mentioned in Table 2.

Antipyretic study

All three test groups of the ethanolic extract of leaves of *T. orientalis* L. showed dose-dependent decrease in body temperature when compared against control as well as against paracetamol (standard) group (Tables 1-3 and Fig. 2).

Antiulcer studies

All three test doses of the ethanolic extract of leaves of *T. orientalis* L. showed dose-dependent decrease in UI when it was compared against control as well as against pantoprazole, which was used as a standard (Tables 4-6 and Figs. 3-5).

LPO

LPO is mentioned in Table 6.

DISCUSSION

T. orientalis showed the inhibition of the increase in rectal temperature in albino Wistar rats in dose-dependent manner; it can be due to inhibition of inflammatory mediators such as cyclooxygenases, interleukins, or prostaglandins.

Ethanol produces massive intracellular accumulation of calcium, which represents a major step in the pathogenesis of gastric mucosal injury. This leads to cell death and exfoliation in the surface epithelium. Ethanol also acts on mitogen-activated protein kinase-II to release inflammatory mediators, which ultimately causes the mucosal injury. The antiulcer property of *T. orientalis* in ethanol-induced ulcer model is evident from its significant reduction in total acidity, gastric volume, LPO, number of ulcers, and UI.

The preliminary phytochemical analysis of *T. orientalis* extract showed the presence of alkaloids, flavonoids, triterpenoids, carbohydrates, and glycosides. The significant increase in the antiulcer activity of

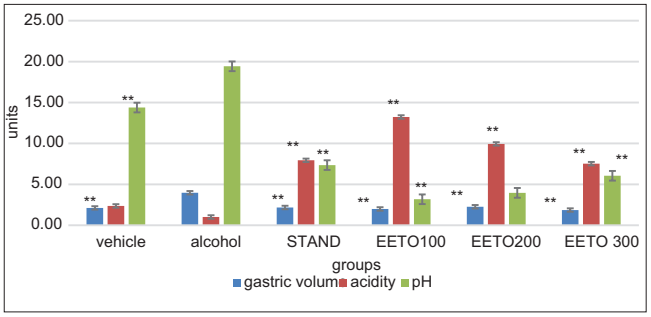

Fig. 4: Effect of ethanolic extract of *Trema orientalis L.* leaves on gastric volume, total acidity, and pH parameter in albino Wistar rats. Significance **p≤0.01, *indicates p≤0.05 when compared with negative control

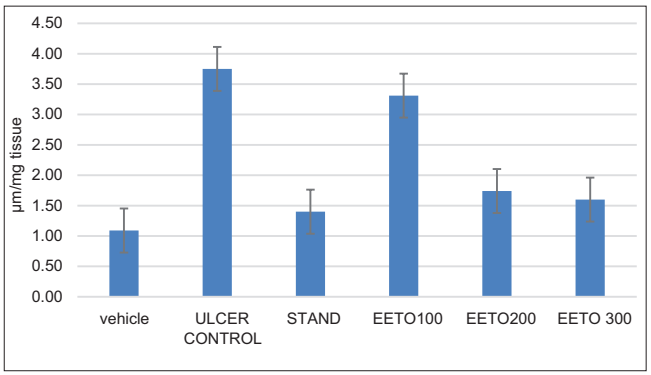

Fig. 5: Effect of ethanolic extract of *Trema orientalis L.* leaves on lipid peroxidation parameter in albino Wistar rats

T. orientalis could be attributed to the presence of flavonoids, alkaloids, tannins, saponins, glycosides, and phenolic compounds. Flavonoids are among the cytoprotective materials for which antiulcerogenic efficacy has been extensively confirmed. It is suggested that these active compounds would be able to stimulate mucus, bicarbonate, and prostaglandin secretion and counteract with the deteriorating effects of reactive oxidants in gastrointestinal lumen. Hence, the antiulcer activity of *T. orientalis* may be attributed to its flavonoids content.

The results of the present study suggest that the ethanol extract of *T. orientalis* leaves may be beneficial in the treatment of gastric ulcer induced by aggressive factors. Further studies to identify the active moieties and elucidation of the mechanism of action are recommended.

CONCLUSION

The present study does confirm that the ethanolic extract of *T. orientalis* does exhibit significant dose-dependent antipyretic and antiulcer activity. The bioactivity-guided phytochemical screening of EETO revealed the presence of flavonoids, tannins, and triterpenoids, which may be responsible for the antiulcer effect and can be further fractionated and investigated for their role and utility in any of the antiulcer mechanisms.

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AUTHORS' CONTRIBUTION

Jyoti Singh collected the clinical samples and carried out antipyretic, antiulcer, and biochemical investigations and drafted the manuscript. Dr. (Mrs.) Vanita Kanase proof-read the whole manuscript and suggested the necessary changes and helped in designing manuscript.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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