

Original Research Article

Evaluation of Antiulcer Activity of Ethanolic Extracts of *Tinospora cordifolia* Aerial Parts and *Psidium guajava* Stem Bark in Pyloric Ligation-Induced Gastric Ulcer Model in Albino Wistar Rats

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ABSTRACT

The present study evaluated the antiulcer potential of ethanolic extracts of the aerial parts of *Tinospora cordifolia* (EETC) and the stem bark of *Psidium guajava* (EEPG) using a pyloric ligation-induced gastric ulcer model in albino Wistar rats. Gastric ulcers were induced by pyloric ligation, a well-established method for assessing ulcerogenesis and gastric acid secretion. The extracts were administered orally as a 1:1 combination of EETC and EEPG at doses of 200 and 400 mg/kg, while ranitidine (150 mg/kg) served as the standard drug. Gastric volume, pH, free acidity, total acidity, bound acidity, ulcer index, gastric lesion length, ulcer severity score, and ulcer number were evaluated, along with percentage protection and percentage inhibition. Treatment with the combined extract produced a dose-dependent reduction in gastric volume and acidity, with a concomitant increase in gastric pH. The 400 mg/kg dose showed pronounced gastroprotective effects compared to the ligation control ($P < 0.01$). Both dose levels significantly reduced ulcer index, ulcer number, and gastric lesion length, showing comparable efficacy to ranitidine. These findings indicate that the combined ethanolic extracts of *Tinospora cordifolia* and *Psidium guajava* exert protective effects against pyloric ligation-induced gastric ulcers, supporting their potential use in ulcer management.

Introduction

Peptic ulcer disease (PUD) is a common gastrointestinal disorder characterized by mucosal damage of the stomach or duodenum resulting from an imbalance between aggressive factors

such as gastric acid, pepsin, reactive oxygen species, and defensive mechanisms including mucus secretion, prostaglandins, and mucosal blood flow [1]. Despite the availability of several conventional antiulcer drugs such as proton pump inhibitors, H_2 -receptor antagonists, and antacids, their long-term use is often associated

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with adverse effects, drug interactions, and relapse of ulcers after discontinuation [2]. Consequently, there is growing interest in identifying safer and more effective antiulcer agents from natural sources. Medicinal plants have been traditionally used for the treatment of gastrointestinal disorders due to their wide spectrum of bioactive phytoconstituents and minimal side effects [3]. Among these, *Tinospora cordifolia* (Menispermaceae), commonly known as Guduchi, is a well-known medicinal plant in Ayurvedic medicine and has been reported to possess antioxidant, anti-inflammatory, immunomodulatory, and gastroprotective activities [4,5]. The aerial parts of *Tinospora cordifolia* contain alkaloids, diterpenoids, glycosides, phenolics, and flavonoids, which are believed to contribute to its therapeutic potential [6]. Similarly, *Psidium guajava* (Myrtaceae), commonly known as guava, has been extensively used in traditional medicine for the management of gastrointestinal ailments such as diarrhea, dysentery, and gastric ulcers [7]. The stem bark of *Psidium guajava* is rich in tannins, flavonoids, triterpenoids, and phenolic compounds, which exhibit potent antioxidant, anti-secretory, and cytoprotective effects on gastric mucosa [8,9]. Previous studies have reported that extracts of *Psidium guajava* exhibit significant ulcer protective activity by reducing gastric acidity and enhancing mucosal defense mechanisms [10]. The pyloric ligation-induced ulcer model is a well-established experimental method for evaluating the antisecretory and gastroprotective effects of drugs and plant extracts [11]. This model induces ulcers primarily through accumulation of gastric acid and pepsin, oxidative stress, and disruption of mucosal integrity, thereby closely mimicking the pathophysiology of human gastric ulcers [12]. Although individual antiulcer effects of *Tinospora cordifolia* and *Psidium guajava* have been reported, scientific evidence supporting their combined therapeutic potential is limited [13]. Combining plant extracts may offer synergistic benefits by targeting multiple ulcerogenic pathways, including acid secretion, oxidative stress, and mucosal defense [14]. Therefore, the present study was designed to evaluate the antiulcer activity of a combined ethanolic extract of *Tinospora cordifolia* aerial

parts and *Psidium guajava* stem bark in a pyloric ligation-induced gastric ulcer model in albino Wistar rats, using ranitidine as a standard reference drug.

Materials and Methods

Collection and authentication of plant materials

The stem bark of *Psidium guajava* L. and aerial parts of *Tinospora cordifolia* L. used in the present study were collected from the premises of Bhaskar Pharmacy College, Hyderabad. The collected plant materials were authenticated by Dr. P. V. Prasanna, Scientist 'F', Botanical Survey of India (BSI), Hyderabad. Voucher specimens were preserved for future reference.

Chemicals and reagents

All chemicals and reagents used in the study were of analytical grade and procured from reputed Indian manufacturers. Ranitidine was used as the standard antiulcer drug. Distilled water was used throughout the experimental procedures.

Experimental animals

Adult male albino Wistar rats weighing 150–200 g were procured from the Central Animal House Facility, Mahaveera Enterprises, Hyderabad. The animals were housed in polypropylene cages (six animals per cage) under standard laboratory conditions with a 12 h light/12 h dark cycle at room temperature. The animals had free access to standard pellet diet and tap water ad libitum.

Ethical approval

The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC), Bhaskar Medical College, Hyderabad, and conducted in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) (Reg. No. 1758/PO/ERe/S/14/CPCSEA).

Preparation of ethanolic extracts

The collected plant materials were shade-dried, coarsely powdered, and subjected to extraction

using ethanol as solvent. The extracts were concentrated under reduced pressure, dried, and stored in airtight containers until further use [15]. The extracts were suspended in 0.5% sodium carboxymethyl cellulose (SCMC) for oral administration.

Phytochemical screening

Preliminary phytochemical screening of the ethanolic extracts was carried out using standard qualitative tests. The ethanolic stem bark extract of *Psidium guajava* L. demonstrated the presence of several bioactive constituents, including alkaloids, saponins, tannins, steroids, cardiac glycosides, reducing sugars, and flavonoids. Similarly, the ethanolic extract of the aerial parts of *Tinospora cordifolia* L. revealed a diverse phytochemical profile, showing positive reactions for alkaloids, diterpenoid lactones, glycosides, steroids, sesquiterpenoids, phenolic compounds, aliphatic constituents, and polysaccharides [16,17].

Acute oral toxicity study

Acute toxicity studies were carried out according to OECD guideline 420 (fixed-dose method). The ethanolic extracts were found to be safe up to a dose of 2,000 mg/kg (p.o.), with no signs of toxicity or mortality observed during the 14-day observation period. Autopsy findings revealed no gross pathological changes in major organs such as liver, heart, kidney, and spleen. Based on these results, 1/10th (200 mg/kg) and 1/5th (400 mg/kg) of the maximum safe dose were selected for the antiulcer study [18–20].

Selection of dose

The selection of dose was based on the acute toxicity study, in which no mortality or observable toxic symptoms were noted at an oral dose of 2,000 mg/kg. Based on these findings, two safe and effective dose levels, namely 200 and 400 mg/kg, were selected for further evaluation using the combined ethanolic extracts in a 1:1 ratio of EETC and EEPG.

Experimental design and pyloric ligation-induced ulcer model

The extracts and ranitidine were administered orally (p.o.) 1 hour prior to pyloric ligation. The rats were fasted for 48 hours before the experiment with free access to water. Animals were housed individually in cages with raised wire mesh floors to prevent coprophagy and cannibalism. Under ether anesthesia, a midline abdominal incision was made, and the pylorus was ligated carefully without disturbing the blood supply [21]. The animals were then allowed to recover and maintained for 4 hours. After this period, the animals were sacrificed using an overdose of ether anesthesia. The stomachs were removed for further analysis.

Evaluation of gastric parameters

Gastric juice volume

Gastric contents were collected into graduated centrifuge tubes through a small incision along the greater curvature and measured for volume.

pH determination

The gastric juice was centrifuged at 3,000 rpm for 10 minutes, and the supernatant was analyzed for pH using a digital pH meter.

Free and total acidity

One milliliter of the supernatant was diluted to 10 mL with distilled water and titrated against 0.01 N NaOH using Topfer's reagent. The endpoint for free acidity was noted by the appearance of an orange color. Titration was continued until a pink color appeared to determine total acidity. Acidity was calculated using the formula (Equation 1):

$$\text{Acidity} = (\text{Volume of NaOH} \times \text{Normality} \times 100) / 0.1 \quad (1)$$

Expressed as mEq/L/100 g body weight

Ulcer evaluation

After pyloric ligation, the stomachs were examined macroscopically for gastric lesions. Ulcer severity was scored based on the number and appearance of lesions using a standard grading scale, and the overall ulcerogenic effect was quantified by calculating the ulcer index

using ulcer number, severity score, and percentage of animals affected [22,23].

Statistical analysis

All results were expressed as mean $\pm$ SEM. Statistical analysis was performed using GraphPad Prism version 8.0. Data were analyzed by one-way ANOVA followed by Dunnett's multiple comparison test. A value of $P < 0.05$ was considered statistically significant

Results and Discussion

Percentage yield of extracts

The percentage yield and physicochemical properties of ethanolic extracts of *Psidium guajava* stem bark and *Tinospora cordifolia* aerial parts are presented in Tables 1 and 2, respectively. The ethanolic extraction yielded 6.7% w/w for *Psidium guajava* and 5.6% w/w for *Tinospora cordifolia*, indicating efficient extraction of phytoconstituents. Both extracts were solid in nature and readily dispersible in 0.5% SCMC, making them suitable for oral administration.

Table 1. Percentage yield and properties of *Psidium guajava* L. stem bark extract

Dried powder wt. (g)	Solvent	Extract wt. (g)	% Yield (w/w)	Color	Nature	Solubility
215.3	Ethanol	14.5	6.7	Dark brown	Solid	0.5% SCMC

Table 2. Percentage yield and properties of *Tinospora cordifolia* L. aerial parts extract

Dried powder wt. (g)	Solvent	Extract wt. (g)	% Yield (w/w)	Color	Nature	Solubility
150	Ethanol	8.5	5.6	Dark greenish brown	Solid	0.5% SCMC

Effect on gastric volume

The effect of EETC + EEPG on gastric volume in pyloric ligation-induced ulcerated rats is shown in Table 3 and Figure 1. Pyloric ligation caused a significant increase in gastric volume compared to the sham control, indicating hypersecretion of gastric juice. Treatment with EETC + EEPG

resulted in a dose-dependent reduction in gastric volume, with the 400 mg/kg dose exhibiting a marked antisecretory effect comparable to that of ranitidine. This reduction in gastric volume suggests inhibition of gastric acid secretion and reduced accumulation of gastric juice.

Table 3. Effect of EETC + EEPG on gastric volume of pyloric ligation-induced ulcer in rats

Groups	Gastric volume (mL) (Mean $\pm$ SEM)
Sham control (Normal saline, 2 mL/kg)	—
Ligation control	5.06 $\pm$ 0.17
Ranitidine (150 mg/kg)	0.90 $\pm$ 0.13**
EETC + EEPG (200 mg/kg)	2.86 $\pm$ 0.15*
EETC + EEPG (400 mg/kg)	1.66 $\pm$ 0.06*

Values are expressed as mean $\pm$ SEM (n = 6).

* $P < 0.05$, ** $P < 0.01$ compared with ligation control, analysed by one-way ANOVA followed by Dunnett's multiple comparison test.

— indicates parameters not measured in that group.

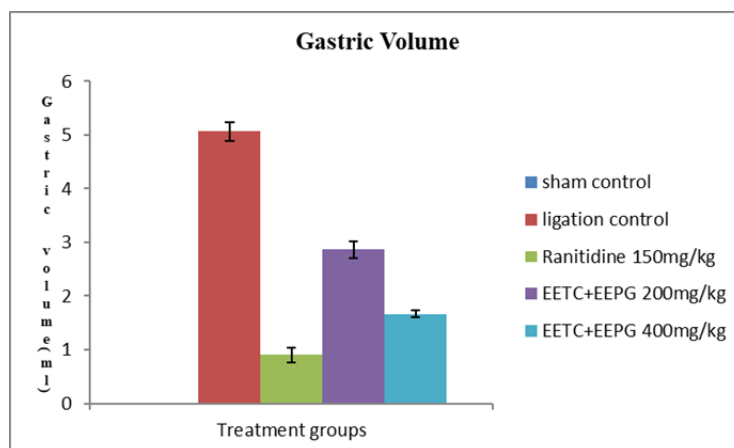


Figure 1. Effect of EETC + EEPG on gastric volume in pyloric ligation-induced ulcer rats. Animals were treated with normal saline (sham control), ligation control, ranitidine (150 mg/kg), or EETC + EEPG (200 and 400 mg/kg, p.o.). Values are mean $\pm$ SEM (n = 6). *P < 0.05, **P < 0.01 vs. ligation control.

Effect on gastric acidity

The effects of the combined extracts on free acidity, total acidity, and bound acidity are summarized in Table 4 and illustrated in Figure 2. Pyloric ligation significantly increased all acidity parameters, reflecting enhanced acid

secretion. Administration of EETC + EEPG significantly reduced free and total acidity in a dose-dependent manner (P < 0.01), with the higher dose showing superior efficacy. This reduction in acidity supports the antisecretory and gastroprotective potential of the combined extracts.

Table 4. Effect of EETC + EEPG on gastric acidity parameters in pyloric ligation-induced ulcer in rats

Groups	Free acidity (meq/L)	Total acidity (meq/L)	Bound acidity (meq/L)
Sham control (Normal saline, 2 mL/kg)	—	—	—
Ligation control	26.96 $\pm$ 0.81	59.65 $\pm$ 1.51	32.67 $\pm$ 1.66
Ranitidine (150 mg/kg)	10.37 $\pm$ 1.50**	35.01 $\pm$ 1.94**	24.63 $\pm$ 3.33**
EETC + EEPG (200 mg/kg)	17.36 $\pm$ 1.12*	29.26 $\pm$ 1.81**	11.89 $\pm$ 1.47**
EETC + EEPG (400 mg/kg)	11.35 $\pm$ 0.95*	24.02 $\pm$ 1.90**	12.66 $\pm$ 0.93**

Values are expressed as mean $\pm$ SEM (n = 6).

*P < 0.05, **P < 0.01 compared with ligation control, analyzed by one-way ANOVA followed by Dunnett's multiple comparison test.

— indicates parameters not measured in that group.

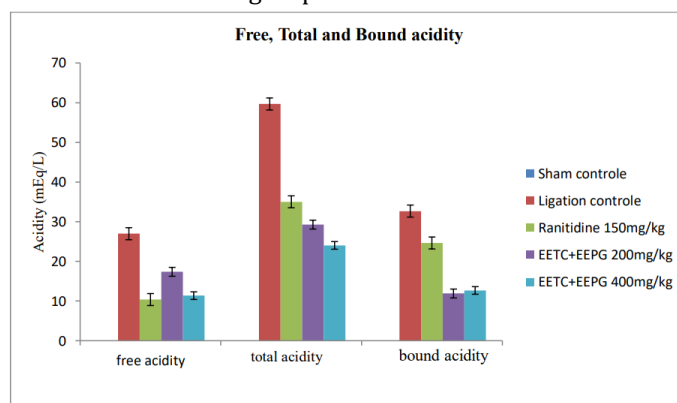


Figure 2. Effect of EETC + EEPG on free, total, and bound gastric acidity in pyloric ligation-induced ulcer rats. Animals received sham control, ligation control, ranitidine (150 mg/kg), or EETC + EEPG (200 and 400 mg/kg, p.o.). Values are mean $\pm$ SEM (n = 6). *P < 0.05, **P < 0.01 vs. ligation control.

Effect on gastric pH

The changes in gastric pH following treatment are presented in Table 5 and Figure 3. Ligation control animals exhibited a significantly low gastric pH, indicating a highly acidic

environment. Treatment with EETC + EEPG significantly increased gastric pH, particularly at 400 mg/kg, indicating suppression of acid secretion. The increase in pH contributes to mucosal protection and ulcer healing.

Table 5. Effect of EETC + EEPG on gastric pH of pyloric ligation-induced ulcer in rats

Groups	pH (Mean $\pm$ SEM)
Sham control (Normal saline, 2 mL/kg)	—
Ligation control	1.60 $\pm$ 0.21
Ranitidine (150 mg/kg)	5.43 $\pm$ 0.17**
EETC + EEPG (200 mg/kg)	4.26 $\pm$ 0.12**
EETC + EEPG (400 mg/kg)	6.28 $\pm$ 0.10**

Values are expressed as mean $\pm$ SEM (n = 6).

**P < 0.01 compared with ligation control, analyzed by one-way ANOVA followed by Dunnett's multiple comparison test.

— indicates parameters not measured in that group.

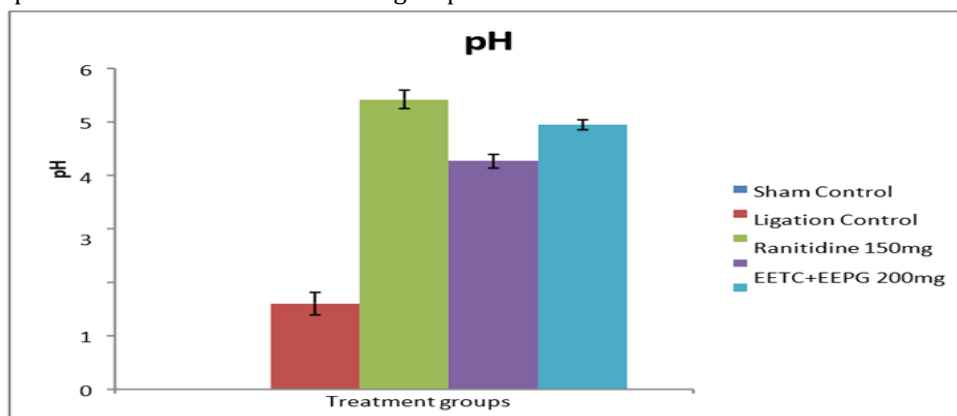


Figure 3. Effect of EETC + EEPG on gastric pH in pyloric ligation-induced ulcer rats. Animals were treated with sham control, ligation control, ranitidine (150 mg/kg), or EETC + EEPG (200 and 400 mg/kg, p.o.). Values are mean $\pm$ SEM (n = 6). **P < 0.01 vs. ligation control.

Effect on ulcer index

The ulcer index values are shown in Table 6 and Figure 4. Pyloric ligation resulted in a significant increase in ulcer index, confirming ulcer

induction. Treatment with EETC + EEPG at 200 mg/kg significantly reduced the ulcer index, indicating reduced ulcer severity and extent. The reduction in ulcer index reflects the protective effect of the extracts on gastric mucosa.

Table 6. Effect of EETC + EEPG on ulcer index of pyloric ligation-induced ulcer in rats

Groups	Ulcer index (Mean $\pm$ SEM)
Sham control (Normal saline, 2 mL/kg)	—
Ligation control	1.00 $\pm$ 0.12
Ranitidine (150 mg/kg)	—
EETC + EEPG (200 mg/kg)	0.40 $\pm$ 0.00*
EETC + EEPG (400 mg/kg)	—

Values are expressed as mean $\pm$ SEM (n = 6).

*P < 0.05 compared with ligation control, analyzed by one-way ANOVA followed by Dunnett's multiple comparison test.

— indicates parameters not measured in that group.

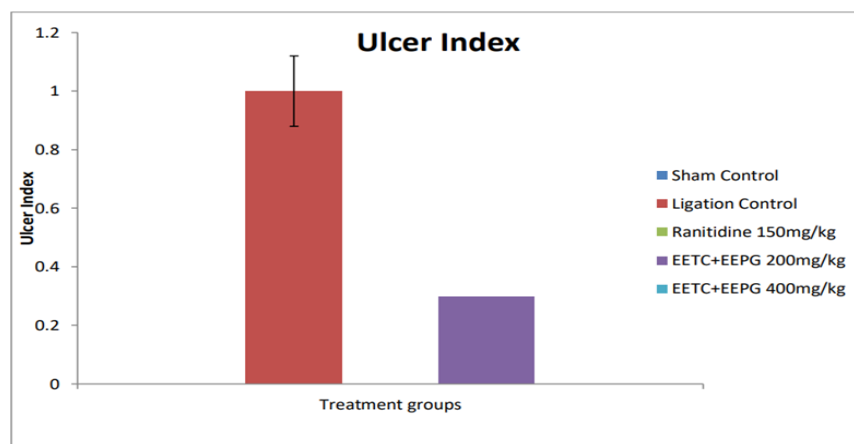


Figure 4. Effect of EETC + EEPG on ulcer index in pyloric ligation-induced ulcer rats. Animals received sham control, ligation control, ranitidine (150 mg/kg), or EETC + EEPG (200 and 400 mg/kg, p.o.). Values are mean $\pm$ SEM (n = 6). *P < 0.05 vs. ligation control.

Effect on gastric lesions

The effect of the extracts on gastric lesion length is presented in Table 7 and Figure 5. Ligation control rats showed extensive gastric lesions,

whereas EETC + EEPG treatment significantly reduced lesion length (P < 0.01). This reduction suggests enhanced mucosal defense and protection against acid-mediated injury.

Table 7. Effect of EETC + EEPG on gastric lesion length in pyloric ligation-induced ulcer in rats

Groups	Gastric lesion (mm) (Mean $\pm$ SEM)
Sham control (Normal saline, 2 mL/kg)	—
Ligation control	16.72 $\pm$ 0.98
Ranitidine (150 mg/kg)	—
EETC + EEPG (200 mg/kg)	4.15 $\pm$ 0.23**
EETC + EEPG (400 mg/kg)	—

Values are expressed as mean $\pm$ SEM (n = 6).

**P < 0.01 compared with ligation control, analyzed by one-way ANOVA followed by Dunnett's multiple comparison test.

— indicates parameters not measured in that group.

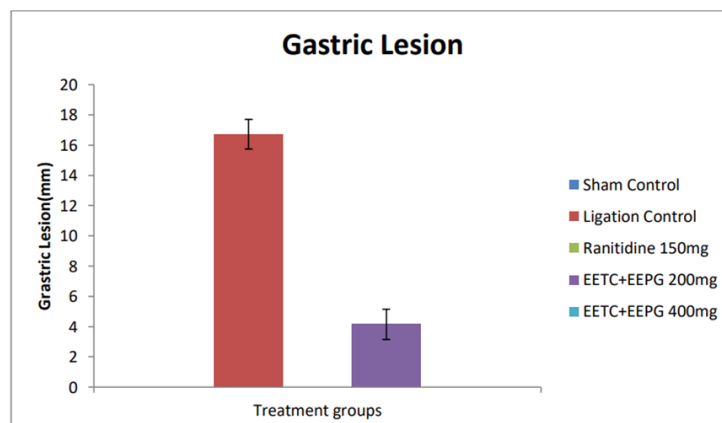


Figure 5. Effect of EETC + EEPG on gastric lesion length in pyloric ligation-induced ulcer rats. Animals were treated with sham control, ligation control, ranitidine (150 mg/kg), or EETC + EEPG (200 and 400 mg/kg, p.o.). Values are mean $\pm$ SEM (n = 6). **P < 0.01 vs. ligation control.

Effect on ulcer number

The ulcer number data are shown in Table 8 and Figure 6. Pyloric ligation caused a significant increase in the number of ulcers. Treatment with

EETC + EEPG significantly reduced ulcer number in a dose-dependent manner, indicating inhibition of ulcer formation. The results further support the antiulcer efficacy of the combined extracts.

Table 8. Effect of EETC + EEPG on the number of pyloric ligations-induced ulcer in rats

Groups	Ulcer number (Mean $\pm$ SEM)
Sham control (Normal saline, 2 mL/kg)	—
Ligation control	3.00 $\pm$ 0.36
Ranitidine (150 mg/kg)	—
EETC + EEPG (200 mg/kg)	1.00 $\pm$ 0.25*
EETC + EEPG (400 mg/kg)	—

Values are expressed as mean $\pm$ SEM (n = 6).

*P < 0.05 compared with ligation control, analysed by one-way ANOVA followed by Dunnett's multiple comparison test.

— indicates parameters not measured in that group.

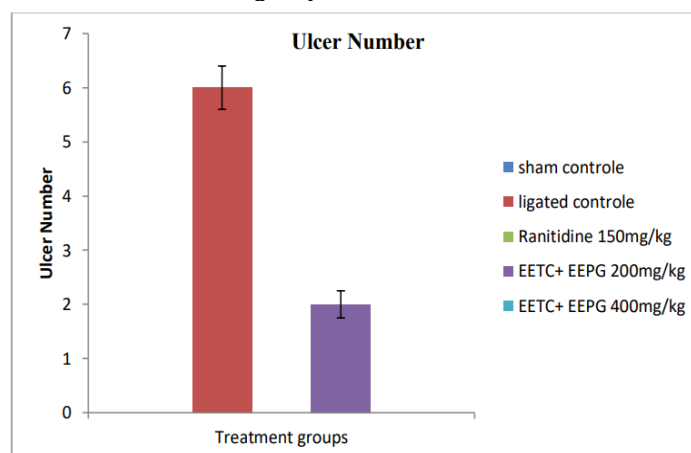


Figure 6. Effect of EETC + EEPG on ulcer number in pyloric ligation-induced ulcer rats. Animals received sham control, ligation control, ranitidine (150 mg/kg), or EETC + EEPG (200 and 400 mg/kg, p.o.). Values are mean $\pm$ SEM (n = 6). *P < 0.05 vs. ligation control.

Effect on ulcer severity score

The ulcer severity scores are summarized in Table 9 and depicted in Figure 7. Ligation control animals exhibited severe gastric

damage, while treatment with EETC + EEPG significantly reduced ulcer severity scores. The decrease in severity score indicates effective protection of the gastric mucosa and reduced hemorrhagic damage.

Table 9. Effect of EETC + EEPG on ulcer severity score of pyloric ligation-induced ulcer in rats

Groups	Ulcer severity score (Mean $\pm$ SEM)
Sham control (Normal saline, 2 mL/kg)	—
Ligation control	2.00 $\pm$ 0.25
Ranitidine (150 mg/kg)	—
EETC + EEPG (200 mg/kg)	0.50 $\pm$ 0.002*
EETC + EEPG (400 mg/kg)	—

Values are expressed as mean $\pm$ SEM (n = 6).

*P < 0.05 compared with ligation control, analysed by one-way ANOVA followed by Dunnett's multiple comparison test.

— indicates parameters not measured in that group.

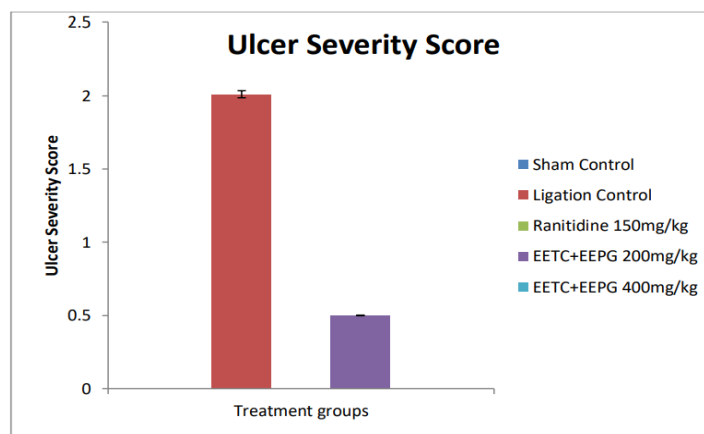


Figure 7. Effect of EETC + EEPG on ulcer severity score in pyloric ligation-induced ulcer rats. Animals were treated with sham control, ligation control, ranitidine (150 mg/kg), or EETC + EEPG (200 and 400 mg/kg, p.o.). Values are mean ± SEM (n = 6). *P < 0.05 vs. ligation control.

Effect on percentage protection (Ulcer index)

The percentage protection based on the ulcer index is presented in Table 10 and Figure 8. The combined extract showed significant protection

against ulcer formation, with 70% protection at 200 mg/kg and complete protection (100%) at 400 mg/kg, comparable to ranitidine. This demonstrates the strong gastroprotective activity of the extract combination.

Table 10. Effect of EETC + EEPG on percentage protection (ulcer index) in pyloric ligation-induced ulcer in rats

Groups	% Protection (Mean ± SEM)
Sham control (Normal saline, 2 mL/kg)	—
Ligation control	0.00 ± 0.00
Ranitidine (150 mg/kg)	100.00 ± 0.00**
EETC + EEPG (200 mg/kg)	70.00 ± 3.21**
EETC + EEPG (400 mg/kg)	100.00 ± 0.00**

Values are expressed as mean ± SEM (n = 6).

**P < 0.01 compared with ligation control, analyzed by one-way ANOVA followed by Dunnett's multiple comparison test.

— indicates parameters not measured in that group.

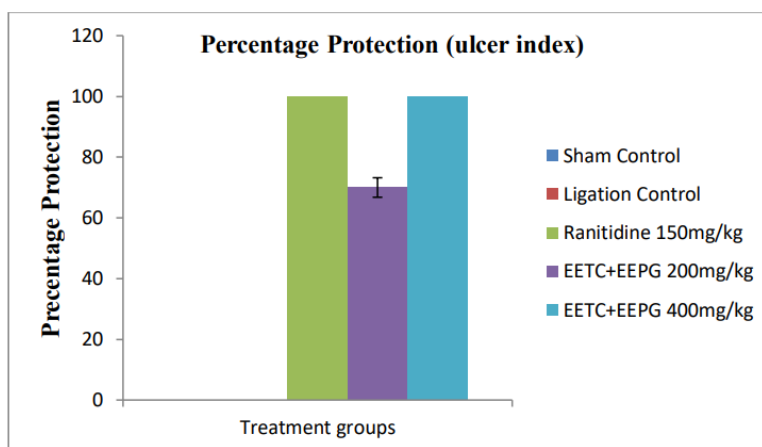


Figure 8. Effect of EETC + EEPG on percentage protection based on ulcer index in pyloric ligation-induced ulcer rats. Animals received sham control, ligation control, ranitidine (150 mg/kg), or EETC + EEPG (200 and 400 mg/kg, p.o.). Values are mean ± SEM (n = 6). **P < 0.01 vs. ligation control.

Effect on percentage inhibition (Ulcer number)

The percentage inhibition of ulcer number is shown in Table 11 and Figure 9. EETC + EEPG significantly inhibited ulcer formation in a dose-

dependent manner, with complete inhibition observed at 400 mg/kg. The results suggest that the combined extracts effectively suppress ulcer development by reducing both ulcer incidence and severity.

Table 11. Effect of EETC + EEPG on percentage inhibition (ulcer number) in pyloric ligation-induced ulcer in rats

Groups	% Inhibition (Mean $\pm$ SEM)
Sham control (Normal saline, 2 mL/kg)	—
Ligation control	0.00 $\pm$ 0.00
Ranitidine (50 mg/kg)	100.00 $\pm$ 0.00**
EETC + EEPG (200 mg/kg)	49.05 $\pm$ 8.47**
EETC + EEPG (400 mg/kg)	100.00 $\pm$ 0.00**

Values are expressed as mean $\pm$ SEM (n = 6).

**P < 0.01 compared with ligation control, analyzed by one-way ANOVA followed by Dunnett's multiple comparison test.

— indicates parameters not measured in that group.

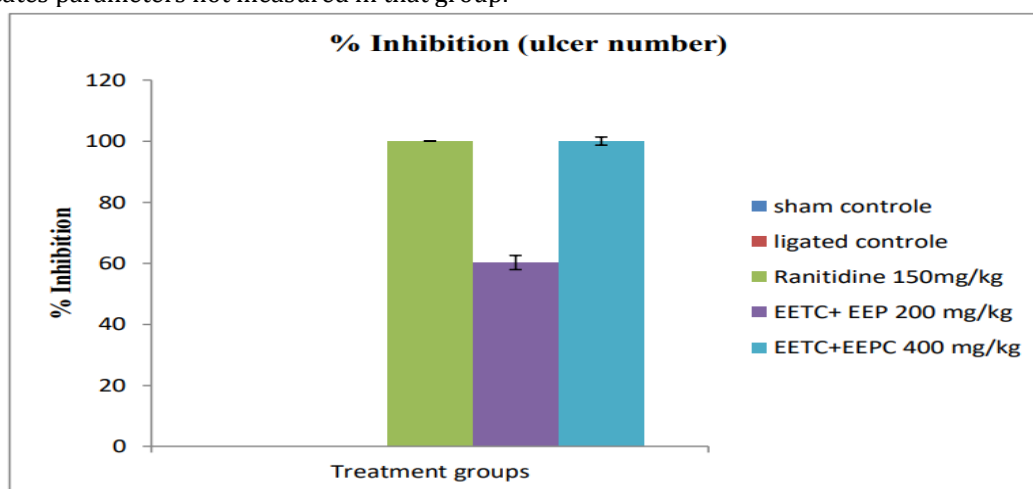


Figure 9. Effect of EETC + EEPG on percentage inhibition of ulcer number in pyloric ligation-induced ulcer rats. Animals were treated with sham control, ligation control, ranitidine (150 mg/kg), or EETC + EEPG (200 and 400 mg/kg, p.o.). Values are mean $\pm$ SEM (n = 6). **P < 0.01 vs. ligation control.

Conclusion

The findings of this study indicate that consumption of ethanol extract of the stem bark of *Psidium guajava* L. and the aerial parts of *Tinospora cordifolia* L extract exerts significant protective action (containing active chemical constituents, e.g., quercetin, gallic acid, tannic acid and guaijavarin in *Psidium guajava* L. and flavonoids, diterpenoid action (Furanolactone), alkaloids (Magnoflorine) against pylorus ligation-induced gastric ulcers. A higher dose (400 mg/kg) of the extract has a greater protective action than a lower dose (200 mg/kg) of the extract. Further investigation with a

longer period of lower doses may show clearer features of these findings.

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None.

Ethical Issues

None.

Competing Interests

The authors declared no conflict of interest in this work.

Authors' Contributions

Conceptualization; data curation; formal analysis; investigation; methodology; project administration; resources; software; supervision; validation; visualization; writing – original draft; and writing – review and editing by CNU, IMS, KAAR, KAI, and MHS.

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Graphical Abstract

