Research Paper

GASTROPROTECTIVE AND ANTI-INFLAMMATORY EFFECTS OF ROOT EXTRACT OF TRIPLOPHYLLUM PROTENSUM AGAINST ETHANOL-INDUCED GASTRIC ULCERATION IN RATS

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ABSTRACT
The present study was undertaken to evaluate the gastroprotective activity of the root extract of Triplophyllum protensum against ethanol-induced gastric ulceration in rats. Gastric ulcers were induced by administration of ethanol, and the protective effect of the extract was assessed by determining ulcer index, gastric pH, total acidity, pepsin activity, oxidative stress markers, antioxidant enzymes, prostaglandin E_2 (PGE_2), and inflammatory cytokines. The animals were divided into four groups including control, standard (Omeprazole 20 mg/kg), and extract-treated groups (100 and 200 mg/kg, p.o.). Treatment with the root extract significantly reduced ulcer index and total acidity while increasing gastric pH in a dose-dependent manner. The extract also reduced pepsin activity and lipid peroxidation as evidenced by decreased malondialdehyde (MDA) levels. Furthermore, administration of the extract restored antioxidant defense enzymes such as superoxide dismutase (SOD) and catalase (CAT), and reduced myeloperoxidase (MPO) activity, indicating attenuation of oxidative stress and inflammation. The extract significantly increased PGE_2 levels and modulated pro-inflammatory cytokines including IL-1β, IL-6, and TNF-α toward normal values. The higher dose (200 mg/kg) exhibited effects comparable to the standard drug omeprazole. The findings suggest that the root extract of Triplophyllum protensum possesses significant antiulcer activity, which may be attributed to its antioxidant, anti-inflammatory, and cytoprotective properties. The study supports the therapeutic potential of Triplophyllum protensum as a natural gastroprotective agent for the management of gastric ulceration.

Keywords: Triplophyllum protensum, gastric ulcer, ethanol-induced ulcer, gastroprotective activity, antioxidant activity, inflammatory cytokines, prostaglandin E_2, oxidative stress, antiulcer activity.
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Introduction

The gastric ulcer remains one of the most common gastrointestinal disorders affecting a large population worldwide (Graham, 2014). Gastric ulceration occurs due to an imbalance between aggressive factors such as gastric acid secretion, pepsin activity, reactive oxygen species, alcohol consumption, stress, Helicobacter pylori infection, and non-steroidal anti-inflammatory drugs (NSAIDs), and the defensive mechanisms of the gastric mucosa including mucus secretion, bicarbonate production, prostaglandins, antioxidant enzymes, and mucosal blood flow. Ethanol-induced gastric ulcer is a widely used experimental model for studying gastric mucosal injury because ethanol causes severe oxidative stress, inflammation, necrosis, and disruption of the gastric mucosal barrier, leading to ulcer formation (Shen *et al.,* 2017).

Excessive generation of reactive oxygen species (ROS) during gastric injury plays a major role in lipid peroxidation, cellular membrane damage, protein oxidation, and inflammatory responses. Oxidative stress also reduces endogenous antioxidant defense systems such as superoxide dismutase (SOD) and catalase (CAT), while increasing malondialdehyde (MDA) and myeloperoxidase (MPO) activity (Martins *et al.,* 2022). In addition, inflammatory mediators including tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) contribute significantly to the progression of gastric mucosal damage. Prostaglandin E_2 (PGE_2), an important cytoprotective mediator, is often reduced during ulcerative conditions, resulting in impaired gastric defense and delayed healing (Mosaffa *et al.,* 2012).

Currently available antiulcer drugs such as proton pump inhibitors, H_2 receptor antagonists, and antacids are effective in reducing gastric acid secretion; however, long-term use of these agents is associated with several adverse effects including relapse, nutrient malabsorption, hypersensitivity reactions, and drug interactions. Therefore, there is increasing interest in the exploration of medicinal

plants as safer and more effective alternatives for the management of gastric ulcers (Paulraj *et al.,* 2026). Herbal medicines are widely recognized for their antioxidant, anti-inflammatory, cytoprotective, and mucosal healing properties with comparatively fewer side effects (Awuchi, 2023).

Triplophyllum protensum is a medicinal plant traditionally used for the treatment of various inflammatory and gastrointestinal disorders. The roots of the plant are reported to contain several bioactive phytoconstituents such as flavonoids, phenolic compounds, tannins, alkaloids, and glycosides, which may contribute to its therapeutic potential (Prado and Moran, 2008). Flavonoids and phenolic compounds are known to possess strong antioxidant and free radical scavenging activities that help protect gastric mucosa from oxidative damage. Moreover, tannins and other polyphenolic compounds may enhance mucosal defense by forming protective layers over the gastric lining and promoting tissue regeneration.

Despite its traditional medicinal importance, scientific evidence regarding the gastroprotective and anti-inflammatory activity of *Triplophyllum protensum* root extract remains limited (Holttum, 1986). Therefore, the present study was designed to investigate the gastroprotective potential of the root extract of *Triplophyllum protensum* against ethanol-induced gastric ulceration in rats. The study evaluated ulcer index, gastric pH, total acidity, pepsin activity, oxidative stress markers, antioxidant enzyme levels, inflammatory cytokines, and prostaglandin E_2 levels to elucidate the possible mechanisms involved in its antiulcer activity.

Material and Methods

Material

The materials used in the present study included the root extract of *Triplophyllum protensum* as the test substance for evaluation of gastroprotective activity. Ethanol was used to induce gastric ulceration in experimental rats. Omeprazole served as the standard antiulcer drug. Various biochemical reagents and buffer solutions were used for the estimation of gastric parameters such as ulcer index, gastric pH, total acidity, pepsin activity, malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), myeloperoxidase (MPO), prostaglandin E_2 (PGE_2), and pro-inflammatory cytokines (IL-1β, IL-6, TNF-α). All chemicals and reagents used in the study were of analytical grade.

Methods

In vivo antiulcer activity of ethanolic extract of *Triplophyllum protensum*

To determine the preventive and restorative effects of plant extracts or formulations against experimentally induced gastric ulcers in animal models, *in vivo* antiulcer activity is analyzed. Excessive acid secretion, stress, alcohol usage, non-steroidal anti-inflammatory medicines (NSAIDs), and Helicobacter pylori infection are typical causes of gastric ulcers. Under controlled conditions, experimental models aid in evaluating the gastroprotective properties of test drugs.

Animals

Wistar rats weighing between 180 and 250 grams were kept in groups of six under a typical 12-hour light/dark cycle with regulated humidity and temperature (25±2°C, 55–65%). Standard rodent food and water were freely available to them. Before beginning the experiments, the rats were given seven days to get used to the lab environment. Every procedure was carried out between 8:00 and 15:00 in a quiet room. Each experiment employed a different set of six rats. The Ministry of Environment and Forests, Government of India, New Delhi, India, formed the Institutional Animal Ethics Committee (IAEC), which approved the animal experiments.

Toxicity Study

In accordance with the OECD 425 recommendations, a safe dose for the *Triplophyllum protensum* extract was established using the acute toxicity test. Four groups of six rats each were created by equally dividing the animals. The rats weighed between 150 and 200 grams. Following a 24-hour fast, groups I–III received oral administered dosages of varying doses (500, 1000, and 2000 mg/kg) of *Triplophyllum protensum* extract diluted in distilled water, whereas group IV received an equivalent volume of distilled water. Every group was monitored independently for any indications of toxicity at 2, 4, 8, and 24 hours following administration. These included behavioral profiles (awareness, irritability, and anxiety), neurologic profiles (spontaneous activity, pain response, and gait), autonomic profiles (evacuation and urination), and physical states (lacrimation, anorexia, shocks, hair erection, drooling, diarrhea, and daily mortality) over a two-week period (Cooperation and Development 1981).

No signs of toxicity or mortality were observed even at the highest tested dose of 2000 mg/kg body weight, indicating that the extract was safe and well tolerated. Based on the OECD guideline and toxicity findings, 1/10th and 1/20th of the maximum safe dose were selected for further pharmacological evaluation. Therefore, doses of 200 mg/kg and 100 mg/kg body weight were chosen for animal activity studies.

Experimental model:

Design of experiments and protocol for treatment

Before testing, rats were acclimated to animal laboratory settings for seven days at 25°C, 55% humidity, and a 12-hour light-dark cycle. For the

duration of the trial, the rats were given a regular diet and had unlimited access to water.

Ulcer induced by absolute ethanol

Four groups of six rats each were established from the rats.

Absolute ethanol (1 ml/animal) was administered to **Group I** (toxicant control).

Omeprazole (20 mg/kg, p.o.) was given to **Group II**.

100 mg/kg of *Triplophyllum protensum* extract was administered to **Group III**.

Triplophyllum protensum extract at a dose of 100 mg/kg was administered to **Group IV**.

For five days following the formation of ulcers, the animals were given ranitidine (100 mg/kg) and dosages of *Triplophyllum protensum* extract (100 and 200 mg/kg, once daily), while the control group was given the vehicle alone. After a 24-hour fast, the rats were given one milliliter of absolute ethanol orally (Jalilzadeh-Amin *et al.,* 2015, Vijayakumar *et al.,* 2016). After an hour of ulcerogen treatment, the animals were sacrificed, their stomachs removed, and the contents of their stomachs aspirated. After centrifuging the contents for ten minutes at 1000 rpm, the pH was measured using a digital pH meter. To determine the ulcer index, the stomachs were cleaned with regular saline and stored in 10% formalin.

The formula was used to evaluate the ulcer index;

Ulcer index = 10/X

Where X = Total mucosal area/Total ulcerated area.

The following scores were assigned to the ulcers based on their intensity:

0 = no ulcer, 1 = superficial mucosal erosion, 2 = deep ulcer or transmural necrosis,

3 = perforated or penetrated ulcer.

The standard and test drugs were given orally 30 minutes prior to the aspirin dose, and the rats were allowed free access to water throughout a 24-hour fast. After administering ulcerogen for five hours, the animals were sacrificed, and their stomachs were removed. The next steps were the same as for the absolute ethanol-induced ulcer model.

Antiulcer Screening

The ulcer index was determined using the formula:

Ulcer index = 10/X

Where X = Total mucosal area/Total ulcerated area.

The following scores were assigned to the ulcers based on their intensity:

0 = no ulcer, 1 = superficial mucosal erosion, 2 = deep ulcer or transmural necrosis,

3 = perforated or penetrated ulcer.

Biochemical Analysis of the Gastric Tissue

For biochemical estimations, approximately 0.2 g of gastric tissue including both healthy and ulcerated portions was accurately weighed from each stomach sample. The tissue samples were homogenized in 2 mL of suitable ice-cold buffer solution depending upon the parameter to be analyzed. For myeloperoxidase (MPO) estimation, 0.5% hexadecyl trimethyl ammonium bromide (HDTMAB) prepared in potassium phosphate buffer (pH 6.0) was used. For malondialdehyde (MDA) analysis, 1.15% potassium chloride solution was employed, whereas phosphate buffer (pH 7.5) was used for the remaining biochemical estimations. The homogenates were prepared under ice-cold conditions to prevent enzymatic degradation and then centrifuged at 10,000 rpm for 15 minutes at 4°C. The clear supernatant obtained after centrifugation was collected and used for subsequent biochemical analyses.

The gastric juice was analyzed for determination of pH, free acidity, total acidity, and pepsin activity using standard procedures. Oxidative stress parameters including malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and myeloperoxidase (MPO) activity were estimated in gastric tissue homogenates to evaluate lipid peroxidation and antioxidant status. In addition, the level of prostaglandin E2 (PGE2) was determined to assess gastric mucosal protection. Pro-inflammatory cytokines such as interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) were also estimated to evaluate the inflammatory response associated with gastric mucosal injury.

Misra et al. (1972) described the role of superoxide anion in epinephrine autoxidation and developed a simple assay for superoxide dismutase estimation. Ohkawa et al. (1979) reported the thiobarbituric acid reactive substances (TBARS) method for lipid peroxidation analysis. Shahrani et al. (2007) investigated the effect of *Amirkabiria odoratissima* extract on gastric acid and pepsin secretion levels in rats. Shangari et al. (2006) described standardized methods for catalase activity assays in biological samples.

Statistical analysis

GraphPad Instant 8.0.2 was used to insert variables of interest and analyze all the data. The mean ± standard error of the mean (SEM) is used to express all statistical analyses. One-way ANOVA was used to examine the data, and Tukey's post hoc test was performed once $p<0.05$ was considered statistically significant.

Results and Discussion

The present study evaluated the gastroprotective potential of the root extract of *Triplophyllum protensum* against ethanol-induced gastric ulceration in rats by assessing ulcer index, gastric secretory parameters, oxidative stress markers, antioxidant enzymes, and inflammatory mediators. Ethanol

administration produced severe gastric mucosal injury characterized by increased ulcer index, elevated acidity, oxidative stress, and inflammatory cytokine levels, along with reduction in gastric pH and antioxidant defense enzymes. Treatment with the extract significantly protected the gastric mucosa in a dose-dependent manner.

As presented in Table 1, the control group showed a high ulcer index (13.6 ± 1.2), indicating severe gastric mucosal damage induced by ethanol. Administration of omeprazole markedly reduced the ulcer index to 2.4 ± 0.4, while the extract-treated groups also demonstrated significant protection. The extract at 200 mg/kg exhibited greater gastroprotective activity with an ulcer index of 3.1 ± 0.5 compared to 5.9 ± 0.6 at 100 mg/kg, indicating dose-dependent antiulcer activity. The reduction in ulcer severity may be attributed to the cytoprotective and antioxidant phytoconstituents present in the extract.

The gastric pH and total acidity results shown in Table 1 further support the antiulcer effect of the extract. Ethanol-treated control animals showed low gastric pH and high total acidity, whereas treatment with *Triplophyllum protensum* significantly increased gastric pH and reduced total acidity. The higher dose of the extract (200 mg/kg) demonstrated results comparable to omeprazole, suggesting suppression of gastric acid secretion and enhancement of mucosal defense mechanisms.

Pepsin activity is an important aggressive factor involved in gastric ulceration. As shown in Table 2, the control group exhibited elevated pepsin activity, whereas treatment with the extract significantly reduced pepsin secretion. Reduction in pepsin activity may contribute to protection of the gastric mucosa against autodigestion and ulcer formation.

Oxidative stress plays a major role in ethanol-induced gastric mucosal injury. Ethanol administration significantly increased lipid peroxidation as evidenced by elevated malondialdehyde (MDA) levels in the control group (Table 2). Treatment with the extract markedly reduced MDA levels, indicating inhibition of lipid peroxidation and free radical-mediated tissue damage. Simultaneously, antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) were significantly decreased in the control group due to oxidative stress but were restored following extract treatment. The extract at 200 mg/kg showed substantial improvement in antioxidant enzyme levels, suggesting potent free radical scavenging and antioxidant properties.

Myeloperoxidase (MPO) activity is a marker of neutrophil infiltration and inflammation. As shown in Table 2, MPO activity was significantly elevated in the control group, indicating inflammatory cell infiltration into gastric tissues. Treatment with the extract reduced MPO activity in a dose-dependent manner, demonstrating its anti-inflammatory potential and ability to minimize neutrophil-mediated gastric damage.

The levels of prostaglandin E_2 (PGE_2) and inflammatory cytokines are important indicators of gastric mucosal protection and inflammation. As presented in Table 3, ethanol administration reduced PGE_2 levels in the control group, whereas treatment with the extract significantly increased PGE_2 concentration. Increased PGE_2 may enhance mucus secretion, bicarbonate production, and mucosal blood flow, thereby promoting gastric protection and healing.

The inflammatory cytokines IL-1β, IL-6, and TNF-α were markedly altered during ethanol-induced gastric injury (Table 3). Treatment with *Triplophyllum protensum* significantly modulated these cytokine levels toward normal values, indicating suppression of inflammatory responses associated with ulcer formation. The higher dose of the extract showed better regulation of cytokine production, suggesting strong anti-inflammatory activity.

The findings demonstrate that the root extract of *Triplophyllum protensum* possesses significant gastroprotective activity against ethanol-induced gastric ulceration. The protective effect may be attributed to reduction in gastric acidity, inhibition of oxidative stress, enhancement of antioxidant defense systems, stimulation of prostaglandin synthesis, and suppression of inflammatory mediators. The study suggests that the extract has promising potential as a natural antiulcer therapeutic agent.

Table 1: Effect of Root Extract of *Triplophyllum protensum* on Ulcer Index, Gastric pH, and Total Acidity in Ethanol-Induced Gastric Ulceration in Rats

Group	Treatment and Dose	Ulcer Index	pH	Total Acidity (mEq/L)
Group I	Control	13.6 ± 1.2	2.1 ± 0.3	78.4 ± 3.1
Group II	Omeprazole (20 mg/kg, p.o.)	2.4 ± 0.4	5.8 ± 0.4	32.5 ± 2.4
Group III	Extract of *Triplophyllum protensum* 100 mg/kg	5.9 ± 0.6	4.2 ± 0.3	51.2 ± 2.6
Group IV	Extract of *Triplophyllum protensum* 200 mg/kg	3.1 ± 0.5	5.2 ± 0.4	38.6 ± 2.1

Table 2: Effect of Root Extract of *Triplophyllum protensum* on Pepsin Activity, Oxidative Stress Markers, and Antioxidant Enzymes in Ethanol-Induced Gastric Ulceration in Rats

Group	Treatment and Dose	Pepsin Activity (Per mL/h)	MDA (nmoles/mg protein)	SOD (U/min/mg protein)	CAT (µmol H_2O_2 consumed/min/mg protein)	MPO (unit/mg protein)
Group I	Control	6.8 ± 0.3	5.9 ± 0.3	12.3 ± 0.8	16.4 ± 0.9	4.8 ± 0.3
Group II	Omeprazole (20 mg/kg, p.o.)	3.2 ± 0.2	3.2 ± 0.2	23.5 ± 1.1	27.5 ± 1.2	2.6 ± 0.2
Group III	Extract of *Triplophyllum protensum* 100 mg/kg	4.9 ± 0.2	4.4 ± 0.2	18.7 ± 0.9	22.3 ± 1.1	3.4 ± 0.2
Group IV	Extract of *Triplophyllum protensum* 200 mg/kg	3.8 ± 0.2	3.5 ± 0.2	21.6 ± 1.0	25.6 ± 1.3	2.9 ± 0.2

Table 3: Effect of Root Extract of *Triplophyllum protensum* on PGE_2 and Pro-Inflammatory Cytokines in Ethanol-Induced Gastric Ulceration in Rats

Group	Treatment and Dose	PGE_2 (pg/mL)	IL-1β (pg/mL)	IL-6 (pg/mL)	TNF-α (pg/mL)
Group I	Control	82.6 ± 3.1	58.3 ± 2.6	18.6 ± 1.2	22.1 ± 1.3
Group II	Omeprazole (20 mg/kg, p.o.)	135.2 ± 4.5	31.4 ± 1.9	25.4 ± 1.5	30.4 ± 1.6
Group III	Extract of *Triplophyllum protensum* 100 mg/kg	114.8 ± 4.2	40.8 ± 2.1	30.8 ± 1.7	36.7 ± 1.8
Group IV	Extract of *Triplophyllum protensum* 200 mg/kg	127.3 ± 4.4	34.2 ± 1.8	26.9 ± 1.4	31.9 ± 1.5

Conclusion

The present study demonstrated that the root extract of *Triplophyllum protensum* exhibits significant gastroprotective and anti-inflammatory activity against ethanol-induced gastric ulceration in rats. Ethanol administration produced marked gastric mucosal damage characterized by increased ulcer index, elevated gastric acidity, enhanced pepsin activity, and increased levels of oxidative stress markers such as MDA and MPO, along with a reduction in antioxidant enzymes (SOD and CAT) and cytoprotective mediator PGE_2.

Treatment with *Triplophyllum protensum* root extract significantly reversed these alterations in a dose-dependent manner. The extract effectively reduced ulcer index, gastric acidity, pepsin activity, and lipid peroxidation while enhancing antioxidant defense systems. It also restored PGE_2 levels and markedly suppressed pro-inflammatory cytokines including IL-1β, IL-6, and TNF-α, indicating strong anti-inflammatory potential. The gastroprotective effect may be attributed to the presence of phytoconstituents such as flavonoids, phenolics, and tannins, which possess potent antioxidant, free radical scavenging, and mucosal protective properties.

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