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Evaluation of Anti-Ulcer Activity of Ethanolic Extract of *Tamarindus Indica* Stem Bark in Experimental Animals

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ABSTRACT:

Tamarindus indica is a tall evergreen tree, native to tropical regions, and has versatile applications in medicine, industry, and cuisine. The bark, rich in phytochemicals such as alkaloids, flavonoids, tannins, and terpenoids, exhibits various pharmacological activities. The fresh bark of Tamarindus indica was collected, washed, dried, and powdered. The obtained powder was used to prepare ethanolic extract using the Soxhlet method. The anti-ulcer activity of the extract was investigated in experimental rats by using Indomethacin and ethanol-induced ulcer models. Two doses (250 mg/kg and 500 mg/kg p.o.) of extract were subjected to study against experimentally induced ulcers by Indomethacin (100 mg/kg p.o.) and ethanol (0.2 ml p.o.) in Wister rats. Omeprazole (5 mg/kg p.o.) and Ranitidine (20 mg/kg p.o.) were served as standard respectively in both models. Two doses of the extract were administered in the experimental animal for 10 days. On the final day, the rats were induced ulcer, sacrificed, and their stomachs isolated for ulcer evaluation and biochemical estimation. Ulcer index, pH, volume of gastric juice, and free and total acidity are the parameters evaluated. The long-term administration of both the 250 mg/kg and 500 mg/kg of extract produced a significant reduction of ulcer index, acidity, and a significant increase of pH in both models when compared with control and induced groups. The results suggest a high dose of ethanolic extract of Tamarindus indica bark (500 mg/kg) could better reverse and control the progression of the disease compared to a low dose (250mg/kg).

Keywords: Anti-ulcer, Ethanol, Indomethacin, *Tamarindus indica*.

Introduction:

The digestive and gastrointestinal diseases are reported as one of the major contributing factors for the high rate of human mortality across the world in both developed and developing countries [1]. Peptic ulcers are sores in the lining of the stomach and duodenum. These ulcers cause significant stomach pain and can often result in gastrointestinal (GI) bleeding [2]. Although advancements in medical treatments have led to a decrease in the overall incidence of PUD, data on the frequency of severe, life-threatening complications remain inconsistent [3]. While current treatments like proton pump inhibitors (PPIs), H_2 receptor antagonists, and antibiotics for *H. pylori* infections are effective, they come with significant side effects, including nutrient malabsorption, increased risk of infections, and long-term dependency [4]. Herbal therapy is still used as primary health care by anywhere between 75% and 80% of the world's population today, especially in less developed countries. The advantage of herbal remedies is that they work without many unpleasant side effects of modern medicines [5].

Tamarindus indica is a large tree belonging to the Caesalpiniaceae family and Leguminosae order. It is native to tropical regions, including Africa and the Americas, and is cultivated in various countries such as China, India, and Spain [6]. Various parts of the tamarind tree, including its leaves, fruit, and seeds, are heavily relied upon in traditional Indian and African medicine [7]. The diverse applications of *Tamarindus indica* underscore its significance in herbal medicine and highlight the need for further scientific investigation into its therapeutic potential. Continued research may unveil additional health benefits and solidify its role in modern medicine. This study aims to investigate the antiulcer potential of *Tamarindus indica*, contributing to the understanding of traditional medicinal plants in treating ulcers.

Methodology:

Collection and authentication of plant material:

The Stem bark of *Tamarindus indica* was collected from the local areas of Valachil, Mangalore, India. The taxonomist Dr. Siddaraju M. N, Dept of Botany, University College Mangalore has authenticated the plant. It is preserved in the department for future reference.

Drugs and chemicals:

Ranitidine and Omeprazole were obtained from Yarrow Chem Products and were used as a reference standard for antiulcer activity. Indomethacin was obtained from Yarrow Chem Products and was used to induce ulcers in rats.

Preparation of extract:

The fresh stem bark of *Tamarindus indica* was washed in running water. Cut into small pieces and shade-dried. The dried sample was ground to powder using a mechanical grinder. The obtained powder was used for the preparation of ethanolic extract. 70g of powder was packed into the Soxhlet extractor with a sufficient volume of ethanol for adequate cycles. The extract was concentrated by evaporation. The final extract was preserved in a refrigerator [8].

Preliminary qualitative phytochemical analysis:

The ethanolic extract of *Tamarindus indica* (EETI) was screened for the presence of various phytoconstituents, such as alkaloids, carbohydrates, flavonoids, glycosides, tannins, and saponins [9].

Experimental animals:

Albino Wistar rats weighing 150 – 250 g were acclimatized to the standard environmental conditions (25 + 2° C room temperature and relative humidity of 45 to 55%) and 12h light and 12h dark cycle. The animals were fed with a standard pellet diet and water ad libitum. The study was approved by the Institutional Animal Ethics Committee (Approval no: SCP/IAEC/F150/P223/2023). CCSEA guidelines were adhered to during the maintenance and experiment. All experiments were carried out between 8:00- 14:00 hours.

Experimental design for Anti-depressant activity:

Albino Wistar rats weighing 150 – 250 g were acclimatized to the standard environmental conditions (25 + 2° C room temperature and relative humidity of 45 to 55%) and 12h light and 12h dark cycle. The animals were fed with a standard pellet diet and water ad libitum. The study was approved by the Institutional Animal Ethics Committee (Approval no: SCP/IAEC/F150/P223/2023). CCSEA guidelines were adhered to during the maintenance and experiment. All experiments were carried out between 8:00- 14:00 hours.

Experimental design for Anti-ulcer activity:

Indomethacin-induced ulcer model [10]:

The animals were divided into five groups of six animals each as follows:

Groups	**Treatment**
Group I: Control	Vehicle
Group II: Toxic control	Indomethacin (100mg/kg p.o.) on the 10^{th} day
Group III: Test drug (low dose)	EETI (250mg/kg p.o.) for 10 days & indomethacin (100mg/kg p.o.) on 10^{th} day
Group IV: Test drug (high dose)	EETI (500mg/kg p.o.) for 10 days & indomethacin (100mg/kg p.o.) on 10^{th} day
Group V: Standard	Omeprazole (5mg/kg p.o.) for 10 days & indomethacin (100mg/kg p.o.) on 10^{th} day

For rats in the pre-treatment groups, omeprazole (5 mg/kg for group-V and Test drug for group-III and group-IV was given orally (gavage) daily for 10 consecutive days; on the final day, these rats were given indomethacin, a single gavage of 100 mg/kg for ulcer induction, 1 hr after the omeprazole and test dosing. Rats in Groups 1 and 2 (that had been dosed with the vehicle in place of either drug for the 10 days) were dosed with, respectively, vehicle or indomethacin in parallel (on the final day). All rats were fasted 24 hr prior to indomethacin oral treatment; over this period, the rats were kept in wide wire mesh-bottom cages to avoid coprophagia; in addition, water access was prevented for 2 hr prior to the indomethacin dosing. Four hours after the indomethacin/vehicle gavage, all rats were euthanized by an overdose of anaesthesia, and their stomachs were excised.

Ethanol-induced ulcer model [11]:

The animals were divided into five groups of six animals each as follows:

Groups	**Treatment**
Group I: Control	Vehicle
Group II: Toxic control	Ethanol (0.2 ml/animal p.o.) on the 10^{th} day
Group III: Test drug (low dose)	EETI (250mg/kg p.o.) for 10 days & ethanol (0.2 ml/animal p.o.) on 10^{th} day
Group IV: Test drug (high dose)	EETI (500mg/kg p.o.) for 10 days & ethanol (0.2 ml/animal p.o.) on 10^{th} day
Group V: Standard	Omeprazole (5mg/kg p.o.) for 10 days & ethanol (0.2 ml/animal p.o.) on 10^{th} day

This is a widely used model that seems to cause gastric ulcers, independently from acid secretion. Acute gastric lesions were induced by intragastric administration of absolute ethanol. Groups 1 (normal control) and 2 (gastric ulcer control) received 0.9% saline at a dose of 50 ml/kg, groups 3 and 4 were given test extract respectively, and the last group obtained ranitidine (20 mg/kg), an antagonist of H_2 receptors, was used as the reference drug. All drugs were administered once daily for 10 days. Drugs were given by gastric gavage, once daily and were suspended in saline. On the last day of treatment, 90 min after drug administration, absolute ethanol (0.2 ml/animal) were administrated orally to all mice except the normal control group. 4 h after ethanol administration, the animals were euthanized with an overdose of anaesthesia, and their stomachs were excised.

Measurement of ulcer index (UI) [12]:

The stomach obtained was opened along the greater curvature. After the removal of the stomach, the contents were poured into a centrifuge tube. Alongside of greater curvature, the stomach was exposed and pinned on a paraffin plate. A 10X magnifying glass checked the mucosa. The lesions appeared mostly in the rumen and antrum region of the stomach. The number of spots of ulcers and the severity were counted with the help of the following scoring method:

0= no ulcer

0.5= red coloration

1= spot ulcer

1.5= hemorrhages streek

2= deep ulcers

3= Perforation

An ulcer index (UI) is calculated: **(UI) = UN + US+ UP $\times 10^{-1}$**

UN= average number of ulcers per animal

US = average of severity score

UP = percentage of animals with ulcers

The ulcer index of treated animals was compared with controls.

Percentage inhibition:

$$\% \text{ inhibition} = \frac{\text{ulcer index of control} - \text{ulcer index of test}}{\text{ulcer index of control}} \times 100$$

Biochemical parameters used to investigate gastric juice:

Gastric juice was collected from experimental rats. The volume and the pH of the gastric juice were measured. For the determinations of the free and the total acidities in the gastric juice, 1 mL of gastric juice was pipetted into a 100-mL conical flask, and two to three drops of Topfer's reagent were added and titrated with 0.01N NaOH until all traces of red colour had disappeared and the solution had become yellowish orange. The volume of alkali added was noted. This volume corresponds to free acidity. Then 2 or 3 drops of phenolphthalein solution was added and titration was continued until a definite red tinge appeared. Again, the total volume of alkali added was noted now this volume corresponds to total acidity.[13].

Statistical analysis:

Results were expressed as mean ± standard error of mean. All the data were analysed using a one-way analysis of variance, followed by Dunnett's test ($*p < 0.05$)

Results

The percentage of yield of the ethanolic extraction was found to be 6.42%. A preliminary phytochemical analysis of the ethanolic extract of *Tamarindus indica* (EETI) identified the presence of several phytoconstituents, including alkaloids, flavonoids, glycosides, tannins, and saponins. To assess the anti-ulcer effects of this extract, doses of 250 mg/kg and 500 mg/kg were administered, and the resulting changes in ulcer index were evaluated across two models, with comparisons made to both a control group and the standard drugs. Additionally, parameters such as pH, volume of gastric juice, free acidity, and total acidity have also been evaluated.

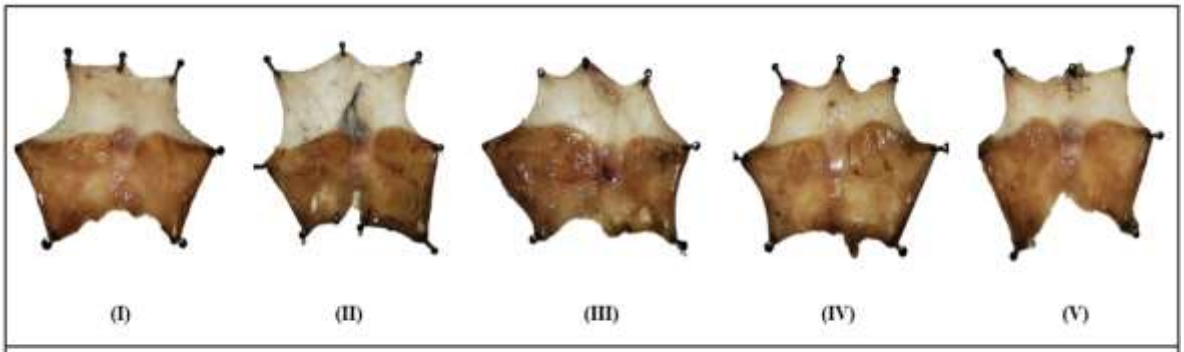

Figure 1: Ulcer protective effect of ethanolic extracts of *Tamarindus indica* bark (EETI) on Indomethacin-induced ulcer in experimental rats in (I) Normal animals treated with vehicle (saline) **(II)** Toxic control (Indomethacin 100mg/kg) **(III)** Indomethacin + Extract of *Tamarindus indica* bark (250mg/kg) **(IV)** Indomethacin + Extract of *Tamarindus indica* bark (500mg/kg) **(V)** Indomethacin + Standard Omeprazole (5mg/kg)

Table 1: Effect of ethanolic extracts of *Tamarindus indica* bark on ulcer index and percentage inhibition in indomethacin and ethanol-induced ulcer in rats

Groups	Indomethacin-induced ulcer model		Ethanol-induced ulcer model	
	Ulcer index	% inhibition	Ulcer index	% inhibition
Vehicle	0	100%	0	100%
Toxic control	22.583±2.067#	0%	22.333±2.174#	0%
Low dose	10.667±1.641*	52.76%	11.083±1.650*	50.37%
High dose	5.833±1.202***	74.17%	5.917±1.221***	73.50%
Standard	4.750±1.055***	78.96%	4.917±1.121***	77.98%
All the Results are expressed as mean ± SEM; (n = 6). Statistical significance was determined using one-way ANOVA followed by Dunnett's test. #p<0.001 when compared with the vehicle-treated control group. ***p<0.001, **p<0.01 and *p<0.05 is statistically significant compared to the control group.				

Table 2: Effect of ethanolic extracts of *Tamarindus indica* bark on Volume of gastric juice, pH, free acidity, and total acidity in indomethacin-induced ulcer in rats

Groups	Volume of gastric juice (ml)	pH	Free acidity	Total acidity
Vehicle	1.150±0.171	4.567±0.228	12.500±1.362	26.667±2.009
Toxic control	3.867±0.158#	1.750±0.287#	66.667±4.912#	115.833±6.509#
Low dose	2.95±0.165*	3.512±0.274**	36.667±3.014*	64.167±4.283*
High dose	2.433±0.254**	3.998±0.306***	27.5±2.531**	60.833±3.651*
Standard	2.283±0.270**	4.223±0.261***	24.435±2.358**	52.500±3.295**
All the Results are expressed as mean ± SEM; (n = 6). Statistical significance was determined using one-way ANOVA followed by Dunnett's test. #p<0.001 when compared with the vehicle-treated control group. ***p<0.001, **p<0.01 and *p<0.05 is statistically significant compared to the control group.				

Table 3: Effect of ethanolic extracts of *Tamarindus indica* bark on Volume of gastric juice, pH, free acidity, and total acidity in ethanol-induced ulcer in rats

Groups	Volume of gastric juice (ml)	pH	Free acidity	Total acidity
Vehicle	1.150±0.171	4.567±0.228	12.500±1.362	26.667±2.009
Toxic control	3.800±0.171#	1.805±0.231#	61.667±4.540#	104.167±5.589#
Low dose	3.067±0.180*	3.467±0.242**	39.167±3.76*	68.333±4.710*
High dose	2.567±0.217**	3.862±0.295**	28.333±2.714**	61.167±3.882*

Standard	2.450±0.246**	4.16±0.273***	25.833±2.729**	53.333±3.601**
All the Results are expressed as mean ± SEM; (n = 6). Statistical significance was determined using one-way ANOVA followed by Dunnett's test. #p<0.001 when compared with the vehicle-treated control group. ***p<0.001, **p<0.01 and *p<0.05 is statistically significant compared to the control group.				

Discussion

Gastric ulcers are the most common disease and are a very common global problem today. Peptic ulcers occur due to an imbalance between gastric acid secretion and gastric mucosal integrity. The aggressive and protective factors in the stomach are acid and pepsin secretion, mucosal barrier, blood flow, cellular regeneration, prostaglandins, and epidermal growth factors. Various non-specific methods are used to restore these imbalances including regular food intake, adequate rest, and avoidance of ulcerogenic agents (e.g. coffee, alcohol, tobacco). They aim to attenuate and possibly block gastric acid secretion or to enhance the mucosal defense mechanisms and the latter can be achieved through increasing mucus production, stabilizing the surface epithelial cells, or interfering with the prostaglandin synthesis [14].

The present study investigated the effect of ethanolic extract of *Tamarindus indica* bark against gastric ulcers induced by indomethacin and ethanol in experimental rats. The phytochemical screening revealed the presence of alkaloids, flavonoids, saponins, tannins, glycosides, triterpenoids, and phenolics.

Indomethacin induces gastric lesions in rats by inhibiting gastric cyclo-oxygenase resulting in less formation of endogenous prostaglandin, also inhibits mucosal blood flow as well as gastroduodenal bicarbonate secretion. The flavonoids present in the bark of *Tamarindus indica* are also known to exhibit antioxidant activity as well as scavenge superoxide radicals. These effects suggested that *Tamarindus indica* bark extract could also increase in COX-1 and COX-2 which leads to an in-prostaglandin synthesis. Several mechanisms have been proposed to explain the gastro-protective effects of flavonoids and alkaloids, these include increased mucosal prostaglandin content, decreased histamine secretion from mast cells by inhibition of decarboxylase, and inhibition of *Helicobacter pylori* growth. The extract showed protection against gastric lesions produced by indomethacin-induced ulcers. The extract of *Tamarindus indic*a showed ulcer protection at 52.76% and 74.17% in 250 mg/kg and 500 mg/kg doses respectively; whereas the standard drug Omeprazole exhibited 78.96% protection against indomethacin-induced gastric ulceration [15].

The ethanol-induced gastric ulcer model was selected due to its well-recognized association with inflammation, oxidative stress, and the disruption of the mucosal barrier through the production of pro-inflammatory cytokines and oxygen-derived free radicals, while ranitidine, a standard drug in this model, is used for its ability to reduce gastric acid secretion, enhance mucosal blood flow, increase bicarbonate and prostaglandin synthesis, and counteract the role of free radicals in ulcer pathogenesis. The extract showed protection against gastric lesions produced by ethanol. The extract of *Tamarindus indica* showed ulcer protection at 50.37% and 73.50% in 250 mg/kg and 500 mg/kg doses respectively; whereas the standard drug Omeprazole. Ranitidine also exhibited 77.98% protection against indomethacin-induced gastric ulceration [16].

The flavonoids are known to antagonize aggressive factors and augment defensive factors to protect the gastric mucosa from injury. Flavonoids decrease histamine secretion from mast cells, by inhibiting histidine decarboxylase and stimulating prostaglandin biosynthesis. Flavonoids also inhibit the H^+/K^+-ATPase and stimulate PGE_2. Additionally, Saponins and tannins are known to affect the integrity of mucus membranes. Some tannins suppress gastric secretion, having a local action of protection of gastric mucosa. It was postulated that the presence of flavonoids, alkaloids, saponins, and other phytoconstituents in the extract of *Tamarindus indica* bark might be responsible for the antiulcer activity [17].

The results of the present study revealed that the presence of various phytoconstituents in the ethanolic extract of *Tamarindus indica* bark might be responsible for gastric ulcer protection against indomethacin and ethanol-induced ulcers by reduction in gastric acid secretion and gastric cytoprotection.

Conclusion

In conclusion, the ethanolic extract of *Tamarindus indica* demonstrates significant antiulcer-like effects in the tested models. These findings suggest that *Tamarindus indica* may be a promising candidate for further research and development for treating peptic ulcers.

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