International Journal of Multidisciplinary Research And Studies

Available online at https://ijmras.com/

INTERNATIONAL JOURNAL OF MULTIDISCIPLINARY RESEARCH AND STUDIES
ISSN: 2640 7272
Volume:06; Issue:09 (2023)

Page no.- 17/17

Evaluation of anti-ulcer activity of leaves extract of *Thunbergia erecta* in ethanol induced ulcer rats

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ABSTRACT

Background

Peptic ulcers are erosions that affect the mucosa of the gastrointestinal tract and may extend to the muscle layer. Their formation is influenced by various factors, disrupting the mucosa's protective and offensive balance. Major contributors include Helicobacter pylori infection and non-steroidal anti-inflammatory drug (NSAID) use. Novel complementary treatments using natural products are being explored to prevent and treat ulcers. This study aims to examine the effects of *Thunbergia erecta* extract on rat gastric mucosa to determine if it has any anti-ulcer activity.

Methods

The methanolic extract of *Thunbergia erecta* was prepared using soxhlet apparatus techniques, and its anti-ulcer effect was tested on ethanol-induced gastric ulcer models at different doses.

Results

The results showed a significant reduction in the total volume of gastric juice and free acidity of gastric secretion ($P < 0.01$), similar to the standard drug ranitidine. Additionally, there was a noteworthy decrease in ulcer index ($P < 0.01$) compared to the control group.

Conclusion

The findings indicate that the methanolic extract of *Thunbergia erecta* possesses promising anti-ulcer activity in the alcohol-induced ulcer model, supporting its traditional use as claimed.

Keywords: *Thunbergia erecta*, Gastric ulcer, Free acidity, Ulcer Index, Ethanol induced ulcer model

1. INTRODUCTION

Gastric ulcer, also called stomach ulcer, is a disruption in the normal integrity of gastric mucosa that extends through the muscularis mucosa into the sub mucosa or deeper. [1]Gastric ulcer disease affects individuals worldwide and leads to severe complications such as hemorrhages, perforations, and malignancy, posing a significant global health issue with high morbidity and mortality. Developing countries have a higher prevalence (around 80%) compared to developed countries (approximately 40%). PUD causes about 15,000 deaths annually, with incidence rates for hemorrhage and perforation varying between 19.4-57 and 3.8-14 per 100,000 individuals, respectively. [2,3] It is characterized by painful sores or lesions in the stomach wall or the first portion of the small intestine, known as the duodenum. It is also termed as peptic ulcer or gastric ulcer, which is a breach in the gastric wall or, some instances, in the lower portions of the esophagus. [4] It is caused by a lack of equilibrium between the gastric aggressive factors (acid, pepsin, H. pylori and non-steroidal anti-inflammatory agents) and the mucosal defensive factors (mucus bicarbonate, blood flow and prostaglandins) [1].

The mucus barrier consists of mucus, bicarbonate anions, and phospholipids forming a surface layer on the gastric mucosa. The bicarbonate maintains the pH near 7 in the mucosa. The mucous layer protects from the proteolytic actions of pepsin. Mucin units to polymerize into large minimalities. [5] The surface epithelial cells form a "barrier" preventing back diffusion of acid and pepsin. [6] Continuous cell renewal from mucosal progenitor cells maintains the structural integrity of the mucosa. [7] PGI2 and NO maintain the viability of endothelial cells and stop platelet and leukocyte adherence to the micro-vascular endothelial cells, thus preventing compromise of the microcirculation. [8]

For centuries, herbals have been used traditionally for the treatment of a large range of ailments, including gastrointestinal disorders. Medicinal plants possessing active principles like flavonoids, saponins, tannins, and terpenoids are found to have anti-ulcer activity. [9] Leaf crude extract of *Jasminium gradiflorum*, [10] *Orbignya phalerata* and *Euterpe edulis*, [11] *Azadirachta indicia*, [12] *Papaya carica*, [13] and ribwort L. [14] possess anti-ulcer activity. Leaf crude extract of *Diodia sarmentosa* (Rubiaceae), *Cassia nigricans* (Celsapinaceae), *Ficus exasperate* (Moraceae) and Synclisias cabrida (Menispermaceae) are the most popularly used as anti-ulcer recipes. [7]

Thunbergia is a genus of flowering plant of *Acanthaceae* family, native to tropical regions of Africa, Madagascar, Australia and South Asia. [15] In most of the places T. erecta is known as bush clockvine and king's-mantle. [16] This shrub has small, ovate leaves with entire

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margins borne opposite on thin, brown stems. The purple flowers have a yellow throat and may appear singly or in small clusters. This plant produces rounded seed capsules that end in a beak.[17] Traditionally, Thunbergia species leaves, stems, and roots used as anti-inflammatory and antipyretics agents.[18] It has been also reported to possess antibacterial activities against gram positive as well as gram negative bacteria such as Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus, Bacillus cereus, Proteus mirabilis and *Streptococcus pyogenes.*[19] Thunbergia species also exhibit antinociceptive and antitumor[20], cytotoxic and antioxidant [21], carminative [22], antidiarrheal[23] activities.

Regarding the previous reports and traditional knowledge, it has been revealed that *Thunbergia erecta* contains several bioactive compounds which have anti-ulceractivity. The scientific basis of Thunbergia protrudes us to investigate the anti-ulcer activity of *T. erecta* in the management of peptic ulcer disorder.

2. Materials and Methods

2.1. Collection, authentication and Extraction of plant

The medicinal plant *Thunbergia erecta* (300gm) was collected locally from Bhopal, M.P. After cleaning, plant parts were dried under shade at room temperature for 3 days and Then in oven at 45°C till complete dryness. Dried plant leaves were stored in air tight glass containers in dry and cool place to avoid contamination and deterioration.

Authentication of selected traditional plant - The medicinal plant *Thunbergia erecta* was authenticated by a plant taxonomist in order to confirm its identity and purity.

The dried leaves were grounded to the coarse powder by blender equipment and before grinding of the sample, the grinder was completely cleaned to restrict contamination with any other materials grounded beforehand. Maceration of 300 g fresh powdered leaves in 3 liters ethanol was performed for seven days under occasional stirring. Seven days later, filtration of the mixture followed by concentrating using rotary evaporator (<40˚C) yielded 30.8 g (yield 10.2%) of semisolid ethanolic extract of *Thunbergia erecta* (EE-TE). Dried extract will weigh and percentage yield for extract was determine using the following formula:

$$Percentage\ of\ yield\ (\%) = \left(\frac{Weight\ of\ extract\ (gm)}{Weight\ of\ power\ are\ taken\ (gm)}\right) \times 100$$

2.2. Experimental Animals

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Wistar rat of 250-300 gm of either sex were selected at random from animal house of PBRI, Bhopal, India. Standard environment condition (relative humidity 55 - 65; temperature 23˚C ± 2˚C; 12 hrs of light-dark cycle) facilitated with standard diet (golden feed, New Delhi) and water were given regularly. Mice were given one week before the experiment to be adapted with the experiment condition. All animals were kept overnight without food prior the experiments. Mice were taken care in accordance with Ethical Principles and Guidelines.

2.3. Experimental design- Anti-ulcer activity in rats:

The gastric ulcers were induced in rats of either sex weighing between 250-300 g by administrating absolute ethanol (90%); (1 ml / 200 g). All the animals were fasted for 36 h before administration of ethanol. The animals (n=30) were randomized into following five groups, each consisting of six rats.

- Group 1- Normal control
- Group 2- Inducer group alcohol 20 mg/kg bw
- Group 3- Treated with *Thunbergia erecta* extract 200 mg/kg bw
- Group 4- Treated with *Thunbergia erecta* extract 400 mg/kg bw
- Group 5- Treated with standard drug (Ranitidine) 20 mg/kg bw

Anti-ulcer activity was assessed using determination of Ulcer Index, pH and Volume of gastric juice and free acidity.

2.4. Experimental Methods

2.4.1. Phytochemical Screening

Freshly ready crude extract of *T. erecta* was used for the qualitative analysis of presence of flavonoids, glycosides, tannins, carbohydrates and alkaloids following the standard procedure as reported previously. [24]

2.4.2. Total phenolic content (TPC)

The total phenolic content of *Thunbergia erecta* extract was determined using the Folin-Ciocalteu Assay. The extracts (0.2 mL from stock solution) were mixed with 2.5 mL of Folin-Ciocalteu Reagent and 2mL of 7.5% sodium carbonate. This mixture was diluted up to 7 mL with distilled water. Then the resulting solutions were allowed to stand at room temperature for 2 hrs before the absorbance was measured spectrophotometrically at 760 nm. Calibration curves were composed using standard solutions of Gallic Acid Equivalent (GAE) mg/gm.

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Concentration of 20, 40, 60, 80, and 100 µg/mL of Gallic aid was prepared. The Folin-ciocalteu reagent is sensitive to reducing compounds including polyphenols. They produce a blue colour upon reaction. This blue colour was measured spectrophotometrically. [25]

2.4.3. Total flavonoid content (TFC)

The flavonoid content was determined using Aluminium chloride method. 0.5 ml of *Thunbergia erecta* extract solution was mixed with 2 ml of distilled water. Then, 0.15 ml of sodium nitrite (5%) was added and mixed properly. After that, wait for 6 minutes before adding 0.15 ml Aluminium chloride (10 %) and allowed to stand for 6 minutes. Then, 2 ml of 4 % sodium hydroxide was added. The mixture was shaken and mixed thoroughly. Absorbance of mixture was estimated at 510 nm using UV spectrophotometer. Calibration curves were composed using standard solutions of Rutin Equivalent (GAE) mg/gm. Concentration of 20, 40, 60, 80, and 100 µg/mL of Rutin was prepared. Total flavonoid content was determined from the calibration curve and results were indicated as mg Rutin equivalent per gram dry extract weight. [26]

2.4.4. In-vitro Antioxidant Assay

The antioxidant activity of the plant extracts against DPPH was determined using the method reported previously. [27] Freshly 1 mg/ml methanol solution of *Thunbergia erecta* extracts/standard was prepared. Different volume of extracts/standard (20 – 100µl) was taken from stock solution in a set of test tubes and methanol was added to make the volume to 1 ml. To this, 2 ml of 0.1mM DPPH reagent was added and mixed thoroughly and absorbance was recorded at 517 nm after 30 minutes incubation in dark at room temperature. For control, take 3 ml of 0.1mM DPPH solution and incubated for 30 min at room temperature in dark condition. Absorbance of the control was taken against methanol (as blank) at 517 nm. Percentage antioxidant activity of sample/standard was calculated by using formula:

$$\% \text{ Inhibition} = [(\text{Ab of control} - \text{Ab of sample} / \text{Ab of control} \times 100]$$

2.4.5. Reducing power assay

3 mg of ascorbic acid was dissolved in 3 ml of distilled water/solvent. Dilutions of this solution with distilled water were prepared to give the concentrations of 20, 40, 60, 80 and 100 µg/ml. Stock solutions of extracts were prepared by dissolving 1mg of dried extracts in 1 ml of

methanol to give a concentration of 1mg/ml. Then sample concentrations of 20, 40, 60, 80 and 100 µg/ml were prepared. According to method, the aliquots of various concentrations of the standard and extracts (20 to 100µg/ml) in 1.0 ml of deionized water were mixed with 2.5 ml of (pH 6.6) phosphate buffer and 2.5 ml of (1%) potassium ferricyanide. The mixture was incubated at 50°C in water bath for 20 min after cooling. Aliquots of 2.5 ml of (10%) trichloroacetic acid were added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution 2.5 ml was mixed with 2.5 ml distilled water and a freshly prepared 0.5 ml of (0.1%) ferric chloride solution. The absorbance was measured at 700 nm in UV spectrometer (Systronic double beam-UV-2201). A blank was prepared without adding extract. Ascorbic acid at various concentrations (20 to 100µg/ml) was used as standard. [27]

2.4.6. Ulcer index

The following arbitrary scoring system was used to grade the incidence and severity of lesion. The stomachs were then cut along the greater curvature, rinsed with normal saline to remove gastric contents, and examined by using a 10x magnifier lens to assess the formation of ulcers. Numbers of ulcers were counted and then scored by using the Kulkarni method (0 = no ulcer, 0.5 = red coloration, 1 = spot ulcers, 2 = Haemorrhagic streaks, and 3 = Ulcers > 3 but < 5 and 5 = Ulcers > 5). [28]

The ulcer Index and percentage of ulcer inhibition were determined as follows:

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

Where, UN = Average number of ulcers per animal, US = Average of severity score, and UP = Percentage of animals with ulcers.

2.4.7. Volume of gastric juice:

The volume of gastric juice of each animal was measured after centrifugation with 1000 rpm for 10 minutes and analysed. The volume of the centrifuged sample was expressed as ml/ 100g body weight. [28]

2.4.8. pH of gastric juice

A pH meter is used for determining pH after diluting 1 ml of gastric juice aliquot with 1 ml of distilled water. [28]

2.4.9. Determination of free acidity

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1 ml of distilled water was used to dilute 1 ml of gastric juice aliquot and then transferred to a conical flask (50 ml) with the addition of 2 drops of phenolphthalein indicator. 0.01 N NaOH was used for titration until a permanent pink color was resulted; its consumed volume was determined. [29] The free acidity was calculated by the formula:

$$Free\ Acidity = \frac{Volume\ of\ NaOH \times Normality\ of\ NaOH \times 100}{0.1}$$

2.5. Statistical Analysis

All the experimental results were expressed as mean ± standard error of mean (SEM). The data of all the groups were analyzed using one-way ANOVA followed by Dunnett's t-test using the software Instat 3.0. In all the tests, the criterion for statistical significance was $P < 0.05$.

3. RESULTS

3.1. Percentage Yield

In phytochemical extraction the percentage yield is very crucial in order to determine the standard efficiency of extraction for a specific plant, various sections of the same plant or different solvents used. The yield of extracts received from the *Thunbergia erecta* is shown in Table 1.

Table 1: Percentage Yield of crude extracts of *Thunbergia erecta* extract

S.No	Solvent	Theoretical weight	Yield(gm)	% yield
1	Pet ether	300	1. 56	0.52%
2	Methanol	237.12	5.58	2.35%

3.2. Preliminary Phytochemical study

The extracts underwent qualitative phytochemical tests to determine their phytoconstituents. On phytochemical screening of methanolic extract of *Thunbergia erecta* extract showed presence of alkaloid, flavonoid, saponins and glycoside. (Table 2)

Table 2: Phytochemical testing of extract

S. No.	Experiment	Presence or absence of phytochemical test	
		Pet. Ether extract	Methanolic extract

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1.	**Alkaloids**		
1.1	Dragendroff's test	Absent	Present
1.2	Mayer's reagent test	Absent	Present
1.3	Wagner's reagent test	Absent	Present
1.3	Hager's reagent test	Absent	Present
2.	Glycoside		
2.1	Borntrager test	Absent	Present
2.2	Legal's test	Absent	Present
2.3	Killer-Killiani test	Absent	Present
3.	**Carbohydrates**		
3.1	Molish's test	Absent	Present
3.2	Fehling's test	Absent	Present
3.3	Benedict's test	Absent	Present
3.4	Barfoed's test	Absent	Present
4.	**Proteins and Amino Acids**		
4.1	Biuret test	Absent	Absent
5.	**Flavonoids**		
5.1	Alkaline reagent test	Absent	Present
5.2	Lead Acetate test	Absent	Present
6.	Tannin and Phenolic Compounds		
6.1	Ferric Chloride test	Absent	Present
7.	**Saponin**		
7.1	Foam test	Absent	Present

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8.	**Test for Triterpenoids and Steroids**		
8.1	Salkowski's test	Absent	Absent
8.2	Libbermann-Burchard's test	Absent	Absent

3.3. Quantitative Analysis of total phenolic (TPC) and total flavonoid content (TFC)

Preliminary phytochemical testing of crude extracts confirmed the presence of phenolics and flavonoids in plant material. To estimate their amount total phenolic (TPC) and total flavonoid content (TFC) assays were performed. Figure 1 show that the methanolic extract of *Thunbergia erecta* contains a considerable amount of phenolic and flavonoid content.

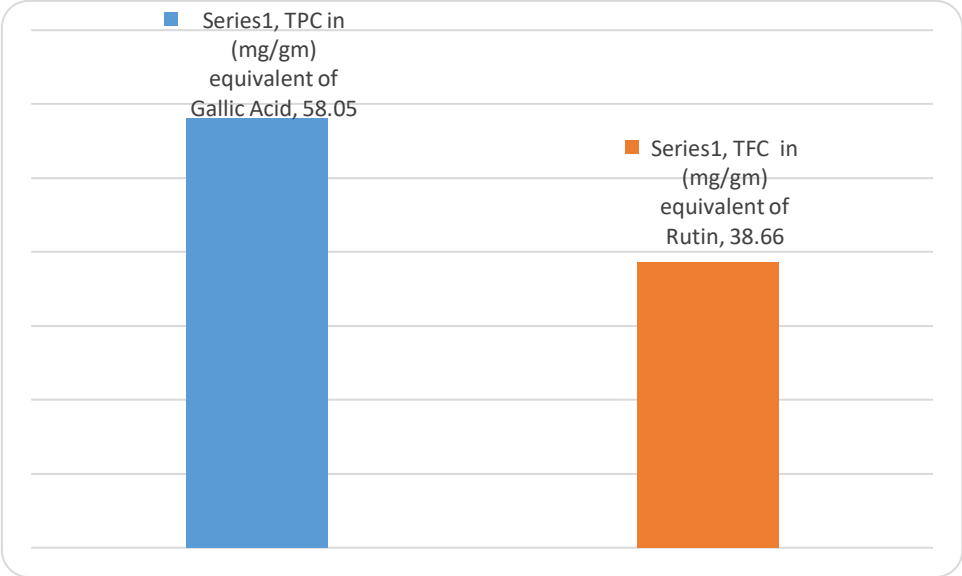

Figure 1: Assay of Total Phenolic Content and total flavonoid content

3.4. In vitro Antioxidant Assays

In the present investigation, the in vitro anti-oxidant activity of methanolic extract of *Thunbergia erecta* was evaluated by DPPH radical scavenging activity. The results are summarized in Table 3. Methanolic extract of *Thunbergia erecta* exhibited a comparable antioxidant activity with that of standard ascorbic acid at varying concentrations tested (20, 40, 60, 80, 100 µg/mL). There was a dose–dependent increase in the percentage antioxidant activity for all concentrations tested. Ascorbic acid was used as the standard drug for the determination of the antioxidant activity by DPPH method. The concentration of ascorbic acid varied from

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20 to 100µg/mL. The IC50 value of ascorbic acid was 22.76µg/mL. IC50 value was observed 57.08µg/mL for the methanolic extract of *Thunbergia erecta*. From Figure 2 and Table 3, it is observed that both extracts show significant DPPH radical scavenging property.

Table 3: Percentage inhibition of standard (ascorbic acid) and test extract

	Standard (ascorbic acid)		Test extract	
Concentration (µg/ml)	**Absorbance**	**% Inhibition**	**Absorbance**	**% Inhibition**
20	0.468	50.9434	0.541	40.22099
40	0.422	55.7652	0.496	45.19337
60	0.329	65.51363	0.448	50.49724
80	0.276	71.06918	0.415	54.14365
100	0.136	85.74423	0.325	64.0884
Control	0.954		0.905	
IC50	22.76		57.08	

3.5. Reducing power

Reducing power assay method is based on the principle that substances, which have reduction potential, react with potassium ferricyanide (Fe3+) to form potassium ferrocyanide (Fe2+), which then reacts with ferric chloride to form ferric–ferrous complex that has an absorption maximum at 700 nm. Table 4 and Figure 2 shows how the reducing power of the test extracts increases with the increase in amount of sample. The reducing power shows comparable linear relationship in both standard (R^2 = 0.9977) and methanolic extract of *Thunbergia erecta* (R^2 = 0.9957).

Table 4: Reducing power scavenging activity of standard (Ascorbic Acid) and methanolic extract of *Thunbergia erecta*

Concentration (µg/ml)	Absorbance of Ascorbic Acid	Absorbance of *Thunbergia erecta*
20	0.102	0.089
40	0.194	0.147
60	0.276	0.226
80	0.384	0.284
100	0.459	0.338

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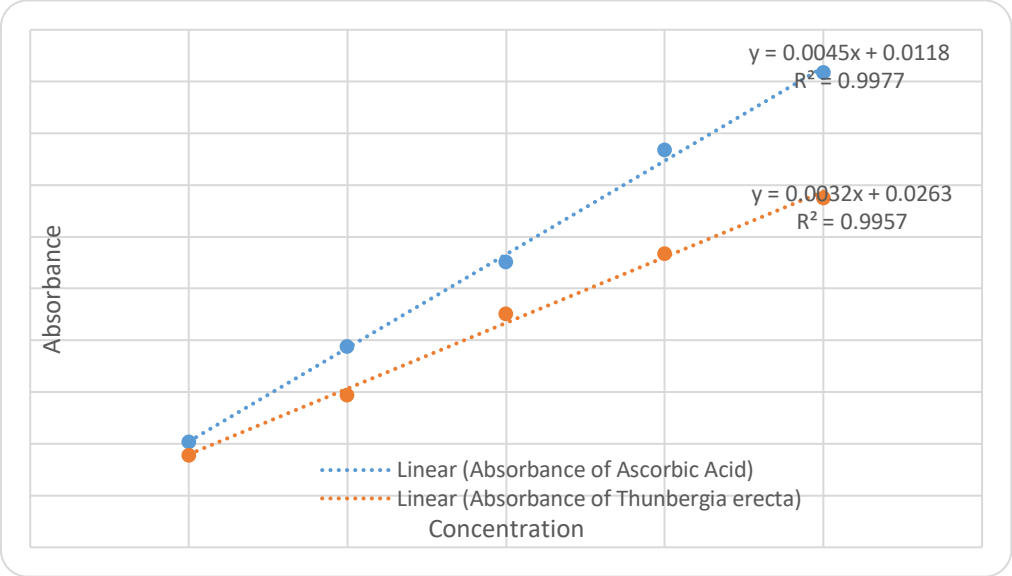

Figure 2: Effect of Standard (Ascorbic acid) and methanolic extract of *Thunbergia erecta* on reducing power

3.6. Determination of Ulcer Index, volume of gastric juice and pH

Figure 3 shows the ulcer index,volume of gastric juice and pH of juice in the alcohol-induced ulcer model. The dose-dependent effect was observed with the of *Thunbergia erecta*. The extract of the plant shows significant protection of ulcer in both doses of 200 and 400 mg/kg ($P < 0.01$) when compared with the control animals. The standard drug, Ranitidine, also shows significant effect in the protection of ulcer in a dose of 50 mg/kg when compared with the control groups ($P < 0.01$). The free acidity and pH were also significantly decreased and increased respectively in a dose of 200 mg/kg of the extract as shown in Figure 3 when compared with the control animals.

Overall the result indicates that the methanolic extract of *Thunbergia erecta* has a potent dose dependent ulcer protective effect against used animal models. A significant decrease in the ulcer index ($P < 0.01$) was observed with the treatment of methanolic extract of *Thunbergia erecta* at a dose of 200 mg/kg.

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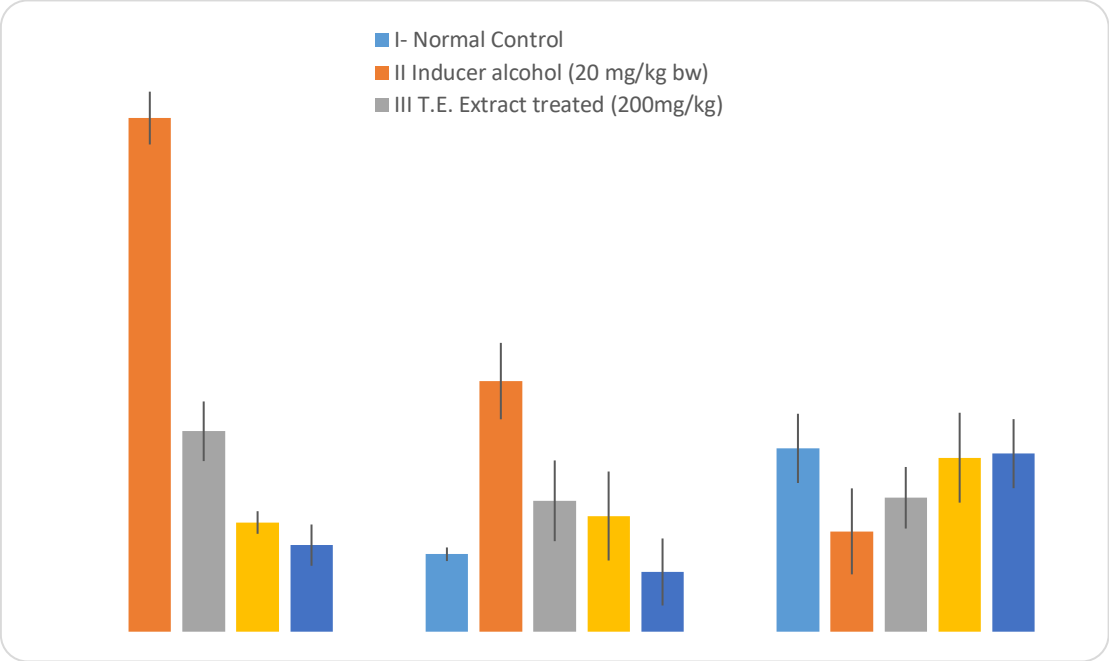

Figure 3: Observation of Ulcer Index, volume of gastric juice and pH of juice

Normal stomach of rat **Ulcer is induced in rat stomach**

Figure 4: Photographs of rat stomach (normal and induced Ulcer)

4. DISCUSSION

Peptic ulceration, a prevalent condition affecting around 10% of the global population, is considered a modern-day epidemic. [30] Previous research has indicated that the development of peptic ulcers arises from an imbalance between acid and pepsin, coupled with the vulnerability of the mucosal barrier. Excessive production of exogenous and endogenous active oxygen and free radicals leads to damage in the stomach's mucosal layer. Chronic consumption of alcohol, prolonged use of anti-inflammatory drugs, stress, and Helicobacter pylori infection are among the main contributing factors to gastric ulcers. [31]

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The aim of this study is to evaluate the anti-ulcer effect of the methanolic extract of *Thunbergia erecta* in experimental ulcer in alcohol model. The plant fraction showed dose-dependent, ulcer-protective effects against all three animal models. In the alcohol-induced model, ulcers are caused due to perturbations of superficial epithelial cells, notably the mucosal mast cells, leading to the release of vasoactive mediators including histamine[32]and reactive oxygen species (ROS),[33]resulting in the damage of rat gastric mucosa. Mucosal blood flow has been considered to be an important factor in the damage caused by alcohol and is modulated by prostaglandin.[34]The effectiveness of the plant fraction in protecting against mucosal damage caused by alcohol is an indication of its effect on prostaglandins synthesis and on the free radical scavenging activity.[35]It has been also accepted that alcohol-induced ulcers are not only inhibited by anti-secretory agents such as ranitidine, but also by agents that enhance mucosal defensive factors.[36] Thus, it could be assumed that the existence of the cytoprotective effect of compound(s) is present in the methanolic extract of *Thunbergia erecta.*

The *Thunbergia erecta* extract demonstrated significant DPPH radical scavenging activity, with a percent inhibition of 64.08% and an IC50 value of 57.08µg/ml. Ascorbic acid, used as a reference compound, exhibited a higher percent inhibition of 85.74% and an IC50 value of 22.76µg/ml. The reducing capacity of the *Thunbergia erecta* extract, indicative of its antioxidant potential, was strong. Compounds with strong reducing power act as electron donors, reducing oxidized intermediates in lipid peroxidation processes and functioning as primary and secondary antioxidants. The extract's potent reducing capacity was comparable to that of dietary antioxidant ascorbic acid. [37]

The extract of *Thunbergia erecta* was evaluated by using alcohol induced peptic ulcer model. Ulcer produced in this model was seen as red sores. The stomachs of rats in the alcohol induced peptic ulcer showed higher inductions of gastric ulcers due to increased levels of gastric juice in the rat's stomachs. There was a significant decrease in the measured gastric ulcer index in the stomach of *Thunbergia erecta* (400 mg/kg bw) treated animals when compared with the *Thunbergia erecta* (200 mg/kg bw) treated. The volume of gastric juice was observed as 2.623 ml of *Thunbergia erecta* (400 mg/kg bw) in decreased level as compared to *Thunbergia erecta* (200 mg/kg bw) treated group showed gastric volume of 2.968. The pH of gastric juice was observed as 3.946 of *Thunbergia erecta* (400 mg/kg bw) treated group and it showed the reduction in acidic pH as compared to *Thunbergia erecta* (200 mg/kg bw) showed 3.037 pH. The free acidity was observed as mE/L of *Thunbergia erecta* (400 mg/kg bw) treated

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group 16.886mE/Land it showed the reduction in acidity as compared to *Thunbergia erecta* (200 mg/kg bw) showed18.648mE/L.

Previous research has indicated that *Thunbergia erecta*'s contains polyphenolic compounds, particularly pocyanidins, epicatechin, and polymeric tannins. [37] These compounds are known for their cytoprotective properties and have been associated with antiulcer effects in other plants. The anti-ulcer potential of these tannins and polyphenolic compounds is believed to arise from their antioxidant and free radical scavenging activities. Tannins may also prevent ulcer development by causing protein precipitation and vasoconstriction. [37] Their astringent action can create a protective layer over the ulcer site, preventing gut secretions and shielding the underlying mucosa from toxins and irritants. [28] Based on our studies, the efficacy of plant extracts in animal ulcer models may be attributed to their cytoprotective effects, which could be linked to free radical scavenging activity or increased mucus secretion.

5. CONCLUSION

Our study findings revealed that the methanolic extract of *Thunbergia erecta* exhibits a significant and dose-dependent anti-ulcer effect in alcohol-induced ulcer models ($P < 0.01$). Therefore, the methanolic extracts derived from *Thunbergia erecta* have the potential to serve as a novel source of anti-ulcer medication for animals.

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