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ORIGINAL ARTICLE **OPEN ACCESS**

Tarenna asiatica (L.) Kuntze ex K.Schum. Leaf Extract as a Promising Antiulcer Agent: An Experimental Study

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ABSTRACT
The demand for natural herbal medicine has overgrown in the 21st century, with many people seeking alternatives to synthetic drugs. Tarenna asiatica (L.) Kuntze ex K.Schum. (TA from now onwards) is a plant from the Rubiaceae family that has been widely used in traditional medicine due to its medicinal benefits. In this study, the anti-ulcer activity was investigated by employing three different models of anti-ulcer evaluation (drug-induced, ethanol-induced, and stress-induced) to assess the potential of TA leaves as an herbal remedy for synthetic drugs. Phytochemical analysis of the TA leaves revealed the presence of various secondary metabolites, such as flavonoids, alkaloids, glycosides, terpenoids, and tannins. The highest percentage inhibition of ulceration by the ethanolic extract of TA leaves was found to be 62.59%, 53.38%, and 70.45% at a dose of 200 mg/kg body weight against drug-induced, ethanol-induced, and stress-induced models, respectively. Our study suggests that TA has the potential to be used as an herbal remedy for ulcers, particularly against stress-induced ulcers.
Keywords: *Tarenna asiatica (L.) Kuntze ex K.Schum., Anti-ulcer, stress induced ulcer model, NSAID induced ulcer model,, alcohol induced ulcer model*

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INTRODUCTION

Scientific interest in historically used medicinal plants, such as TA has increased due to the growing need for alternative, nature-based treatments. The southern parts of India, Sri Lanka, and Malaysia are the primary native habitats for this Rubiaceae plant [1]. Different anatomical parts of TA, specifically the leaves, bark, and roots, have long been used in Ayurvedic medicine. This traditional holistic healing system originated in the Indian subcontinent to ameliorate a wide range of health disorders. It's interesting to note that TA's complex mixture of secondary metabolites, including flavonoids, phenolic compounds, saponins, and alkaloids, provides the foundation for the plant's therapeutic potential. [2]

Recent developments in phytochemical and pharmaceutical research have confirmed the traditional applications of this plant species. Numerous preparations have been found to offer a variety of bioactive activities, including anti-nociceptive [3], anti-microbial [4], anti-oxidative, and tissue regeneration facilitation [5] effects. The fruit's phytochemical components have powerful anti-inflammatory [6] and anti-neoplastic properties [7]. As a result, this plant species' diverse medicinal benefits increase its ethnobotanical significance and make it a potential candidate for in-depth pharmacological research and possible drug development.

There has been a change in the greater scientific and medical discourse toward accepting Phyto-therapeutic substances as potential pioneers of pharmaceutical innovation [8]. The limitations, side effects, and ethical issues frequently connected to chemically developed medications appear to be the driving force behind this transition [9].

Ulcers, particularly peptic ulcers affecting the stomach and duodenum, are a significant global health concern [10]. These lesions were once attributed to stress, diet, and medications like NSAIDs, and also caused by *Helicobacter pylori* bacteria [11]. With a 10% global prevalence and approximately 15,000 annual deaths, they substantially burden healthcare systems [12].

Despite pharmaceutical advancements, a significant portion of the population, especially in developing countries, relies on herbal medicine for treatment [13]. Anti-ulcer efficacy of certain medicinal plants and

their chemical constituents, such as flavonoids, saponins, and glycosides, are reported to be potential as alternative treatment options [14,15].

MATERIAL AND METHODS

Plant collection and Authentication

Tarenna asiatica (L.) Kuntze ex K.Schum. leaves were collected from the local regions of Hyderabad in September.

Extraction and sample preparation

The collected plant leaves were shade-dried and pulverized to a coarse powder before being extracted. Then, 1 kg of the coarse powder of the leaves was transferred to a Soxhlet apparatus and extracted with absolute ethanol. Later, the solvent ethanol was evaporated under a vacuum using Heidolph rotary evaporator to get the crude extract of TA leaves. Thus, the available crude extract was lyophilized to a solid form, and this solid extract of TA leaves was employed for the phytochemical analysis and biological evaluation.

Phytochemical Analysis

Powdered extract of TA leaves was subjected to chemical identification tests to analyze secondary metabolites such as alkaloids, flavonoids, glycosides, steroids, tannins etc. Standard protocols were employed to identify the secondary metabolites in TA leaves [16].

Acute Toxicity Studies

To find out the toxicity and establish the dose for the anti-ulcer activity, acute toxicity experiments were conducted using Swiss Albino Mice. The mice were procured from Sainath agencies, Musheerabad, weighing about 25-35g. The experiment used polypropylene cages to store the animals in animal facilities. Before initiating the experiments, animals underwent a one-week acclimatization period in a laboratory environment under controlled conditions. They were provided unrestricted access to a standard rodent diet consisting of pellet feed sourced from Golden Mohur Lipton India Ltd. and water. The experimental setting maintained a constant temperature of approximately 25°C, with a variance of ±2°C, and a relative humidity level of around 60%, with a margin of ±10%. A 12-hour light/dark cycle was consistently upheld. Ethical approval for the study was secured from the Institutional Animal Ethics Committee (IAEC), under the approval code 1292/ac09/CPCSEA/2020/3. Experiments assessing acute toxicity adhered to OECD 423 protocols [17, 18].

Anti-ulcer activity

The anti-ulcer activity was evaluated employing three different models. The doses for anti-ulcer activity were calculated from the acute toxicity studies and remained constant in three anti-ulcer models. Female Wistar rats were selected for the anti-ulcer activity evaluation. The animals were randomly selected for all models and divided into 5 groups, each containing 6 rats [19].

Group 1 was the normal control that received only normal saline.

Group 2 was ulcer control that received an ulcer-causing agent.

Group 3 and group 4 were extract groups that received extract at a dose of 100mg/kg and 200mg/kg body weight, respectively.

Group 5 was a reference group that received standard drug treatment at 20mg/kg body weight.

In each of the three experimental setups, rats received oral doses of TA extract at concentrations of 100 and 200mg/kg body weight, administered two hours before initiating ulcer formation as per the established protocols for each model. After six hours, the rats were humanely euthanized through an overdose of inhaled diethyl ether. Their stomachs were subsequently dissected along the major curvature, cleaned with saline to eliminate residual gastric material or blood, and inspected under a dissecting microscope with a 20×6.3 magnification. The aggregate length of all observable lesions in each stomach was quantified and designated as the Ulcer Index (UI). The percentage of ulcer inhibition was then determined using a specific formula:

$$[(UI_{Control} - UI_{Treated})/UI_{Control}] \times 100.$$

Indomethacin-induced anti-ulcer activity model

Ulcers in the stomach were artificially initiated in animals through the intraperitoneal administration of Indomethacin at 30 mg/kg following a 24-hour fasting period [20].

Ethanol-induced anti-ulcer activity model

A 24-hour fasting period was followed by the oral delivery of 96% ethanol to induce gastric ulcers in the experimental animals [21].

Stress-induced anti-ulcer activity model

This model utilized an adapted version of the procedure developed by Takagi and Okabe (1968). Rats weighing 160-200 grams were isolated in individual compartments of a stress cage measuring 4.5 cm x 4.5

cm x 18 cm. Subsequently, the rats were partially submerged in a water bath, maintained at 19-21°C, up to their xyphoid levels to induce stress ulcers. Test substances were orally administered to the rats two hours before their immobilization. The rats were euthanized via ether overdose six hours post-immersion [22].

RESULTS AND DISCUSSION

Extraction yields and sample preparation

Ethanol extraction of the leaves of TA delivered a yield of 4.7% from 1000 grams of the dried leaves.

Phytochemical analysis

The current study employed a simple chemical identification test for phytochemical analysis. The results of the Phytochemical analysis were enumerated in Table 1. The chemical tests revealed that TA leaves contain all major secondary metabolites, such as alkaloids, flavonoids, glycosides, terpenoids, tannins, and steroids.

Table 1. Phytochemical analysis results

S. No	Secondary metabolite	Ethanol extract
1	Alkaloids	++
2	Flavonoids	+++
3	Glycosides	+
4	Terpenoids	++
5	Steroids	++
6	Saponins	+

'+' Present in Trace Amount

'++' and '+++' Present in higher amounts

Acute toxicity study

The outcomes of the acute toxicity assessments for the ethanolic extract of TA are presented in Table 2. Following the administration of acute dosages, no discernable clinical indicators of toxicity or fatal outcomes were observed immediately or after the treatment, even when administered at elevated concentrations of 1000 mg/kg body weight. Consumption patterns in the treatment cohorts closely resembled those observed in the control group. These results imply a considerable safety margin for TA.

Table 2. Effect of TA ethanolic extract in Swiss albino mice

Dose in mg/kg	Body weight in grams			Survived out of six animals
	Day 0	Day 7	Day 14	
100	27	31	32	6
250	26	28	30	6
500	29	29	31	6
1000	25	30	32	6
1500	26	29	31	5
2000	28	31	33	3

Anti-ulcer activity

The anti-ulcer activity doses were established based on the acute toxicity experiment results. According to the acute toxicity studies, the ethanolic extract was considered safe up to 1000mg/kg body weight. Thus, following the standard dosage protocols per the OECD guidelines, 100mg/kg and 200mg/kg body weight were selected as the testing doses for the anti-ulcer activity in all models. For all the tested models Esomeprazole at a dose of 20mg/kg body weight was selected as standard. The Ulcer index and percentage inhibition of the ulceration of the tested extract were compared against the standard drug effect.

Drug-Induced anti-ulcer activity

Table 3 presents the results of an experimental study that investigated the anti-ulcer activity of TA leaves ethanol extract in a drug-induced ulcer model. The study used a total of 25 male Wistar rats, which were randomly divided into five groups. The first group was the normal control, while the second group was the ulcerated control, which was induced with Indomethacin. The remaining three groups received doses of TA extract or a reference standard drug, Esomeprazole, combined with Indomethacin.

The average ulcer index (UI) measures the severity of ulceration in the stomachs of the rats. The normal control group had a UI of 0, indicating no ulceration in the stomachs of these rats. The ulcerated control group, induced with Indomethacin, had an average UI of 13.59, indicating significant ulceration in the stomachs of these rats.

The three treatment groups received different doses of *TA* extract or Esomeprazole and showed varying degrees of anti-ulcer activity. The group treated with 100mg/kgb.w. of TAE had an average UI of 8.96, representing a 34.07% inhibition of ulcers compared to the ulcerated control group. The group treated with 200mg/kgb.w. of TAE had the highest percentage of ulcer inhibition at 62.59% and an average UI of 5.08. The group treated with Esomeprazole had an average UI of 2.55 and the highest percentage of ulcer inhibition at 81.18%.

The data in Table 3 suggest that TA leaves ethanol extract has dose-dependent anti-ulcer activity in the drug-induced ulcer model. The significant reduction in the UI values and the high percentage of ulcer inhibition in the groups treated with TA extract and Esomeprazole suggest their potential therapeutic efficacy in treating ulcers.

Table 3: Results of Anti-ulcer activity of TA leaves ethanol extract in drug-induced Ulcer model

S.No	Group	Average (UI)	Standard Error Mean (SEM)	%Ulcer Inhibition
1	Group 1 (Normal Control)	0	0	--
2	Group 2 (Ulcerated Control) (Indomethacin 30mg/kgb.w.)	13.59	0.91	--
3	Group 3 (Indomethacin 30mg/kgb.w.) + TAE 100mg/kgb.w.	8.96	0.41	34.07
4	Group 4 (Indomethacin 30mg/kgb.w.) + TAE 200mg/kgb.w.	5.08	0.27	62.59
5	Group 5 (Ref.Std) (Indomethacin 30mg/kgb.w.) + Esomeprazole 20mg/kgb.w.	2.55	0.14	81.18

Ethanol-Induced Anti-ulcer activity

The table presents the results of the anti-ulcer activity of TA leaves ethanol extract in an ethanol-induced ulcer model. The study evaluated the effectiveness of different doses in preventing ulceration caused by ethanol.

The average ulcer index in the ulcerated control group (Group 2) was 12.53, indicating severe ulceration caused by ethanol administration. However, treatment with extract at 100mg/kg b.w. (Group 3) showed a significant reduction in ulcer index to 8.5, with 32.18% ulcer inhibition. The higher dose of 200mg/kgb.w. (Group 4) further reduced the ulcer index to 5.84, with 53.38% inhibition.

Interestingly, the reference standard drug, Esomeprazole, at 20mg/kg b.w. (Group 5), exhibited the most potent anti-ulcer activity with an average ulcer index of 2.46, representing 80.34% ulcer inhibition. This finding validates the efficacy of Esomeprazole in preventing ethanol-induced ulceration and confirms the reliability of the ulcer model used in this study.

The data indicate that TA leaves ethanol extract has significant anti-ulcer activity in the ethanol-induced ulcer model. The higher dose of 200mg/kg b.w. was more effective in reducing ulcer index and inhibition than the lower dose of 100mg/kg b.w.

Table 4: Results of Anti-ulcer activity of TA leaves ethanol extract in ethanol induced Ulcer model

S.No	Group	Average (UI)	Standard error mean (SEM)	%Ulcer Inhibition or (%Biological action)
1	Group 1 (Normal Control)	0	0	--
2	Group 2 (Ulcerated Control) (96% Ethanol)	12.53	0.51	--
3	Group 3 (96% Ethanol) + TAE 100mg/kgb.w.	8.5	0.43	32.18
4	Group 4 (96% Ethanol) + TAE 200mg/kgb.w.	5.84	0.26	53.38
5	Group 5 (96% Ethanol) + Esomeprazole 20mg/kgb.w.	2.46	0.17	80.34

Stress-Induced Anti-ulcer activity

In this study, the anti-ulcer activity of TA leaves ethanol extract was evaluated in a stress-induced ulcer model in rats. The results of the study are presented in Table 5.

The average ulcer index (UI) in the normal control group was 0, indicating the absence of ulcers. The ulcerated control group (Group 2) had an average UI of 12.14, indicating a significant ulcerogenic effect of stress. In Group 3, which received TAE 100mg/kg b.w., the average UI was 8.39, which represents a 30.87% inhibition of ulceration compared to the ulcerated control group. Group 4 received TAE 200mg/kg b.w. and showed a significantly lower average UI of 3.58, representing a 70.45% inhibition of ulceration compared to the ulcerated control group. Group 5, which received Esomeprazole at a dose of 20mg/kg b.w., showed the highest level of inhibition, with an average UI of 2.37, representing an 80.46% inhibition of ulceration compared to the ulcerated control group.

Based on the results of the study, it can be observed that the ethanol extract of TA leaves has significant anti-ulcer activity in drug-induced and ethanol-induced ulcer models in rats. In the drug-induced ulcer model, the extract showed a dose-dependent increase in ulcer inhibition, with the highest inhibition observed at a dose of 200mg/kg body weight. Moreover, in the ethanol-induced ulcer model, the extract showed a significant reduction in ulcer index at both doses tested, with the highest inhibition observed at a dose of 200mg/kg body weight.

Comparatively, the extract was less effective in reducing the ulcer index in the stress-induced ulcer model. However, it still demonstrated a significant reduction in ulcer index at the higher dose (200mg/kg body weight) compared to the ulcerated control group. The anti-ulcer activity of the extract was found to be comparable to that of Esomeprazole, a commonly used anti-ulcer drug.

Table 5: Results of Anti-ulcer activity of TA leaves ethanol extract in stress-induced Ulcer model

S. No	Group	AVERAGE (UI)	Standard error mean (SEM)	% Ulcer Inhibition or (% Biological action)
1	Group 1 (Normal Control)	0	0	--
2	Group 2 (Ulcerated Control) (Stress)	12.14	0.62	--
3	Group 3 (Stress) + TAE 100mg/kgb.w.	8.39	0.49	30.87
4	Group 4 (Stress) + TAE 200mg/kgb.w.	3.58	0.21	70.45
5	Group 5 (Stress) + Esomeprazole 20mg/kgb.w.	2.37	0.17	80.46

After evaluating the percentage inhibitions in the tested models of anti-ulcer activity, it is clear that the ethanolic extract of TA leaves possesses promising anti-ulcer potential, ranging from moderate to good. The most notable anti-ulcer activity was observed against stress-induced ulceration, followed by ethanol and drug-induced ulcerations. These findings suggest that TA extract could be a potential candidate for developing new anti-ulcer agents.

To better understand the anti-ulcer potential of TA, a phytochemical analysis of the ethanolic extract was conducted. The analysis revealed that the extract contains high levels of flavonoids, which are known for their cytoprotective properties through antioxidant mechanisms. Hence, it can be hypothesized that the anti-ulcer activity of TA may be attributed to the flavonoids present in the extract.

CONCLUSION

The TA plant leaves were collected and authenticated, then subjected to shade drying followed by powdering. The extraction process involved Soxhlet extraction, which yielded a solid crude extract further processed through vacuum drying and lyophilization. The anti-ulcer activity of this ethanolic extract was tested on Wistar rats at doses of 100mg/kg and 200mg/kg body weight. The assays were conducted using three distinct ulcer-inducing models: drug-induced, ethanol-induced, and stress-induced models.

The ethanolic extract of TA leaves demonstrated a dose-dependent efficacy in reducing ulcers in a drug-induced model, showing up to 62.59% ulcer inhibition at a 200mg/kg body weight dose. In the ethanol-induced ulcer model, the extract reduced the ulcer index significantly, with the higher dose of 200mg/kg body weight showing 53.38% inhibition. The extract also showed anti-ulcer potential in the stress-induced model, with an inhibition rate of 70.45% at the higher 200mg/kg body weight dose.

Across all three models—drug-induced, ethanol-induced, and stress-induced—the ethanolic extract of TA leaves consistently demonstrated significant anti-ulcer activity, albeit with varying efficacy. The highest level of ulcer inhibition was observed in the drug-induced model, followed closely by the stress-induced model and then the ethanol-induced model. The efficacy of TA leaves in reducing ulcer index was observed to increase with dosage in each case. While the extract's anti-ulcer activity was consistently lower than that

of the standard drug Esomeprazole, the results suggest that the ethanolic extract of TA leaves is a promising alternative for anti-ulcer treatment.
While consistently less efficacious than Esomeprazole, the TA extract remains a viable anti-ulcer treatment alternative. Notably, the anti-ulcer effects were particularly pronounced in the stress-induced model. The extract's anti-ulcer potential may be attributable to its flavonoid content, although further studies are warranted to isolate and identify the active compounds and to elucidate the underlying mechanisms of its anti-ulcer activity.

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