Antiulcer Activity of Stem Extracts of *Tinospora malabarica* (Lamk.)

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Summary

The objective of the study was to investigate anti ulcer activity of petroleum ether, alcohol and aqueous extracts of stem of *Tinospora malabarica* (Lamk.). All the three extracts were subjected to preliminary phytochemical investigation and also for acute oral toxicity study. The preliminary phytochemical screening revealed the presence of flavonoids, carbohydrates and amino acids in aqueous extract, flavonoids, alkaloids and carbohydrates in alcoholic extract, while petroleum ether extract contains only steroids. Aqueous and alcohol extracts found to be non toxic upto a dose of 5000 mg/kg while petroleum ether extract was safe up to a dose of 2000 mg/kg after single dose administration of the extract. Present study showed that, there was significant reduction in the ulcer index with aqueous and alcoholic extract and with that of petroleum ether it was less significant, also their was a considerable reduction in the free as well as the total acidity in pylorus ligated rats. In case of stress induced ulcers there was a significant reduction in ulcer index wit all the three extracts and percentage protection of aqueous extract was nearly equivalent with that of standard.

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Introduction

Stress is a psycho-physiological response to a change in the environment[1]. Stress has been postulated to be involved in the etiopathogenesis of variety of disease states, ranging from psychiatric disorder, indigestion, gastritis, bowel disturbance, muscle pain, imunosupression, endocrine disorder, male impotency, cognitive dysfunction, peptic ulcer, hypertension and ulcerative colitis[2].

Peptic ulcer is a benign lesion of gastric or duodenal mucosa occurring at the site where the mucosal epithelium is exposed to acid and pepsin. There is constant confrontation in the stomach and upper small bowel between acid-pepsin aggression and mucosal defense. Usually, the mucosa can withstand the acid-pepsin attack and remain healthy. That is, a mucosal "barrier" to back diffusion of acid is maintained[3]. However, an excess of acid production or an intrinsic defect in the barrier functions of the mucosa can allow the defense mechanism to fail and ulcer to result.

Since its recognition of the peptic ulcer as an important chemical entity, various efforts have been made to find a suitable remedial measure. For several decades the adage "no acid-no ulcer" and the drugs used to reduce acid secretion have dominated the pharmacological basis of ulcer therapy[4]. More recently, the role of mucosal factor in peptic ulceration has received much attention and the term "cytoprotection" has been coined[5]. It now well established that peptic ulcer disease can be prevented by strengthening the defensive mechanisms of gastric and mucosa rather than attenuating factors of aggression causing ulceration.

Because of the problem of being highly complex, expensive and toxic, efforts were made to find a suitable palliative and/or curative agent for the treatment of peptic ulcer disease in natural products of plants and animal origin. A large section of the world's population relies on traditional remedies to treat a plethora of diseases. Medicinal herbs are an indispensible part of traditional medicine practiced all over the world due to low costs, easy access and ancestral experience[6]. In the last few years, efforts have been taken to identify new antiulcer drugs from natural sources. Mechanism of *T. malabarica* as anti stress in restraint stress was explored[7] wherein the stress period was of short duration.

Recently *T. malabarica* have been reported to possess anti stress or adaptogenic activity in animals used to overcome stress related disorders in different models of stress[8]. In the northern Karnataka regions this plant is traditionally used to treat stomach ache and ulcers. As this plant is having its potential to develop adaptation in animals it is also expected to have anti ulcer activity

Materials and Methods

Drugs and Chemicals

Standard ranitidine injection procured from the market (Aciloc Injection, Cadila Healthcare Ltd, Mumbai. Absolute alcohol, petroleum ether, EDTA solution, anesthetic ether, formaline, tween-80 were of laboratory grade purchased from local market.

Animals

Adult Swiss albino mice (20-25g) and Wistar rats (160-200g) procured from Sri Venkateshwara enterprises, Bangalore, were used to study anti stress activity. All animals were maintained under standard husbandry conditions (Light / dark period of 12 hrs day / night and temperature 24^0 C ± 2^0C) with free access to food and water ad *libitum*. The experimental protocols were approved by Institutional Animal Ethics Committee and all experiments were performed between 10:00 -17:00hrs.

Preparation of plant extracts

T. malabarica (Lamk.) was purchased from Alwa Pharmacy, Moodbidre, Mangalore, Dakshin Karnataka. The dried stems were reduced to coarse powder and were successively extracted in soxhlet apparatus using petroleum ether and absolute alcohol for 18 hrs. The same marc was extracted by maceration process with water for 48 h to get aqueous extract and percentage yield for all the three extracts were 2.43. 4.53 and 6.5 g respectively.

Preliminary Phytochemical screening

All the three extracts were subjected to preliminary phytochemical investigation for the presence of various phytochemical constituents as described by Khandewal[9].

Acute oral toxicity studies (LD_{50}):

The acute toxicity of petroleum ether, alcoholic and aqueous extracts of *Tinospora malabarica (Lamk.)* were determined in 3 h fasted female albino mice by OECD guide lines No. 425. The LD_{50} of the test extracts were calculated using AOT 425 software provided by Environmental Protection Agency, USA.

ANTIULCER ACTIVITY.

Pylorus ligation induced gastric ulcers in rats (SHAY rat)

Rats of either sex weighing 150–170 g are starved for 48h having access to drinking water ad libitum. During this time they are housed single in cages with raised bottoms of wide wire mesh in order to avoid cannibalism and coprophagy[10]. The rats were divided into 5 groups with 6 animal in each group. Group I received vehicle (tween 80), Group II received standard (ranitidine 20 mg/kg), Group III received aqueous extract (500mg/kg), Group IV received alcoholic extract (500mg/kg) and Group V received petroleum ether extract (500mg/kg). All the drugs were administered by oral route.

Under ether anesthesia a midline abdominal incision was made. The pylorus was ligated, care being exercised that neither damage to the blood supply nor traction on the pylorus occurs. Grasping the stomach with instruments was avoided, to prevent ulceration invariably developing at such points. The abdominal wall was closed by sutures. The test compounds were given orally by gavage. The animals were placed for 6 h in plastic cylinders with an inner diameter of 45mm being closed on both ends by wire mesh. Afterwards, the animals are sacrificed in ether anesthesia. The abdomen was opened and a ligature was placed around the esophagus close to the diaphragm. The stomach was removed, and the contents were drained in a centrifuge tube. Along the greater curvature the stomach was opened and pinned on a cork plate. The mucosa was examined with a dissection microscope. The number of ulcers was noted and the severity recorded with the following scores:

0 = no ulcer, 0.5= spot ulcers, 1 = superficial ulcers, 2 = deep ulcers, 3 = perforation.

Mean ulcer score for each animal was expressed as ulcer index and the percentage was calculated using the formula,

Percentage protection =100 –Ut/Uc x 100

Where, Ut = Ulcer index of treated group.
Uc = Ulcer index of control group.

The volume of the gastric content was measured. After centrifugation, acidity was determined by titration with 0.01 N NaOH using toppfer's reagent and phenolphthalein as indicators[11].

Water immersion Stress induced ulcer.

Stress ulcers were induced by forced swimming in the glass cylinder[12] with a height 45 cm; diameter 25 cm containing water to the height of 35 cm maintained at 25^{o}C for 1h. Animals were fasted for 24h prior to the experiment and divided into 5 groups with 6 animals in each group. Group I received vehicle (tween 80), Group II received standard (ranitidine 20 mg/kg), Group III received aqueous extract (500mg/kg), Group IV received alcoholic extract (500mg/kg) and Group V received petroleum ether extract (500mg/kg). After the drug treatment animals were subjected to swim in water for 1h. Then, they are removed, dried and injected intravenously via the tail vein with 30 mg/kg Evans blue. Ten min later, they were sacrificed in ether anesthesia and their stomachs removed. Formolsaline (2% v/v) was then injected into the totally ligated stomachs for storage overnight. The next day, the stomachs were opened along the greatest curvature, washed in warm water, and examined under a dissection microscope. The number of ulcers was noted and the severity recorded as mentioned above and the ulcer index was calculated.

Statistical Analysis

All the values are expressed as mean ± SEM. Statistical differences between means were determined by one-way ANOVA followed by Dunnett's post hoc test. P <0.05 was considered as significant. Difference in means of ulcer index were tested with Kruskal-Wallis followed by Dunn' s test. All the statistical analysis was performed using Instat ® software (Graph pad Inc., Santabarba, CA)

Results

Preliminary phytochemical investigation

The preliminary phytochemical screening with stem extracts of *Tinospora malabarica (Lamk.)* revealed the presence of flavonoids, carbohydrates and amino acids in aqueous extract, flavonoids, alkaloids and carbohydrates in alcoholic extract and only steroids in petroleum ether extract.

Acute toxicity study:

Aqueous and alcohol extracts up to a dose of 5000 mg/kg and petroleum ether extract up to a dose of 2000 mg/kg were found to be safe.

Effect of *T. malabarica* extracts in Pylorus ligation induced gastric ulcers

Pretreatment with extracts significantly decreased ulcer index; $p<0.001$, with aqueous and alcoholic extract while $p<0.01$; with petroleum ether extract. There was significant rise in pH with reduction in volume of gastric contents, free acidity, total acidity with extract treated group as compared to extract untreated rats. significant with aqueous and alcoholic extract and percentage protection was comparable with that of standard (ranitidine). (Table no. 1)

Table no. 1. Effect of *T. malabarica* on ulcer index, pH, volume of gastric juice, free acidity, total acidity and percentage protection in pylorus ligated rats.

Treatment	**Dose mg/kg**	**Ulcer index**	**pH**	**Vol. of gastric juice**	**Free acidity**	**Total acidity**	**% protection**
Control		4.25±1.04	1.33±0.33	4.83±0.31	89.17±8.40	150.83±11.13	---------
Ranitidine	20	0.5±0.22***	4.83±0.74**	2.25±0.46**	35±15.81*	45.5±20.97***	88.23
Aqueous extract	500	0.75±0.40***	5.16±0.47*	2.67±0.17**	32.83±10.69*	67.83±6.48**	82.35
Alcoholic extract	500	0.83±0.10***	4.66±0.61**	5.08±0.49	47.5±13.95	76.83±21.23*	80.47
Pet extract	500	1.17±0.28**	3.66±0.95	3.66±0.49	51.66±14.59	110.5±14.42	72.47

Results are expressed as Mean ±SEM; *Significance at* $P<0.05$*, $P<0.01$** and $P<0.001$***as compared to control

Water immersion Stress induced ulcer.

Pretreatment with all the extracts has significantly reduced ulcer index $p<0.001$ and percentage protection of ulcerogenic effects with aqueous extract (85.65) was more than the standard (71.520 and extract like alcoholic and petroleum ether extracts did not show much protection. (Table no. 2).

Table no. 2. Effect of *T. malabarica* on ulcer index and percentage protection in stress induced ulcers in rats.

Treatment	Ulcer index	% protection
Control	4.67+0.494	------
Ranitidine	1.33+0.333**	71.52
Aqueous extract	0.67 + 0.210***	85.65
Alcoholic extract	3.5 + 0.224	25.05
Pet extract	4.5 + 0.428	3.64

Results are expressed as Mean ±SEM; *Significance at* $P<0.05$*, $P<0.01$** and $P<0.001$***as compared to stress control

Discussion

In pylorus ligation induced ulcer model all the extracts of *Tinospora malbarica* reduced gastric volume, free acidity, total acidity and ulcer index thus showing the antisecretory mechanism involved in the extracts for their antiulcerogenic activity. Ulcer index parameter was used for the evaluation of anti-ulcer activity since ulcer formation is directly related to factors such as gastric volume, free and total acidity.

In case of vehicle control, pylorus ligation increased the acid secretion, which in turn caused increase in gastric volume, low pH, increased free and total acidity resulting into increase in ulcer index.

Water immersion test one of the best models of stress in rats to induce ulcer. The model provides both emotional stress as well as physiological stress to the animal. In case of water immersion induced stress in rats, all the extracts showed significant ($p<0.001$) ulcer inhibition.

The results indicate that *T. malabarica* extracts produced antiulcerogenic effects possessing antisecretory, cytoprotective and H_2 blocking/ proton pump inhibition mechanism.

This study indicates that *T. malabarica* extracts has a potential anti ulcer activity especially the aqueous extract. However, further study is required to isolate the active molecule responsible for the activity.

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