Research Article

Antiulcer and Antisecretory Activity of *Trychosanthes lobata*

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ABSTRACT

Objectives: The objective was to investigate antiulcer and antisecretory activity of Ethanolic and aqueous extract of *Trychosanthes lobata* on different experimental ulcer models. **Methods**: Anti ulcer activities were evaluated against pyloric ligation and aspirin induced ulcer models. Anti-ulcer effect of both extracts of 250 and 500 mg/kg were evaluated in different ulcer models using Wistar rats compared with ranitidine as standard in terms of inhibition of release of gastric juice, hydrochloric acid and neutralization activity. The observation was made for ulcer sores, ulcer index, free acidity, total acidity and P^H. **Results:** This study give results that aqueous and methanolic extract of *Trychosanthes lobata* possess potentially useful anti-ulcer activity in different ulcer model in rats. **Conclusion:** This result suggests the usefulness of ethanolic and aqueous extract of *Trychosanthes lobata* as antiulcer agent.

Keywords: Anti-ulcer, pyloric ligation, Helicobacter pylori.

INTRODUCTION

Peptic ulcers are a deep gastrointestinal erosion disorder that involves the entire mucosal thickness, penetrating the muscular mucosa[1]. For decades it was believed that gastrointestinal ulcerations were caused by the excessive secretion of gastric acid, but many patients presenting such ulcerations had normal acid secretion rates[2]. Then, researchers reported that peptic ulcers were been caused by an imbalance between the aggressive factors and a number of known defense mechanisms. Exogenous aggressive factors such as smoke, anti-inflammatory drugs, alcohol, stress, fatty foods and *Helicobacter pylori* infections triggered tissue necrosis through mucosal ischemia, free radical generation and cessation of nutrient delivery, hydrochloric acid together with pepsin, pancreatic enzymes and bile decreased the defense mechanisms of gastrointestinal mucosa such as the intercellular junctions, local blood flow, mucus/bicarbonate secretion and cellular growth[3,4]. Acid peptic activity and a collapse of mucosal defense mechanism have been implicated in the genesis of gastro-duodenal ulcers. Efforts are being made to find a suitable agent for the treatment of peptic ulcer Disease[5]. The extracts and compounds from medicinal plants and other natural products have become the widely acceptable source of therapeutic agents for the treatment of peptic ulcers. A potential of hepatoprotective property underlying *Trychosanthes lobata* may be attributed to the anti-oxidative constituents due to the presence of flavonoids, saponin, and tannins in ethanolic extract of *Trychosanthes lobata* was confirmed by phytochemical analysis and TLC, and these compounds are reported to have antioxidant properties[6]. The Aqueous extract of leaves of *Trychosanthes dioica* Roxb was evaluated for its antiulcer activity against different ulcer models in Wistar rats with comparison to the standard Ranitidine (25 mg/kg)[7]. In reference to above our aim is to investigate antiulcer and antisecretory activity of *Trychosanthes lobata* in different ulcer model.

MATERIALS AND METHODS

Adult male albino mice 20 – 25 gms and rats 150 – 200 gms were used for the study. Animals were kept in the animal house of GIET School of Pharmacy, Rajahmundry, maintained under standard husbandry condition with free access to food and water *ad libitum.* All the experiments in this study were approved by institutional animal ethical committee with CPCSEA registration number 1069/PO/ac/07/CPCSEA, GIET School of pharmacy.

Collection of plant material

The plant was collected from rural belt of Bhubaneswar, Orissa and was authenticated in the department of Botany, Utkal University, Bhubaneswar. The plant was collected in bulk

and washed with tap water to remove the soil and dirt particles and then shade dried. The dried plant materials were milled into coarse powder by a mechanical grinder and sieved in sieve 20. The coarse powder was taken for extraction in soxhlet apparatus and fine powder for maceration.

Preparation of extract

Alcoholic Extract

The powdered plant (2.5 kg) was exhausting extracted by soxhlet apparatus with 95% ethanol. The total ethanol extract was then concentrated in vacuum to syrupy mass.

Aqueous Extract

The powdered plant material (25 kg) was macerated with chloroform water (1:9) for seven days. The extract was filtered and concentrated over a water bath and further dried in vacuums oven till constant weight.

Acute toxicity studies

Oral acute toxicity studies were carried out with Albino mice weighing 20 – 25 gm, with 2 mice per dose group. The extracts were administered as per the staircase method[8]**.** The mice were fed with alcoholic and aqueous extract of Trychosanthes lobata separately suspended in 2% of gum acacia at dose 1000, 2000, 3000, 4000, 5000 mg/kg bodyweight. The animals were observed continuously for 2 h for the gross behavioral changes and then intermittently once in every 2 h and finally at the end of 24 and 72 h to note for any signs of toxicity including death.

Pyloric Ligation Induced Gastric Ulcer Method

Albino Rats were weighing between 100-200gms were fasted for 24hrs but were allowed free access to water. Under light ether anesthesia, the pylorus was ligated through a midline abdominal incision with care not to damage any blood vessels. After wound closure, the animals were caged individually without food and water during this period. At the end of experiment, the animals were sacrificed by euthanasia and the stomach was removed. Gastric contents were drained from the stomach and centrifuged at 3000 rpm for 5 min, and the volume of supernatant solution was measured. Free and total acidity were estimated by titration with 0.1 N NaOH using topfer reagent and phenolphthalein as indicator. Acid output was expressed in mEq by multiplying the volume in ml by the acid concentration in mEq/l[9]. The result has been depicted in **Table-1.**

Aspirin induced ulceration

The selected animals (rats) were divided randomly into four groups of six animals each. Each group of animals received the test samples twice daily for two days for the dose level 500mg/kg and the standard drug ranitidine was administered once daily orally at a dose of 25mg/kg body weight. For two days prior to and one hour before administration of aspirin. On second day (37th hour) aspirin was administered at a dose of 200 mg/kg orally as a suspension prepared in 0.5% w/v CMC with distilled water for all groups, one hour prior to pyloric ligation. The animals were deprived of food and water during the post-operative period. Ranitidine (25 mg/kg) was used as reference standard. After 30 minute, each animal was administered 200 mg/kg aspirin through oral route. After 1hr, pylorus ligation was made as per the procedure[11]. The animals were killed after 4hr; the stomachs were opened along the greater curvature and carefully observed for severity of ulceration as described earlier and subjected to analysis for free acidity, Total acidity, Total protein, Pepsin. The result has been depicted in **Table-2.**

Determination of Free Acidity of Gastric Juice

One milliliter of the supernatant liquid was pipetted out and diluted to 10 ml with distilled water. The solution was titrated against 0.01N NaOH solution using topfer's reagent as indicator, to the end point the solution turned to orange color. The volume of NaOH needed was taken as corresponding to the free acidity. Acidity was determined by using

$$\text{Acidity} = \frac{\text{volume of sodium hydroxide} \times \text{normality} \times 100\text{mEq/L/100gm}}{0.1}$$

Determination of Total Acidity of Gastric Juice

An aliquot of 1 ml of gastric juice was taken in to a 50 ml conical flask and two drops of phenolphthalein indicator was added and titrated with 0.01N NaOH until a permanent pink color was established .The volume of 0.01N NaOH consumed was noted, total acidity was calculated and expressed as mEq/l.

Determination of pH

The pH of the gastric juice was determined by using pen type pH meter.

Measurement of Ulcer Index

Immediately after the animals were sacrificed, their stomach was dissected out, incised along the greater curvature and the mucosa was rinsed with cold normal saline to remove blood contaminant, ulcers were examined under a magnifying lens. The ulcers were measured with the help of vernier caliper using the following arbitrary scale.

The scale was as follows, if,

Score 0 = no ulcers; Normal stomach,
Score 0.5 = red coloration
Score 1 =spot ulcer; petechial hemorrhage,
Score1.5 = hemorrhagic
Score 2 = ulcers<2mm,
Score 3 = ulcers>2<4; perforation
Score 4 =ulcers > 4m.

Estimation of Pepsin

For each determination four tubes were placed and numbered as 1-4 in which 1 and 2 containing 5 ml of substrate 3 and 4 containing 10 ml of trichloroacetic acid. The gastric juice was mixed with an equal volume of HCl at pH 2.1 warmed to 370c and 1ml of mixture was added to each tubes 1 and 4. After 25 min. incubation, 1+3 gives test and 2+4 gives blank. Filtered the content of the test tubes and kept for 30min. 2 ml of the filtrate was pipetted into 10 ml of sodium hydroxide and mixed by gentle rotation. After 30 minutes the intensity of color was measured at 680 nm in Hitachi 15-20 spectrophotometer. The difference between test and blank gives a measure of peptic activity[10].

RESULTS

From the above study it was indicated that Reduction in volume of gastric juice was significant (P <0.01) compared to control for aqueous extract (500mg/kg) indicating their good antisecretory potential. The acid neutralizing capacity as indicated by pH of the gastric fluid was found to be more for aqueous extract (500mg/kg) as compared to standard drug ranitidine. This extract also reduced the free acidity and total acidity as compared to ranitidine. From **Table-1** it concludes that all the extracts produced a significant (P<0.01) reduction in the ulcer index. In pylorus-ligated model, free acidity decreased with no significant change in the gastric volume for alcoholic extract. Reduction in pepsin content was significant in aqueous extract rather than alcoholic indicates gastric protective activity. From T**able-2** in aspirin induced gastric ulcer model ethanolic extract shows maximum reduction in acid volume which is significant. In pH, also a significant difference was observed between ethanol extract and Control group. There was a significant change in pepsin content for aqueous extract as compared to alcoholic one. The anti-ulcer effect was also supported by the decreases in the aggressive factors like pepsin. The cytoprotective effect of Trychosanthes lobata appears due to increase in pH and decrease in gastric volume, total acidity and free acidity and pepsin content. In pyloric ligation model(PL), aspirin induced model(ASP), Ethanolic extract showed protection index of 70.51% and 69.37% and aqueous extract showed maximum inhibition of 80.28% and 70.9% respectively, whereas ranitidine exhibited 85.86% and 84.64% respectively (**Fig 1**).

DISCUSSION

In pylorus ligated model, the ulcer is developed due to increased metabolism of carbohydrates and increased synthesis of nucleic acid and also exhaustion of carbohydrates and other compensatory mechanisms[12]**.** The increased carbohydrate content by the test extract may presumed to be responsible for altering mucous secretion which in turn alter status of mucosal barrier. The aqueous extract of Trychosanthes lobata shows maximum ulcer inhibition in pylorus ligated model. Aspirin induced ulceration is attributed principally to inhibition of biosynthesis of cytoprotective prostaglandins, e.g. PGE's and PGI_2 (by inhibition of cyclooxygenase pathway of arachidonic acid metabolism), resulting in overproduction of leukotrienes and other products of 5-lipoxygenase pathway[13]**.** In this model it has been confirmed that aqueous extract may inhibits the cyclooxygenase pathway of arachidonic acid metabolism.

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Table 1: Effect of leaves of *Trychosanthes lobata* on pyloric ligated ulcer in rat

Treatment	Dose	Gastric Volume(ml)	pH	Ulcer Index	Free acidity (mEq/l)	Total acidity (mEq/l)	% Protection	Pepsin (µg/ml)
Solvent	2ml/kg	10.6±0.49	1.86±0.29	21.3±1.41	57.67±2.34	62.82±3.07	----------	18.85±3.11
Ranitidine	25mg/kg	3.28±0.26***	6.30±0.32***	3.01±0.12***	10.2±0.95***	12.5±0.47***	85.86	6.35±0.34**
Ethanolic extract	500mg/kg	3.9±0.54	2.90±0.37	6.28±0.42**	32.17±3.89	39.0±3.83	62.60	13.67±0.29
Aqueous extract	500mg/kg	2.3±0.25**	4.1±0.15**	4.2±0.28**	8.81±0.88**	15.8±1.8**	74.70	10.67±0.88

Data are represented as mean±S.E.M. Statistical analysis was done with one way analysis of variance (ANOVA). * p<0.01, ** p<0.001 as compared to control (n=6 in each group).

Table 2: Effect of leaves of *Trychosanthes lobata* on aspirin induced ulcer model in rat

Treatment	Dose	Gastric Volume(ml)	pH	Ulcer Index	Free acidity (mEq/l)	Total acidity (mEq/l)	% Protection	Pepsin (µg/ml)
Solvent	2ml/kg	10.3±0.54	2.34±0.24	20.9±1.01	53.67±2.34	59.43±2.835	----------	21.88±1.12
Ranitidine	25mg/kg	2.89±0.44***	6.02±0.72***	3.21±0.45***	7.89±0.87***	10.21±0.32***	84.64	5.32±0.36**
Ethanolic extract	500mg/kg	3.23±0.42*	3.24±0.28	6.4±0.38*	36.50±3.58	42.17±3.46	61.90	16.28±1.19
Aqueous extract	500mg/kg	4.7±0.25	3.3±0.25	6.08±0.50*	38.3±4.29	42.0±4.18	63.79	10.58±2.46*

Data are represented as mean±S.E.M. Statistical analysis was done with one way analysis of variance (ANOVA). * p<0.01, ** p<0.001 as compared to control (n=6 in each group).

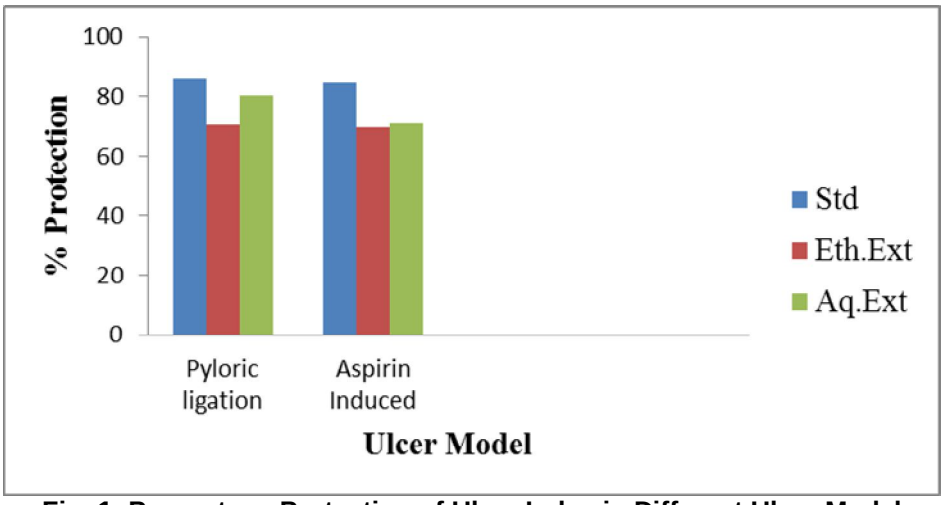

Fig. 1: Percentage Protection of Ulcer Index in Different Ulcer Model

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