

EVALUATION OF BIOACTIVE CONSTITUENTS AND IN VIVO ANTI ULCER ACTIVITY OF *TRICHOPUS ZEYLANICUS* (STEM)**Surjeet Kumar*, Sailesh Kumar Ghatuary, Satkar Prasad, Amit Dubey****RKDF School of Pharmaceutical Sciences, Bhopal (M.P.)***Corresponding Author's E mail: sitmdirector@rediffmail.com

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ABSTRACT

Over the past 200 years, peptic ulcer disease which includes both stomach and duodenal ulcers has posed a serious threat to the world's population due to its high morbidity and significant mortality. The incidence and frequency of this disease and its consequences have exhibited notable geographic variations according to epidemiological data. A little herbaceous plant from the family Trichopodaceae called *Trichopus zeylanicus* subsp. *travancoricus* is only found in the Western Ghats of South India. The study on the evaluation of the bioactive components and in vivo anti-ulcer activity of *Trichopus zeylanicus* stem extract is the subject of the current communication. Hydroalcoholic leaves extract of *Trichopus zeylanicus* show slight CNS depression for a few hours after treatment at the dose of 3000 mg /kg. However, there was no sign of toxicity or mortality up to 14 days indicates that the extract is relatively safe. Any substance that is not toxic at 300 mg /kg is considered relatively safe.

Keywords: *Trichopus zeylanicus*, Anti-ulcer activity, Phytochemical analysis.

INTRODUCTION

Peptic ulcer disease embraces both gastric and duodenal ulcers and has been a major threat to the world's population over the past two centuries, with a high morbidity and substantial mortality. Epidemiological data for this disease and its complications have shown striking geographical variations in incidence and prevalence. Development of ulcer disease and death from it has been associated with the birth of urbanisation and was interpreted as a birth-cohort event with the peak of disease in those born during the late 19th century.¹⁻²

Our understanding of the disease changed greatly with the discovery of *Campylobacter pyloridis* (renamed *Helicobacter pylori* in 1989 because of a revised taxonomic classification) in 1982 by Warren and Marshall.³⁻⁴

This discovery switched the notion from an acid-driven disease to an infectious disease, opening a huge area for intensive research that resulted in the reconciliation of previously suggested mechanisms of pathogenesis. The fall of the acid dogma in peptic ulcer disease, which had found its undisputed acceptance during and after the introduction of histamine H₂-receptor antagonists, led to the present therapeutic principle. Maintenance acid suppressive therapy for duodenal ulcer, which followed decades of dominance of surgical interventions (subtotal gastric resections, several forms of vagotomy), was replaced with a short-term antibiotic regimen targeting eradication of *H. pylori* infection.⁵⁻⁶

H. pylori eradication as cure of peptic ulcer received its full recognition when the Nobel Prize for Medicine and Physiology was awarded to Warren and Marshall in 2005. This recognition has not, however, closed the chapter on peptic ulcers. The management of ulcer disease and its complications remains a clinical challenge. Additionally, non-steroidal anti-inflammatory drugs (NSAIDs) and low-dose aspirin are an increasingly important cause of ulcers and their complications even in *H. pylori*-negative patients. Other rare causes of ulcer disease in the absence of *H. pylori*, NSAIDs, and aspirin also exist.

Trichopus zeylanicus subsp. *travancoricus*, belonging to the family Trichopodaceae, is a small herbaceous plant exclusively present in Western Ghats of South India. The antifatigue and instant energy giving properties experienced after eating the fresh seed kernels by the scientists who made the expedition to these tribal areas¹ are very significant and therefore, deserve scientific investigation.

Being a very rare plant with absolutely no information on its medicinal value prior to the very little chemical and pharmacological work has ever been carried out on this plant. It was in this context and against this background that the present authors initiated an in depth investigation on this plant. The present communication relates to the study on the Evaluation of bioactive constituents and in vivo anti ulcer activity of stem extract of *Trichopus zeylanicus*.

MATERIAL AND METHODS

Preparation of crude extracts

Powdered material of (Stem) *Trichopus zeylanicus* was subjected to solvent extraction using appropriate methods.

Extraction by Soxhletion Method⁷

100 gram of powdered stems of *Trichopus zeylanicus* was exhaustively extracted with different solvent petroleum ether and Hydroalcohol (Ethanol: water;70:30) by Soxhletion method. The extract was evaporated above their boiling points. Finally, the percentage yields were calculated of the dried extracts.

Phytochemical analysis⁸⁻⁹

Qualitative photochemical analysis

Photochemical examinations were carried out extracts as per the following standard methods.

1. Detection of alkaloids: Extracts dissolved individually in dilute Hydrochloric acid and filtered.

a) Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Alkaloids confirmed by the formation of yellow coloured precipitate.

2. Detection of carbohydrates: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

a) Fehling's Test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

3. Detection of glycosides: Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

a) Legal's Test: Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Finding of pink to blood red colour indicates the presence of cardiac glycosides.

4. Detection of saponins

a) Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the incidence of saponins.

5. Detection of phenols

a) Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

6. Detection of flavonoids

a) Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the occurrence of flavonoids.

7. Detection of proteins

a) Xanthoproteic Test: The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

8. Detection of diterpenes

a) Copper acetate Test: Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

Quantitative studies of phytoconstituents¹⁰

Total phenol content estimation

The total phenol content of the extract was determined by the modified folin-ciocalteu method. 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10- 50µg/ml was prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol. 2 ml of extract and each standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

Total flavonoids content estimation

Determination of total flavonoids content was based on aluminum chloride method. 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids. 1 ml of 2% AlCl₃ solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm¹¹.

Pharmacological activity

Wistar rats (180-200 g) and Swiss albino mice (males; 20–25 g) were used in the present study. The animals were procured from College of Veterinary Science and Animal Husbandry Mhow, Indore (M.P), India. They were provided normal diet and tap water ad libitum and were exposed to 12-h light and 12-h dark cycle. The animals were acclimatized to the laboratory conditions before experiments. Experimental protocol was approved by Institutional Animal Ethics Committee. Care of the animals was taken as per guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals

(CPCSEA), Ministry of Environment and Forests, Government of India. Experiment protocol was approved by Institutional Animal Ethics Committee.

Acute toxicity study

Five groups (n = 5) of male albino mice were used in the acute toxicity study of *Trichopus zeylanicus* Hydroalcoholic extract. Animals from all groups were fasted overnight and administered (p.o) with single dose (250, 500, and 3000 mg /kg) of the extract. A group of animals which received equal volume of PBS served as control. Changes in the behavior of animals were observed for 24 h after extract administration. For any signs of toxicity and mortality, animals were observed for 14 days¹².

Anti-ulcer (ulcer-preventive) activity study

Five groups (n = 6) of Wistar rats were used to study the anti-ulcer activity of *Trichopus zeylanicus* Hydroalcoholic extract. PBS, Hydroalcoholic extract, omeprazole and ethanol were administered to the animals per orally (p.o). Group 1 received PBS (10 mL/ kg) all over the experimental period (11 days) and served as control. Group 2 received PBS (10 mL/ kg) for 10 days and on the 11th day received absolute ethanol (5 mL/ kg) and served as ulcer control. Group 3 and 4 were respectively administered with 300 and 400 mg/kg *Trichopus zeylanicus* Hydroalcoholic extract, group 5 with omeprazole (8 mg /kg) for 10 days. All the groups were fasted for 24 h and again administered with the extract or drug at respective dose. After 30 min of this treatment, animals of groups 2-5 were administered with 5 mL/ kg ethanol to induce ulcer. After 15 min of ethanol administration, all the animals were sacrificed using anesthetic ether. Gastric volume was measured by pylorus ligation method. Each stomach was opened along the greater curvature and examined macroscopically for gastric erosions under a dissecting microscope (20 X). The length and width (mm) of ulcer on the gastric mucosa were measured by plane glass square (10×10 mm). The Ulcer Area (UA) was calculated¹³. The % of protection (P %) availed to the animals through various treatments were calculated using the formula:

$$P\% = \frac{UA \text{ ulcer control} - UA \text{ treatment}}{UA \text{ ulcer control}} \times 100$$

Grouping of animals:

Group-I Control Received PBS 10 ml/kg, p.o

Group-II Ulcer Control Received PBS 10 ml/kg, p.o up to 10 days and on 11th day receives absolute alcohol 5 ml/kg, p.o

Group-III Received HATZ 300 mg/kg, p.o

Group-IV Received HATZ 400 mg/kg, p.o

Group-V Received Omeprazole 8 mg/kg, p.o

RESULTS AND DISCUSSION

Ulcer has long been recognized as one of the most important gastrointestinal problem. With the ever growing interest in natural medicine, many plants have been screened and reported to be useful in treating and managing ulcer. *Trichopus zeylanicus* has several pharmacological properties including anti-inflammatory and anti-diarrhoeal. In spite of its uses in the traditional medicine against various ailments, this plant has so far not been screened for anti-ulcer activity. We report on the anti-ulcer activity of Hydroalcoholic leaves extract of *Trichopus zeylanicus*. The results of the present study have shown that Hydroalcoholic leaves extract of *Trichopus zeylanicus* possess gastro protective activity, as evidenced by its significant inhibition in the formation of ulcers induced by ethanol.

The protective effect was confirmed by histological examination showing prevention of mucosal lesions and sub-mucosal edema. As flavonoids have been identified in the methanolic extract, we believe that the anti-ulcer activity of this extract is probably due to the antioxidant activity of the extract. Antioxidant activities of flavonoids have been well documented in the literature. Moreover, flavonoids have been reported for their anti-ulcerogenic activity and gastric protection already. Sub-acute toxicological studies have revealed that the methanolic extract of Hydroalcoholic leaves extract of *Trichopus zeylanicus* show slight CNS depression for a few hours after treatment at the dose of 3000 mg /kg. However, there was no sign of toxicity or mortality up to 14 days indicates that the extract is relatively safe. Any substance that is not toxic at 300 mg /kg is considered relatively safe.

Table 1: % Yield (W/W) of *Trichopus zeylanicus* (Stem)

S. No.	Extract	% Yield
1.	Pet. Ether	7.36 %
3.	Hydro alcohol	11.59 %

Table 2: Result of photochemical screening of *Trichopus zeylanicus* (Stem).

S. No.	Constituents	Methanol extract
1.	Alkaloids	
	Hager's Test:	+ve
2.	Glycosides	
	Legal's Test:	-ve
3.	Flavonoids	
	Lead acetate Test:	+ve
4.	Diterpenes	
	Copper acetate Test:	-ve
5.	Phenol	
	Ferric Chloride Test:	+ve
6.	Proteins	
	Xanthoproteic Test:	+ve
7.	Carbohydrate	
	Fehling's Test:	+ve
8.	Saponins	
	Froth Test:	+ve

Table 3: Estimation of total phenolic and flavonoids content of *Trichopus zeylanicus* (Stem)

S. No	Extract	Total phenolic content (mg/100mg of dried extract)	Total flavonoids content (mg/ 100 mg of dried extract)
1.	Hydroalcohol extract	3.45	8.44

Table 4: Effect of the Ethanolic leaves extract of *Trichopus zeylanicus* on Ethanol Induced Gastric Ulcers in Rats

Treatment	Dose (mg/kg b.w)	Gastric Volume(ml)	Ulcer area(mm ²)	Protection (%)
Control	PBS 10 ml/kg	1.54±0.08	0.00±0.00	NA
HATZ	300 mg/kg	3.47±0.29	485.36±6.00	28.25%
HATZ	400 mg/kg	2.10±0.29	297.42±0.27	54.44%
Omeprazole	8 mg/kg	2.45±0.31	154.87±4.65	69.23%

Data are expressed as mean SEM., n=6 in each group. *p<0.05, **p<0.01 - One way ANOVA followed by Dunnett's test

Table 5: Effect of Hydroalcoholic extract of *Trichopus zeylanicus* on Antisecretory Parameters

Treatment	Dose (mg/kg b.w)	pH	Free Acidity (mEq/l/ 100g)	Total Acidity (mEq/l/ 100g)
Control	PBS 10 ml/kg	1.58±0.45	59.01±4.6	78.32±2.5
HATZ	300 mg/kg	3.05±0.35	37.32±0.47	37.22±2.61
HATZ	400 mg/kg	3.87±0.61	33.32±2.65	33.55±2.55
Omeprazole	8 mg/kg	6.32±1.21	19.13±5.2	27.25±2.58

Values are mean ± S.E.M, n=6, NS-not significant, *p < 0.05 and **p < 0.01 Vs control, One way ANOVA followed by Dunnett's test

Table 6: Effect of Hydroalcoholic extract of *Trichopus zeylanicus* on Total Proteins and C/P

Treatment	Dose (mg/kg b.w)	Total proteins (µg/ml)	C/P
Control	PBS 10 ml/kg	450.21±0.36	0.65
HATZ	300 mg/kg	410.19±2.14**	1.8
HATZ	400 mg/kg	301.54±2.01**	3.54
Omeprazole	8 mg/kg	233.47±2.5**	4.36

Values are expressed in terms of mean ± S.E.M, **p<0.01- One way ANOVA followed by Dunnett's test

Table 7: Effect of Hydroalcoholic extracts of *Trichopus zeylanicus* on Total Carbohydrates

Treatment & Dose (mg/kg)	Total Carbohydrates (µg/ml)			
	Total Hexose	Hexosamine	Fucose	Sialic acid
Control	150.21±0.33	170.23±0.87	70.87±0.58	23.02±0.10
HATZ	280.54±1.25**	230.46±0.58**	80.55±1.21**	43.66±1.29**
HATZ	310.21±1.11**	260.54±0.12**	86.89±1.22**	38.25±1.55**
Omeprazole	480.56±0.36**	420.11±0.82**	160.36±0.27**	51.36±0.98**

Values are expressed in terms of mean ± S.E.M, **p<0.01 Vs control- One way ANOVA followed by Dunnett's test

CONCLUSION

From this study, it is clear that *Trichopus zeylanicus* stem extract have significant anti-ulcer activity in animal models. It has muco-protective activity and gastric antisecretory when compared with that of reference drug omeprazole. The extract is non-toxic even at relatively high concentrations. The anti-ulcer activity is probably due to the presence of flavonoids. Further studies are being carried out to characterize and explore the biological activity of the compounds present in the extract.

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