

Anti-ulcer potential of saponin fraction of *Trichopus zeylanicus* on a various experimental animal models

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Abstract

Background: *Trichopus zeylanicus* Gaertn is a perennial herb, belongs to the family Trichopodaceae, is wild plant, a rare genus, small glabrous herb growing in the Agasthyar hilly forest of Kerala. The Kani tribes of this area call this plant as “Arogyapacha” or “Arokyapachilai” in Malayalam. **Objective:** The present study was investigated for anti-ulcer potential of Saponin fraction of the whole plant of *T. zeylanicus* on various experimental animal models. **Materials and Methods:** Acute toxicity study of saponin fraction of *T. zeylanicus* (SFTZ) was carried out on female albino rat up to 2000 mg/kg as per OECD guideline No.423. The experimental animal models, i.e., ethanol induced ulcer, restrained stress induced and pyloric ligation (PL) induced ulcers model were tested for anti-ulcer activity SFTZ at three various doses of (75,150 and 300 mg/kg, p.o.) for 5 days to Wistar Albino rat. Esomeprazole (10 mg/kg, p.o.) was used as a reference standard for the present study. **Results:** Treatment of SFTZ (75,150 and 300 mg/kg, p.o.) showed a significant dose-dependent effect in lowering ulcer index with significantly increased in percentage protection against ethanol, restrained stress and PL induced ulcer model in rats. Biochemical parameter like gastric volume, pH, free acidity, total acidity total proteins, total hexoses, hexosamine, fucose, sialic acid, and pepsin were determined in PL induce ulcer models. The result showed significantly increased in level of defensive mucin secretion in terms of total carbohydrates: Protein ratio after SFTZ treated rats in PL induce ulcer models. **Conclusion:** The action potential of SFTZ is positively found to be more active in alleviating the ulcer by chemical and physical induced models.

Key words: Anti-ulcer, saponin, *Trichopus zeylanicus*, ulcer index

INTRODUCTION

Peptic ulcer being the most prevalent gastrointestinal disorder continues to occupy the key position in concern of both clinical practitioner and researchers. As a result, more and more drugs, both herbal and synthetic drugs are coming up in a market, offering newer and better options for treatment of peptic ulcer. The type of drug varies from being a proton pump inhibitors to H₂ receptor antagonists, and cytoprotective agents as sucralfate. At the same time, each of these drugs confers simpler to severe side effects such as arrhythmias, gynecomastia, enterochromaffin-like cells hyperplasia and hematopoietic changes.^[1] This marks the foremost thrust area of research at present that mainly revolves around the search of an indigenous drug possessing fewer side

effects to have a better and safer alternative for the treatment of peptic ulcer.

Trichopus zeylanicus Gaertn is a perennial herb, belongs to the family Trichopodaceae popularly known as “Arogyapacha” or “Arokyapachilai” in Malayalam literally meaning “the green that gives strength.” The Kani tribes are using this plant for increasing the stamina.^[2] The plant also possesses

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immunomodulatory activity,^[3] scabies and ringworm infection.^[4] Aqueous extract of whole plant of *T. zeylanicus* saponin fraction of *T. zeylanicus* (SFTZ) showed the presence of polyphenols, and sulfhydryl compounds.^[5] Earlier there was study reported on anti-ulcer activity of seed of *T. zeylanicus*.^[6] On the basis of traditional use and literatures, the aim of the present study was to evaluate the antiulcer effect of saponin fraction of *T. zeylanicus* of whole plant material.

MATERIALS AND METHODS

Plant Material

T. zeylanicus Gaertn, collected from Agasthyar hills of Kerala in the month of September 2008, and authenticated by taxonomist of National Institute of Pharmaceutical Education and Research nursery, Mohali, Chandigarh, India. A voucher specimen No. NIP-159 was deposited in their laboratory for future reference.

Extraction and Phytochemical Screening

The whole plant material of *T. zeylanicus* (2.0 kg) was dried. It was ground to coarse powder. The powder materials were defatted 3 times with (15 l) of petroleum ether (40-60°C) using soxhlet apparatus. The defatted plant material (1.2 kg) was extracted 3 times with 10 l of 90% methanol at room temperature. The methanol extracts were filtered, concentrated under reduced pressure and dried in freeze dryer. The (350 g) of methanol extract was further tested for the presence of carbohydrates, proteins, saponin glycosides, alkaloids, flavonoids, steroids and triterpenoids.^[7,8] After phytochemical tests, the methanol extract was used as starting material for isolation of saponins.

Isolation of Saponins

The (210 g) of methanol extract was suspended in (3.0 l) of water and extracted with 3 times with an equal volume of n-butanol. The (75 g) butanol extract precipitated by addition of large quantity of acetone (1.0 l). The resulting precipitate was filtered and dried to give (50 g) of crude saponins fraction of *T. zeylanicus*^[9] and further it was confirmed by hemolytic and foam test for detection of saponins.^[7] It was qualitatively analyzed by using thin layer chromatography using a mixture of chloroform:methanol:water (6:4:1) as an eluent and 5% vanillin-H₂SO₄ reagent for detection of saponins.

Animals and Treatments

Wistar albino rat of either sex weighing between 120-200 g were used for the study. The animals were housed in well ventilated colony cages in the departmental animal house

at (25°C ± 2°C, 12:12 h light and Dark cycle). The animals were fed with standard rodent pellet diet (Amrut Feed Pvt. Ltd. Sangali) and water *ad libitum*. All the experimental procedure and protocols used in the study were approved by IAEC (887/ac/05/Committee for the purpose of Control and Supervision of Experiments on Animals [CPCSEA]) of JKK Nataraja college of Pharmacy, as per CPCSEA guidelines.

Acute Toxicity

Acute toxicity assay was performed as per OECD guidelines 423 (limit test). Six female Wistar albino rats (three animals in each step) were randomly selected. The animals were kept fasting for overnight providing only water. The test drug was administered orally at one dose level of 2000 mg/kg b.w. After that rats were observed continuously for the 1st 4 h and then periodically up to 24 h for toxic symptoms and mortality.^[10]

Anti-ulcer Studies

Experimental protocol

The anti-ulcer activity of SFTZ was studied using three experimental models. In each model rats were divided into 5 groups of 6 animals each. Each were placed in cages with grating floor to avoid corphrophagy and fasted for 24 h allowing free access to water. For anti-ulcer study different doses of SFTZ (75,150 and 300 mg/kg, p.o.), esomeprazole (ESO 10 mg/kg, p.o.) (Sun Pharmaceuticals, Ltd.) prepared in 1% carboxymethyl cellulose (1%) suspension was used for treated groups. All the drugs and chemicals are used for the experiment of analytical grade. The various doses of SFTZ were administered in twice a daily for 5 days.

Ethanol induced ulcer

On the 5th day, the test samples were given 30 min before ethanol administration. After 30 min, ethanol (1 ml/200 g) was administered to every group. The animals were deprived of both food and water after administration of above test samples and ethanol. The animals were sacrificed after 1 h by cervical dislocation. The abdomen of rats were opened and stomachs were examined for ulcer index and percentage (%) protection.^[11,12]

Restrained stress induced ulcer

On the 5th day, all the groups were treated with the test samples 30 min before subjecting the animals to restraint stress ulcerogen. Each rat was placed in PVC pipe having diameter 5 cm. The limbs were putted together with adhesive tape so that the animal cannot move. At the end of 24 h animal was removed from the pipes and sacrificed using overdose of anaesthetic ether and each stomach was examined for ulcer index and percentage protection.^[13]

Modified pyloric ligated (shay) rats

Pre-treatment were given to animal groups for 5 days before subjecting to ulcerogen. After 48 h fasting period the rats were anaesthetized with anaesthetic ether. Pyloric ligation (PL) was applied by ligating the pyloric end of stomach. On same day, the test samples were given 30 min. p.o., in all groups before PL.^[11,14] After 4 h of surgery, rats were sacrificed with over dose of anaesthetic ether and gastric juice was collected for performing gastric secretion studies such as free, total acidity,^[15] pepsin,^[16] protein,^[17] total hexose,^[18] fucose,^[19] sialic acid,^[20] and hexosamine,^[21] then each stomachs were examined for ulcer index and percentage protection.

Ulcer score

Ulcers were scored according to Bhargava *et al.* (1988). 10 = shedding of epithelium, 20 = frank or petechial hemorrhage, 30 = one or two ulcers, 40 = many ulcers, 50 = perforated ulcers. Mean ulcer scores of each group were calculated, which was designated as the ulcer index and percentage protection was calculated as, ulcer index = $(C-T/C) \times 100$. Where, C = ulcer index in control group, T = ulcer index in treated groups.

Statistical Analysis

All the results were expressed as mean \pm standard error of the mean of 6 animals in each group. The results were analyzed statistically using one-way analysis of variance followed by Dunnett's comparison test, $P < 0.05$ was considered as significant, as compared to the respective control group. All calculation were performed using Graph pad Prism software version 5.01.

RESULTS

Phytochemical Screening

Phytochemical screening of the methanolic extract of *T. zeylanicus* showed the presence of carbohydrate, proteins, saponin, alkaloids, flavonoids, steroid, and triterpenoids. The SFTZ showed positive for foam and hemolytic tests. The thin-layer chromatography plate was observed in daylight the gray-blue spot with R_f value 0.15-2. Further SFTZ was used for anti-ulcer studies.

Acute Toxicity

The SFTZ was evaluated for acute toxicity in female rat as per the OECD Guidelines No.423. As the SFTZ did not show any mortality and toxicity up to dose of 2000 mg/kg, b.w. p.o. In female rat. Hence, SFTZ (75,150 and 300 mg/kg, p.o.) were selected for anti-ulcer activity.

Anti-ulcer Studies

Ethanol-induced ulcers

In ethanol induced ulcer model, [Figure 1], SFTZ at various doses like (75,150 and 300 mg/kg; p.o.) showed significant ($P < 0.01$) decrease in ulcer index and percentage inhibition was found as 64.09%, 68.19%, and 75.00%, respectively, as compared to control [Table 1]. The rat stomach mucosa from control group shows shedding of epithelium, reddening of gastric mucosa, petechial hemorrhage and perforated ulcer whereas the rat stomach from the standard group (ESO 10 mg/kg, p.o.) neither shows shedding of epithelium nor petechial hemorrhage nor perforated ulcer. SFTZ shows dose-dependent decrease in shedding of epithelium and petechial hemorrhages compared to standard group [Figure 1].

Restrained stress-induced ulcer

SFTZ in doses (75,150 and 300 mg/kg; p.o.) given daily for 5 days showed significant protection against the experimental ulcer induced by restrained stress [Table 1]. SFTZ (75,150 and 300 mg/kg, p.o.) was effective in lowering ulcer index with percentage protection of 53.12%, 56.25% and 62.49% respectively in restrained stress induced ulcer as compared to control.

Modified pyloric ligated rats

SFTZ in doses (75,150, and 300 mg/kg, p.o.) given daily for 5 days showed significant protection against the experimental ulcer induced by PL. SFTZ at various doses of (75,150 and 300 mg/kg, p.o.) and standard drug ESO (10 mg/kg, p.o.) showed dose-dependent significant percentage protection 40.00, 43.34, 56.66, and 78.44%, respectively as compared to control group [Table 1]. SFTZ in doses (75,150 and 300 mg/kg, p.o.) and ESO (10 mg/kg, p.o.) shows significant

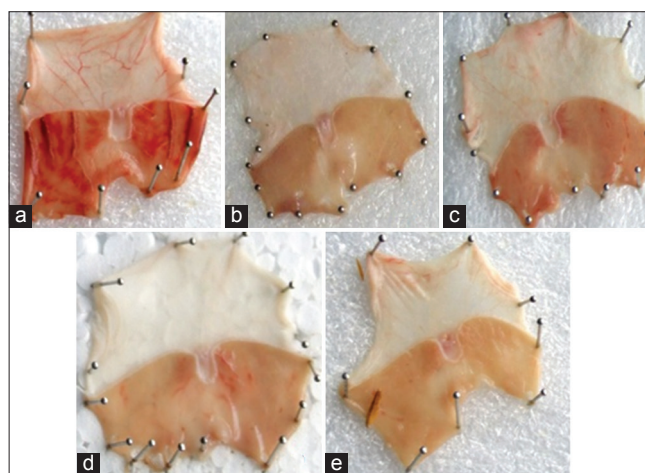


Figure 1: Stomach of rats (a-e). (a) - Stomach treated with 1 ml absolute ethanol, (b) - the stomach treated with esomeprazole 10 mg/kg and 1 ml of absolute ethanol p.o., (c-e) - treatment with 75, 150 and 300 mg/kg of Saponin (fraction) of *Trichopus zeylanicus* and 1 ml absolute ethanol, p.o., respectively

decreased free, total acidity, and pepsin activity as compared to control group ($^bP < 0.01$) and also shows significant increased in the level of total hexoses, hexosamine, fucose, sialic acid ($^bP < 0.01$) and also shows significant decrease in ($^bP < 0.01$). It shows significant increased ($^bP < 0.01$) in level of defensive mucin secretion in terms of Total carbohydrate: Protein (TC: P) ratio [Table 2].

DISCUSSION

Saponin mainly those of triterpene type like glycyrrhetic acid and carbenoxolone, have also been found as anti-ulcer agent, whose action is mediated by the formation of mucous on the gastric mucosa.^[22] Since saponins have been present in *T. zeylanicus*, show significant gastroprotective effect in ethanol, pyloric ligated rat and restrained stress induced ulcerogenesis. The incidence of ethanol induced ulcer is predominant in the glandular part of the stomach and was reported to stimulate the production of leukotrienes,^[23] mast cell secretory products; and reactive oxygen species^[24]

resulting in the damage of rat gastric mucosa.^[25] Ethanol-induced depletion of gastric wall mucus was prevented by SFTZ; it implies that a concomitant increase in prostaglandins^[26] or sulfhydryl compound^[27] contribute to protect the stomach from ethanol injury.

Stress is known to alter the physiological homeostasis of the organism and resultant breakdown in the adaptation processes in response to such extreme environmental demand, in fact, elicit various endocrinal and visceral response in a variety of experimental situation. Stress play an important role in etiopathology of gastroduodenal ulceration, increased in gastric motility, vagal overactivity,^[28] mast cell degranulation;^[29] decreased gastric mucosal blood flow,^[30] and decreased prostaglandin synthesis^[31] are involved in stress-induced ulcerogenesis. Changes in plasma corticosterone and gastric mucosal integrity are widely reported during stress. Various stress induced ulcer models reported that adrenergic, cholinergic and serotonergic mechanisms are facilitatory to stress ulcerogenesis, whereas central gamma-aminobutyric acid (GABA)-ergic mechanism and opioid receptors are

Table 1: Effect of SFTZ on ethanol induced ulcer, restrained stress induced ulcer and PL induced ulcer models

| Treatment group | Ulcer index (% protection) | | |
|-----------------|----------------------------------|----------------------------------|---------------------------------|
| Models | Ethanol-induced ulcer | Restrained stress induced ulcer | Pyloric-ligation induced ulcer |
| I | 73.33±8.433 | 53.33±4.216 | 50.00±6.32 |
| II | 13.33±2.108 ^b (81.82) | 11.67±1.667 ^b (78.44) | 10.78±2.23 ^a (78.44) |
| III | 30.00±4.472 ^b (64.09) | 25.00±4.428 ^b (53.12) | 30.00±6.83 ^a (40.00) |
| IV | 23.33±3.333 ^b (68.19) | 23.33±3.333 ^b (56.25) | 28.33±6.54 ^a (43.34) |
| V | 18.33±4.014 ^b (75.00) | 20.00±3.651 ^b (62.49) | 21.67±4.01 ^b (56.66) |

Values are expressed as means±standard error of the mean (SEM) ($n=6$), one-way ANOVA followed by Dunnett's test. ^a $P<0.05$, ^b $P<0.01$ compared with control group, SFTZ: Saponin fraction of *Trichopus zeylanicus*. Group-I: Control, Group-II: ESO (Esomeprazole) (10 mg/kg; p.o.), Group-III: SFTZ 75 mg/kg, p.o., Group-IV: SFTZ 150 mg/kg, p.o., Group-V: SFTZ 300 mg/kg, p.o., ANOVA: Analysis of variance, PL: Pyloric ligation

Table 2: Effect of Saponin fraction of *T. zeylanicus* (SFTZ) on biochemical parameters of gastric juice in PL induced ulcer in rat model

| Parameters | Group-I | Group-II | Group-III | Group-IV | Group-V |
|---------------------------------|------------|--------------------------|--------------------------|--------------------------|---------------------------|
| Gastric Juice (ml/100 g b.w.) | 3.75±0.16 | 2.33±0.06 ^b | 2.55±0.12 ^b | 2.48±0.12 ^b | 2.35±0.07 ^b |
| pH (Units) | 2.59±0.06 | 3.31±0.05 ^b | 3.25±0.03 ^b | 3.25±0.06 ^b | 3.36±0.05 ^b |
| Free acidity (MEq/l/100 b.w.) | 53.63±3.3 | 31.58±0.55 ^b | 36.25±0.42 ^b | 35.01±0.68 ^b | 32.58±0.49 ^b |
| Total acidity (mEq/l/100g b.w.) | 92.33±9.7 | 52.68±9.57 ^b | 67.45±1.22 ^b | 64.83±0.30 ^b | 61.67±1.01 ^b |
| Pepsin (µg/ml) | 37.90±2.6 | 19.61±1.97 ^b | 29.12±3.11 ^b | 23.35±1.03 ^b | 22.67±0.58 ^b |
| Total hexoses (µg/ml) | 136.40±2.1 | 232.6±10.38 ^b | 207.3±11.28 ^b | 197.9±13.27 ^b | 158.40±17.80 ^b |
| Hexosamine (µg/ml) | 213.7±7.57 | 347.8±16.63 ^b | 290.2±18.92 ^b | 276.0±13.90 ^a | 289.80±20.04 ^b |
| Fucose (µg/ml) | 59.10±3.74 | 24.76±0.79 ^b | 11.68±0.71 ^b | 11.78±0.93 ^b | 11.91±0.69 ^b |
| Sialic acid (µg/ml) | 80.03±2.16 | 87.57±1.04 ^b | 84.61±0.66 ^b | 84.75±0.31 ^b | 84.59±0.43 ^b |
| Total protein (µg/ml) | 687.5±8.93 | 555.5±1.23 ^b | 579.3±3.76 ^b | 564.6±1.55 ^b | 551.3±1.57 ^b |
| TC: P | 0.68±0.07 | 1.13±0.02 ^b | 1.02±0.04 ^b | 1.01±0.04 ^b | 1.01±0.05 ^b |

Values are expressed as means±standard error of the mean (SEM) ($n=6$), one-way ANOVA followed by Dunnett's test. ^a $P<0.05$, ^b $P<0.01$ compared with control group, Group-I: Control, Group-II: Esomeprazole (ESO) 10 mg/kg, p.o., Group-III: SFTZ 75 mg/kg, p.o., Group-IV: SFTZ 150 mg/kg, p.o., Group-V: SFTZ 300 mg/kg, p.o., Total carbohydrate: Protein (TC: P), SFTZ: Saponin fraction of *T. zeylanicus*, ANOVA: Analysis of variance, PL: Pyloric ligation

inhibitory in nature.^[13] We can conclude that SFTZ shows anti-ulcer activity in restrained stress induced ulcer model probably because of its central GABA-ergic mechanism.

In pylorus ligated animals, the genesis of ulceration is based on an increased presence of acid and pepsin in the stomach.^[14] Biochemical analysis of gastric juice for marker parameters such as total hexose, hexosamine, fucose, sialic acid, total carbohydrate, and protein will be highly useful in accessing the efficacy of anti-ulcer drug. The increased level of total hexose and sialic acid in the gastric mucosa may contribute towards its anti-ulcerogenic, and cytoprotective effects by increasing viscosity of gastric mucus in SFTZ treated animal at various doses^[32] and decrease in protein content also signifies decrease in leakage from mucosal cells, indicating increased mucosal resistance.^[33] An increased in (TC: P) ratio is also taken as a reliable index for mucin secretion.^[34]

From the phytochemical investigation, we can predict that SFTZ showed anti-ulcer activity due to the presence of saponins in the extract that can protect the rat stomach mucosa by various underlying mechanisms.

CONCLUSION

The action potential of SFTZ is positively found to be more active in alleviating the ulcer by chemical and physical induced models. The potency of anti-ulcer activity can be judged by the findings of promising action in all the various models for the screening. Every studied model has a different mechanism of action for their ulcer production. In case of ethanol induced ulcer model SFTZ showed protection against free radicals formed by ethanol which is responsible necrosis of gastric mucosa whereas of restrained stress induced ulcer SFTZ showed significant protection against ulcer may be due to reducing stress precipitating mechanism. In PL model the SFTZ may reduce acid secretion by inhibiting acid producing cells. All these animal models for anti-ulcer study may act through the specific kind of stress. The finding of this study could lead to further isolation of active saponins constituent with its pharmacological action.

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