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Anti-ulcer activity of stem bark of Shorea tumbuggaia

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Abstract

<u>Objective:</u> To study the anti-ulcer activity of stem bark of *Shorea tumbuggaia* in pylorus ligation method. <u>Materials and method:</u> Petroleum ether (60-80°), chloroform, ethanol and aqueous extract of *Shorea tumbuggaia* stem bark were tested for various preliminary phytoconstituents and were screened for anti-ulcer activities in pylorus ligation method in albino rats. Doses for the different extracts were selected based on the results of acute toxicity studies in mice. The effect was assessed by ulcer index, total acidity, free acidity, pH of gastric contents, serum calcium levels and serum alkaline phosphatase levels in pyloric ligation method. <u>Result:</u> Lipids, sterols, triterpenoids, tannins and phenol were found to be present in various extracts of *Shorea tumbuggaia* stem barks. The ethanolic extract of stem bark of *Shorea tumbuggaia* significantly raised the pH of gastric contents. It lowered the free acidity, total acidity and ulcer index as compared to control. It significantly raised the serum calcium levels and to control. It estimates the serum calcium levels and the serum calcium levels and the tehanolic extract of stem bark of *Shorea tumbuggaia* has significant anti-ulcer property.

Key words: Shorea tumbuggaia, anti-ulcer, pyloric ligation.

1. Introduction

Peptic ulcer is a common gastrointestinal disease and it affects particularly the working years of a patient's life and its social implications are considerable [1].

Peptic ulcer disease is defined as pathological lesions and ulcers of any portion of the gastrointestinal tract exposed to acid activated pepsin [2]. Peptic ulcer is a breach in the gastric

or duodenal epithelium associated with acute or chronic inflammation. Efforts have been made to find suitable alternative remedies from plant for the treatment of peptic ulcers. *Shorea tumbuggaia* family Dipterocarpaceae is a large deciduous tree.

The tree is distributed in the forests of Cuddopah, N.Arcot, and Chingleput Hills of South India [3]. Traditionally the tribal people in Sheshachalam hills use half a glass of stem bark decoction of *Shorea tumbuggaia* daily until the ulcer is cured [4]. In view of this folklore claim, the present study has been undertaken to evaluate in detail the anti-ulcer activity of stem bark of *Shorea tumbuggaia*.

2. Materials and methods

2.1 Plant material

The stem bark of *Shorea tumbuggaia* was procured from local areas of Tirumalla Hills and was identified and authenticated at Herbal Research Folklore Centre, Tirupati.

2.2 Preparation of extracts

A coarsely powdered, air-dried bark of *Shorea tumbuggaia* was subjected to hot continuous extraction (soxhlet) with petroleum ether (60-80°) in a soxhlet extractor [5]. After complete extraction, the solvent was distilled off and concentrated on a water bath to a dry residue. The marc was dried completely at 50°C and again loaded in the extractor and further extracted successively with chloroform and ethanol.

Finally, the marc was macerated with distilled water to obtain the aqueous extract. Each extract is concentrated by distilling off the solvent and then evaporating to dryness on the water-bath. The different extracts were subjected to qualitative chemical investigation and were taken for pharmacological studies.

2.3 Animals

Albino rats of either sex weighing 150-200g were selected for the pharmacological study. The study protocol was approved from Animal Ethical Committee of the Institution.

2.4 Drug treatment

Gum acacia 1% was used as a vehicle to suspend the various extracts. Six groups of animals having six rats in each group were used. The first group served as control and was given the vehicle (gum acacia 1%) orally. The second group received the standard drug ranitidine at a dose of (2mg/kg). The third, fourth, fifth and sixth groups received petroleum ether, chloroform, ethanolic and aqueous extracts respectively by oral route at a dose of 190, 179, 199 and 173 mg/kg.

2.5 Anti-ulcer studies

2.5.1 Pylorus Ligation Method [6]

Albino rats weighing 150-200 g were starved for 48 h having access to drinking water. During this time they were housed single in cages with raised bottoms of wide wire mesh in order to avoid cannibalism and coprophagy. Six animals were used per extract, six animals were used for standard drug and six animals were used as control.

Under ether anaesthesia, 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. The abdominal wall was closed by suturing it. The test compounds were given orally by gavage after the animals recovered from anaesthesia.

The animals were deprived of food and water post operatively and the animals were sacrificed after 19 h of pyloric ligation. Blood samples were withdrawn from the marginal tail vein and subjected to estimation of serum alkaline phosphatase and serum calcium. The stomachs were dissected out along the greater curvature and examined for lesions and the contents were collected. The mucosa was then washed and extent of ulceration was scored as per the system suggested by Kunchandy *et al.* [7].

The gastric juice collected from each stomach was centrifuged and its pH was measured. Free and total acidity were estimated titrimetrically with 0.1N NaOH using Topfer's reagent and phenolphthalein as indicator. Acidity of the gastric juice was expressed as mEq/L [8].

Ulcer index was calculated using formula: Ulcer Index = 10/x

Where, $X = \frac{\text{Total mucosal area}}{\text{Total ulcerated area}}$

Alkaline phosphatase is an enzyme capable of catalysing the hydrolysis of various phosphate esters at alkaline pH. Alkaline phosphatase activity has been reported to be increased in bone diseases, liver diseases and gastrointestinal lumen. The release of this enzyme has been suggested to play a role in tissue necrosis, associated with various models of gastrointestinal ulceration [9]. Alkaline phosphatase activity in serum is determined using Kind and King's method [10]. Serum calcium is estimated by Ortho Cresolphthalein Complexone (OCPC) method. [11,12]

2.6 Statistical Analysis

Results were expressed as mean \pm SE. The data was analysed by one-way ANOVA

followed by Dunnett's test at a level of significance p<0.01.

3. Results and discussion

Sterols were present in ethanolic extract, lipids in petroleum ether extract, sterols and triterpenoids in chloroform extract and tannins and phenols in aqueous extract as observed by the qualitative tests.

The results of *Shorea tumbuggaia* stem bark extracts are illustrated in Table 1. The severity of gastric ulceration in Pylorus-ligation model was assessed based on the means of ulcer index. The ulcer index in control animals was (4.51). Ethanolic extract (2.26) significantly reduced the ulcer index (P<0.01) as compared to control. The reduction in ulcer index by chloroform, aqueous and petroleum ether extract are 2.46, 3.12 and 3.29 respectively. Ranitidine, a standard anti-ulcer drug showed no ulcer production.

The serum calcium level in control animals is (9.62 mg/dl). Ethanolic extract (12.60 mg/dl)

Table 1.

Effect of different extract of *Shorea tumbuggaia* stem bark for anti-ulcer activity in pyloric ligation method

Treatment	Dose mg/kg	рН	Free Acidity mEq/litre	Total Acidity mEq/litre	Ulcer index Ul / L	Serum alkaline phosphate mg / dl	Serum calcium
Vehicle	1ml gum acacia (1%)	2.50 ± 0.035	$27.75\pm$ 0.28	46.6± 0.43	4.51± 0.14	47.96± 0.38	9.62± 0.09
Pet-ether Extract	190	$2.90^{*\pm}$ 0.016	22.83*± 0.33	43.0*± 0.99	3.29*± 0.01	34.92*± 0.12	11.43*± 0.04
Chloroform Extract	179	$3.68^{\pm}{0.008}$	18.08*± 0.15	36.91*± 0.83	2.46*± 0.03	29.75*± 0.16	10.61* ± 0.089
Ethanol Extract	199	3.75*± 0.013	16.13*± 0.19	34.08*± 0.15	$2.26^{*}\pm 0.05$	25.91*± 0.17	12.60*± 0.11
Aqueous Extract	173	3.44*± 0.027	19.83*± 0.16	40.4*± 0.23	3.12*± 0.03	32.67*± 0.009	9.79*± 0.05
Ranitidine	2	3.85*± 0.016	12.50*± 0.25	30.4*± 0.48	25.43*± 0.007	12.67*± 0.009	

n=6 *p<0.01 vs. control; values are in Mean ± SEM

showed highly significant increase (P<0.01) in serum calcium levels as compared to control. The increase in serum calcium levels by chloroform, aqueous and petroleum ether extracts is 10.61, 9.79 and 11.43 mg/dl respectively. The increase in serum calcium levels by standard drug Ranitidine is 12.67 mg/dl.

Serum alkaline phosphatase level is increased in damaged tissue. The serum alkaline phosphatase level in control animals is (47.96 Ul/L). Ethanolic extract (25.91 Ul/L) showed highly significant decrease (P<0.001) in serum alkaline phosphatase as compared to control. The decrease in serum alkaline phosphatase levels by chloroform, aqueous and petroleum ether extract is 29.75, 32.67 and 34.92 Ul/L respectively. When compared to ranitidine (25-43 Ul/L), ethanolic extract is equipotent in decreasing serum alkaline phosphatase levels.

In control animals, without any drug the mean pH is 2.5. All the four extracts showed rise in pH of gastric contents. Ethanolic extract showed maximum rise in pH (3.755) as compared to control. The rise in pH shown by chloroform, aqueous and petroleum ether extract are 3.68, 3.44 and 2.95 respectively. Ranitidine, a standard drug raised the pH to (3.85) which is statistically significant (p<0.01).

Gastric free acidity is increased to (27.75 mEq/ litre) in control animals due to pylorus ligation. Ethanolic extract (16.13 mEq/litre) showed significant decrease in free acidity (p<0.01) as compared to control. The decrease in free acidity by chloroform, aqueous and petroleum ether extracts are 18.08, 19.83 and 22.83 mEq/ litre respectively. When compared with Ranitidine, a known anti-ulcer drug, ethanolic extract is equipotent to Ranitidine (12.5 mEq/ litre) whereas; petroleum ether extract is less potent in decreasing gastric acidity.

Gastric total acidity is increased to (46.6 mEq/ litre) in control animals. Ethanolic extract (34.08 mEq/litre) showed highly significant decrease in total acidity (P<0.01) as compared to control. The decrease in total acidity by chloroform, aqueous and petroleum ether extracts are 36.91, 40.4 and 43.0 mEq/litre respectively. While decrease in total acidity by Ranitidine is 30.4 mEq/litre.

The ethanolic extract of *Shorea tumbuggaia* stem bark exhibited potent anti-ulcer activity. The activity may be attributed to one or more of the bioactive constituents present in the *Shorea tumbuggaia* stem bark. The results confirm the folklore claim for the stem bark of *Shorea tumbuggaia* as anti-ulcer.

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