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EVALUATION OF ANTIULCER ACTIVITY OF *TINOSPORA SINENSIS* LEAVES

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ABSTRACT

The antiulcer activity of ethanolic extract of *Tinospora sinensis* leaves (EETS) was investigated in pylorus ligation and ethanol induced ulcer models in experimental rats. In both models the common parameter determined was ulcer index. Ethanolic extract of *Tinospora sinensis* at a dose of 150 and 300mg/kg produced significant inhibition of the gastric lesions induced by pylorus ligation induced ulcer and ethanol induced gastric ulcer. The extract (150mg/kg and 300mg/kg) showed significant ($p<0.05$) reduction in gastric volume, free acidity and ulcer index as compared to control. This present study indicates that EETS have potential anti-ulcer activity in both models. These results may further suggest that the extract was found to possess antiulcerogenic as well as ulcer healing properties, which might be due to its antisecretory activity.

INTRODUCTION

Peptic ulcer is one of the most common gastrointestinal diseases [1]. The exact causes of peptic ulcer disease are not known but it may be result from an imbalance between acid-pepsin secretions and mucosal defence factors [2]. Peptic ulcer disease occurs mainly due to consumption of NSAIDS, infection by H.pylori, stress or due to pathological condition such as Zollinger-Ellison Syndrome [3]. *Tinospora sinensis* (syn: *Tinospora malabarica*) is a plant that grows almost throughout India and other South East Asian countries and belongs to the family Menispermaceae.

The stem of this plant has great therapeutic value traditionally in treating debility, dyspepsia, fever, inflammation, syphilis, ulcer, bronchitis, jaundice, urinary disease, skin disease and liver disease [5] and known for its adaptogenic and immunomodulatory properties [6]. The aqueous and alcoholic extracts of this species are reported to have many biological potential, such as anti-inflammatory, anti-diabetic, hepotoprotective, and immuno modulatory, and adaptogenic [7]. Previous phytochemical investigations have discovered that this species contains steroids, flavonoides and alkaloids. In India, cassava is used for the treatment of ringworm, tumor, conjunctivitis, sores and abscesses [4-6]. So for no systematic study has been reported for antiulcer properties of *Tinospora sinensis* leaf extracts. In the present study effort has been made to establish the scientific validity to the anti ulcer property of *Tinospora sinensis* leaves extracts using pyloric ligation and ethanol induced ulceration models in albino rats.

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MATERIALS AND METHODS

Plant materials

The leaves of *Tinospora sinensis* were collected in

Tirumala hills, Chittoor district, Andhra Pradesh in the month of Dec 2012. The specimen was identified and authenticated by Prof. Dr. P. Jayaraman, Director, Plant Anatomy Research Center (PARC), Tambaram, Chennai. The specimen was deposited to herbarium of Bhaskar Pharmacy College, Moinabad, Rangareddy-500075, Andhra Pradesh. After authentication, fresh leaves collected in bulk from plants, washed, shade dried and then milled to a coarse powder by a mechanical grinder.

Preparation of extract

The powders of dried leaves were packed in to soxhlet column and extract with ethanol. The extract was filter through a Whatman filter paper no.1 and concentrated under reduced pressure (yield of extract was 9.40% with respect to dry material). Just prior to use, the substance was dissolved in physiological saline solution.

Animals

The study was conducted on male Wister rats (175-200gm) housed in polypropylene cages under standard conditions of temperature (22 ± 2°C), relative humidity (60 ± 5%) and light (12h light/dark cycle) were used. They were fed with standard diet and water. The food was withdrawn 18 hours before the experiment but allowed free access of water. All animal experiments were carried out in accordance with the guidelines of CPCSEA.

Acute oral toxicity studies

Acute toxicity was carried out according to Organization of Economic Co-Operation and Development (OECD) guidelines [7], No mortality was observed and all the test doses were found to be safe.

Pyloric ligation in rats

The animals were divided into 5 groups, each consisting of six rats. Control group received distilled water only. Second group of rats are pyloric ligated. Third and fourth groups received EETS in a dose of 150 and 300 mg/kg. The fifth group of animals received Ranitidine in the dose of 20mg/kg as a reference drug for ulcer protective studies. After 45 min of the treatment, pyloric ligation was done by ligating the pyloric end of stomach of rats of respective groups under ether anesthesia at a dose of 35mg/kg of body weight. Ligation was done without causing any damage to the blood supply of the stomach. Animals were allowed to recover and stabilize in individual cages and were deprived of water during post-operative period. Rats were sacrificed after 4hr of surgery and ulcer scoring was done. Gastric juice was collected and gastric secretion studies were performed according to the standard procedure [8].

Ethanol induced ulcer model

The ulcer was induced by administering absolute ethanol (1ml/200g). All the animals were fasted for 36 hours and then ethanol was administered to induce ulcer. The animals were divided into five groups, each consisting of six rats. The control group received distilled water, second group received ethanol. Third and fourth groups received EETS in a dose of 150 and 300 mg/kg. The fifth group of animals received Ranitidine in the dose of 20 mg/kg as a reference drug. They were kept in specially constructed cages to prevent coprophagia during and after the experiment. The animals were anaesthetized 1 hr later with anaesthetic ether and stomach was incised along the greater curvature and ulceration was scored. A score for the ulcer was studied to pyloric ligation induced ulcer model [9].

Scoring of ulcers

Normal stomach	-0
Red coloration	-0.5
Spot ulcer	-1
Hemorrhagic streak	-1.5
Ulcers (< 2mm)	-2
Ulcers (>2 < 4 mm) perforation	-3
Ulcers (< 4mm)	-4

Mean ulcer score for each animal was expressed as ulcer index. The percentage of ulcer protection was determined by

$$\% \text{ of ulcer protection} = \frac{\text{Control mean ulcer index} - \text{Test mean ulcer index}}{\text{Control mean ulcer index}} \times 100$$

Determination of free acidity

$$\text{Acidity} = \frac{\text{Volume of sodium hydroxide} \times \text{Normality} \times 100\text{mEq/L}/100\text{g}}{0.1}$$

Stastical analysis

The values are represented as mean ± S.E.M, and Stastical significance between treated and control groups was analyzed using of one way ANOVA, followed by Dennett's test where $P<0.05$ was considered stastically significant.

RESULTS

Pyloric ligation induced gastric ulcer

In pyloric ligation induced ulcer model, oral administration of EETS in two different doses showed significant reduction in ulcer index, gastric volume, free acidity, total acidity compared to the central group. EETS exhibited a protection index of 68.7% and 81.2% at the dose of 150 and 300 mg/kg respectively, whereas Ranitidine as reference standard exhibited a protection index of 85.2% (Table 1).

Ethanol-induced gastric ulcer

In control animal, oral administration of absolute ethanol produced characteristic lesions in the glandular

portion of rat stomach which appeared as elongated bands of thick, blackish red lesions. EETS has shown significant protection index of 67.7% and 71.2% with the dose of 150 and 300 mg/kg respectively whereas Ranitidine as reference standard showed protection index of 79.6% (Table 2).

Table1. Effect of EETS on various parameters in pyloric ligation induced gastric ulcers

Group	Treatment	Ulcer index	Free acidity meq/ltr	P^H of gastric juice	Gastric juice	Total acidity meq/ltr	Protection (%)
I	Normal (distilled water)	---	41.3 ± 0.3	5.41 ± 0.3	3.8 ± 0.4	62.3 ± 0.2	---
II	Control (pyloric ligation)	14.2 ± 1.2	95.6 ± 1.4	2.51 ± 0.2	8.2 ± 0.2	112.5 ± 0.2	---
III	EETS (150mg/kg)	4.5 ± 0.5	43.7 ± 0.3	4.87 ± 0.2*	5.3 ±1.2	75.3 ± 0.4	68.7 %
IV	EETS (300mg/kg)	2.8 ± 0.4*	39.8 ± 0.2*	5.51 ± 0.4*	4.2±0.4*	61.7 ± 0.6*	81.2%
V	Ranitidine (20mg/kg)	2.2 ± 0.5*	37.4 ± 0.2*	5.71 ± 0.4*	3.9 ± 0.2*	60.1 ± 1.4*	85.2%

Table 2. Effect of EETS on various parameters in ethanol induced gastric ulcers

Group	Treatment	Ulcer index	P^H of gastric juice	Protection (%)
I	Normal (distilled water)	---	5.42 ± 0.3	---
II	Control (pyloric ligation)	12.3 ± 0.2	2,83 ± 0.6	---
III	EETS (150mg/kg)	4,3 ± 0.5	3.68 ± 0.6	67.7%
IV	EETS (300mg/kg)	3.6 ± 0.4*	4.86 ± 0.7*	71.2%
V	Omeprazole (20mg/kg)	2.7 ± 0.4*	5.62 ± 0.7*	79.6%

Values are expressed as mean ± SEM of observations, Statistical comparisons as follows: Significant *P <0.005 compared to control group.

DISCUSSION

The etiology of peptic ulcer is unknown in most of the cases, it is generally accepted that gastric ulcer results from an imbalance between aggressive factors and the maintenance of the mucosal integrity through the endogenous defence mechanism [10]. Different therapeutic agents are used to inhibit the gastric acid secretion or to boost the mucosal defence mechanisms by increasing mucosal production, stabilizing the surface epithelial cells or interfering with the prostaglandin synthesis [11]. The prostaglandins can provide gastric cytoprotection in rats against strong necrotizing irritants without reducing gastric acid secretion [12].

The causes of gastric ulcer by pyloric ligation are believed to be due to stress induced increase in gastric hydrochloric acid secretion and/or stasis of acid and the volume of secretion is also an important factor in the formation of ulcer due to exposure of the unprotected lumen of the stomach to the accumulating of acid. The ligation of the pyloric end of the stomach causes accumulation of gastric acid in the stomach. This increase in the gastric acid secretion causes ulcers in the stomach. The lesions produced by this method are located in the lumen region of the stomach [13].

Ethanol induced gastric lesion formation may be due to stasis in gastric blood flow which contributes to the development of the haemorrhage and narcotic aspects of tissue injury [14]. Alcohol rapidly penetrates the gastric mucosa apparently causing cell and plasma membrane damage leading to increased intra cellular membrane permeability to sodium and water. The massive intracellular accumulation of calcium represents a major step in the pathogenesis of gastric mucosal injury. This leads to cell death and exfoliation in the surface epithelium [15].

In the present study EETS showed protection against gastric lesions in the experimental rats, reduced gastric volume, free acidity, total acidity and ulcer index thus showing the anti-secretory mechanism involved in the extracts for their anti-ulcerogenic 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 [16].

The protection of EETS against characteristic lesions may be due to both reductions in gastric acid secretion and gastric cycloprotein or enhancement of the mucosal barrier through the increase production of prostaglandin and this may be due to the presence of glycosides. Further studies are needed for their exact mechanism of action on gastric acid secretion and gastric cytoprotection.

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REFERENCES

1. Dandiya PC, Kulkarni SK. (2005). Introduction to Pharmacology. Vallabh Prakashan, New Delhi, 247.
2. Padmaja U. (2005). Textbook of medical Pharmacology. CBS publishers, New Delhi, 317.
3. Mohammed A, Ravi Kumar J, Santosh HY and Nagashruthi MH. (2008). Antiulcer activity of anisochilus carnosus leaf extracts in pylorus ligation rats. *Indian Drugs*, 45(12), 979.
4. Akhar MS, Zafar I, Khan MN and Muhammad L. (2000). Anthelmintic activity of medicinal plants with Particular reference to their use in animals in indo Pakistan sub continent. 38, 99-107.
5. Wealth of India. (1976). Raw materials: Publication and Information Directorate, CSIR, New Delhi, X, 251.
6. Kirtikar KR, Basu BD. (1993). Indian medicinal plants. International Book Distributors, Dehradune, India. 77.
7. Ecobion DJ. (1997). The basis of toxicity testing. CRS Press New York, 43-49.
8. Shay H, Komarav SA, Fele SS, Merarnze D, Gruenstin H and Siplet H. (1975). A simple method for uniform production of gastric ulceration in rats. *Gastroenterol*, 5, 43-61.
9. Mahmod AA. (2005). Antiulcer and gastrprotective effect of honey in combination with Trigonella foenum graecum seeds extracts on experimental gastric ulcer in rats. *Int J Mol Adv Sci*, 1, 225.
10. Hikino H. (1985). Recent research on oriental medicinal plants in economic and medicinal plants, Academic press London, 53.
11. Tan PV, Dimo T and Dongo E. Effects of methanol cyclohexane and methylene chloride extracts of Bidens pilosa on various gastric ulcer models in rats. *J. Ethanopharmacol*, 73, 415-421.
12. Yamamoto K, Kakegawa H, Matsumto H, Sudo T and Satoh T. (1992). Gastric cytoprotective antiulcerogenications of hydroxychalcones in rats. *Planta medica*, 58, 389-393.
13. Dhuley JN. (1991). Protective effect of Rhinax a herbal formulation against physical and chemical factors induced gastric and duodenal ulcers in rats. *Indian J Pharmacol*, 31, 128-132.
14. Soll AH (1990). Pathogenesis of peptic ulcers and implications for therapy. *New Eng J Med*, 322, 909-16.
15. Surendra S. (1999). Evaluation of gastric anti ulcer activity of Fixed oil of tulsi and possible mechanism. *Indian J Exp Biol*, 36(3), 253-257.
16. Goel RK and Bhattacharya SK. (1991). Gastro duodenal mucosal defence and mucosal protective agents. *Indian J Exp Biol*, 29, 701-714.