

EVALUATION OF ANTIULCER ACTIVITY OF ETHANOLIC EXTRACT OF LEAVES OF VITEX NEGUNDO ON PYLORUS LIGATURE INDUCED AND ASPIRIN INDUCED ULCER IN ALBINO RATS

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ABSTRACT

Objective: The aim of the present study is to evaluate the anti-ulcer activity of Ethanolic extract of *Vitex negundo* in pylorus ligation and aspirin induced gastric ulcers in rats.

Methods: The antiulcer activity was studied in rats in which gastric ulcer was induced by oral administration of aspirin (200mg/Kg) and by pylorus ligation method. The animals used for the experiment were divided into 4 groups for each model, 6 rats for each group. In pylorus ligation model, all groups of rats were pre-treated with test drugs, Group-I(control) received 2%gum acacia-2ml/100g, Group-II (standard) received Ranitidine (60mg/kg.) and group-III, IV were treated with Ethanolic extracts 150mg/kg, 300mg/kg. respectively orally 30mins prior to pylorus ligation. The antiulcer activity of Vitex was assessed by determining and comparing gastric volume, free acidity, total acidity, pH, percentage of ulcer protection, , ulcer index. In aspirin induced ulcer model, all groups of rats were treated with test drugs for 7 days prior to Aspirin induced ulcer. Animals were divided into 4 groups and treated with drugs as in above model. After 7 days of treatment, animals were fasted for 24 hrs. Ulcers were produced by administration of Aspirin (200mg/Kg) on the day of sacrifice. The animals were sacrificed 4 hours later and stomachs were open along the greater curvature and ulcers were graded. Percentage of ulcer protection, ulcer index were observed and calculated.

Results: The extract of Vitex negundo in pylorus ligation model, it decreased the ulcer index (1.66) and there was a decrease in total gastric acid and free acid ($p < 0.0001$), and increases the pH value ($p < 0.0001$) and also reduces the total gastric volume ($p < 0.0003$), increases the percentage of ulcer protection (61.66%). In aspirin induced ulcer model, it decreases the ulcer index (10.66) and increases the ulcer protection (72.09%).

Conclusion: The ethanolic extract of vitex negundo was clearly shows a protective effect against total acid, free acid, gastric volume and ulcer index and also increases pH and percentage of protection against ulcers in both models.

Keywords: Vitex negundo, Gastric protection, Aspirin, Ulcer.

INTRODUCTION

Peptic ulcer remains a prevalent condition affecting up to 10% of the population and is responsible for considerable morbidity and loss of work time [1]. The high incidence and chronicity of the suffering and decreased ability to work associated with it, has made peptic ulcer an important health problem. An ulcer is thought to develop when the equilibrium is disturbed either by enhanced aggressiveness or by lessened mucosal resistance [2]. Drugs that affect acid and enhance defensive mechanisms are available. Of late, the concept is now changing from chemical pH to microbial Hp (pH= acidity, Hp= H.pylori)[3]. Flood gates of research on Helicobacter pylori actually opened up when Marshall and Warren in 1984 demonstrated the association between this organism with active chronic gastritis and peptic ulcer[4]. Long term use of NSAIDs can also cause gastric ulcer. Treatment cost is estimated more than 2-4\$ billion per year. So most of the ulcers heal by using synthetic drugs. After 6-8 weeks there is a problem of recurrence of side effects. Therefore people prefer natural product drugs for disease treatment. Over 3 quarters of the world population relieved by plants and plant extracts for health care. More than 30% of the entire plant species at one time or other has been used for medicinal purpose [5]. *Vitex negundo* is an important medicinal plant with potent anti-inflammatory activity. It contains the various chemical classes such as alkaloids, tannins, flavonoids, carbohydrates. Vitex negundo, commonly known as the five-leaved chaste tree, is a large aromatic shrub with quadrangular, densely whitish, tomentose branchlets. The major constituents of the leaf oil were α -pinene (35.9%), limonene (12.2%) and

bicyclogermacrene (9.5%), while the fruit oil contained α -pinene (31.7%), bicyclogermacrene (14.5%) and limonene (11.5%) and the flower oil contained α -pinene (14.7%), bicyclogermacrene (8.3%) and limonene (5.8%). The leaves are reported to show anti-inflammatory, anti-ulcer, anti-oxidant, and hepatoprotective [6]. Hence the present study was aimed to investigate the anti ulcer effect of vitex negundo in experimental animal models.

MATERIALS AND METHODS

Plant material

Fresh green leaves of Vitex negundo popularly known as vaavili were obtained in sufficient quantity from local forest in Nandyal, AP in August 2012. They were carefully washed to remove dust particles and other foreign materials and dried in shaded area.

Preparation of alcohol extract

The dried fine powder of the Vitex negundo was weighed on balance 30g. and taken into the sac like cloth material and placed in the Soxhlet basket. 300ml of ethyl alcohol was taken as solvent into the Soxhlet flask. Cold tap water must flow through the inlet and outlet of the condenser. The Soxhlet apparatus kept running for 24hours for proper extraction. The extract laden solvent falling from the Soxhlet basket is dark in colour and it becomes clearer, that indicates the extraction process is finished. At the end of the extraction process the solvent is then evaporated and the remaining mass is measured [7].

Table 1: The percentage yields are calculated as mg per gm dried powder.

S. No	Weight in gm.			Percentage Yields
	Solvent	Dry Momordica Powder	Extract	
1.	Ethyl Alcohol	30gm.	5gm.	16.6%

The yield of the ethyl alcohol extract is 16.6%. The extract was suspended in 2ml of 2% Gum acacia and used for the oral.

Experimental animals

The animals were supplied from Sainath Agencies, Hyderabad, AP, India. They were randomly distributed into groups and housed in cages (6/cage) and kept under standard conditions at $26 \pm 2^\circ\text{C}$ and

relative humidity 44-56% and 10 hours light: 14 hrs dark cycles each day for 1 week before and during the experiments. All animals were fed the standard rodent pellet diet and water. So this study was cleared by institutional animal ethical committee.

Experimental design

The animals used for the experiment were divided into 4 groups for each model, 6 rats for each group.

In Pylorus ligation model

All groups of rats were pre-treated with test drugs, Group-I (control) received 2% gumacacia-2ml/100g, Group-II (standard) received Ranitidine (60mg/kg) [8] and group-III, IV were treated with Ethanolic extracts 150mg/kg, 300mg/kg. respectively orally 30mins prior to pylorus ligation. After giving drugs all group of animals were anaesthetized with ether and lower abdominal wall was opened and pyloric end was ligated. Then abdominal wall was closed with interrupted sutures. During this procedure strict aseptic conditions were maintained to prevent infections. After 4 hours of pylorus ligation, animals were sacrificed and stomach was opened along with the greater curvature and total gastric contents was collected and centrifuged. The antiulcer activity of Vitex was assessed by determining and comparing gastric volume, free acidity, total acidity, pH, percentage of ulcer protection, ulcer index.

In Aspirin induced ulcer model, all groups of rats were treated with test drugs for 7 days prior to Aspirin induced ulcer. Animals were divided into 4 groups and treated with drugs as in above model. After 7 days of treatment, animals were fasted for 24 hrs. Ulcers were produced by administration of Aspirin (200mg/Kg) [9] on the day of sacrifice. The animals were sacrificed 4 hours later and stomachs were open along the greater curvature and ulcers were graded. Percentage of ulcer protection, ulcer index were observed and calculated.

Statistical analysis

The results obtained were expressed as Mean±SEM and were analyzed by the application of One-way Analysis of Variance (ANOVA)

RESULTS

Pylorus ligation method

Extract of *Vitex negundo* (150mg/Kg, 300mg/Kg) treated animals has showed significant reduction in Ulcer Index, Volume of Gastric juice ($p<0.001$), total acid and free acid ($p<0.001$) and also it increases pH ($p<0.001$), percentage protection (61.66%) when compared with control group. Standard drug treatment with ranitidine (60mg/Kg) also showed significant reduction in Ulcer Index, Volume of Gastric juice, total acid and free acid and also increases in pH and percentage of protection (92.37%).

Table 2: Effect of Vitex negundo on pylorus ligation ulcer model (n=6, Mean±SEM)

Groups	Treatment	Dose	Gastric contents (Mean±SEM)				Ulcer index	% of ulcer protection
			Vol. of gastric juice	Total acid	Free acid	pH		
1	Control-2% GA	2ml/100g	4.70±0.34	94.33±3.59	32.33±1.56	2.03±0.12	4.33	0%
2	Standard-ranitidine	60mg/kg	2.98±0.15***	53.33±4.07**	19±1.36*	4.79±0.14**	0.33	92.37%
3	Test-T ₁ -extract	150mg/kg	4.15±0.27*	79.83±4.14*	27.83±0.98**	3.46±0.26*	2.8	35.33
4	Test-T ₂ -extract	300mg/kg	3.18±0.21**	63±2.54**	25.50±1.89*	3.98±0.13**	1.66	61.66

* $P<0.05$, ** $P<0.01$, *** $P<0.001$ compared to Control.

Aspirin induced ulcer model

Extract of *Vitex negundo* (150mg/Kg, 300mg/Kg) treated animals has showed significant reduction in Ulcer Grades ($p<0.001$), Ulcer Index (10.66) with high dose (300mg/Kg) and increase in

percentage of protection (72.09%) when compared to control group. Standard drug treatment with ranitidine (60mg/Kg) also showed significant reduction in Ulcer Index and increase in percentage of protection (95.65%).

Table 3: Effect of vitex negundo in Aspirin induced ulcer model

Groups	Treatment	Dose	Ulcer grade (Mean±SEM)	Ulcer index	% of ulcer protection
1	Control-2% GA	2ml/100g	33.33±3.33	38.2	0
2	Standard-ranitidine	60mg/kg	1.66±1.66**	1.66	95.65
3	Test-T ₁ -extract	150mg/kg	26.66±2.10*	27.99	26.72
4	Test-T ₂ -extract	300mg/kg	10±4.47.	10.66	72.09

* $P<0.05$, ** $P<0.01$, *** $P<0.001$ compared to Control.

DISCUSSION

In spite of tremendous development in the field of synthetic drugs during recent era, they are found to have some or other side effects, whereas plant products or homeo drugs still hold their own unique place by the way of having no side effects. Peptic ulcer disease is a chronic inflammatory disease characterized by ulceration in the upper GI [10]. The pathophysiology of ulcers is due to an imbalance between aggressive factors (acid, pepsin, H.pylori and NSAIDs) and local mucosal defensive factors (mucous, bicarbonate, blood flow and prostaglandins). The integrity of gastro duodenal mucosa is maintained through a hemostatic balance between these aggressive and defensive factors. The major cause of gastric ulcer is the chronic use of NSAIDs. Therapeutic & adverse effects of NSAIDs have been attributed to the ability of these drugs to inhibit the action of cyclooxygenase (COX). COX is responsible for the synthesis of prostaglandins that normally inhibit acid secretion, as well as having a protective effect on gastric mucosa. Infection of the stomach mucosa with H.pylori—a gram negative spiral shaped bacterium is now generally

considered as the major cause of gastro intestinal ulcers. Treatment includes H₂-receptor antagonists (Cimetidine), proton pump inhibitors (Omeprazole) and cytoprotectives (Misoprostol). Antacids like Aluminium hydroxide & magnesium hydroxide are used often to neutralize excess gastric acidity in the stomach. Due to problems associated with recurrence after treatment, there is a need to seek an alternative drug against gastro intestinal ulcers [11]. The present investigation demonstrated the efficacy of *vitex negundo* plant extract against gastric ulceration induced by two experimental models viz.. Pylorus ligation induced gastric ulceration and Aspirin induced ulceration.

In pylorus ligation model, the plant extract of *vitex negundo* produces a decrease in the ulcer number, total gastric volume, total acid, free acid and increases the pH and percentage of ulcer protection (61.66%) and in standard drug (Ranitidine) treated animals the percentage of ulcer protection (92.37%) when compared with control group (0%). High dose of extract (300mg/Kg) more active than low dose (150mg/Kg) but less active than standard drug (Ranitidine).

In Aspirin induced ulcer model, the plant extract of *vitex negundo* produces a decrease in the ulcer number and increase in percentage of ulcer protection (72.09%) and with standard drug the percentage of ulcer protection (95.65%) when compared to control group (0%). High dose of extract (300mg/Kg) more active than low dose (150mg/Kg) but less active than standard drug (Ranitidine).

The anti ulcer property of *vitex negundo* in both the experimental models explained above, is due to presence of flavonoids, terpenoids, tannins, and triterpenes. The triterpenes are known as an anti-ulcer agent and their action has been mentioned to be due to activation of cellular proteins, reduction of mucosal prostaglandin metabolism, cytoprotective actions and reduction of gastric vascular permeability and remaining compounds have been shown to scavenger free radicals [12-14]. The results in the present study seem to provide support for the use of *vitex negundo* as an anti-ulcer drug in folk medicine. Therefore, also in view of its large use in India further investigations are needed to know about its anti-ulcer activity in human beings.

CONCLUSION

The present study indicates that the plant *vitex negundo* has potential anti-ulcer activity against pylorus ligation and Aspirin induced ulcers in experimental animals. So this activity of plant probably due to the compounds present such as Flavonoids and triterpenes. So the plant *vitex negundo* uses for both Ayurvedic and Modern drug development areas because of its phyto-medicinal uses.

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