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ANTI -ULCER ACTIVITY OF ETHANOLIC EXTRACT OF *DIOSPYROS VIRGINIANA* IN RATS

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ABSTRACT

Ulcer disease is one of the common diseases in the world. Treatment of ulcer with synthetic drugs such as proton pump inhibitors, H_2 receptor antagonists and other non-steroidal anti-inflammatory drugs has shown adverse effects, relapses, drug interactions. Medicinal plants containing active chemical constituents are useful in prevention and treatment of various diseases. Literatures suggest that herbal formulations of medicinal plants are considered to be potential source for the treatment of ulcers. This study was focused to evaluate the protective activity of *Diospyros virginiana* leaf and bark ethanolic extracts against HCL-ethanol induced ulcers. This plant both extracts significantly reduced the ulceration induced by acid alcohol when compared to that of standard ranitidine.

KEYWORDS: *Diospyros virginiana,* antiulcer, ethanolic extracts ranitidine.

INTRODUCTION

Ulcer disease is the term used to describe a heterogeneous group of condition with ulcerations. It is characterized by the disruption of the mucosal integrity of the esophagus, stomach, or duodenum.[1,2] As the most common gastrointestinal disturbance, it affects 10%-15% of the population at any one time. Ulcers are primarily caused by an imbalance between some endogenous aggressive and protective factors in the stomach such as acid-pepsin secretion, integrity of the mucosal barrier, mucus secretion, blood flow, cellular regeneration, prostaglandins, and growth factors.[3] Acid, peptic activity and a collapse of mucosal defense

mechanism have been implicated in the genesis of gastro-duodenal ulcers. Efforts are being made to find a suitable agent for the treatment of peptic ulcers and ulceral diseases. The extracts and compounds from medicinal plants and other natural products have become the widely acceptable source of therapeutic agents for the treatment of peptic ulcers.[4]

Diospyros virginiana is a persimmon species commonly called the American Persimmon, Common Persimmon, Eastern Persimmon, "'Simmon", "Possumwood", or "Sugar-plum". This is a well-known indigenous trees[5], growing in woods and fields. The parts of the *D.virginiana* have a long history of use in the new world. Persimmon have been used medicinally as an astringent and antiseptic and for the treatment of uterine hemorrhage, diarrhea and dysentery, diphtheria, dropsy, fevers, gonorrhea, hemorrhoids, syphilis and thrush. Persimmons have been used to lubricate the lungs and strengthen the spleen and pancreas. They improve energy and contain enzymes that help damaged cells and foreign microbes be broken down. Persimmons have a special affinity for the large intestines and heart.[6] Persimmons have been also used to treat bronchitis, catarrh, cough, , goiter, hangover and hiccoughs. The bark has been used in intermittent and both it and the unripe fruit have been beneficial in various forms of disease of the bowels, chronic dysentery, and uterine hemorrhage; used in infusion, syrup, or vinous tincture.[7] This study reports the antiulcer activity of leaf and bark ethanolic extracts of *Diospyros virginiana* using experimental models in rats.

MATERIALS AND METHODS

Plant collection and preparation of the extract

D.virginiana belongs to the family *Ebenaceae* was collected from Coonoor, Nilgiris District, Tamil Nadu, India and identified by the special key given Cambell flora.[8] The leaf and bark of *D.virginiana* were washed with sterile distilled water. After, the leaves and bark were shade dried and powdered by using pestle and mortar. 25g of powder was filled in the thimble and extracted successively with ethanol using a Soxhlet extractor for 48 h. The extracts were concentrated using rotary flash evaporator and preserved at 5°C in airtight bottle until further use. The ethanolic extracts of the plant was diluted with distilled water and was administered orally to mice.

Experimental design

The rats were divided into five groups each comprising of six rats (both sex) each. Gastric ulcers were induced in rats by administrating absolute acid alcohol (0.3M Hydrochloric acid

in 60% ethanol). They were kept in specially constructed cages to prevent coprophagia during and after the experiment. The animals were anaesthetized 1 h later with anaesthetic ether and the stomach was incised along the greater curvature and ulceration was scored. Scores for ulcer was studied similar to pyloric ligation induced ulcer model.[9]

The experimental design given below has been followed for the present study.

Group I: Normal control

Group II: Ulcer induced disease control- ethanol-acid (25 mL/Kg of 0.3 M HCl in 60 % ethanol)

Group III: Ulcer induced + *Diospyros virginiana* bark (500mg/kg)

Group IV: Ulcer induced + *Diospyros virginiana* leaves (500mg/kg)

Group V: Ulcer induced + received ranitidine 32/mg Kg

After the experimental period animals were sacrificed by cervical decapitation. Blood was collected. Liver tissue was dissected out and washed in ice-cold saline. Liver tissues were homogenized in 0.1M phosphate buffer (pH 7.4) and used for studying various parameters. Mean ulcer score for each animal was expressed as ulcer index.

Calculation of ulcer index and percentage inhibition

$U1 = (UN + US + UP)\ X10^{-1}$

Ulcer index of each animal was calculated by adding the values and their mean values were determined and percentage inhibition was calculated.

$$\text{Percentage inhibition} = \frac{UA_{\text{control}} - UA_{\text{treated}}}{UA_{\text{control}}} \times 100$$

Determination of gastric pH and acidity

The gastric contents were collected in a test tube and centrifuged at 3000 revolutions per minute (rpm) for 10 minutes. The volume of supernatant was measured and expressed as ml/100 g body weight. The pH of the supernatant was measured using digital pH meter. An aliquot of 1.0 ml of gastric juice was pipette out to a 50 ml conical flask and 2/3 drops of Topfer's reagent were added to it and titrated with 0.01 N Sodium Hydroxide (NaOH) until all traces of the red color disappeared, and the color of the solution turned yellowish orange. The volume of 0.01N NaOH was noted, which corresponded to the free acidity. Then 2-3 drops of phenolphthalein were added and titration was continued until a permanent

pink color was developed. The volume of total alkali consumed was noted which corresponded to the total acidity.[10]

RESULTS AND DISCUSSION

Antiulcer study of ethanolic extracts of *Diospyros virginiana* leaves and bark were performed as per the standard methods. The effect of *D.virginiana* on acid alcohol induced ulceration was studied and the results were tabulated in table-1. For antiulcer activity, volume of gastric juice, pH, total acidity, free acidity, ulcer lesion and ulcer index were estimated both from control and tested animals. *D.virginiana* both extracts significantly reduced the ulceration induced by acid alcohol. Ulceration due to administration of acid alcohol is shown in the stomach section of albino rats. The gastric damage as thick red lines and lesions as red areas were observed in the stomach. A significant ($P< 0.001$) reduction in volume of gastric juice was observed in both leaves, bark extracts and ranitidine treated animals when compared to control. Similarly, administration of drug and leaves, bark extracts elevated the pH level of gastric juice over control.

Gastric ulceration has been attributed to various causes such as stress, hormones, drugs, alcohols, smoking and ligestion of certain foods.[11] In gastrointestinal disorder, ulcer requires a well targeted therapeutic strategy. A number of drugs including proton pump inhibitors and H_2 receptor antagonists are available for the treatment, but clinical evaluation of these drugs interactions.[12,13] This medication has been the cause for the development of new anti-ulcer drugs and the search for novel molecules has been extended to herbal drugs that offer better protection and decreased relapse. It is generally accepted that ulcer results from an imbalance between aggressive factors and the defence mechanism.[14] To regain the balance, drugs of plant origin are investigated to inhibit the gastric acid secretion or to activate the mucosal defence mechanism by increasing mucus production.[15]

Plant extracts are some of the most attractive sources of new drugs, and have shown promising results for the treatment of gastric ulcer in several experiment models for evaluating anti-ulcer drugs.[16]

Moreover, these results showed that the anti-ulcer activity of this extracts was not only related to a local neutralization of gastric content, but also it was effective after the absorption of the extracts; indicating a systemic effect. This effect is also indicative of antihistaminic activity.[17] The antiulcerogenic activity of methanolic extract of *Bauhinia*

recemosa (flower buds) in acid alcohol induced gastric ulcers in rats. Their effects were studied on the volume of gastric juice secreted; acid output, peptic activity, mucin activity and curative ratio were recorded. *Bauhinia tomentosa* leaves also decreased the ulcer index significantly.[18]

Table 1: Effect of *D.virginiana* leaf and bark extracts on gastric ulcer in experimental animals

Groups	Volume of Gastric juice (ml/100g)	pH	Total Acidity (mEg/L)	Free Acidity (mEg/L)	Ulcer Lesion	Ulcer Index
Normal	0.3 ± 0.02	4.2 ± 0.16	30.1± 1.0	15.9 ± 0.9	-	-
Control	2.5 ± 0.04	1.3 ± 0.07	96 ± 7.3	77 ± 6.3	35.4 ± 3.2	2.32 ± 0.08*
Leaf extract (500mg/kg)	0.61 ± 0.03**	3.5 ± 0.25*	37.3 ± 2.8*	33.8 ± 1.8*	15.8 ± 1.8	2.24 ± 0.08*
Bark extract (500mg/kg)	0.78 ± 0.03	3.8 ± 0.10*	47.2 ±0.0*	33.2 ± 0.1*	21.5 ± 1.0*	2.03 ± 0.01*
Ranitidine (32/mg Kg)	0.50 ± 0.02*	4.10±0.18**	29.3 ± 1.0*	16.2 ± 0.9*	10.5 ± 0.8*	2.00 ± 0.01

Values are expressed as S.E.M; *P<0.01 Vs control; **P< 0.001 Vs control;

6 Number of animals were used in each group.

CONCLUSION

The present study established potent antiulcer potential in ethanolic extracts of both leaf and bark of *D.virginiana* acid-ethanol induced gastric ulceration animal models in rats. When the results are compared, both plant extracts showed better activity in pylorus ligation than in ranitidine induced ulcers.

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