

EVALUATION OF ANTI ULCER ACTIVITY OF ETHANOL EXTRACT OF *DIOSPYROS MELANOXYLON* (ROXB). BARK

KIRAN KUMAR. ANDE*, GOWRISHANKAR N.L, MANJU BHARGAVI. V, NAGARJUNA. M, RAJANI. G, SWETHA. Y AND VINAY REDDY. P

*Department of Pharmacology, Swami Vivekananda Institute of Pharmaceutical Sciences, Vangapally (V), Yadagirigutta (M), Nalgonda (D), Pin 508286, Andhra Pradesh, India. Email: andekiranpharmacology@gmail.com

Received: 23 May 2012, Revised and Accepted: 06 Aug 2012

ABSTRACT

Diospyros melanoxyton is a species of flowering tree in the family Ebenaceae that is native to India and Sri Lanka. Traditionally, various parts of this plant have been used in the treatment of diabetes, anemia, inflammation of spleen, dyspepsia, diarrhea, hypotensive and used as carminative, laxative, diuretic, and astringent. Antiulcer activity of ethanol extract (200 mg/kg) of *Diospyros melanoxyton* (EEDM) bark was evaluated against positive control (forced swim stress), famotidine 40 mg/kg body weight was used as reference standard in antiulcer testing. Acute and short term toxicity studies were performed initially in order to ascertain the safety of EEDM.

Treatment with EEDM bark possesses significant anti-ulcer activity in forced swim stress induced gastric ulcer model. It has a gastric antisecretory and acid neutralizing effect that are comparable to reference drug famotidine. *Diospyros melanoxyton* exhibited significant antiulcer activity.

Keywords: Ethanol extract of *Diospyros melanoxyton* (EEDM), Anti-ulcer activity, Famotidine.

INTRODUCTION

Gastric ulcer, one of the most widespread, is believed to be due to an imbalance between aggressive and protective factors¹. The gastric mucosa is continuously exposed to potentially injurious agents such as acid, pepsin, bile acids, food ingredients, bacterial products (*Helicobacter pylori*) and drugs². These agents have been implicated in the pathogenesis of gastric ulcer, including enhanced gastric acid and pepsin secretion, inhibition of prostaglandin synthesis and cell proliferation growth, diminished gastric blood flow and gastric motility³. Drug treatment of peptic ulcers is targeted at either counteracting aggressive factors (acid, pepsin, active oxidants, platelet aggravating factor "PAF", leukotrienes, endothelins, bile or exogenous factors including NSAIDs) or stimulating the mucosal defences (mucus, bicarbonate, normal blood flow, prostaglandins (PG), nitric oxide)⁴. The goals of treating peptic ulcer disease are to relieve pain, heal the ulcer and prevent ulcer recurrence. Currently there is no cost-effective treatment that meets all these goals. Hence, efforts are on to find a suitable treatment from natural product sources.

In the light of *Diospyros melanoxyton* use in the management of diverse diseases and treatment of diabetes⁵, anaemia, inflammation of spleen, dyspepsia, diarrhoea, scabies, hypotensive and used as carminative, laxative, diuretic, astringent, the present study was conducted to evaluate the antiulcer activity. Preliminary phytochemical review shows the presence of alkaloids, carbohydrates, flavonoids, aminoacids, glycosides, tannins, proteins, steroids, saponins⁶.

MATERIALS AND METHODS

Collection and authentication of *Diospyros melanoxyton* (Roxb.) leaves

Diospyros melanoxyton (Roxb.) bark was collected from Station ghanpur village of Warangal District, Andhra Pradesh. This plant is identified and authenticated by Dr. S. Srinivas Rao M. Sc., Ph.D, Dept of Botany, S.L.N.S Degree and PG College, Bhongir, Andhra Pradesh, India. The bark of *Diospyros melanoxyton* (Roxb.) were isolated, chopped into small pieces and dried under shade at room temperature for seven days.

Preparation of ethanol extract of bark of *Diospyros melanoxyton* (Roxb.)

The bark was washed with absolute ethanol to avoid the microbial growth, and were dried at open air under the shade, cut in to small

pieces and powdered mechanically. The powdered material of bark of *Diospyros melanoxyton* (Roxb.) was refluxed successively with the 250 ml of ethanol in a Soxhlet extractor for 48 hrs. The solution so obtained was transferred to china dish and then allowed for drying. The extract so obtained dried by using desiccators in order to remove the moisture content. The extract so obtained from ethanol was labeled, weighed and used for various studies.

Acute oral toxicity studies

A safe oral dose of the extract was determined by acute oral toxic class method of Organization of Economic Co-Operation and Development (OECD) as per 425 guidelines⁷.

Experimental animals

Adult Wistar Albino rats of either sex weighing between 150- 175 g were used to study the antiulcer activity. All the animals were housed for a week in a ventilated standard lab condition (room temperature 26 ± 2 °C and 12 hours ± day night cycle). The animals used in this study was approved from Institutional Animal Ethical Committee (IAEC) of SASTRA University, Thanjavur, Tamilnadu. The experiments were conducted as per the guidelines of CPCSEA (Committee for the Purposes of Control Supervision of Experiments on Animals), Chennai, India. (Approval no: 86/ SASTRA/ IAEC/ RPP).

Chemicals

All chemicals and reagents used were of analytical grade.

Preparation of test sample

One concentration (200 mg) of the extract was prepared by using 0.1 % tween 80. Famotidine (20 mg) suspended in 0.1 % tween 80 solution used as standard drug.

Forced swim stressed induced ulcer in rat^{8,9,10,11}

Stress ulcers were introduced by forcing swimming in the glass cylinder (height 35 cm, diameter 25 cm) containing water to the height of 35 cm maintained at 25 °C for 3 hours. Animals were fasted for 24 hours prior to the experiment and divided into 4 groups 6 animals in each group. Group 1 received 0.1 % tween 80 as vehicle control, group 2 treated with Swim stress (3 hours) Positive control, group 3 treated with Swim stress (3 hours) + *Diospyros melanoxyton* ethanol bark extract (200 mg/kg) p.o and group 4 treated with Swim stress (3 hours) + famotidine (20 mg/kg) p.o. after the drug treatment animals were allowed to swim in this water for 3 hours.

The stomach of each animal was removed and the extent of gastric damage was assessed. A score of ulcer will be made as follows:

- Normal stomach..... (0)
- Red coloration..... (0.5)
- Spot ulcer..... (1)
- Hemorrhagic streak... (1.5)
- Ulcers..... (2)
- Perforation..... (3)

Statistical analysis

All data will be expressed as mean ± SEM. The statistical significance between groups will be compared using one way ANOVA, followed by Dunnet’s t-test (multiple comparisons). P < 0.05 will be considered as significant.

RESULTS

Acute toxicity studies

Acute toxicity studies were conducted for the ethanol extract of

Diospyros melanoxylon bark. The maximum tolerated dose was found to be 2000 mg/kg b.w when the extract was administered orally. As per the OECD (International toxicity testing) guidelines the maximum therapeutic dose is 1/10th of maximum tolerated dose, hence the therapeutic dose selected for the extracts was 200 mg/kg body weight.

Antiulcer activity - Forced swim stress induced ulcer

Forced swim stress induced ulcer is one of the best models of stress in rats to induce ulcer. The model provides both emotional stress as well as physiological stress to the animal. Famotidine was used here to study the histamine (H₂) receptor inhibitory mechanism. In forced swim stress induced ulcer, the plant extract at a dose of 200 mg/kg showed significant gastro protective activity shown in table-1.

Figure1 and figure 2 shows potent ulcerogenic effect after 3 hours of forced swim stress in positive control group and potent anti-ulcer activity after 3 hours of swim stress in *Diospyros melanoxylon* bark (200 mg/kg) famotidine (40 mg/kg) treated group.

Table I: Ulcer index estimation in forced swim stress induced ulcer

Group	Treatment	Ulcer index (UI) (Mean + SEM)	% Inhibition of ulcer index
I	Vehicle control (0.1 % Tween 80)	0.280 + 0.052	-----
II	Positive control, Swim stress (3 hours)	3.125 + 0.080***	-----
III	Swim stress (3 hours) + <i>Diospyros melanoxylon</i> bark extract (200 mg/kg)	1.043 + 0.054***	55.91
IV	Swim stress (3 hours) + Famotidine (40 mg/kg)	0.626 + 0.044***	75.54

Values are expressed as mean ± SEM. n = 6 for each group. Significance between various groups at *P < 0.0001 by one way ANOVA followed by Dunnet’s t-test.

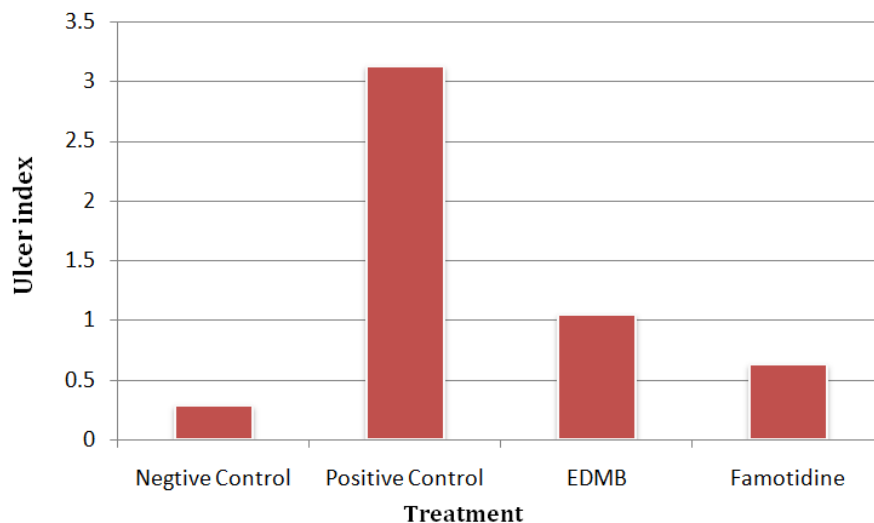
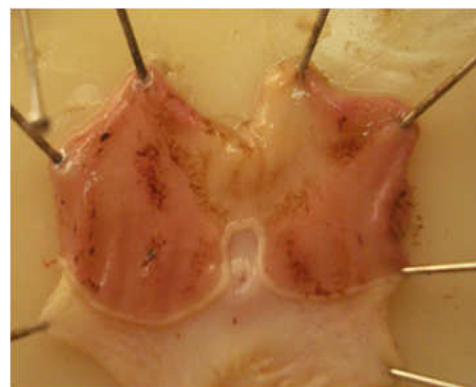


Fig. 1: Ulcer index of forced swim stress induced ulcer



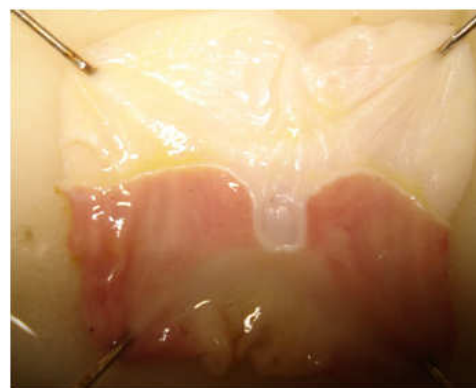
Negative control



Positive control treated group



Diospyros melanoxydon bark extract (200 mg/kg) treated group



Famotidine (40 mg/kg) treated group

Fig. 2: Macroscopical view of rat stomach using forced swim stress induced ulcer

DISCUSSION

In forced swim stress induced model, stress increases histamine release with enhanced acid secretion which causes ulcer and reduces mucous production. Stress induced ulcers can be prevented partly or fully by vagotomy. Vagal overactivity is suggested as the principle effector in stress induced ulcers.

Preliminary phytochemical investigations of the ethanol bark extract of *Diospyros melanoxydon* (200 mg/kg) showed positive test for flavanoids, steroids, pink colour indicates the presence of terpenoids, hence the antiulcer activity of *Diospyros melanoxydon* Roxb. bark in this experiment may be due to flavanoids and steroids. Flavonoids decrease histamine secretion from mast cells by inhibition of histidine decarboxylase and stimulate prostaglandin biosynthesis, it was postulated that these mechanisms of action may be responsible for the anti-ulcer activity of flavonoids¹². The results demonstrated that *Diospyros melanoxydon* Roxb. bark extract produced antiulcerogenic effects possessing antisecretory, cytoprotective and proton pump inhibition mechanism. The interesting observation indicates that *Diospyros melanoxydon* Roxb. bark extract can be a potential source for the treatment of ulcer. However, detailed study like isolation of active molecule and characterization is required to confirm the phytochemical responsible for the activity.

ACKNOWLEDGEMENT

The management of Swami Vivekananda Institute of Pharmaceutical Sciences, Vangapally (V), Yadagirigutta (M), Nalgonda (D), for providing all the necessary facilities to conduct this work.

REFERENCES

1. AlKofahi A, Atta AH., Pharmacological screening of the antiulcerogenic effects of some Jordanian Medicinal Plants in rats, *J Ethnopharmacol*, 1999, 65, 341-5.
2. Peskar BM., Maricic N., Role of prostaglandins in gastroprotection, *Dig Dis Sci*, 1998, 43, S23-9.
3. Toma W., Hiruma-Lima CA., Guerrer RO., Souza AR. Preliminary studies of *Mammea Americana* L (Guttiferae) bark/latex extract point to an effective antiulcer effect on gastric ulcer models in mice, *Phytomedicine*, 2005, 12, 345-50.
4. Borelli F., Izzo AA., The plant kingdom as a source of anti-ulcer remedies, *Phytother Res*, 2000, 14, 581-91.
5. Topno K.K., Plants used by tribals of Chotta Nagpur against diabetes. *Botanica*, Topno 47, 1997: 99-101.
6. Maridass M., Ghanthikumar S and Raju G. Preliminary Phytochemical Analysis of *Diospyros Species*. *Ethnobotanical Leaflets* 2008, 12: 868-72.
7. OECD 425 guidelines. OECD Guidelines for testing animals 2001; Decl/26:1-26.
8. Katzung BG. Basic and Clinical Pharmacology. 10th Ed. New York: McGraw- Hill companies; 2007.
9. Goodman, Gilman. The Pharmacological Basis of Therapeutics. 11th Ed. New York: McGraw-Hill; 2006.
10. Shenoy AM, Shastry CS, Sridevi, Gopkumar P. Anti-ulcer activity of *Naravelia zeylanica* leaves extract. *J Pharma Res* 2009; 2(7): 1218-1220.
11. Alder R. In Breakdown in human adaptation to stress. Boston: Martinus Nijhoff: 1984, 653.
12. Beil W., Birkholz C., Sewing K. Effect of Flavanoids on parietal cell acid secretion, gastric mucosal prostaglandin production and *Helicobacter pylori* growth. *Arzneimittel for schung / Drug Res* 1995; 45(1): 697-700.