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ANTI-ULCER ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *DIOSPYROS MELANOXYLON* (ROXB). LEAVES

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ABSTRACT

Plants 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 hydroalcoholic extract (200 mg/kg) of *Diospyros melanoxyton* (HDM) leaves was evaluated against positive control (forced swim stress), Famotidine 20 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 HDM. Treatment with HEDM leaves possesses significant anti-ulcer activity in forced swim stress induced gastric ulcer model. It has gastric anti-secretory and acid neutralizing effects that are comparable to reference drug Famotidine. *Diospyros melanoxyton* exhibited significant antiulcer activity.

KEY WORDS

Hydroalcoholic extract of *Diospyros melanoxyton* (HDM), Anti-ulcer activity and Famotidine.

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INTRODUCTION

Gastric ulcer is one of the most widespread 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 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 melanoxylon* 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

Preparation of hydroalcoholic extract of leaves of *Diospyros melanoxylon* (Roxb.)

The leaves were 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 leaves of *Diospyros melanoxylon* (Roxb.) was refluxed successively with the 250 ml of hydroalcoholic in a Soxhlet extractor for 48 hrs. The solution so obtained was transferred to china dish and then allowed for drying. The extract obtained was dried by using desiccators in order to remove the moisture content. The extract so obtained from hydroalcohol 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

synthesis and cell proliferation growth, diminished 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 \pm day night cycle). The animals used in this study were 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 rat⁸⁻¹¹

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 melanoxylon* hydroalcoholic leaves 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 AND DISCUSSION

Acute toxicity studies

Acute toxicity studies were conducted for the hydroalcoholic extract of *Diospyros melanoxylon* leaves. 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 No.1.

Figure No.1 shows potent ulcer genic 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* leaves (200 mg/kg) Famotidine (20 mg/kg) treated group.

The 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 over activity is suggested as the principle effector in stress induced ulcers. Preliminary phytochemical investigations of the hydroalcoholic leaves extract of *Diospyros melanoxylon* (200 mg/kg) showed positive test for flavanoids, steroids, pink colour indicates the presence of terpenoids, hence the antiulcer activity of *Diospyros melanoxylon* Roxb. leaves 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.

Table No.1: Ulcer index estimation in forced swim stress induced ulcer

S.No	Group	Treatment	Ulcer index (UI) (Mean ± SEM)	% Inhibition of ulcer index
1	I	Vehicle control (0.1 % Tween 80)	0.280 ± 0.052	–
2	II	Positive control, Swim stress (3 hours)	3.125 ± 0.080***	–
3	III	Swim stress (3 hours) + <i>Diospyros melanoxylon</i> leaves extract (200 mg/kg)	1.043 ± 0.054***	55.91
4	IV	Swim stress (3 hours) + Famotidine (20 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.

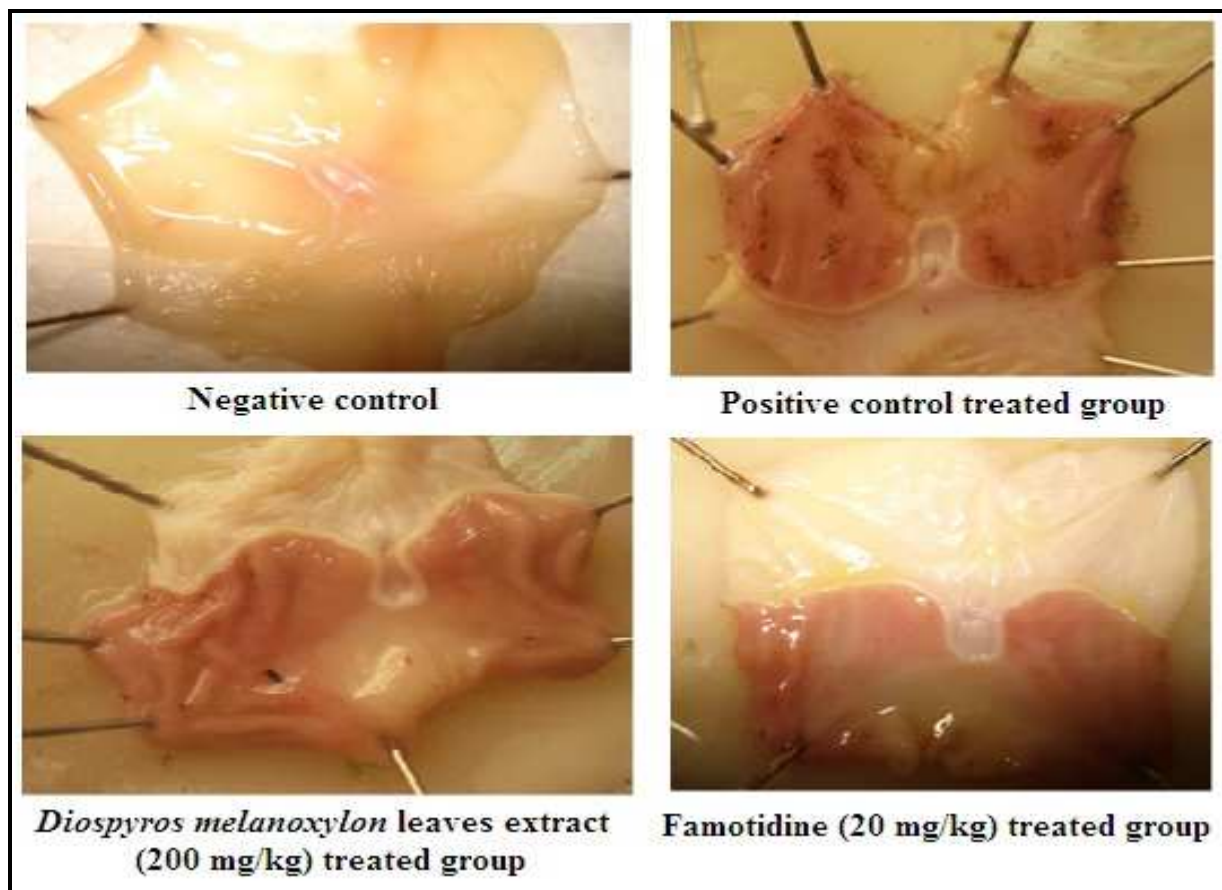


Figure No.1: Macroscopical view of rat stomach using forced swim stress induced ulcer

CONCLUSION

This study concluded that *Diospyros melanoxylon* Roxb. leaves extract produced antiulcerogenic effects possessing antisecretory, cytoprotective and proton pump inhibition mechanism. The interesting observation indicates that *Diospyros melanoxylon* Roxb. Leaves 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.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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