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GASTRIC ULCER PROTECTIVE EFFECT OF *MADHUCA LATIFOLIA* ROXB BARK IN WISTAR RATS

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ABSTRACT: The present study was carried out to investigate the protective effect of ethanolic extract of *Madhuca latifolia* (Roxb.) bark (MLE) and aqueous extract of *Madhuca latifolia* (Roxb.) bark (MLA) in aspirin-induced gastric ulcer. In both extract, acute toxicity performed according to guidelines for the selection of doses. The oral administration (400 mg/kg) of MLE & MLA extract reduced the ulcer index and prevents the development of gastric lesions by 76.57% and 81.14% (% of protection), respectively. The present studies provide preliminary data on the antiulcer potential of bark and support the traditional uses of the plant for the treatment of gastric ulcer.

INTRODUCTION: Herbal extracts gathered lots of attention of scientist working for the development of safe and effective delivery of traditional medicines. They have played a significant role in maintaining human health and improving the quality of human life for thousands of years [1]. The demand for herbal medicine is increasing because of their therapeutic effectiveness wide, higher safety margin, and economical. *Madhuca latifolia Roxb,* synonyms *Madhuca indica* Gmel., *Madhuca longifolia* Macb. commonly known as Mahua in Hindi [2].

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Madhuca latifolia Roxb is a large, much branched fast-growing deciduous tree that grows up to 18m high and 80cm diameter at breast height**.** It is considered a holy tree in India by many tribal communities because of their tremendous benefits. In the tribal belt of Central India, it is used for cultural and economic reasons. It provides live hood security to poor households who collect it both for self-consumption and sale [3]. Its various parts are used for nutritive, medicinal and other valuable products. Corolla, fruits, flower are used for nutritional purpose [4].

Phytochemical values of the plant include the presence of triterpenoids in fruit pulp; beta-sitosterol glucoside, quercetin, and dihydro-quercetin in a nutshell; sugars, vitamins, phosphorus, calcium, iron, magnesium, and copper present in corolla. The sugars identified are sucrose, maltose, glucose, fructose, arabinose, and

rhamnose. The seeds yielded Saponins-2, 3-di-O-glucopyranoside of basic acid (saponin A and saponin B). Saponins mixture obtained from seeds shows spermicidal activity. Trunk bark contains lupeol, beta-amyrin acetate, alpha-spinasterol, erythrodiol monocaprylate, betulinic acid, and oleanolic acid caprylates [5, 6].

The pharmacological activities reported are antioxidant activity of methanolic extract of bark,[7] antihyperglycemic and antioxidant activity of ethanolic extract of bark, [8] antioxidant and hepatoprotective effect of bark, [9] antihyper-glycemic activity of methanolic extract of bark, [10] nephro and hepatoprotective effect and antioxidant activity of leaves, [11] anti-inflammatory, analgesic and antipyretic activity of aerial parts, [12] analgesic effect of flower, [13] antiepileptic activity of heartwood, [14] anticancer activity of ethanol extract of leaves, [15] wound healing activity of leaves, [16] anti Inflammatory, antiulcer and hypoglycemic activities of Seed Cake, [17] antioxidant and antimicrobial activity [18].

Gastric ulcer is a localized area of erosion in the stomach mucous membrane that is exposed to gastric acid and pepsin. In gastric ulcer, one of the key defensive mechanisms is the secretion of a mucus layer that protects gastric epithelial cells. Gastric mucus coats the mucosal surface of the stomach slows ion diffusion and prevents the irritation, autodigestion mucosal damage by gastric acid and pepsin [19]. It results probably due to an imbalance between the aggressive/injurious factors and the mucosal defensive factors [20]. Inhibitions of gastric acid secretion most commonly by proton pump inhibitors followed by H_2-blockers, anticholinergics, and ulcer protective drugs by sucralfate and bismuth compounds are current recommendations for drug therapy of gastric ulcers [11]. One of the major problems in the treatment of gastroduodenal ulcer is that, despite a healing rate of 80-100% after 4-8 weeks of therapy with H_2 – antagonists and in proton pump inhibitors (potent suppressors of gastric acid secretion), the rate of ulcer recurrence (40-80%) within 1 year after suspending the treatment [21]. The currently used these type of antiulcer drugs have adverse reactions such as gynecomastia, joint pain, menstrual disorder, sore throat *etc*.[20]

There is a need for the search of newer therapeutic antiulcer agents from plant sources which are traditionally used in many tribal areas and from the alternative therapy. So, *Maduca latifolia* Roxb. was selected for the study.

MATERIALS AND METHODS:

Materials:

Plant Material: The fresh bark of plant *Madhuca latifolia* Roxb. was collected from Kharsia, Raigarh district of Chhattisgarh (India) in July. The species was identified and authenticated by Dr. Shiddamallayya N, National Ayurveda Dietetics Research Institute, Bangalore-560011. (Reference no. is Drug Authentication/SMPU/NADRI/ BNG/2010-11/341) The collected bark was thoroughly washed with water to remove the adherent impurities and shade dried at room temperature and then reduced into a coarse powder with a mechanical grinder and sifted through sieve no. 22 and stored in an airtight container.

Chemicals*:* Aspirin and Ranitidine hydrochloride were purchased from Healthy Life Pharma Pvt. Ltd. Mumbai, India.

Methods:

Preparation of Extract: The coarsely powdered material (300gm) was subjected to extraction with ethanol (1000 ml) in a Soxhlet apparatus by using hot continuous extraction method at 60 ºC and separately 300gm powdered drug was extracted with distilled water (1500 ml), by cold maceration method [22]. The extracts obtained were evaporated to dryness at a temperature below 30 ºC to yield ethanolic extract (6.82% w/w) and aqueous extract (4.36% w/w).

High-Performance Thin Layer Chromatography (HPTLC) Studies: HPTLC studies of the MLE and MLA were carried out using HPTLC applicator Camag Linomat IV, HPTLC scanner Camag TLC scanner II and software for the interpretation of data. An aluminum plate (10cm × 10cm) precoated with silica gel (Merck 60 F 254) was used as adsorbent. The volume of sample loaded 10 µl. The plates were developed using hexane: ethyl acetate (8:2) and ethyl acetate: methanol: toluene: (6:2:2) for MLE and MLA respectively in a development mode Camag Twin trough chamber.

Antiulcer Activity (*in-vivo*):

Animals: Adult albino male Wistar rats (180-200 gm) were obtained from the animal house of School of Pharmacy, Chouksey Engineering College, Bilaspur (C.G.), India. All the animals were housed under standard laboratory condition at controlled room temperature 22 ± 3 ºC and relative humidity 60 ± 5% with 12 h light and 12 h dark cycle. Animals were allowed free access to a standard dry pellet diet and water *ad libitum* [23]. The animals were randomly select and grouped in cages and acclimatized to laboratory conditions for seven days before commencement of the experiment. All experiments were performed in accordance with the guidelines of CPCSEA (Committee for Control and Supervision of Experiments on Animal). All experimental animal procedure was reviewed and approved by IAEC (reg. no.: 1257/AC/09/ CPCSEA/2010/15).

Acute Toxicity Studies: The acute toxicity studies of MLE and MLA were carried out in Female Wistar rats (weighing 180-200 gm) by fixed dose method of OECD guideline no 420. [23]

Aspirin-Induced Antiulcer Activity: Twenty four male Wistar rats (weighing180-200gm) were taken. They were divided into four groups of six rats each (n=6). The groups were as follows:

Group I: Vehicle (1ml/kg) + aspirin (200mg/kg) and was kept as control.

Group II: Ranitidine (50mg/kg) + aspirin (200mg/kg) and was kept as standard.

Group III: MLE (400mg/kg) + aspirin (200mg/kg).

Group IV: MLA (400mg/kg) + aspirin (200mg/kg).

All the animals were fasted for 24 h before the study but had free access to water. After the fasting period ranitidine and all the sample of plant extracts were given orally. After 30 min of treatment with the drug, aspirin was given orally. The animals were then sacrificed by cervical dislocation 5 h after the treatment. The Stomachs were cut open along the greater curvature and rinsed with water to remove the gastric contents and blood clots & examined grossly.

The ulcer index was evaluated according to the severity of lesions formed and ulceration was scored using magnifying lens and the ulcer scored according to its severity in comparison with that of standard by using the described scale. *i.e.* 0= Normal stomach; 0.5= Red colouration; 1= Spot ulcer; 1.5= Hemorrhagic streak, 2.0= Ulcers, 3.0= Perforation [24, 25]. The mean ulcer index in each group was calculated and expressed the percentage of inhibition using the following formula:

% Inhibition = Control mean ulcer index – Test mean ulcer index × 100 / Control mean ulcer index

Histopathological parameters were studies and compared between all four groups to confirm the ulcer score.

Statistical Analysis: All data obtained were expressed as the Mean ± Standard error of the mean (SEM). Statistical significance was analyzed by using one-way Analysis of Variance (ANOVA) followed by Dunnett's test, with the level of significance set at $p<0.05$ and $p<0.01$ was considered highly significant.

RESULTS:

HPTLC Studies: The preliminary HPTLC studies of ethanolic and aqueous extract of *Madhuca latifolia* Roxb bark revealed that hexane: ethyl acetate (8:2) solvent system was ideal for MLE and gave six phytoconstituents having R_f values 0.06, 0.35, 0.58, 0.62, 0.72 and 0.82 **Fig. 1** while solvent system ethyl acetate: methanol: toluene: (6:2:2) was ideal for MLA which gave nine phyto-constituents having R_f values 0.07, 0.09, 0.18, 0.31, 0.36, 0.45, 0.56, 0.69 and 0.81 **Fig. 2**.

Acute Toxicity Studies: The acute toxicity studies of MLE and MLA showed no animal died even at 2000 mg/kg and hence both the extracts were treated as non- toxic and 1/5th (400 mg/kg) of the 2000 mg/kg was selected for further investigations.

Aspirin-Induced Antiulcer Activity: The antiulcer activity was assessed by determining and comparing the ulcer index in the test drug groups with that of the vehicle control and standard ranitidine. In the case of aspirin-induced ulcers, the both extract showed significant reduction of ulcers **Table 1**. Results are also supported by histological studies **Fig. 3**, which showed that rats pre-treated

with MLE, MLA and ranitidine significantly (P<0.01) inhibited the gastric lesions formation and erosion, induced by aspirin compared to rats pre-treated with the vehicle. When compared the both extract MLA showed high % of protection (81.14%) as compared to extract MLE (76.57%).

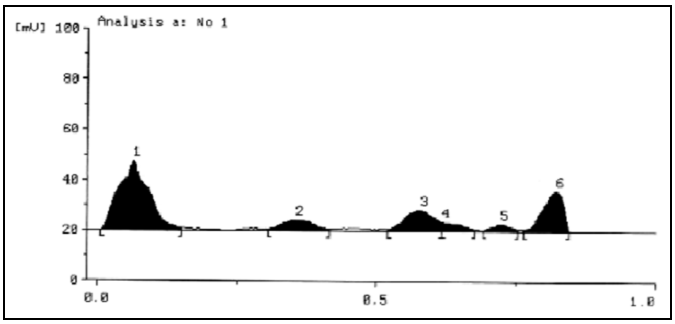

FIG. 1: HPTLC FINGERPRINT PROFILE OF MLE

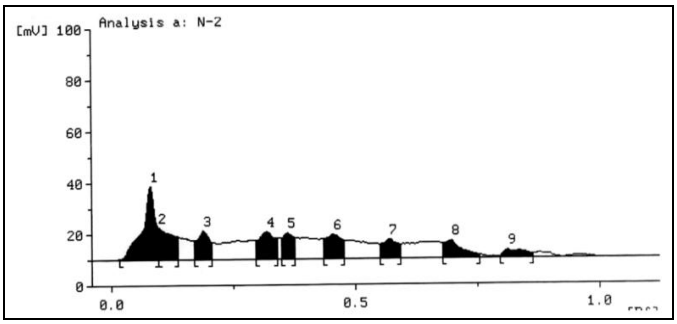

FIG. 2: HPTLC FINGERPRINT PROFILE OF MLA

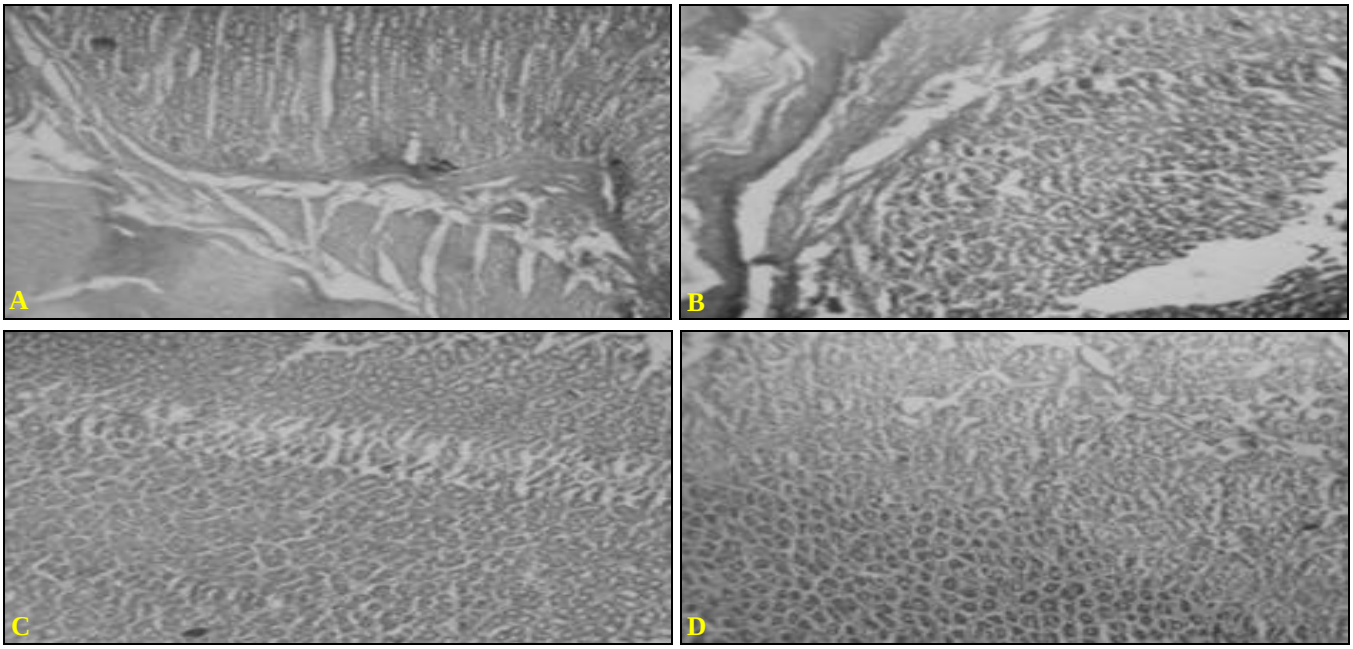

FIG. 3: HISTOPATHOLOGICAL PROFILE OF STOMACH SECTION; A: CONTROL (SHOWING NECROSIS AND DEGENERATED EPITHELIAL CELLS), B: STANDARD (SHOWING INTACT MUCOSA), C: MLE (SHOWING MILD SUBMUCOSAL EDEMA) AND D: MLA (SHOWING INTACT MUCOSA WITH REGENERATIVE CELLS)

DISCUSSION: Present study emphasized to focus the protective effect of *Madhuca latifolia* (Roxb) bark in gastric ulcer. The antiulcer activity of MLE and MLA was assessed in aspirin-induced gastric ulcer. Twenty four male *Wistar* rats were divided into four groups; six rats in each. Pre-treatment with (MLE) (400 mg/kg), (MLA) (400 mg/kg) extract of *Madhuca latifolia* (Roxb.) bark reduced gastric ulceration when compared with vehicle control group rats. When compared both extract MLA showed high % of protection (81.14%) as compared to extract MLE (76.57%) **Table 1**. It may be due to the presence of more number of phytoconstituents in extract MLA, which are identified by HPTLC fingerprint.

TABLE 1: EFFECT OF *MADHUCA LATIFOLIA* BARK EXTRACT ON ASPIRIN-INDUCED GASTRIC ULCER

Group	Treatment	Oral dose	Ulcer index	Ulcer index
I	Vehicle	1ml/kg	1.75±0.3	-
II	Ranitidine	50mg/kg	0.25±0.11**	85.71
III	MLE	400mg/kg	0.41±0.15**	76.57
IV	MLA	400mg/kg	0.33±0.16**	81.14

Values in Mean ± SEM with n=6, * Symbol represent statically significance**p<0.01. One way ANOVA which came with a significant difference in column means, supported by Dunnett test which compared control *vs.* std. & extract & found highly significant at p<0.01. SEM: Standard error means, ANOVA: Analysis of variance, *vs.*: Versus, Std.: Standard

CONCLUSION: *Madhuca latifolia* Roxb. bark extracts (MLE and MLA) showed good anti-ulcer (*in-vivo*) activities. Both the plant extracts showed significant prevention of the formation of lesions and decrease ulcer index, which indicates that plant is potential for the NSAIDs induced gastric ulcer. Further HPTLC chromatogram of extracts showed the good separation of phytoconstituents at different R_f in optimizing the solvent system. This study can be extended for the isolation of pure compounds from the extracts and their biological activity.

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CONFLICT OF INTEREST: Nil

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