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Anti-ulcer properties of 70% ethanolic extract of leaves of *Albizzia lebbeck*

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ABSTRACT

This study was designed to determine the gastro-protective effect of 70% ethanolic extract of leaves of *Albizzia lebbeck* in pylorus ligation, ethanol and indomethacin induced models in rat. The 70% ethanolic extract of leaves of *Albizzia lebbeck* was prepared and subjected to acute toxicity study as per CPCSEA guideline no. 420. Two doses i.e. 100 mg/kg and 200 mg/kg were selected for the further study. In pylorus ligation induced ulcer model, the parameters studied were gastric volume, pH, free acidity, total acidity and ulcer index. Ulcer index was also determined in ethanol and indomethacin induced ulcer models. Pretreatment with the extract (100mg/kg, 200 mg/kg) has shown dose dependant decrease in ulcer index in all the experimental models of ulcers (indomethacin, ethanol and pylorus ligation induced ulcers). The prior administration of extract (100 mg/kg and 200 mg/kg doses) also reduced the total acidity (58.50 and 46.17), free acidity (51.48 and 40.05) and increased the pH (2.58 and 5.15). However, the gastric volume was not reduced with 100mg/kg dose and significantly reduced with 200mg/kg dose. The 70% ethanolic extract of leaves of the plant possess antiulcer properties. The antiulcer properties of the extract may be attributed to the polyphenolic compounds that are present in it.

KEY WORDS: *Albizzia lebbeck*, Antiulcer, indomethacin.

INTRODUCTION

Peptic ulcer occurs due to an imbalance between the aggressive (acid, pepsin and *Helicobacter pylori*) and the defensive (gastric mucus and bicarbonate secretion, prostaglandins, innate resistance of the mucosal cells) factors (1). Number of drugs including proton pump inhibitors, prostaglandins analogs, histamine receptor antagonists and cytoprotective agents are available for the treatment of peptic ulcer. But most of these drugs produce several adverse reactions including toxicities and even may alter biochemical mechanisms of the body upon chronic usage. (2). Hence, herbal medicines are generally used in such cases when drugs are to be used for chronic periods. Several natural drugs have been reported to poses anti-ulcerogenic activity by virtue of their predominant effect on mucosal defensive factors (3, 4).

The present study is planned to exploit the safety and efficacy of a plant named *Albizzia Lebbeck* (benth) family; Mimosaceae as a gastroprotective agent. Upon literature review it was found that, the leaves are used in ophthalmia (5). The bark is used in bronchial asthma and other allergic disorders (6). The flowers are useful in chronic cough and bronchitis (7). The seeds are

aphrodisiac (5), useful in inflammation, scrofula, skin disease, leprosy, leucoderma, chronic catarrh, seminal weakness, ophthalmopathy and poisoning (6). The root is used in hemicrania (8).

The modern literature revealed that the plant is reported to possess nootropic (9,10), anxiolytic(10), anticunvulsant(11,12), antifertility(13) and antidiarrhoeal activity(14). The leaves of the plant *Albizzia Lebbeck* are rich in flavon, echinocystic acid, β -sitosterol and vicienin II(6). A plant flavonoid has been found to be effective against ulcer in experimental animals (15). The present study was undertaken with the aim to assess the antiulcerogenic properties claimed by traditional system of medicine.

MATERIALS AND METHODS

Collection of Plant Material

The leaves of plant *Albizzia lebbeck* were collected from fields of Harapanahalli, Karnataka in the month of May 2007. It was identified and authenticated by Prof. K. Prabhu, Dept of Pharmacognosy, S.C.S. College of Pharmacy. A herbarium specimen was kept in the college herbal museum.

Preparation of 70% EELAL - The leaves were shade dried at room temperature and pulverized. The 70%

ethanolic extract (12.82%) was prepared by using 70% ethanol in a soxhlet apparatus after defatting with pet.ether. Preliminary phytochemical investigation showed the presence of flavonoid and tannin in 70% ethanolic extract (EELAL). So, 70% EELAL was selected for the present activity.

Animals

Albino Wister rats (150-200g) and Swiss albino mice (18-25 g) of either sex were used for the study, obtained from Venkateshwara Enterprise, Bangalore, Karnataka. After one week of acclimatization the animals were used for further experiments. All the animal protocols were approved by institutional animal Ethics committee (Reg. no.157/1999/CPCSEA) as per the Indian CPCSEA guidelines.

Acute Toxicity Studies

The acute toxicity was determined on Swiss albino mice by fixed dose method of OECD Guide line no 420 given by CPCSEA. Groups of 6 mice were administered test drug by oral route in the range of 2000-300 mg/kg and mortality was observed after 24 hr.

Anti- Ulcer Activity

Indomethacin induced ulcer

The albino rats of either sex weighing between 180 - 200 gm were divided into 4 groups of 6 animals each and fasted for 24 hrs with water *ad libitum* prior to experiment. The animals of group 1 were pretreated with vehicle and the animals of group 2 were treated with standard i.e. lansoprazole 8mg/kg. Similarly the animals of group 3 and 4 were pre-treated with ethanolic extract 100 mg/kg and 200mg/kg respectively. Indomethacin (30mg/kg p o) was administered to the animals of group 2 - 4, 60 minutes after the respective treatments. The animals were then sacrificed by cervical dislocation after 4 hrs. The stomach was taken out and cut open along the greater curvature of stomach. (17). The number of ulcers per stomach were noted and severity of the ulcers were observed microscopically and scoring was done as described before (16): 0 for normal coloured stomach, 0.5 for red colouration, 1 for spot ulcer, 1.5 for hemorrhagic streaks, 2 for ulcer between > 3 but < 5mm and 3 for ulcer > 5mm. Mean ulcer score for each animal is expressed as ulcer index. The percentage protection was calculated.

Ethanol induced (EtOH) induced ulcer

The albino rats of either sex weighing between 180 - 200 gm were divided into 4 groups of 6 animals each and fasted for 24 hrs with water *ad libitum* prior to experiment. The animals of group 1 were pretreated with vehicle and the animals of group 2 were treated

with standard i.e. lansoprazole 8mg/kg. Similarly the animals of group 3 and 4 were pre-treated with ethanolic extract 100 mg/kg and 200mg/kg respectively. Ethanol (100% 1ml/200 g, po) was administered to all the animals of group 2 - 4, 60 minutes after the respective treatments. The animals were sacrificed by cervical dislocation after one hour of EtOH administration and stomach was incised along the greater curvature and examined for ulcers (18). The ulcer index was scored as mentioned above (16) and percentage protection was also reported.

Pylorus - ligated (PL) rats

Albino rats of either sex weighing between 180 - 220 g were divided into 4 groups of 6 animals each and fasted for 18 hrs and care was taken to avoid coprophagy. Control vehicle (group-1) or standard drug (group-2) or extracts (group - 3 and 4) were administered 60 minutes prior to pyloric ligation under light ether anesthesia. The abdomen was opened and pyloric ligation was done without causing any damage to its blood supply. The stomach was replaced carefully and the abdomen wall was closed in two layers with interrupted sutures. The animals were deprived of water during the post operative period. After 6 hrs, stomach was dissected out; contents were collected into tubes for estimation of biochemical parameters. The stomach was taken out and cut open along the greater curvature and ulcers were scored and % protection was reported as mentioned in the above explained models (10, 19).

Gastric Secretion

The gastric juice was collected 6 hrs after pylorus ligation and centrifuged for 5 minutes at 2000 rpm and the volume of supernatant was noted. The pH of the gastric juice was recorded by the pH meter. Then the contents were subjected to analysis for free and total acidity. Free acidity and total acidity were determined using 0.01N NaoH and Topfer's reagent containing phenolphthalein as indicator (10).

RESULTS

The observations of positive control group indicated that indomethacin (30mg/kg) induced gastric ulcerations to the extent of 6.58 ± 0.583 (ulcer index). Pretreatment with test extracts reduced the ulceration in a dose dependant manner. The extent of gastro-protective effect of the test extracts is 45.59% and 62.00% at 100mg/kg and 200mg/kg doses respectively, which is comparable to that of standard lansoprazole 8mg/kg. Similar results were obtained with ethanol induced ulcer model also. The test extract has shown gastro-protection in a dose dependant manner i.e.

Table No.1 – Effect of 70% ethanolic extract of leaves of *Albizzia lebbeck* on Indomethacin, Ethanol (1ml/200gm) and 6 hrs Pylorus ligation (PL) induced gastric ulcers in rats.

Treatment	Dose	ULCER INDEX			% OF PROTECTION		
		Indomethacin	Ethanol	Pylorus ligation	Indomethacin	Ethanol	Pylorus ligation
Positive Control	--	6.58±0.58	6.91±0.61	6.16±0.60	-	-	-
Lansoprazole	8mg/kg BW	0.91±.27***	1.16±0.21***	0.5±0.31***	86.17	83.21	91.88
70% EELAL	100mg/kg BW	3.58±0.53***	3.16±0.16***	2.67±0.40***	45.59	54.27	56.65
70% EELAL	200mg/kg BW	2.50±0.25***	2.25±0.21***	2.08±0.23***	62.00	67.43	66.23

Values are the mean ± S.E.M. of six rats /treatment. Significance * $p < 0.05$, ** $p < 0.001$ and *** $p < 0.001$ Vs. Control

Table No.2 – Effect of 70% ethanolic extract of leaves of *Albizzia lebbeck* on Gastric Secretion following Pyloric Ligation induced Ulcer in Rats.

Treatment	Dose	Volume(ml)	pH	Free Acidity(Eq/l)	Total Acidity(Eq/l)
Control	--	8.88±0.64	2.43±0.10	97.2±6.24	104.22±5.95
Lansoprazole	8mg/kg BW	1.58±0.16***	7.02±0.10***	30.15±2.06***	37.08±1.87***
70% EELAL	100mg/kg BW	8.83±0.55	2.58±0.15	51.48±3.96***	58.50±4.11***
70% EELAL	200mg/kg BW	4.88±0.40***	5.15±0.39***	40.05±3.07***	46.17±3.88***

Values are the mean ± S.E.M. of six rats / treatment. Significance * $p < 0.05$, ** $p < 0.001$ and *** $p < 0.001$ Vs. Control

54.27% and 67.43% protection at 100 and 200 mg/kg doses respectively. The test extracts at the doses mentioned above has shown significant protection even to that of standard lansoprazole (8mg/kg). The results are compiled in table 1.

The pyloric ligation has caused the accumulation of gastric secretions (8.88ml) with pH 2.43. The total acidity and free acidity of the gastric secretions were 104.22±5.95 and 97.2±6.24 respectively. Pretreatment with the test extracts reduced the volume of gastric secretion (5.15ml at 200 mg/kg dose) and the pH was elevated up to 5.15. In addition, total acidity and free acidity were also reduced significantly in a dose dependant manner. The results are compiled in table no.2. Further it is observed that pyloric ligation has caused gastric ulcerations and pretreatment with test extracts has reduced them significantly in a dose dependently (table no.1). In this model also the gastro-protection offered by the test extracts was comparable to that of the standard lansoprazole 8mg/kg.

DISCUSSION

It has been proposed that in pyloric ligation, ulcers are developed due to accumulation of gastric acid and pepsin, which leads to auto-digestion of gastric mucosa (20). The Anti-ulcer property of 70% EELAL in pylorus ligation model is evident from its significant reduction in free acidity, total acidity and ulcer index. Because 70% EELAL treated animals significantly inhibited the formation of ulcers in the pylorus ligated rats and also decreased both the concentration and increased the pH, it is suggested that 70% EELAL can suppress gastric damage induced by aggressive factors.

The incidence of ethanol-induced ulcers which is

predominant in the glandular part of the stomach has been reported to stimulate the formation of leukotriene C₄ (LTC₄) resulting in the damage of the gastric mucosa (21, 22). 70% EELAL significantly protected the gastric mucosa against ethanol challenge as shown by reduced values of ulcer index as compared to solvent control group suggesting its potent gastroprotective effect. Similarly NSAID'S like indomethacin inhibits COX1 thereby inhibits the prostaglandin synthesis, consequently lipooxygenase pathway is enhanced liberating leukotrienes and these leukotrienes are reported to have a role in ulcerogenesis. In addition there is some evidence that NSAIDs may induce ulcer by causing the back diffusion of H⁺ ion in to mucosal cells (23). Therefore the gastroprotective effect of the test extract may be due to its ability to inhibit the synthesis of prostaglandins/leukotrienes. In addition 70% ethanolic extract of leaves of *Albizzia Lebbeck* was significantly effective in protecting gastric mucosa against all the ulcerogenic models of the study. Hence, it may be inferred that 70% ethanolic extract of leaves of *Albizzia Lebbeck* affords effective protection to gastric mucosa against various insults may be by increasing gastric mucin content and increased the pH and decreased the free and total acidity in rats, which in turn reduces the activity of pepsin and prevent mucolysis. This in turn protects the stomach from all the above mentioned challenges. Medical treatment of peptic ulcer is dependent on correcting the imbalance between the offensive and defensive factors. The test extracts acts on both the parameters of equation which govern the treatment of peptic ulcer and thus

can be useful clinically. However further studies are needed to assess its safety profile before it is put into use clinically

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