

Anti-Ulcer Activity of Albizia Procera Leaves In Different Growth Phases

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ABSTRACT: Clinical research has confirmed the efficacy of several plants for the treatment of gastroduodenal disease, while basic scientific research has uncovered the mechanisms by which some plants exert their therapeutic effects. Ulcers are lesions on the surface of the skin or a mucous membrane characterized by a superficial loss of tissue. Preliminary photochemical screening of this medicinal plant identified the presence of important secondary metabolites like flavonoids and tannins which are the active principles of antiulcer activity. Present study was conducted to analyze the plant *Albizia procera* leaves considered as gastroprotective and healing agents on ulcers in medicinal resources and beside that to gather evidence for their effectiveness. The test substances were administered as a single dose by orally. Prior to dosing, animals were kept for 12 h of fasting. Ulceration due to oral administration of ethanol was shown in the stomach section of animal models. The gastric damage as thick red lines and lesions as red areas were observed in the stomach. It was significant to note that increase in the volume, total acidity and free acidity and decreased pH of gastric juice were observed in ulcer treated rats compared to untreated control rats. The present investigation thus established pharmacopeia standard for leaf of *A. procera*, based on phytochemical and pharmacological studies of leaves collected at three different growth phases.

KEY WORDS: *Albizia procera*, Antiulcer activity, tannins, flavanoids, gastroprotective agent.

I. INTRODUCTION

Medicinal properties of plants were known even to pre-historic men and many of these plants have been used in traditional medicine for hundreds of years with reputation as efficacious remedies (Ghani, 1998). Phytotherapy seems to be an alternate system of medicine for the people residing in the Suburban/rural areas (Nandankunjidam, 2006). According to the WHO, about 80% of the World's population relies on traditional medicine for their primary health care (Behera, 2006). Over the last century, ethnobotany has evolved into a specific discipline that looks at the people-plant relationship in a multi-disciplinary way such as ecology, economic botany, Pharmacology, public health and other disciplines as needed (Balick, 1996). Medicinal plants containing active chemical constituents are useful in prevention and treatment of various diseases. Herbalists and indigenous healers have used Botanical medicines traditionally worldwide for the prevention and treatment of different pathologies. Clinical research has confirmed the efficacy of several plants for the treatment of gastroduodenal disease, while basic scientific research has uncovered the mechanisms by which some plants exert their therapeutic effects (Kanner and Lapidot, 2001; Gurbuz et al., 2000).

Ulcers are an open sore of the skin or mucus membrane characterized by sloughing of inflamed dead tissue (Chan and Graham, 2004). Ulcers are lesions on the surface of the skin or a mucous membrane characterized by a superficial loss of tissue. Ulcers are most common on the skin of the lower extremities and in the gastrointestinal tract, although they may be encountered at almost any site. There are many types of ulcer such as mouth ulcer, esophagus ulcer, peptic ulcer, and genital ulcer. Of these peptic ulcer is seen among many people. The peptic ulcers are erosion of lining of stomach or the duodenum (Debjit et al., 2010; Vimala and Gricilda Shoba, 2014). Peptic ulcer is one of the

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world's major gastrointestinal disorders and affecting 10% of the world population (Zapata-Colindres et al., 2006). About 19 out of 20 peptic ulcers are duodenal. An estimated 15000 deaths occur each year as a consequence of peptic ulcer. Annual incidence estimates of peptic ulcer hemorrhage and perforation were 19.4–57 and 3.8–14 per 100,000 individuals, respectively. The average 7-day recurrence of hemorrhage was 13.9% and the average long-term recurrence of perforation was 12.2% (Lau et al., 2011).

In this modern era also 75–80% of the world populations still use herbal medicine mainly in developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effects (Kumar, 2011). Histological studies revealed that these medicinal plants did not show any acute toxicity. Preliminary photochemical screening of this medicinal plant identified the presence of important secondary metabolites like flavonoids and tannins which are the active principles of antiulcer activity (Patel and Jain, 2010). Present study was conducted to analyze the plant *Albizia procera* leaves considered as gastroprotective and healing agents on ulcers in medicinal resources and beside that to gather evidence for their effectiveness and biochemical mechanisms in modern investigation.

II. MATERIALS AND METHODS

Plant Collection

Collection of plant materials Leaves of *Albizia procera* (Roxb.) Benth was collected in and around Thanjavur City, Tamil Nadu. Collected specimen were carefully examined and identified with the help of Regional Floras (Gamble, 1967; Kirtikar and Basu, 1980; Matthew, 1983; Nair and Hendry, 1983) and the Botanical identification was authenticated by Dr.M. Jegadeesan, Formerly Professor and Head, Department of Environmental and Herbal sciences, Tamil University, Thanjavur. Specimen was further confirmed with reference to Herbarium sheets available in the Botanical Survey to India, Southern Circle, Coimbatore. Voucher specimen has been deposited at Tamil University Herbarium for future reference. The collected leaves were shade dried and pulverized fine powdered. The leaf powder of *A. procera* was extracted successive by using Soxhlet apparatus with 95% Ethanol. The extracted sample dried and used for antiulcer activity assessment.

Animal Maintenance

Albino rats were purchased and maintained under standard experimental conditions (temperature $27 \pm 2^{\circ}\text{C}$, relative humidity $60 \pm 5\%$ 12 hours light / dark cycle) and the weight of animal between 130 – 140g approximately. The average life span of albino rats in this strain is ~31 months on an *ad libitum* diet and also had free access to tap water *ad libitum*. Pharmacological experiments involving animals described in the present work were carried out and get approved by local animal ethical committee (CBSCEA/265) of Muthayammal College of Arts and Science, Namakkal.

Anti-Ulcer Study (Ethanol induced model)

The gastric ulcer was induced in rats of either sex weighing between 130-140g by administrating absolute ethanol (8 ml/kg B.W). They were kept in specially constructed cages to prevent coprophagia during and after the experiment. The rats were divided into nine groups each containing six animals and fasted for 24 hrs and allowed free access to water only and second group received ethanol orally. The third group received ethanol and standard antiulcer drugs Omeprazole (150 mg/kg B.W). The fourth, fifth, sixth, seventh, eighth and ninth groups were given absolute alcohol and 95 percent alcoholic extracts of *A. procera*, vegetative, flowering and fruiting at a dose of 5, 10, 20 mg/kg B.W respectively. The drugs were administered orally 30 min prior to the oral administration of absolute ethanol. The animals were anesthetized 6 h later with ether and stomachs were incised along the grates curvature. Collected the gastric juice and ulceration was recorded.

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Collection of gastric juice

Gastric juice was collected from the pylorus-ligated rats. The gastric juice thus collected was centrifuged and the volume of gastric juice as well pH of gastric juice was measured. The sodium (Na⁺) and Potassium (K⁺) ion concentration of gastric juice was carried out in flame photometer (Jeffery *et al.*, 1991). Finally gastric juice was subjected to Biochemical estimation.

Biochemical Estimation

The collected gastric juice were analyzed for gastric volume (Hawk, 1947), pH, free acidity (Jeffery *et al.*, 1992). Biochemical estimation like total protein (Lowery *et al.*, 1951), hexose (Winzler, 1958), hexosamine (Disch and Boren treund, 1983), Pepsin (Debnath *et al.*, 1974), fucose (Dische and Schettles, 1948) Sialic acid (Warren, 1959) were also estimated. The mucosa was flushed with saline and stomach pinned on a frog board and scored. The number of ulcer and length of each ulcer were measured.

Statistical Analysis

Results of each value are expressed as mean \pm standard deviation. Turkey Kramer Multiple comparison test was used to compare groups for Biochemical analysis, which were compared between groups by the use of an analysis of convariance using antiulcer activity estimation us a convariable. Values of $p^* < 0.05$ were considered statistically significant.

III. RESULTS

The test substances were administered as a single dose by orally. Prior to dosing, animals were kept for 12 h of fasting. Ulceration due to oral administration of ethanol was shown in the stomach section of animal models. The gastric damage as thick red lines and lesions as red areas were observed in the stomach. It was significant to note that increase in the volume, total acidity and free acidity and decreased pH of gastric juice were observed in ulcer treated rats compared to untreated control rats.



Fig 1: Normal **Fig 2: Control** **Fig 3: Vegetative** **Fig 4: Flowering** **Fig 5: Fruiting**
Fig 6: Standard
(Ulcer induced)

The mean free acid with negative Control, standard, Vegetative, Fl, and Fr were 71 ± 0.49 , 62 ± 0.44 , 60 ± 0.42 , 66 ± 0.48 , and 68 ± 0.43 respectively. When using vegetative extract, 11Meq/l of free acidic acid was reduced. Other extracts also exhibited a significant gastro protective effect but they showed less than standard. The result was tabulated in (Table 1 and 2).

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TABLE - 1
Anti-ulcer activity of leaf extract of *Albizia procera* in ethanol induced ulcer in rats.

Values are expressed as Mean \pm SD for six rats

Groups	Volume (ml)	pH	Pepsin (μ mole/ml)	Free acidity (Meq/l)	Total acidity (Meq/l)	Sodium (mg/l)	Potassium (mg/l)
Normal	1.65 \pm 0.11	3.60 \pm 0.25	8.41 \pm 0.57	60 \pm 0.42	64 \pm 0.43	3.85 \pm 0.26	0.256 \pm 0.013
Ulcer rats	2.10 \pm 0.147	2.40 \pm 0.18	12.32 \pm 0.84	71 \pm 0.49	76 \pm 0.52	2.95 \pm 0.21	0.167 \pm 0.011
Vegetative	1.70 \pm 0.11	4.29 \pm 0.28	8.4 \pm 0.57	60 \pm 0.42	63 \pm 0.44	4.20 \pm 0.32	0.284 \pm 0.019
Flowering	1.80 \pm 0.10	3.75 \pm 0.26	9.23 \pm 0.52	66 \pm 0.48	69 \pm 0.47	4.12 \pm 0.29	0.219 \pm 0.015
Fruiting	2.00 \pm 0.14	3.70 \pm 0.25	10.48 \pm 0.70	68 \pm 0.43	72 \pm 0.50	3.93 \pm 0.28	0.209 \pm 0.014
Standard (Omeperazole)	1.60 \pm 0.11	3.58 \pm 0.25	9.10 \pm 0.57	62 \pm 0.44	62 \pm 0.44	4.16 \pm 0.31	0.267 \pm 0.018

The mean value of pH of gastric secretion with Negative Control, standard, Vegetative, Fl and Fr were 2.40 \pm 0.18, 3.58 \pm 0.25, 4.29 \pm 0.28, 3.75 \pm 0.26 and 3.70 \pm 0.25 respectively. Among the three phases of extracts, pH was increased using the vegetative extract and second most Flowering extract.

The mean total acidity with Control, standard, Vegetative, Fl and Fr were 76 \pm 0.52, 62 \pm 0.44, 63 \pm 0.44, 69 \pm 0.47 and 72 \pm 0.50 Meq/l respectively. Among the three phases of extracts, total acidity was reduced by vegetative and second was Flowering extract and followed by fruiting. In this investigation next part of the study was estimation of pepsin. The mean pepsin with control, standard was 12.32 \pm 0.84, 9.10 \pm 0.57 μ mole/ml respectively. Among the 3 extracts better activity was observed using vegetative extract (8.4 \pm 0.5757 μ mole/ml). The protective factor of sodium and potassium also increased when using vegetative extract. The result was tabulated in (Table. 2).

TABLE- 2
Anti- ulcer activity of leaf extract of *Albizia procera* in ethanol induced ulcer rats.

Groups	Hexose (μ g/ml)	Hexosamine (μ g/ml)	Fucose (μ g/ml)	Sialic acid (μ g/ml)	Total carbohydrate (μ g/ml)	Protein (μ g/ml)
Normal	332.43 \pm 23.24	150.56 \pm 10.53	172.19 \pm 12.05	56 \pm 3.92	821.64 \pm 62.51	715.46 \pm 50.08
Ulcer rats	262 \pm 18.34	132.4 \pm 11.36	134.14 \pm 9.38	48 \pm 3.36	747.72 \pm 52.29	620.13 \pm 43.40
Vegetative	334.67 \pm 0.11	158.14 \pm 11.06	173.76 \pm 12.16	58 \pm 4.06	823.64 \pm 57.65	710.76 \pm 79.75
Flowering	321.43 \pm 22.47	155.32 \pm 10.81	156.89 \pm 10.98	54 \pm 3.78	810.72 \pm 56.75	664.73 \pm 46.53
Fruiting	318.13 \pm 22.26	150.72 \pm 10.55	141.32 \pm 9.89	52 \pm 3.64	782.92 \pm 54.80	648.32 \pm 45.38
Standard (Omeperazole)	330.15 \pm 23.10	152.52 \pm 10.67	170.47 \pm 11.93	55 \pm 3.85	818.63 \pm 57.30	712.63 \pm 49.88

Values are expressed as Mean \pm SD for six rats

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Anti-ulcer activity of ethanol extracts of three growth phases of *A. procera* leaves was tested by measuring protective factors like Hexose, Hexosamine, Fucose, sialic acid, total carbohydrate and protein (Table 2). Among the vegetative, flowering and fruiting extracts, vegetative extracts treated rats showed maximum amount of these protective factors, followed by flowering and fruiting phase for the leaves of hexosamine was even more than that in standard drug- treated rats (15.14µg/ml). Hence, it is inferred that ethanol leaf extract of *A. procera* in vegetative phase extracted anti-ulcer activity equal or more than that of standard drug (Omeprazole).

IV.DISCUSSION

In anti-ulcer activity of leaf extracts of *A. procera* was tested on rats at 200mg /kg BW dose. The anti-ulcer activity was in the following order vegetative > Flowering > Fruiting. Both the neutralizing factor and protective factor was maximum in leaf extract from vegetative stage. Leaf extract (200mg/kg BW) also was found to have anti-oxidant, analgesic activity (Sivaramakrishnan and Kottai Muthu, 2013a and 2013b; Mahfza Kharoom *et al.*, 2014) Methanol leaf extract of *A. chevalieri* showed anti – oxidant activity (Aliyu *et al.*, 2009). Presence of saponins, flavonoids, tannins in the leaf could be attributed to anti-ulcer activity of the extract. Rutin extract of the stem and leaf of *A. procera* (Wankhade and Mulani, 2015) could also exert anti-ulcer activity (Mulani, 2015) could also exert anti-ulcer activity. Leaf collected during vegetative stage, had more anti-ulcer activity because of the presence of more active biological compounds (having anti- oxidant and anti inflammatory activity) in the vegetative phase than leaf from flowering and fruiting phase (Mahfuza Khatun *et al.*, 2002) also isolated high concentration of saponin, steroids, tannins, glycosides and phenolics in ethyl acetate fraction of leaf of *A. procera*.

V. SUMMARY AND CONCLUSION

Pharmacological studies for anti-ulcer activities are carried out for leaves of *A. procera* collected in three different growth phases. It was found that leaves of *A. procera* collected during vegetative growth phase exhibited maximum antiulcer activities. Quality and quantities of biological compounds vary with different growth phase and these are attributed to their pharmacological activities. The present investigation thus established pharmacopeia standard for leaf of *A. procera*, based on phytochemical and pharmacological studies of leaves collected at three different growth phases, it is recommended that leaves of *A. procera* collected during vegetative phase are therapeutically superior to that collected during flowering and fruiting phases.

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