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Antiulcer activity of *Melia azedarach* linn in aspirin induced and pylorus ligated rats

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ABSTRACT

In the present study aqueous and alcohol extracts of leaves of *Melia azedarach* Linn. (Meliaceae) were screened for antiulcer activity in aspirin induced and pylorus – ligated rats. The effect was assessed by parameters like pH, free acidity, total acidity and ulcer index. Aqueous extract and alcohol extract of *M. azedarach* leaves (250 mg/kg b.w., p.o.) were administered orally. Antiulcer effects were compared with standard drug Omeprazole (20 mg/kg b.w., p.o.). These observations helped us to conclude that aqueous extract (250 mg/kg b.w., p.o.) is endowed with antiulcer property.

Keywords: Antiulcer; Aspirin; *Melia azedarach*; Omeprazole; Pylorus - ligation.

INTRODUCTION

A peptic ulcer is a hole in the gut lining of the stomach, duodenum, or esophagus. A peptic ulcer of the stomach called a gastric ulcer, of the duodenum, a duodenal ulcer and of the esophagus, an esophageal ulcer. An ulcer occurs when the lining of these organs is corroded by the acidic digestive juices that are secreted by the stomach cells. For many years, excess acid believed to be the major cause of ulcer disease. Accordingly, treatment emphasis was on neutralizing and inhibiting the secretion of stomach acid. While acid considered significant in ulcer formation, the leading cause of ulcer disease currently believed to be infection of the stomach by bacteria called *Helicobacter pyloridus* (*H. pylori*). Another major cause of ulcers is the chronic use of anti-inflammatory medications, commonly referred to as NSAIDs (non-steroidal anti-inflammatory drugs), including aspirin. Cigarette smoking is also an important cause of ulcer formation and ulcer treatment failure **[1]**.

Melia azedarach Linn (Meliaceae) commonly known as Mahanimba, Ramyaka, Dreka, Karmuka, Keshamushti, Persian lilac and White cedar. *M. azedarach* is a native of west Asia and now naturalized throughout the warm countries. In India, it grows wild in the Sub - Himalayan tract up to 1800 m. The various parts of the tree are reputed to have the same therapeutic values as those of nim tree **[2]**.

Various other medicinal values are recorded **[3, 4, 5]**. The major constituents include Azaridine, bakayanin, bakalactone, margosine, azadirone, vanillin and cinnamic acid (fruits) proteins, limonoid glycosides, nimbinene, salannin and meldenin (seeds) vanillic acid and dl-catechin (cortex) nimbinene, meliacin, quercetrin, quercetin-3-O-β-rutinoside, kaempferol-3-O-β-rutinoside, rutin and kaempferol-3-L-rhamno-D-glucoside (leaves) nimbinene, azaridine, paraisine, isochuanliansu, kuline, kulactone, kulolactone, kulinone, (stem bark) bakayanin, bakalactone, tannin (heart wood) salannin, a limonoid (root) azedarachol, a steroid ester (root-bark) **[6]**.

The literature reviews revealed that antiviral **[7, 8, 9]**, insecticidal **[10, 11]**, antiparasitic **[12]**, pregnancy interceptive activity **[13]**, pesticidal **[14, 15]**, antiurolithiatic **[16]** property of *M. azedarach* have been studied scientifically.

So far no systematic study has reported for antiulcer properties of leaf extracts of *M. azedarach*. In the present study, effort has made to establish the scientific validity to the antiulcer property of *M. azedarach* leaves extracts using aspirin and pylorus - ligation induced ulceration models in rats.

MATERIALS AND METHODS

Plant Material and Extraction Procedure: The fresh leaves of *M. azedarach* collected from local areas of Belgaum, Karnataka, India during May-2006 and authenticated by P.S.N.Rao, Joint Director at Botanical Survey of India (BSI), Pune, India. A voucher specimen of the plant was deposited in the Botanical Survey of India herbarium under the number BSI/WC/Tech/275.

The air-dried leaves of *M. azedarach* reduced to fine powder (#40 mesh) and around 200 gms of dried powder was extracted separately with 70% v/v alcohol by continuous hot percolation process using Soxhlet apparatus **[17]**. Finally fresh leaves of *M. azedarach* were macerated in chloroform water I.P. for aqueous extract. After the effective extraction, solvent were concentrated at room temperature in reduced pressure using a rotary evaporator and water was removed by freeze drying to become semi-solid residue **[18]**.

Animals: Albino rats (150-250 g each) of either sex kept under standard environmental conditions (25 ± 2°C under 12 h light and 12 h dark cycles). They were housed in cages and fed with regular rat chow (Lipton India Ltd) and drinking water *ad libitum*. For experi-

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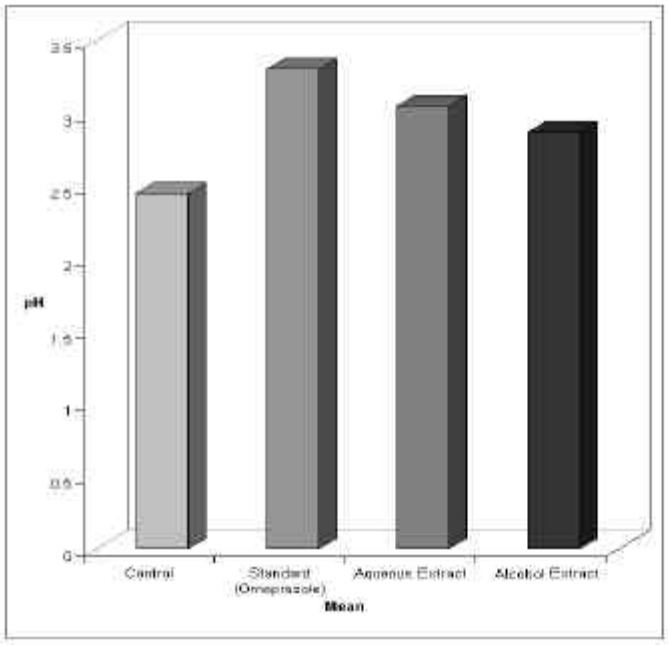
Table I.Effect of Different Extracts of *Melia azedarach* Leaves for Antiulcer Activity in Pyloric Ligation Method.

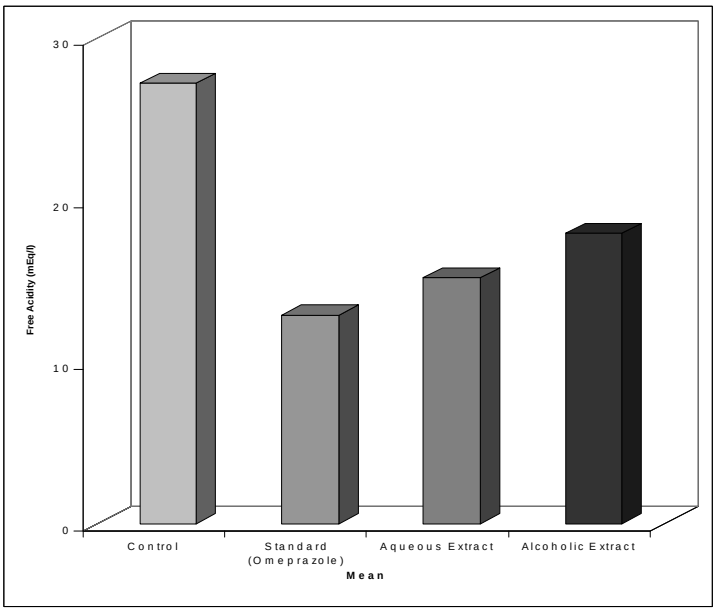
Treatment	Dose mg/kg	pH	Free acidity mEq/litre	Total Acidity mEq/litre	Ulcer index UI/L
Vehicle	1ml CMC (1%)	2.45 ± 0.042	27.23 ± 0.273	45.67 ± 1.215	4.27 ± 0.359
Aqueous Extract	250	3.05* ± 0.188	15.18* ± 0.424	33.52* ± 0.376	2.39* ± 0.150
Alcohol Extract	250	2.87* ± 0.267	17.95* ± 0.214	34.22* ± 0.268	2.50* ± 0.09
Omeprazole	20	3.305* ± 0.105	12.9* ± 0.195	30.23* ± 0.879	0.995* ± 0.346

*n=6 *p<0.01 vs. control; values are in Mean ± SEM*

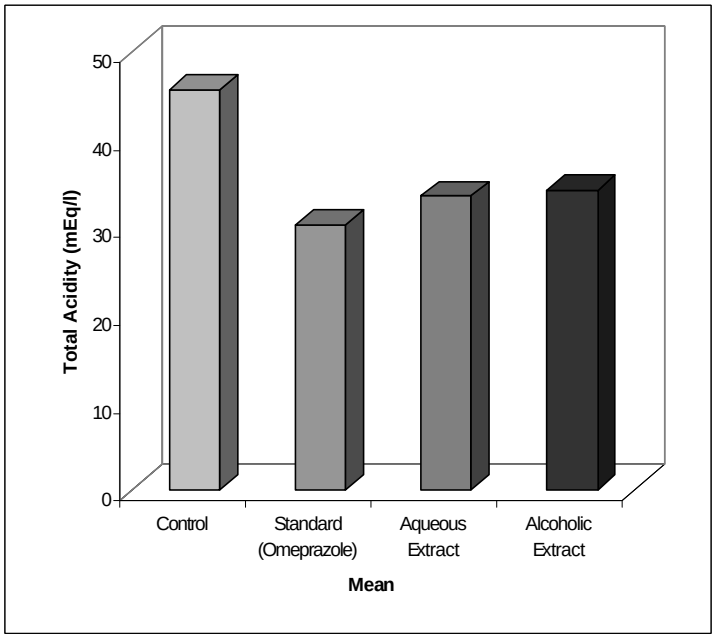
Graph I. Histogram showing the effect of aqueous extract and alcohol extract of *Melia azedarach* on pH of gastric content



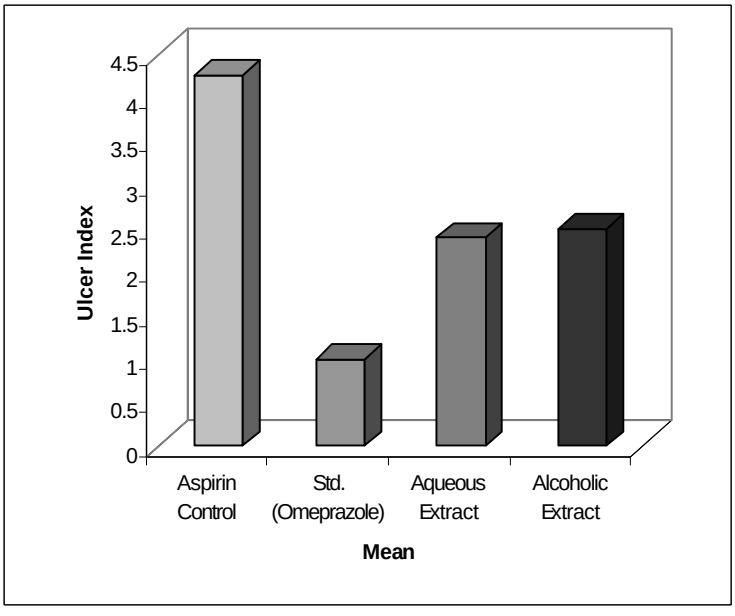
Graph II .Histogram showing the effect of aqueous extract and alcohol extract of *Melia azedarach* on free acidity of gastric content.



Graph III.Histogram showing the effect of aqueous extract and alcohol extract of *Melia azedarach* on total acidity of gastric content



Graph IV.Histogram showing the effect of aqueous extract and alcohol extract of *Melia azedarach* on ulcer index.



mentation, the animals were fasted overnight and 5-10 animals were included in each group. The experimental protocols were approved by the standard regulations.

Acute Toxicity Studies: The acute oral toxicity study was carried out as per guidelines set by organization for economic cooperation and development (OECD) revised draft guidelines 423 B. The LD_{50} cut-off dose for aqueous and alcohol extracts of leaves was found to be 2500 mg/kg body weight for all the extracts, hence, $1/10^{th}$ of the median lethal dose (LD_{50}) was taken as an effective dose (i.e. 250 mg/kg, body weight) **[19]**.

ANTIULCER STUDIES

Aspirin induced ulcers in rats: Four groups of albino rats weighing 150 - 200 g are used. Each group contains six animals. The test drugs administered orally in 2% acacia solution 10 min prior to oral aspirin in a dose of 200 mg/kg (20 mg/ml). Six hours later, the rats sacrificed in ether anesthesia and their stomachs removed. Formal - Saline (2% v/v) is then injected into the totally ligated stomachs for storage overnight. The next day, the stomachs were opened along the greater curvature, then washed in warm water and examined under a 3-fold magnifier. The lengths of the longest diameter of the lesions measured and summated to give a total lesion score (in mm) for each animal, the mean count for each group being calculated **[20, 21]**.

Pylorus ligation induced gastric ulcers: Twenty rats of either sex randomly divided into four groups and fasted for 48 h with free access to water. Pyloric ligation was performed under light ether anesthesia to each animal. Animals were given 1% Carboxy methyl cellulose (CMC) solution or leaves extracts 250 or 20 mg/kg Omeprazole orally immediately after pylorus ligation. Animals were sacrificed 4 h later. The stomach was carefully removed and gastric contents were collected. The gastric juice centrifuged at 3000 rpm for 30 min and the volume of the gastric juice was measured. Free and total acidities in the supernatant were determined by titration with 0.1 N NaOH and expressed as mEq/L/100 g. The stomach was cut open along the greater curvature and pinned on a soft board for evaluating gastric ulcers and ulcer index was calculated. The percentage inhibition of ulcers was calculated as mean ulcer index of control-mean ulcer index of test/ mean ulcer index of control x 100 **[22, 23, 24]**.

EVALUATION OF ANTIULCER ACTIVITY

Determination of Free Acidity and Total Acidity: The gastric contents were centrifuged at 1000 rpm for 10 min. 1ml of supernatant was diluted with 9 ml of distilled water. A volume of 2 ml diluted gastric juice was titrated with 0.1 N Sodium hydroxide run from a micro burette using 3-4 drops of Topfer's reagent as indicator until canary yellow color was observed. Volume of NaOH required was noted. This corresponds to free acidity. Further 2-3 drops of phenolphthalein was added and titrated with NaOH until pink color was restored. This gives total acidity.

Free acidity and total acidity is expressed in terms of ml of 0.1N HCl per 100 gms of gastric contents. This is the same as mEq/L. To obtain this figure multiply the burette reading obtained from titration by 10 **[25]**.

Ulcer Scoring & Ulcer Index Determination

Each stomach was examined grossly and the ulcers were graded using the following system suggested by Kunchandy et al., 1985.

0	-	Normal Mucosa
0.5	-	Red coloration
1.0	-	Spot ulcers
1.5	-	Hemorrhagic streaks
2.0	-	Ulcers >3 but <5
2.5	-	Ulcer >5

Ulcer index was calculated using following formula:

Ulcer Index = 10/x

Where, X = Total mucosal area / Total ulcerated area **[26, 27]**.

Statistical Analysis: Results were expressed as mean ± SE. The data obtained by the various parameters statistically evaluated by one way analysis of variance (ANOVA) followed by Dunnett test at a level of significance $p<0.01$ **[28]**.

RESULTS

Pharmacological activity led to the conclusion that the aqueous extract exhibited more significant activity than the alcohol extract. The results of pharmacological activity also concluded that the aqueous extract significantly raised the pH of gastric contents; it lowered the free and total acidity and ulcer index as compared to standard drug Omeprazole. In control animals, without any drug the mean pH is 2.45. Both the extracts showed rise in pH of gastric contents. Aqueous extract showed rise in pH (3.05) as compared to control. The rise in pH shown by alcohol extract is 2.87. Omeprazole, a standard drug raised the pH to 3.305, which is statistically significant ($p<0.01$).

Gastric free acidity is increased to (27.23 mEq/L) in control animals due to Pylorus ligation. Aqueous extract (15.18 mEq/L) showed significant decrease in free acidity ($p<0.01$) as compared to control. The decrease in free acidity by alcohol extract was 17.95 mEq/L. When compared with Omeprazole, a known antiulcer drug, aqueous extract is equipotent (12.90 mEq/L), whereas other extracts are less potent in decreasing gastric acidity.

Gastric total acidity is increased to (45.67 mEq/L) in control animals. Aqueous extract (33.52 mEq/L) showed highly significant decrease in total acidity ($p<0.01$) as compared to control and standard Omeprazole (30.23 mEq/L). The decrease in total acidity by alcohol extract is (34.22 mEq/L).

Aspirin administration (200 mg/kg) resulted in the production of gastric mucosal damage. The ulcer index in control animals was (4.36). Aqueous extract (2.39) significantly reduced the ulcer index ($p<0.01$) as compared to control. The reduction in ulcer index by alcohol extracts (2.50). Omeprazole, a standard antiulcer drug showed no ulcer production. The results are tabulated in table I.

DISCUSSION

Melia azedarach antiulcer property was evaluated by employing Pylorus-ligation and Aspirin induced ulceration models in albino rats. Aspirin induced ulceration models were used because they represent some of the most common causes of gastric ulcers in humans. Many factors and mechanisms are implicated in the ulcerogenesis and gastric mucosal damage induced by models employed in the present study involving the increase of gastric acid output, vascular injury, depletion of gastric wall mucin and mucosal damage induced by Non-Steroidal Anti-Inflammatory drugs **[29]**. Gastric acid and Pepsin are important factors for the formation of ulcers in pylorus-legated rats **[30]**. Increased synthesis of nucleic acids and metabolism of carbohydrates and other compensatory mechanisms could also be responsible for the ulceration due to Pylorus-ligation **[31]**.

It has been proposed that in Pylorus-ligation, the digestive effect of accumulated gastric juice and interference of gastric blood circulation are responsible for induction of ulceration **[32]**. *Melia azedarach* antiulcer activity in Pylorus-ligation model is evident from its significant reduction in free acidity, total acidity and ulcer index along with significant increase in the gastric pH in drug treated animals. *Melia azedarach* leaves extracts treated animals significantly inhibited the formation of Pylorus-ligated ulcers in the stomach and also decreased acid concentration, therefore it is suggested that *Melia azedarach* can suppress gastric damage induced by aggressive factors. Non-steroidal anti-Inflammatory drugs like Aspirin cause gastric mucosal damage by decreasing prostaglandin levels through inhibition of prostaglandin synthesis **[33]**. *Melia azedarach* was significantly effective in protecting gastric mucosa against Aspirin induced ulcers.

CONCLUSIONS

It is concluded that aqueous extract of leaves of *Melia azedarach* possess significant antiulcer activity against experimentally induced ulcers in rats. There was an inhibitory effect on acid secretory mechanisms, which may be due to the presence of venal compounds. Further research to isolate antiulcer principle is needed.

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