

GASTROPROTECTIVE ACTIVITY OF *PONGAMIA PINNATA* STEM BARK IN WISTAR ALBINO RATS

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ABSTRACT

The present study was carried out with the stem bark of *Pongamia pinnata* Linn, belonging to the family Fabaceae. It is one of the fast growing, glabrous, deciduous tree and has recently gained importance as a commercial source of alkaloids like kaempferol, karanjin. Methanolic extract of *Pongamia pinnata* was subjected to phytochemical screening. Acute gastric ulceration was produced by oral administration of various noxious chemicals including Ethanol, Indomethacin, Pylorus ligation and Cold restraint stress. In pharmacological screening, the effect of methanolic extract of stem bark of *Pongamia pinnata* Linn was evaluated in Wistar Albino Rats of either sex (150-200g) for Antiulcer activity at a dose of 200mg/kg and 400mg/kg (p.o) and the effect was compared with Omeprazole (10mg/kg p.o) as standard drug. The extract decreased the ulcer index thereby increasing the percentage ulcer protection. Thus from the study and literature, it can be concluded that *Pongamia pinnata* Linn have potent antiulcer activity.

KEYWORDS: Antiulcer activity, *Pongamia pinnata*, Screening methods, Omeprazole.

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INTRODUCTION

A peptic ulcer refers to a group of disorders characterized by circumscribed lesions of the mucosa of the upper gastrointestinal tract (especially of the stomach and the duodenum). The lesions occur in regions exposed to gastric juices. Peptic ulcer occurs when there is an imbalance between offensive and defensive factors. Ulceration in the mucosa can be because of either breakdown of mucosa with the development of surface defects or failure of restitution of mucosal integrity resulting in retardation or failure of healing of the ulcers¹.

Pongamia pinnata (L) Pierre (Fabaceae) synonym (*Pongamia glabra* Vent.) popularly known as 'Karanja' in hindi, is a medium sized glabrous tree found throughout India and further distributed eastwards, mainly in the littoral regions of south eastern Asia and Australia. In the Ayurvedic literature of India, different parts of this plant have been used in the treatment of several ailments. Traditionally used in treatment of bronchitis, whooping cough, rheumatic joints, diabetes^{2,3}. The roots, seeds, fruits, flowers have been reported in treatment of various diseases⁴. Phytochemical

examination of this plant has indicated the presence of furanoflavones, furanoflavonols, chromenoflavones, furanodiketones and flavonoid glycosides^{5,6}. The objective of the present study was to investigate the gastroprotective activity of methanolic extract of stem bark extract as it is traditionally reported to cure ulcer.

MATERIALS AND METHODS

Plant material

Stem bark of *Pongamia pinnata* was collected from Erode (TN) during September. It was authenticated by Botanical survey of India, Coimbatore.

Preparation of extract

Shade dried and powdered bark (1kg) was extracted with 70% methanol in Soxhlet apparatus. Solvent evaporation under reduced pressure yielded the semisolid extract.

Animal used

Wistar Albino rats of either sex weighing between 180-250g were used. Animals were housed under standard conditions of temperature (24±2°C) and relative humidity (30-70%) with a 12:12 (light: dark) cycle. The animals were given standard diet and water *ad libitum*. All procedures involving animals were carried out under

the Institutional Animal Ethics Committee approval (688/02/C-CPCSEA) of NCP.

Toxicity studies

Toxicity studies of the stem bark extract were carried out in Swiss Albino mice of either sex weighing between 20 and 25 g. The LD₅₀ of the stem bark extract was found to be safe till 2000 mg/kg (p.o).

Methods

Ethanol induced ulcer

The gastric ulcers were induced in rats of either sex weighing between (180-250g) by administering absolute ethanol (8ml/kg). They were kept in specially constructed cages to prevent coprophagia during and after the experiment. The rats were divided into four groups each containing six animals and fasted for 24h and allowed free access of water. The first group received control vehicle only and the second group standard Omeprazole at the dose of 10mg/kg; third and fourth group received methanolic extract of *Pongamia*

pinnata at the dose of 200 and 400mg/kg orally, daily, respectively for five days for ulcer protective studies. On the sixth day of experiment the drugs were administered orally 30min prior to the oral administration of absolute ethanol. The animals were anaesthetized 6h later with ether and stomach was incised along the greater curvature and ulceration was scored. The number of ulcers and the length of each ulcer were scored⁷. A score for the ulcer was made as

| Ulcer Score | Descriptive Observation |
|-------------|-----------------------------|
| 1 | Less than 1 mm (Pin point) |
| 2 | 1-2 mm |
| 3 | Greater than 2 mm and above |

Mean ulcer score for each animal was expressed as ulcer index. The percentage of ulcer protection was determined as follows:

$$\% \text{ protection} = \frac{\text{control mean ulcer index} - \text{test mean ulcer index}}{\text{Control mean ulcer index}} \times 100$$

Indomethacin (IND) induced ulcers

Animals were divided into four groups of six animals each. First group received vehicle only, second group received Omeprazole (10mg/kg), third and fourth group received methanolic extract of *Pongamia pinnata* at a dose of 200 and 400mg/kg orally for 5 days. On the sixth day of experiment the drugs were administered orally 30 min prior to the oral administration of Indomethacin (25mg/kg). The animals were anaesthetized 4h later with ether and rats were sacrificed. The glandular portion of stomach was taken and used for estimation of ulcer index. The number of ulcers and the length of each ulcer were measured. A score for the ulcer was made as mentioned above⁷.

Pylorus Ligation Method

In this method, Wistar Albino rats were fasted in individual cages for 18h. First group received control vehicle, second group received Omeprazole (10mg/kg, p.o) and animals of third and fourth group received methanolic extract of *Pongamia pinnata* at a dose of 200 and 400mg/kg, 1h before pylorus ligation. Under ether anaesthesia, the abdomen was opened and pylorus was ligated. The abdomen was then sutured. At the end of 4h after ligation, the animals were sacrificed with excess of anaesthetic ether, and the stomach was dissected out. The glandular portion was exposed and examined for ulceration. Ulcer index was determined as mentioned above⁷.

Biochemical estimation

The various biochemical parameters like carbohydrate content viz. fucose, hexosamine, total hexoses and total protein were evaluated. Secretions and enzymes viz. gastric volume, pH, free and total acidity were evaluated.

Gastric Volume

This was measured after centrifuging the gastric fluid, allowed to stand, decant, and poured into the measuring cylinder.

Determination of pH

The pH of the gastric juice was measured using the pH meter.

Determination of Free Acidity and Total Acidity

1 ml of gastric juice was pipetted into a 100 ml conical flask. 2 drops of Topfer's reagent was added and titrated with 0.01N sodium hydroxide (NaOH) (which was previously standardized with 0.01 N of oxalic acid) until all traces of the red colour disappears and the colour of the solution become yellowish orange. The volume of alkali added was noted. This volume corresponds to free acidity. Then 2 drops of phenolphthalein solution was added and titration was continued until a definite red tinge reappears. Again the total volume of alkali added was noted⁸. This volume corresponds to total acidity.

Acidity was calculated by using the formula:

$$\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Actual Normality of NaOH} \times 100}{0.1} \mu\text{eq/l/100g}$$

Estimation of Total Proteins

Lowry reagent was prepared by mixing sodium carbonate 100ml, sodium potassium tartrate 1 ml and copper sulphate 1 ml. To 1ml tissue homogenate 5ml of Lowry reagent was added and mixed. It was incubated for 10 minutes at 25°C. Then 0.5ml of folin's reagent was added and incubated for 30 minutes at 25°C. The absorbance of the sample was measured at 750nm spectrophotometrically⁹. The amount of total protein in the tissue was expressed in μg protein/ml.

Estimation of Total Carbohydrates

The dissolved muco-substances in gastric juice were estimated in the alcoholic precipitate obtained by adding 1 ml of gastric juice to 9 ml of 90% alcohol and the mixture was kept for 10 min and the supernatant was discarded. The precipitate separated was dissolved in 0.5ml of 0.1 N NaOH. To this 1.8 ml of 6N HCl was added. This mixture was hydrolyzed in the boiling water bath for 2 hours. The hydrolysate was neutralized by 5N sodium hydroxide using phenolphthalein as indicator and the volume was made up to 4.5 ml with distilled water and used for the estimation of total hexoses, hexosamine and fucose as described below¹⁰.

$$\text{True optical density} = \frac{(\text{OD}_{396} - \text{OD}_{430})_{\text{unknown}} - (\text{OD}_{396} - \text{OD}_{430})_{\text{unknown blank}}}{(\text{OD}_{396} - \text{OD}_{430})_{\text{water blank}}}$$

Estimation of Hexosamine

0.5 ml of the hydrolysate fraction was taken. To this 0.5 ml of acetyl-acetone reagent was added. The mixture was heated in boiling water bath for 20 min and it was cooled under running tap water. 1.5 ml of 90% alcohol was then added followed by an addition of 0.5 ml of Ehrlich's reagent. The reaction was allowed for 30 min. The colour intensity was measured in spectrophotometer at 530 nm against the blank prepared by using distilled water instead of hydrolysate. Hexosamine content of the sample was determined from the standard curve prepared by using D (+) glucosamine hydrochloride and concentration has been expressed in $\mu\text{g/ml}$ of gastric juice.

Estimation of Total Hexoses

To 0.4 ml of hydrolysate, 3.4 ml of orcinol reagent was added. The mixture was then heated in the boiling water

Estimation of Fucose

In this method, three test tubes were taken. In one tube 0.4 ml of distilled water was taken and serves as control and in each of the other two tubes 0.4 ml of hydrolysates were taken. To all three tubes 1.8 ml of sulphuric acid: water (6:1) was added by keeping the tubes in ice cold water bath to prevent breakage due to strong exothermic reaction. This mixture was then heated in boiling water bath for exactly 3 min. The tubes were then taken out and cooled. To the blank and to one of the hydrolysate containing tube (unknown) 0.1 ml of cysteine reagent was added and to the another hydrolysate containing tube cysteine reagent was not added (unknown blank). It was then allowed for 90 min to complete the reaction. The reading was taken in spectrophotometer at 396 and 430 nm setting the zero with the distilled water. The optical density for the fucose in the hydrolysate was calculated from the differences in the reading obtained at 296 and 430 nm and subtracting the values without cysteine. This was read against standard curve prepared with D (+) – fucose. The fucose content was expressed in terms of $\mu\text{g/ml}$ of gastric juice.

bath for 15 minutes. This was then cooled under running tap water and intensity of the colour was read in spectrophotometer at 540 nm against the blank by using distilled water instead of hydrolysate. Total hexoses content was determined from the standard curve of D (+) – galactose –mannose and has been expressed in $\mu\text{g/ml}$ of gastric juice.

Cold Restraint induced ulcers

Four groups of Wistar Albino rats (n=6) were selected. In this model, Group I served as Normal control (vehicle) received 1% CMC, p. o., and Group II received Standard Omeprazole (10mg/kg, p.o) whereas Groups III and IV animals received methanolic extract of *Pongamia pinnata* at the dose of 200 and 400 mg/kg, p.o. respectively daily for 3 days. Animals were fasted overnight prior to the experiment, and water *ad libitum*. On day 3, after 30 min of methanolic extract of

Pongamia pinnata or Omeprazole treatment, rats were immobilized in a stress cage and were placed at 4–6 °C in an environmental cage¹⁰. The animals were sacrificed 2h later and ulcer index was calculated following the method as described earlier.

Statistical analysis

All the results were expressed as mean ± standard error. The data was analysed statistically using ANOVA followed by Dunnett's T test.

RESULTS

Present study shows that the methanolic extract of *Pongamia pinnata* stem bark possess good antiulcer activity. Ulcer index were decreased and percentage protection were significantly increased but it was less than Omeprazole treated animals (**Table 1**).

DISCUSSION

Peptic ulcers are thought to be due to an imbalance between offensive acid-pepsin secretions versus impaired mucosal resistance¹¹. The defense mechanism of the gastrointestinal mucosa against aggressive factors, such as Hydrochloric acid, Bile acid, Free radicals, *Helicobacter pylori* colonization, Non-steroidal anti-inflammatory drugs etc., mainly consists of functional, humoral and neuronal factors. Mucus-alkaline secretion, mucosal microcirculation, cellular mucus, life span of mucosal cells and motility act as functional factors, while prostaglandins and nitric oxide act as humoral factors, and capsaicin sensitive sensory neurons act as neuronal factors. All the above factors are known to contribute to mucosal protection¹². The defensive factors like mucus secretion, life span of mucosal cells and its proliferation leading to early repair, prostaglandins (PGs), mucosal blood flow and antioxidants status of the mucosal cells offering resistance to damage. A good understanding of pathogenesis and etiology can lead to effective prevention and treatment of the cause and the most effective therapeutic interventions we can design. Commonly used antisecretory drugs produce several adverse reactions such as Gynaecomastia, Hematopoietic changes, acute interstitial nephritis, Anaphylaxis reactions¹³, Nephrotoxicity and Hepatotoxicity¹⁴. Thus, there is a need for more effective, less toxic and less expensive antiulcer (ulcer protective and healing) agent. Hence the search for an ideal anti-ulcer drug continues and has also been extended to herbal drugs in search for new and novel molecules, which can afford better protection and decrease the incidence of relapse.

In the present study, it indicates that the pharmacological evaluation of antiulcer activity of methanolic extract of stem bark of *Pongamia pinnata* was screened at the dose of 200kg/kg and 400mg/kg per oral. All the parameters

were observed for antiulcer activity. It was compared with standard Omeprazole. It shows equipotent action as that of Omeprazole. Gastric volume, pH, total acidity and free acidity were calculated for the anti secretory model. Previous studies have demonstrated that several compounds which could be responsible for the plant antiulcer effect such as flavonoids, saponins, alkaloids(karanjin, kaempferol)¹⁵. These results tends to suggest that the methanolic extract of *Pongamia pinnata* stem bark possess antiulcer activity and it was traditionally used as antiulcer justified.

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Table 1: Effect of *Pongamia pinnata* on Antiulcer models indicating ulcer index (Ui) and percentage protection (% Pro)

| Treatment | Ethanol induced ulcer | | IND induced ulcer | | Pylorus ligation | | Cold restraint ulcer | |
|------------------------------------|-----------------------|-------|-------------------|-------|------------------|-------|----------------------|-------|
| | Ui | % Pro | Ui | % Pro | Ui | % Pro | Ui | % Pro |
| Solvent control (1% CMC) | 58.33±3.80 | - | 37±1.23 | - | 62±4.28 | - | 39.33±1.38 | - |
| <i>Pongamia pinnata</i> (200mg/kg) | 28.33±2.66** | 51.43 | 21.00±1.15** | 43.24 | 28.33±2.66** | 54.30 | 27.50±1.83** | 30.07 |
| <i>Pongamia pinnata</i> (400mg/kg) | 24.50±1.47** | 57.99 | 15.66±0.33** | 57.67 | 24.66±1.80** | 60.22 | 22.66±1.05** | 42.38 |
| Omeprazole (10mg/kg) | 23.33±0.88** | 60.00 | 14.50±0.34** | 60.81 | 23.66±0.66** | 61.83 | 20.16±1.07** | 48.74 |

Values are mean ± SEM, Number of animals in each group = 6
 * P value <0.05, **p<0.01 compared with the corresponding control group

Table 2: Effect of *Pongamia pinnata* on Pylorus Ligated (Shay) Rat Model Indicating Gastric volume, pH, Free acidity & Total Acidity

| Sl.No. | Treatment | Gastric volume (ml) | pH | Free acidity (µeq/ml/100g) | Total acidity (µeq/ml/100g) |
|--------|------------------------------------|---------------------|-------------|----------------------------|-----------------------------|
| 1. | Solvent Control (1% CMC) | 6.33±0.13 | 3.23±0.10 | 73.18±0.90 | 133.68±1.57 |
| 2. | Omeprazole (10mg/kg) | 3.26±0.13** | 4.43±0.04** | 44.53±1.13** | 95.17±1.11** |
| 3. | <i>Pongamia pinnata</i> (200mg/kg) | 3.28±0.17** | 3.15±0.15** | 44.16±1.25** | 95.49±1.20** |
| 4. | <i>Pongamia pinnata</i> (400mg/kg) | 3.02±0.02** | 4.1±0.10** | 43.97±0.22** | 81.57±0.77** |

Values are mean ± SEM, No. of animals in each group = 6
 * P value <0.05, ** P value <0.01 compared with the corresponding control

Table 3: Effect of *Pongamia pinnata* on Pylorus Ligated (Shay) Rat Model Indicating Total Hexose, Hexosamine, Fucose & Total Protein

| Sl. No. | Treatment | Total Hexoses (µg/ml) | Hexosamine (µg/ml) | Fucose (µg/ml) | Total Protein (µg/ml) |
|---------|------------------------------------|-----------------------|--------------------|----------------|-----------------------|
| 1. | Solvent Control (1% CMC) | 256.50±2.29 | 259.00±17.09 | 76.83±2.13 | 138.33±1.45 |
| 2. | Omeprazole (10mg/kg) | 367.71±1.38** | 459.19±4.67** | 174.18±2.44** | 516.20±3.79** |
| 3. | <i>Pongamia pinnata</i> (200mg/kg) | 341.36±0.79** | 430.34±2.45** | 117.18±2.13** | 553.54±3.06** |
| 4. | <i>Pongamia pinnata</i> (400mg/kg) | 317.87±2.01** | 451.38±7.09** | 168.73±1.59** | 548.53±3.23** |

Values are mean ± SEM; No. of animals in each group = 6.
 * P value <0.05, ** P value <0.01 compared with the corresponding control.

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