

## RESEARCH ARTICLE

ANTIULCER ACTIVITY OF *PONGAMIA PINNATA* IN RATS\*Anupriya Pandey<sup>1</sup> Vaibhavi Jakhethia<sup>1</sup> and Sonu Sharma<sup>2</sup><sup>1</sup>Department of Pharmacognosy, Suresh Gyan Vihar University, Jaipur, Rajasthan, India<sup>2</sup>Nims Institute of Pharmacy, Nims University, Shobhanagar, Jaipur, Rajasthan, India\* Corresponding Author's Email id: [anupriya.pandey09@gmail.com](mailto:anupriya.pandey09@gmail.com)

## ABSTRACT

*Pongamia pinnata* (Linn) belonging to the family *Fabaceae* (*Papilionaceae*) commonly known as **Karanj**. It is a small evergreen tree, which is widely distributed in India, Bangladesh, China, and Australia. The anti-ulcer activity of methanolic extract of *Pongamia pinnata* (L) (MEPP) was investigated in HCl/ethanol and aspirin induced ulcer models in wistar rats. In both models the common parameter determined was ulcer index. MEPP at doses of 50 and 500 mg/kg p.o produced significant inhibition of the gastric lesions induced by aspirin induced ulcer & HCL/ethanol induced gastric ulcer. The extract (50 mg/kg & 500 mg/kg) showed significant ( $P < 0.05$ ) reduction in ulcer index as compared to control. This present study indicates that *Pongamia pinnata* (L.) extract have potential anti ulcer activity in the both models. These results may further suggest that methanolic extract was found to possess antiulcerogenic as well as ulcer healing properties.

**Key words:** *Pongamia pinnata* (L.), HCl/ethanol induced ulcer, Aspirin induced ulcer, Ulcer index.

## INTRODUCTION

*Pongamia pinnata* (L.) belonging to the family Fabaceae is a mangrove plant known 'Karanj' in Hindi. According to literature survey, it is an important medicinal plant. It has been found to possess major therapeutic activity such as antibacterial,<sup>1</sup> anti-inflammatory and analgesic,<sup>2</sup> antihyperammonemic,<sup>3</sup> antinociceptive and antipyretic activities,<sup>4</sup> anti-dyslipidemic<sup>5</sup> and anticonvulsant activity<sup>6</sup> and many more. We have studied the antiulcer activity of *Pongamia pinnata* (L.) plant leaves on different models on gastric ulceration.

## MATERIALS AND METHODS

## Plant material

The leaves of *Pongamia pinnata* were collected from Jaipur in August 2010. A voucher specimen (Voucher no. RUBL20817) was kept at the Department of Botany, University of Rajasthan by Mr. Vinod Sharma Botanist after identification of the plant.

## Preparation of extract

The powder of dried leaves of *Pongamia pinnata* was subjected to successively soxhlet extraction with various organic solvents such as petroleum ether (60-80°C) and methanol. The extract was cool at room temperature, filtered, concentrated to dryness of extract.<sup>7</sup>

## Preliminary phytochemical screening

The phytochemical examination of the methanolic extract of leaves of *Pongamia pinnata* (L.) was performed by the standard methods.<sup>8</sup>

## Animal used

Wistar rats of either sex weighing between 150-250g were used. Animals were housed under standard conditions of temperature ( $24 \pm 2^\circ\text{C}$ ) and relative humidity (30-70%) with a 12:12 light: dark cycle. The animals were given

standard diet supplied and water *ad libitum*. All procedures involving animals were carried out under the institute ethics committee approval (1234/A/08/CPCSEA).

## HCl - Ethanol induced ulcer

Rats were allotted into different groups; each group was containing 6 animals and fasted for 24 hours, then first control group receiving an oral dose of saline (9% NaCl, 5 ml/kg), next second group was received methanolic extract 50mg/kg as test drug, next third group receive methanolic extract 500mg/kg as test drug and other groups were receive Ranitidine (100 mg/kg, p.o) as reference compounds. After 1 hour, all groups were orally treated with 1ml of 150 mM HCl/EtOH (40:60, v/v) solution for gastric ulcer induction. Animals were killed 4 hours after the administration of ulcerogenic agent; their stomach were excised and opened along the great curvature, washed and stretched on cork plates. The surfaces were examined for the presence of lesions and the extent of the lesions was measured. The numbers of the lesions along the stomach were recorded as ulcer index and % I.<sup>9</sup>

## Aspirin induced ulcer in rats

Rats were allotted into different groups; each group was containing 6 animals weighed 150–200 g were used, Then the test and standard drugs were administered orally in 0.1% Tween 80 solution 10 min prior to oral Aspirin in a dose of 20 mg/kg (4 mg/ml dissolved in 0.1% Tween 80 solution). After six hours, the rats were sacrificed in CO<sub>2</sub> anesthesia and their stomachs were removed. Then the totally ligated stomachs were injected into Formol-saline (2% v/v) for storage overnight. Then next day, the stomachs were opened along the greater curvature and were washed in warm water, and examined under a 3- fold magnifier. Number of lesions along the stomach was

recorded as ulcer index and % I was also calculated using the formula.<sup>10</sup>

$$\%I = \frac{U_{sc} - U_{st}}{U_{sc}} \times 100$$

U<sub>sc</sub> = Control mean ulcer index

U<sub>st</sub> = Test mean ulcer index

### Ulcer index (UI) and percent inhibition (% I)

Mean ulcer score for each animal will be expressed as ulcer index.<sup>11</sup>

Macroscopic evaluation was carried out and the presence of lesions was scored. Scoring of ulcer is shown in table.

**Table 1: Macroscopic evaluation for ulcer score**

Observation	Scores
Normal stomach	0.0
Red coloration	0.5
Spot ulcer	1.0
Hemorrhagic streak	1.5
Ulcer	2
Perforation	3

### Statistical analysis

The values are represented as mean  $\pm$  S.E.M, and statistical significance between treated and control groups was analyzed using of One way ANOVA, followed by Dennett's test where P<0.05 was considered statistically significant.

### Histopathological evaluation

#### Microtomy of rat's stomach

After the success of pharmacological studies for antiulcer models. Treated groups were selected for histological studies. Rats from the most active groups were taken and their stomach were dissected out after euthanizing the animals under deep ether anesthesia and preserved (fixed) in 10% neutral formalin buffer.<sup>12</sup>

#### Dehydration

Transverse sections of epithelium layer of stomachs were cut into and placed in absolute alcohol for dehydration. Four changes were given in absolute alcohol each for 15 minutes viz. 30, 50, 70 and 90 % alcohol.

#### Preparation of Tissues for Embedding

After four changes in alcohol, parts were transferred in a mixture of absolute alcohol + xylene (1:1) for 15-20 minutes. Then mixture was decanted off and parts were put in xylene for 30 minutes. Further 30 minutes later, scrapings of wax were added to xylene till saturation and kept for 24 hours.

#### Paraffin Infiltration and Embedding

The matured wax was filtered off to remove any suspended particles and it was kept in molten state for 24 hours at 62-64°C. The material was transferred directly in molten wax in the first infiltration pan for 45 minutes at 62°C in an oven. After the first embedding, tissue pieces were removed and placed in second infiltration and were kept as such at controlled temperature.

### Block Preparation

The lid of cuff ling jar was applied on upper and side surface of lid. The filtered matured wax was poured in the lid up to 4/5<sup>th</sup> of the total height. The tissues were removed immediately from the infiltration pan and placed gently into the lid. It was allowed to stand at room temperature until solidification. The lid was placed in a tray containing water. It was kept as such until the block separated and floated in the water. The block was cut and trimmed to remove excess wax.

### Microtomy

The block was then cut serially into ribbons of section with the help of microtome. The ribbon sections were transferred to a glass slide on which a fixative had been applied previously.

### Staining of slides

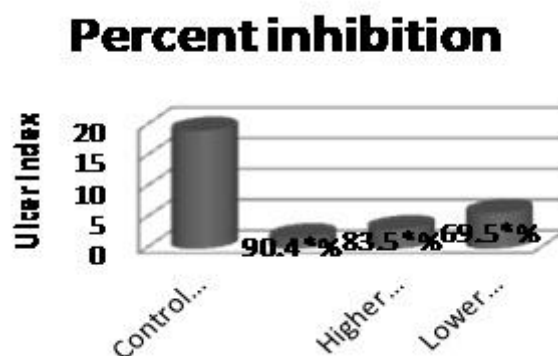
The sections on slides were de waxed with xylol. Aqueous haematoxylin and alcoholic eosin were used for staining. The sections were dehydrated with different concentration of alcohol and xylol. Sections were mounted with Canada balsam on the slides carefully with glass rod, covered with cover slip, viewed (10x) and photographed.<sup>13</sup>

## RESULTS

### 3.1 Phytochemical screening

The results of preliminary phytochemical screening of the Methanolic extract of *Pongamia pinnata* (L.) (MEPP) revealed that presence of alkaloids, flavonoids, carbohydrates, glycosides, tannins, triterpenoids and steroids.

#### HCl - Ethanol induced ulcer



**Graph 1: Effect of methanolic extract of *Pongamia pinnata* (L.) Family Fabaceae on HCL/ETOH induced ulcer**

**Table 2: Effect of the methanolic extract of *Pongamia pinnata* (L.) Family Fabaceae on HCL/ETOH induced ulcer**

Group no.	Treatment	Dose	Ulcer index	%I
1.	Control	-	19.16±	-
2.	Lower dose	50mg/kg	*5.83±	69.5%
3.	Higher dose	500mg/kg	*3.16±	83.5%
4.	Standard	100mg/kg	*1.83±	90.4%

Significant at \* $P < 0.05$  compared to control.

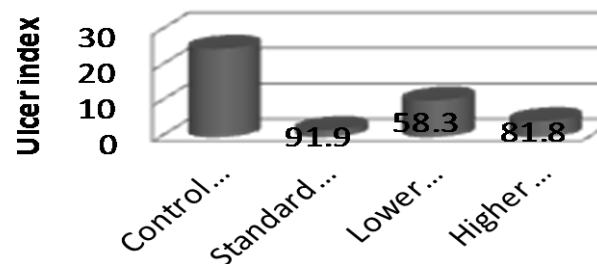
### Aspirin induced ulcer in rats

**Table 3: Effect of the methanolic extract of *Pongamia pinnata* (L.) Family Fabaceae on Aspirin induced ulcer**

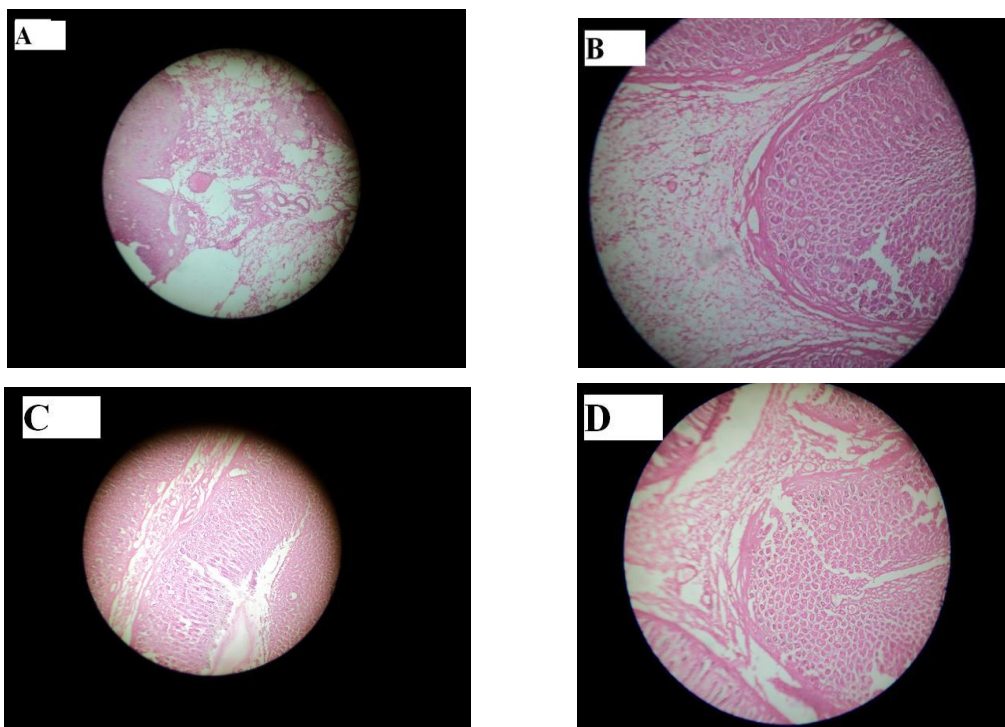
Group no.	Treatment	Dose	Ulcer index	%I
1.	Control	-	24.83±	-
2.	Lower dose	50mg/kg	10.33±	58.3%
3.	Higher dose	500mg/kg	*4.5±	81.8%
4.	Standard	8.0mg/kg	*2.00±	91.9%

Significant at \* $P < 0.05$  compared to control.

## Percent inhibition

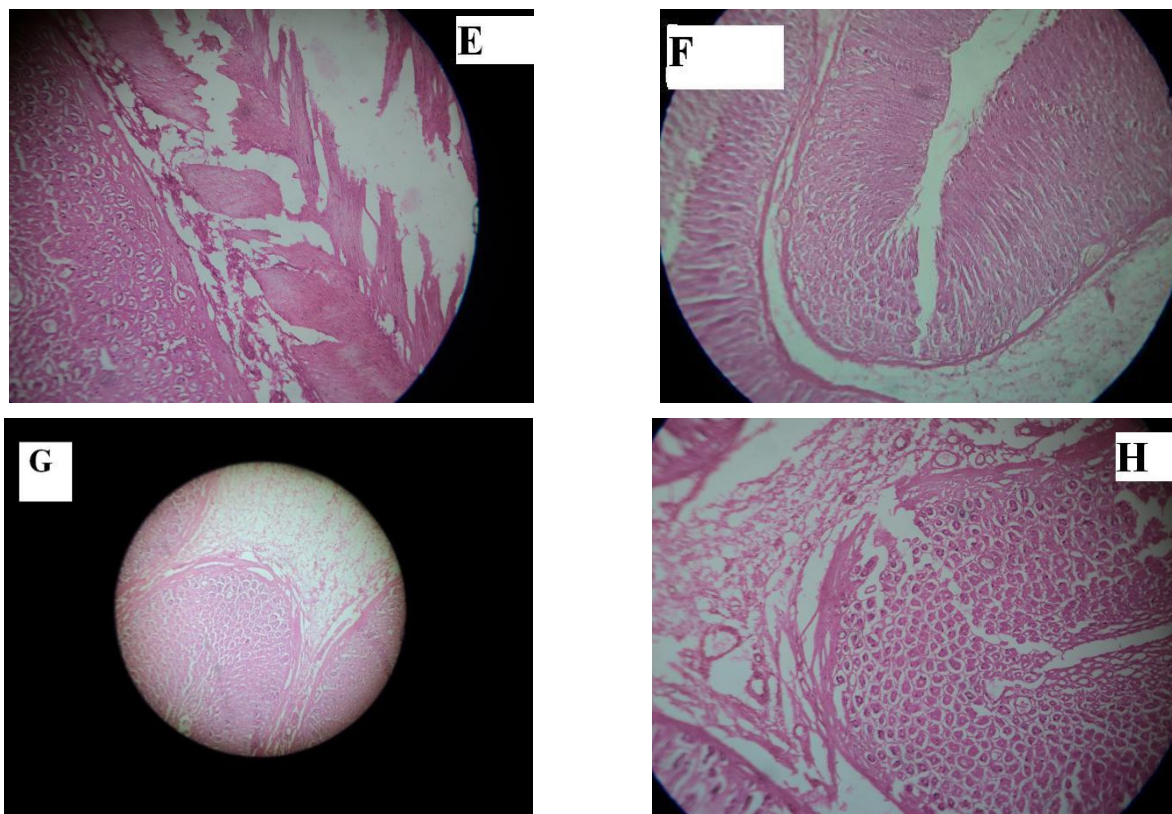
**Graph 2: Effect of the methanolic extract of *Pongamia pinnata* (L.) Family Fabaceae on Aspirin induced ulcer**

### Macroscopical and Histopathological Evaluation



**Figure 1: Rats stomach HCL/ETOH induced ulcer: (A) Control, (B) Standard, (C) Extract higher dose, (D) Extract lower dose.**





**Figure 2: Rats stomach Aspirin induced ulcer: (E) Control, (F) Extract higher dose, (G) Standard, (H) Extract lower dose.**

## DISCUSSION

Peptic ulcer is caused by a lack of equilibrium between the gastric aggressive factors and the mucosal defensive factors. Gastric ulcer is among the most serious diseases in the world. The etiology of gastroduodenal ulcers is influenced by various aggressive and defensive factors such as acid-pepsin secretion, parietal cell, mucosal disease barrier, mucus secretion, blood flow, cellular regeneration and endogenous protective agents such as prostaglandins and epidermic growth factors. Some other factors, such as inadequate dietary habits, excessive ingestion of non-steroidal anti-inflammatory agents, stress, hereditary predisposition and infection by *Helicobacter pylori*, may be responsible for the development of peptic ulcer.<sup>14</sup>

### In HCl - Ethanol induced ulcer

Oral administration of methanolic extract of leaves of *Pongamia pinnata* (L.) in two different dose showed significant reduction in ulcer index as compared to the control group. It was showing %I of 69.5 % and 83.5 % at the dose of 50 and 500 mg/kg respectively in comparison to control whereas Ranitidine as reference standard drug was reduction of ulcer 90.4% (Results are tabulated in Table-2 and graph 1).

### In Aspirin induced ulcer in rats

Oral administration of methanolic extract of leaves of *Pongamia pinnata* (L.) in two different dose showed significant reduction in ulcer index as compared to the control group. It was showing %I of 58.3 % and 81.8 % at the dose of 50 and 500 mg/kg respectively in comparison to control whereas Omeprazole reference standard drug was reduction of ulcer 91.9%. (Results are tabulated in Table-3 and graph 2).

### Macroscopical and Histopathological Evaluation

The prepared slides were viewed under light microscope (10X). The erosion in the epithelium was severe in case of control group. It is quite evident from the sections seen. The more number of ulcers was found in control group as compared to the other groups. The less numbers and severity of ulcer was found in groups treated with methanolic extract. The presence of few ulcer sites in case of higher dose of methanolic extract. Treated groups signify that these drug treatments have potential ulcer protective effects are shown in figure 1 and 2.

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