

**GASTROPROTECTIVE EFFECT OF HYDROALCOHOLIC LEAVES EXTRACT OF
*PONGAMIA PINNATA*****MAHENDRA A. GIRI^A, RASIKA D. BHALKE^A AND SUBODH C.PAL^B**^A Sanjivani College of Pharmaceutical Education and Research Kopergaon,
M.S., India, Pin code-423603,^B N. D. M. V. P. S's College of Pharmacy, Nashik, (MS), India-422002***Corresponding Author** rasikabhalke@yahoo.co.in**ABSTRACT**

Pongamia pinnata has long been used in folk medicine in treatment for bronchitis, whooping cough, rheumatic arthritis, and diabetes, various inflammatory conditions and dyspepsia. Therefore, Present study was designed to investigate the antiulcer effect of hydroalcoholic extract of leaves of *P. pinnata* (HLEPP) using different models of gastric ulceration in rats. Hydroalcoholic extract of leaves of *P. pinnata* (HLEPP) was subjected to preliminary phytochemical screening. Acute gastric ulceration in rats was produced by oral administration of various noxious chemicals including aspirin or ethanol or indomethacin or pylorus-ligated technique. Gastric tissue was also examined histologically. HLEPP was administered in the dose of 400 mg/kg orally in all experiments; dose was calculated on the basis of acute toxicity study. Omeprazole, ranitidine, misoprostol were used as a reference drug. The antiulcer activity was assessed by determining and comparing the ulcer index in the test drug group with that of the vehicle (negative) control group and standard (positive) control group. The qualitative phytochemical study reveals the presence of alkaloids, carbohydrates, phytosterols, saponins, tannins and flavonoids. HLEPP at the dose of 400 mg/kg produced a significant ($P < 0.05$) reduction in the ulcer index. HLEPP significantly inhibited gastric mucosal damage induced by aspirin, ethanol, and indomethacin and in pylorus-ligated rats. The anti-ulcer effect was further confirmed histologically. The anti-ulcer activity of HLEPP was however, less than that of standard drugs. Moreover, further insight into the precise mechanism of action is essential to exploit the complete potency of HLEPP and increase its usage in contemporary medicine. The above effects of HLEPP may also be due to the presence of tannins and flavonoids in the extract.

Keywords: *Pongamia pinnata*, hydroalcoholic extract, aspirin induced ulcer, ethanol induced ulcer, indomethacin induced ulcer, pylorus ligation technique

INTRODUCTION

Pongamia pinnata (L.) Pierre (Leguminosae, Papilionaceae; synonym *Pongamia glabra* Vent.), popularly known as 'Karanj' or '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 recommended as a remedy for various ailments, and have been used in traditional medicines for bronchitis, whooping

cough, rheumatic joints and to quench dipsia in diabetes^{1,2,3}. The seed and seed oil have been used for treating various inflammatory and infectious diseases such as leucoderma, leprosy, lumbago, and muscular and articular rheumatism. The leaves are hot, digestive, laxative, anthelmintic and cure piles, wounds and other inflammations. A hot infusion of leaves is used as a medicated bath for relieving rheumatic pains and for cleaning ulcers in gonorrhea and scrofulous enlargement. Different extracts of roots and seeds (ethanol, petroleum, ether, benzene extracts and others) of *Pongamia pinnata* have been reported to have anti-inflammatory activity⁴. In addition, phytochemical examinations of this plant have indicated the presence of furanoflavones, furanoflavonols, chromenoflavones, flavones, furanodiketones and flavonoid glucosides^{5,6}. The objective of the present study was to investigate the antiulcer activity of hydroalcoholic leaves extract as it is traditionally reported to cure ulcer.

MATERIAL AND METHODS

Plant material

Leaves of *P. pinnata* were collected from the western rural area of the kopargaon, Ahmednagar District (M. S.) during June at the flowering stage of the plant. It was authenticated by the Botanical Survey of India, Pune. (Voucher specimen no. AABPP1)

Preparation of extract

Shade-dried and powdered leaves were defatted with petroleum ether and then extracted with hydroalcohol (70%) in Soxhlet apparatus. Solvent evaporation under reduced pressure yielded the dried hydroalcohol extract (6.5%).

Animal used

Albino Wistar rats of either sex weighing between 150-250g 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 supplied by Pranav agro industries Ltd. (Sangli) and water *ad libitum*. All procedures involving animals were carried out under the institute ethics committee approval (997/c/06/CPCSEA).

Toxicity studies

Toxicity studies of the hydroalcohol (70%) leaf extract were carried out in Swiss Albino mice of either sex weighing between 20 and 25 g. The LD₅₀ of the hydroalcohol (70%) leaf extract was found to be safe till 2000 mg/kg (i.p. and p.o.).

Antiulcer activity

Aspirin induced ulcer

Animals were divided in three groups of six animals each. Group I served as negative control received distilled water, Group II served as positive controls and received omeprazole at the dose of 20 mg/kg, and animals of Group III received hydroalcoholic extract of leaves of *P. pinnata* at the dose of 400 mg/kg, orally daily, respectively, for five days for ulcer protective studies. Aspirin in dose of 20 mg/kg was administration to the animals on the day of the experiment and ulcers were scored after 4 h. The animals were anaesthetized, sacrificed and the stomach was then excised and cut along the greater curvature, washed carefully with 5.0 ml of 0.9 % NaCl and ulcers were scored by a person unaware of the experimental protocol in the glandular portion of the stomach. Ulcer index was then calculated by adding the total number of ulcers per stomach and the total severity of ulcers per stomach⁷⁻⁹.

A score for the ulcer was made as: 0.5-Hemorrhage, 1-Streaks, 2-Spot ulcer, 3-Sever ulcer or Sever steaks, 4-Erosions, 5-Perforation.

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

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

Ethanol induced ulcer

The gastric ulcers were induced in rats of either sex weighing between 130-150 g by administering absolute ethanol (8 ml/kg). They were kept in specially constructed cages to prevent coprophagia during and after the experiment. The rats were divided into three groups each containing six animals and fasted for 24 h and allowed free access to water. The first group received control vehicle only and the second group received standard ranitidine in the dose of 20 mg/kg, third group received hydroalcoholic extract of leaves of *P. pinnata* at the dose of 400 mg/kg, orally daily, respectively, for five days for ulcer protective studies. On the sixth day of experiment the drugs were administered orally 30 min prior to the oral administration of absolute ethanol. The animals were anaesthetized 6 h later with ether and stomach was incised along the greater curvature and ulceration was scored¹⁰⁻¹³. The number of ulcers and the length of each ulcer were measured. A score for the ulcer was made as mentioned above.

Indomethacin induced ulcer

Animals are divided into three groups of six animals each. Group I served as negative control received distilled water, Group II served as positive controls received misoprostol (100 µg/kg, p.o), and animals of Group III received hydroalcoholic extract of leaves of *P. pinnata* at the dose of 400 mg/kg, orally daily, respectively, for five days. On the sixth day of experiment the drugs were administered orally 30 min prior to the oral administration of indomethacin (5 mg/kg, p.o). The animals were anaesthetized 4 h later with ether and rats were sacrificed. The glandular portion of the 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 above¹⁰⁻¹³.

Pyloric ligation method

In this method albino rats were fasted in individual cages for 24 h. Group I served as negative control received distilled water, Group II

served as positive controls received ranitidine (20 µg/kg, p.o), and animals of Group III received hydroalcoholic extract of leaves of *P. pinnata* at the dose of 400 mg/kg, 1 h before pylorus ligation. Under thiopental sodium anesthesia, the abdomen was opened and the pylorus was ligated. The abdomen was then sutured. At the end of 4 h after ligation, the animals were sacrificed with excess of anesthetic ether, and the stomach was dissected out¹⁴. The glandular portion was then exposed and examined for ulceration. Ulcer index was determined as mentioned above.

Statistical analysis

Mean values \pm S. E. M. were calculated for each parameter. For the determination of significant intergroup differences, each parameter was analyzed separately and one-way analysis of variance (ANOVA) was carried out. $p < 0.05$ was considered significant.

RESULT AND DISCUSSION

Ulcer index parameter was used for the evaluation of anti-ulcer activity since ulcer formation is directly related to factors such as reduction in gastric volume, decrease in free and total acidity. Hydroalcoholic extract of leaves of *P. pinnata* at the dose of 400 mg/kg, has decreased the intensity of gastric mucosal damage induced by ulcerogenic agents.

HLEPP at the dose of 400 mg/kg and omeprazole at 20mg/kg produced a significant ($P < 0.05$) reduction in the ulcer index 41.00 and 18.5 and has protection index of 71.19 % and 87.00 % respectively as shown in table 1. Aspirin has been reported to produce ulcers by both local and systemic effects¹⁵. Aspirin causes direct irritant effect and mucosal damage by interfering with prostaglandin synthesis, increasing acid secretion by increasing the H⁺ ion transport/back diffusion of H⁺ ions, resulting overproduction of leukotrienes and other products of 5-lipoxygenase pathway. It decreases mucin, surface active phospholipids bicarbonate secretion, mucosal proliferation and also produces damage by formation of free radicals¹⁶. The possible protective effect of

HLEPP against aspirin-induced gastric lesions could be due to prevention of direct irritation, increased mucus secretion and due to its 5-lipoxygenase inhibitory effect.

HLEPP showed the ability to reduce significantly the severity of ulceration of stomach induced by absolute ethanol. HLEPP at dose of 400 mg/kg and ranitidine at 20mg/kg has shown significant ($P < 0.05$) reduction in ulcer index upto 41.00 and 32.0 with protection index of 65.10% and 72.76% respectively as shown in table 1. The results of histopathological investigation revealed that the pretreatment with HLEPP absolutely prevented the ethanol-induced congestion, hemorrhage, edema, necrosis, inflammatory and dysplastic changes, erosions and ulceration in the gastric mucosa of rats. The stomach appearance was normal. The incidence

of ethanol-induced ulcers predominant in the glandular part of stomach was reported to stimulate the formation of leukotriene C4 (LTC4), mast cell secretory products¹⁷, and reactive oxygen species resulting in the damage of rat gastric mucosa¹⁸. In ethanol model, ulcers are caused due to perturbations of superficial epithelial cells, notably the mucosal mast cells leading to the release of the vasoactive mediators including histamine, thus causing damage to gastric mucosa¹⁹. Mucosal blood flow has been attributed to be an important factor in the damage caused by alcohol and is modulated by prostaglandin²⁰. The effectiveness of HLEPP protection against mucosal damage caused by ethanol is indication of its effect on prostaglandins. The results are presented in table 1.

Table I
Anti-ulcer activity of hydroalcoholic extract of leaves of *P. pinnata* on different ulcer-induced models:

| Treatment | Aspirin induced ulcer | | Ethanol induced ulcer | | Indomethacin induced ulcer | | Pylorus ligated ulcer | |
|-----------------------|-----------------------|--------------|-----------------------|--------------|----------------------------|--------------|-----------------------|--------------|
| | Ulcer index | % protection | ulcer index | % protection | ulcer index | % protection | Ulcer index | % protection |
| Vehicle | 142.33 ± 3.667 | -- | 117.50 ± 4.463 | -- | 87.83 ± 2.928 | -- | 124.00 ± 2.733 | -- |
| Test (HLEPP) 400mg/kg | 41.00 ± 2.216* | 71.19 | 41.00 ± 4.211* | 65.10 | 26.50 ± 2.141* | 69.82 | 29.00 ± 1.155* | 76.61 |
| Ranitidine 20µg/kg | § | § | 32.00 ± 1.461* | 72.76 | § | § | 32.0 ± 1.461* | 74.19 |
| Omeprazole 20mg/kg | 18.50 ± 2.566* | 87.00 | § | § | § | § | § | § |
| Mesoprostol 100µg/kg | § | § | § | § | 16.83* ± 1.138 | 80.83 | § | § |

Values are mean ± S.E.M. * $p < 0.05$, ** $P < 0.01$, *** $P < 0.0001$ compared to control group.

-- The group treated for the negative control and receive vehicle.

§ -not done.

HLEPP - hydroalcoholic extract of leaves of *P. pinnata*

HLEPP at dose of 400 mg/kg has shown significant effect in indomethacin induced ulcer model with a protection index of 69.82 % ($P < 0.05$) whereas standard drug misoprostol has 80.83 %. Indomethacin causes generation of reactive oxygen metabolites (such as superoxide

anion, hydrogen peroxide and hydroxyl radical), which damages the gastric tissue and causes ulcer formation. The pathogenesis of gastric mucosal lesions by indomethacin is associated with increased lipid peroxidation. Oral administration of HLEPP prevents gastric ulcers.

Reduced glutathione in the gastric mucosa acts as the major scavenger of the oxygen-derived free radicals. It may be concluded that HLEPP has preventive action on indomethacin induced ulcer in rats. It is possible that the antioxidant effect of HLEPP might also play a role in the mechanism of antiulcer activity²¹. The results are summarized in table 1.

Pylorus ligation induced gastric ulcers occur because of an increase in acid-pepsin accumulation due to pyloric obstruction and subsequent mucosal digestion and breakdown of the gastric mucosal barrier²². A copious amount of mucus is secreted during superficial damage and provides favorable microenvironment in repair. Hence estimation of acid secretion, pepsin secretion and mucus secretion is a valuable part of the study to clarify the mechanism of action of the hydroalcoholic extract is under trial. The results are summarized in table 1.

The qualitative phytochemical study reveals the presence of alkaloids, carbohydrates, phytosterols, cardiac glycosides, saponins, tannins and flavonoids. The above effects of HLEPP may also be due to the presence of tannins and flavonoids in the extract. Tannins have astringent action, precipitating proteins of mucosal membranes and skin. According to Tani *et al.* (1979) and Esaki *et al.* (1986) some tannins suppresses the gastric secretion, having a local action of protection of the gastric mucosa^{23, 24}.

Overall, HLEPP has shown a substantial and significant protection against gastric ulcers in all the models. Further insight into the precise mechanism of action is essential to exploit the complete potency of HLEPP and increase its usage in contemporary medicine.

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