ANTIULCER, ANTI-INFLAMMATORY AND ANALGESIC ACTIVITIES OF LEAF EXTRACTS OF *PONGANIA PINNATA* LINN (FABACEAE)

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Summary

Leaf extracts of *Pongamia pinnata i.e,* Petroleum ether (PEPP) and ethyl alcohol (EAPP) extracts, prepared by successive extraction in soxhlet apparatus and aqueous extract (AQPP) by maceration process were evaluated for the antiulcer, anti-inflammatory and analgesic activities in rats. LD_{50} studies of all the three extracts were carried out in albino mice up to the dose limit of 2000mg/kg. One- fifth of the maximum dose tested for LD_{50} of each extract was selected to study the anti-ulcer, anti inflammatory and analgesic activities in different experimental models such as pylorus ligated, aspirin and swimming stress induced ulcer models in rats. Carrageenin induced paw edema model for anti-inflammatory and radiant heat (tail flick) induced nociceptive model for analgesic activity were adopted. In pylorus ligation model parameters like ulcer index, volume of gastric juice, free acidity, total acidity, p^H and total protein content of gastric juice were estimated. In carrageeenin induced inflammatory model percentage inhibition of paw edema and with radiant heat analgesiometer delay in tail flick response were recorded. No mortality was observed with any of the three extracts up to the maximum dose of 2000mg/kg. Further all the three extracts (PEPP, EAPP and AQPP) at 200mg/kg, 100mg/kg and 200 mg/kg, p.o respectively had significantly ($P<0.001$) reduced the ulcer index in pylorus ligated, aspirin and swimming stress induce ulcers. A significant anti- inflammatory activity was noted with all the three extracts and also a significant analgesic activity was observed with ethanol and petroleum ether extracts but not with aqueous extract. The present study revealed the anti-ulcer, anti-inflammatory with all the three extracts but analgesic activity only with ethanol and petroleum ether extracts of *P.pinnata.* The above activities observed may be due to the presence of phytochemical constituents such as alkaloids, flavonoids, triterpenes, steroids, glycosides and tannins.

Key words: *P. pinnata*, leaf extracts, antiulcer, anti-inflammatory, analgesic activity

Introduction

Plants have been one of rich and important sources of medicines and are the gifts of nature to the mankind for treating different types of diseases. The medicinal properties of plants are due to the presence of phytochemicals, for example; vincristine and vinblastine which are the chemical constituents of *Catharanthus roseus*, are used as anticancer agents in the treatment of Hodgkin's diseases[1]. Considering the importance of plants as a source of medicine, we have selected a plant *P. pinnata* Linn (Fabaceae) which is used for centuries for ulcers, as an analgesic, anti diabetic, for wounds and tapeworm infestations[2]. *P. pinnata* synonymous to *P. glabra* is a handsome tree which is normally found growing along river and stream sides. Earlier studies have indicated that *P. pinnata* Linn has anti-viral[3], anti-fungal[4], anti-diarrhoeal[5], anti-parasitic[6], anti-nematode[7], and anti-nephrotoxic[8]activity. Leaf extracts were reported with anti inflammatory activity in acute, sub acute and chronic models[9], and analgesic activity with hot plate, acetic acid writhing models[10]. Roots are used for anti-inflammatory[11], analgesic and antiulcer activities[12]. In the present study, we have attempted to investigate the anti-ulcer, anti-inflammatory and analgesic activities of various extracts of the leaves of *P. pinnata (*Plate-1).

Peptic ulcer is a benign lesion of gastric or duodenal mucosa occurring at a site where the mucosal epithelium is exposed to acid and pepsin. So it's a major health problem in terms of morbidity and mortality. A number of factors responsible for pathogenesis of ulcer diseases are increased parietal cell mass, basal secretory drive, post prondial acid secretion, gastrin release, sensitivity of secretogogues and decreased inhibition of acid and gastrin secretion, delayed gastric emptying and decreased mucosal resistance and genetic, environmental factors are also thought to play a role in ulcer formation. Acute upper gastrointestinal erosions, ulcers in cancer patients, stress due to disease states and anxiety also contribute the production of ulcers. In general stress induced ulcers due to mucosal damage and ischemic conditions are more common than ulcers due to hyper secretion of gastric acid in cancer patients.

Methods

Plant Material and Extracts: The leaves of *P. pinnata* were collected in the month of October (2003) from the herbal garden of our college and were identified by a botanist. The leaves were dried under shade, powdered and extracted with petroleum ether (60-80°C) for 48hrs by soxhlet process and same marc was successively extracted with ethyl alcohol (60-80°C) for 48 hours. The aqueous extract was prepared by using fresh powder by maceration process (60-80°C). i.e 100gm of the powdered drug was taken in a 2000ml round bottom flask and to which 500ml of distilled water was added along with 10ml of chloroform used as preservative. The extraction process was carried out for a week with occasional stirring and the resultant obtained so after filtration at the end of 7th day was subjected for drying at 45ºC to get a solid mass.

Phytochemical tests:

Preliminary Phytochemical tests were carried out with all the three extracts adopting standard procedures (Kokate, 1994)[13].

Experimental Animals:

Adult albino mice (18-22g), Wistar rats (150-200g) of either sex were used for the study. The mice and rats procured from Sri Venkateshwara Enterprises, Bangalore were kept in the animal house for 7 days maintained under standard husbandry conditions with free access to food and water supplied ad libitum.

Acute toxicity (LD_{50}) test:

The acute toxicity of PEPP, EAPP and AQPP was determined in albino mice. After administration with different doses of these extracts, the mortality with each dose was noted up to 48 hours (acute study) and to 14 days (chronic study). LD_{50} was calculated as per OECD guidelines NO. 425. $1/5^{th}$ of dose (maximum dose) tested for LD_{50} of individual extracts was considered for the testing of antiulcer, anti-inflammatory and analgesic activities. The extracts were administered p.o at doses of 200mg/kg (PEPP), 100mg/kg (EAPP) and 200mg/kg (AQPP) in all the experiments.

Drugs:

Ranitidine and Indomethacine were procured from Dr.Reddy's Laboratory, Hyderabad, Aspirin form Intermed, Bangalore, Pentazocine was procured from Ranbaxy laboratory, Ahamadabad and Indomethacine from Dr. Reddys laboratory. The solvents such as Ethyl alcohol and Pertroleum ether were procured from Nice Chemical Limited and Carrageenin from Genuine Chemicals Company- Mumbai.

ANTI ULCER ACTIVITY

A. Pylorus ligated ulcer model

Wistar rats of either sex weighing between 150-200gms were divided into five groups of six animals in each and all the animals were fasted for 24 hours. (Group I) animals were administered with 1ml of 2% gum acacia suspension which was served as control, the standard (Group II) with Ranitidine 30mg/kg, p.o ethanol extracts of 100mg/kg, po (Group III), petroleum ether extract of 200mg/kg, po (Group IV), and aqueous extract of 200mg/kg, po in (Group V). After 30 minutes of drug administration the pylorus part of the stomach of all animals was ligated under light ether anesthesia and was sacrificed 6 hours after ligation. The contents of the stomach were collected for estimation of free acid, total acid, pH, volume of gastric juice and glycoprotein content. The stomach were washed and severity of the ulcers were scored under 10X as per ulcer index by kulkarni[14] (i.e.0= normal colored stomach, 0.5= red colouration, 1=spot ulcer, 1.5= hemorrhagic streaks, 2= ulcers> 3mm but<5mm, 3=ulcers>5mm). The percentage protection was calculated using the formula[15].

$$100-(U_t/U_c) \times 100.$$

Where U_t = Ulcer index of treated group

U_c =Ulcer index of control group.

B. Aspirin induced ulcers

Five groups of animals each consists of six are used here like the previous study. The vehicle, standard drug and all the three extracts i.e PEPP, EAPP and AQPP were administered 30 minutes prior to aspirin 500mg/kg p.o. Six hours later rats were sacrificed, stomachs isolated, contents were collected for estimation of different parameters as mentioned above.

C. Swimming stress induced ulcer model

All the animals were deprived of food for 24 hours and the test drugs i.e vehicle, ranitidine, PEPP, EAPP and AQPP extracts were given 30 minutes prior to the stress. They are forced to swim inside a vertical trough of 30 cm height and 15 cm diameter containing water 15 cm height which is maintained at 25 °C for 4 hours. After the stress, they were removed from the trough and sacrificed for testing the ulcer index.

ANTI-INFLAMMATORY ACTIVITY

Five groups of animals each consisting of six were selected. At '0' hr left hind paw volume of all the animals were recorded plethysmographically. Group I was administered with 0.2ml of normal saline orally which served as normal control,. Group II with 20 mg/kg of Diclofenac sodium orally served as positive control Group III, IV and V were administered with three extracts at dose levels 200mg 100mg, and 200 mg/kg respectively. After 30 minutes 0.1 ml of 1% suspension of carrageenan was administered at sub plantar region of hind paws of all rats. Paw volume of the control, standard and test groups were measured plethysmographically at prefixed time intervals i.e ½,1, 2 and 3hrs.

ANALGESIC ACTIVITY

The analgesic activity of different extracts of *P. pinnata* was carried out by radiant heat analgesiometer in rats[16]. In this study rats were subjected to noxious stimuli by exposing tip of the tail (last 1-2 cm to radiant heat source) by using Radiant heat analgesimometer and the withdrawal of the tail from the heat source is taken as the cut off time, or the end pint. The animals were divided into five groups each consisting of six animals. Initially normal reaction time in all the animals were recorded. Group I served as normal control which received normal saline, Group II with 10mg /kg of pentazocine i.p. which served as reference standard. Group III, IV and V were received with petroleum ether (200mg/kg) ethanol (100mg/kg) and aqueous extract (200mg/kg) respectively which served as test groups. The reaction time was recorded at 15, 30 and 45 minutes.

Statistical Analysis:

Data was shown as the mean ± SEM and analysed by one way ANNOVA followed by students't' test. The level of significance for all experiments was $P<0.05$*, 0.01** and 0.001***.

Results

Phytochemical investigation:

Phytochemical investigation revealed the presence of alkaloids, flavonoids, triterpenes and steroids in petroleum ether, alkaloids, glycosides, triterpeneids, flavanoids and tannins in ethyl alcohol and alkaloids, glycosides, tannins in aqueous extracts.

Anti-ulcer activity:

A significant anti-ulcer activity was observed with all the three extracts in pylorus ligated rats and percent protection exhibited by PEPP, EAPP and AQPP were 72.11%, 64.02% and 43.09% respectively (Plate-2). All the three extracts were significantly reduced Aspirin induced ulcers in rat model as reduction in ulcer index were 79.03%, 60.27% and 42.62% respectively (Plate-3). In swimming stress a significant reduction in ulcer index i.e. 66.75%, 36.25% and 39.75% were observed with PEPP, EAPP and AQPP extracts respectively(Plate-4). Gastric juice volume in pylorus ligated rats was significantly reduced (32.00%, 20.86%) by EAPP and PEPP extracts respectively but not with AQPP extract and pH of the gastric acid is significantly increased with all the three extracts. In pylorus ligated rats free acid, total acidity and glycoprotein content of gastric juice were significantly reduced by all three extracts.

Anti-inflammatory and analgesic activity:

A significant anti-inflammatory activity was noted with all the three extracts at different time intervals (i.e 1/2, 1, 2 and 3 hour) i.e, reduction in edema volume with all the 3 extracts noted were PEPP (13%, 34.14%, 38.34%, 59.59%) EAPP (15%, 38.71%, 43.60%, 61.46%) and AQPP (6%, 10.75%, 12.78%, 19.69%) extracts at four time intervals respectively. More anti-inflammatory effect was recorded with EAPP and PEPP but not with AQPP extract . The anti-inflammatory of the former two extracts were almost equal to standard diclofenac sodium at 3rd hour. A significant analgesic activity was observed with EAPP and PEPP extracts but not with AQPP extract at prefixed time intervals.

Table I: Effect of *Pongamia pinnata* leaf extracts on pylorus ligated ulcers

Groups	**Dose mg/ kg**	**Total score**	**Mean ulcer index ± SEM**	**Vol.of gastric juice ml/100gm mean ± SEM**	**Free acidity (eq/l) 100gm mean ± SEM**	**Total acidity mEq/l/100 gm mean ± SEM**	**Total Protein mean ± SEM**	**Percentage Protection**	**pH mean± SEM**
G I	1	43.0	7.17 ± 0.54	6.28 ± 0.107	90.17 ± 5.28	184.17 ± 0.22	644.2 ± 12.78	-	2.58 ± 0.15
G II	30	5.5	1.0 ± 0.22	3.37 ±0.201	39.17 ± 6.19	81 ± 15.09	596.78 ± 4.43	86.05	4.9 ± 0.316
G III	100	12.0	2.0 ± 0.22	-	-	-	-	72.11	5.23 ± 5.48
G IV	200	15.5	2.58 ± 0.74	4.97 ±0.23	51.83 ± 6.84	111.67 ±15.28	346.02 ±11.69	64.02	3.22 ± 0.020
G V	200	24.5	4.08 ± 0.81	6.14 ± 0.20	29.5 ± 1045	99.5 ± 5.67	497.73	43.09	3.23 ± 1.32
One way ANNOVA F df P			25.88 29 P<0.001	38.80 29 P<0.001	14.44 29 P<0.001	5.91 29 P<0.001	38.33 29 P<0.001		31.98 29 P<0.001

Group I- control 2 % gum acacia Group II- Ranitidine standard
Group III- EAPP extract Group IV- PEPP extract
Group V- AQPP extract
Each group comprises of six animals; Significance at p< 0.05*, 0.001**, 0.001***.

Table II: Effect of *Pongamia pinnata* leaf extracts on aspirin induced ulcers

Group	Dose mg/kg	Total scores	Mean ulcer Index ± SEM	Percentage Protection
Group I	1	43.5	7.25 ± 0.51	-
Group II	30	4.0	0.667 ± 0.17	90.80 ***
Group III	100	9.0	1.5 ± 0.22	79.31 ***
Group IV	200	17.0	2.83 ± 0.038	60.27 **
Group V	200	28.0	4.66 ± 0.86	-
One way ANNOVA		F df P	28,68 29 P<0.001	

Group I- control 2 % gum acacia Group II- Ranitidine standard
Group III- EAPP extract Group IV- PEPP extract
Group V- AQPP extract
Each group comprises of six animals; Significance at p< 0.05*, 0.001**, 0.001***.

Table III: Effect of *Pongamia pinnata* leaf extracts on Swimming stress induced ulcers

Group	Dose mg/kg	Total scores	Mean ulcer Index ± SEM	Percentage Protection
Group I	1	24.0	4.0 ± 0.45	-
Group II	30	4.0	0.67 ± 0.16	83.25 ***
Group III	100	6.0	1.33 ± 0.28	66.75***
Group IV	200	10.5	1.75 ± 0.34	56.25 ***
Group V	200	14.5	2.41 ± 0.271	39.75 *
One way ANNOVA		F df P	16.49 29 P<0.001	

Group I- control 2 % gum acacia Group II- Ranitidine standard
Group III- EAPP extract Group IV- PEPP extract
Group V- AQPP extract
Each group comprises of six animals; Significance at p< 0.05*, 0.001**, 0.001***.

Table IV: Anti inflammatory activity of *Pongamia pinnata* leaf extracts on carrageenin induced edema in rat hind paw

Group	Dose	Paw oedema volume after							
	Mg/kg	0hr	% ROV	1 hr	% ROV	2 hr	% ROV	3hr	% ROV
I	2	0.45	-	0.93 ± 0.009	-	1.33 ±0.07	-	1.93 ± 0.006	-
II	20	0.54	20%	0.56 ± 0.01	39.79% ***	0.64 ± 0.006	51.88% ***	0.71 ± 0.005	63.21%***
III	100	0.52	15%	0.57 ± 0.007	38.71% ***	0.75 ±0.007	43.60% ***	0.74 ± 0.003	61.66%***
IV	200	0.51	13%	0.61 ± 0.004	34.14% ***	0.82 ± 0.006	38.34% ***	0.78 ±0.005	59.59%***
V	200	0.48	06%	0.83 ± 0.007	10.75%	1.16 ± 0.004	12.78% ***	1.55 ± 0.01	19.69%***
One way ANNOVA			F df P	417.86 29 <0.001		2.3073 29 <0.001		1292.9 29 <0.001	

Group I- control 2 % gum acacia; Group II- Diclofenac sodium standard ; Group III- EAPP extract Group IV- PEPP extract; Group V- AQPP extract; Each group comprises of six animals; Significance at p< 0.05*, 0.001**, 0.001***.

Table V: Analgesic activity of *Pongamia pinnata* leaf extracts by tail flick methods

Group	Dose mg/kg	Basal reaction time (second) after			
		0 min	15 min	30 min	45 min
Group I	0.2 ml	4.00 ± 0.26	4.00 ± 0.26	4.00 ± 0.26	4.00 ± 0.26
Group II	10 mg/kg	4.33 ± 0.21	7.83 ±0.31***	8.67 ± 0.42 ***	10.50 ± 0.50 ***
Group III	100 mg//kg	4.67 ± 0.21	7.33 ± 0.21***	8.17± 0.31***	9.33 ± 0.33***
Group IV	200 mg/kg	4.67 ± 0.21	5.5 ±0.22	6.33 ±0.21***	6.83 ± 0.30***
Group V	200 mg /kg	4.00 ± 0.26	4.50 ± 0.34	4.50 ±0.02	4.71 ± 0.30
One way ANNOVA		F df P	38.62 29 <0.001	50.80 29 <0.001	70.43 29 <0.001

Group I- control 2 % gum acacia; Group II-Pentozocine standard; Group III- EAPP extract; Group IV- PEPP extract; Group V- AQPP extract; Each group comprises of six animals; Significance at p< 0.05*, 0.001**, 0.001***.

Discussion

Although in most of the cases etiology of ulcers is unknown but it is due to imbalance between offensive (acid and pepsin) and defensive factors (mucin secretion, cell proliferation, PG'S deficiency). An accepted notion that an imbalance between aggressive factors and maintenance of mucosal integrity through the endogenous mechanism also results in ulcers. Mucosal defense against the luminal pepsin complicated by the mucosal action of pepsin which does not diffuses through the mucous, attacks the gut at luminal surface to produce degraded glycoprotein in the gastric juice. Thickness and turn- over rate of the mucosal layer might be great value in protecting the mucosal layers underlying the epithelial cells as in mucosal layer surface cells are continuously renewed. Increased acid secretion and decreased and poor quality of mucus production renders easy degradation by the enzyme attacks also leads the lesion on gastric mucosa and ultimately the ulcers. It is postulated that dopamine imbalance is one of the several factors for pathogenesis of gastric ulceration.

A number of drugs also readily induces ulcers i.e dopamine receptor antagonists like pimozide, spiperone, metacloperamide, haloperidol and cysteamine Hcl. Excess of gastrin secretion also results in the enhanced secretion of gastric acid. Motility of duodenum is also one of the factors responsible for impaired delivery of endogenous acid neutralizing bicarbonate secretion from distal to proximal duodenum. So collection of acid at duodenal bulb ultimately leads to ulceration. A decrease in gastro-duodenal blood flow caused by sympathetic excitation also involves in the formation of peptic ulcers.

Anti-ulcer activity was produced by the decrease in leakage and damage in mucosa and by increasing mucoprotein (mucosal) and mucus secretion i.e increased total hexose and hexsosamine. To regain the balance, different therapeutic agents including plant extracts are used to inhibit the gastric acid secretion or to boost the mucosal defense mechanism by increasing mucus production, stabilization of surface epithelial ulcer and interfere with the PG synthesis. An increase in free acid and a decrease in total acid content is a positive indication for the antiulcer activity of the administered compound. All the three extracts showed the presence of flavonoids, alkaloids, tri-terpenes, glycosides as active principles and these may be responsible for antiulcer activity as these are reported with antiulcer action. Antiinflammatory activity of this plant was not associated with any ulcerogenic effect which most of NSAIDS like aspirin show commonly. Alkaloids, flavonoids are reported with anti-inflammatory activity. All the three extracts on phytochemical studies revealed the presence of the above two principles. This confirms their anti-inflammatory activity in animal studies. Tri-terpene, alkaloids and steroidal principal present with plant products are reported with analgesic activity. PEPP and EAPP but not AQPP extracts have produced analgesic activity as they are positively reported with alkaloids, flavonoids and steroids. All the three extracts had produced significant anti ulcer(increased PG production), anti-inflammatory activities(decreased PG activity) and also analgesic activity except with AQPP extract. This indicates that in plant kingdom multiple activities may /can run side by side and these drugs could be used for their anti inflammatory activities without any danger of gastric ulceration[17].

Conclusion

According to the activities observed with all the three extracts (i.e. Petroleum ether, ethanol and aqueous extracts) it was found that ethanol extract showed maximum antiulcer activity than other two in pylorus ligated, aspirin induced and swimming stress induced ulcers in rat model. So anti ulcer activity is standard> ethanol extract >petroleum ether> aqueous extract. A significant anti-inflammatory activity was observed as EAPP>PEPP>AQPP. A significant analgesic activity observed with EAPP and PEPP extracts only but more so with EAPP extract. Results obtained with leaf extracts of *Pongamia pinnata* (Linn) revealed the anti-ulcer, analgesic and anti-inflammatory activities.

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