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Antiulcer Activity of Leaf Extracts of *Pongamia pinnata* Linn.

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Preliminary phytopharmacological investigations of different extracts of leaves of *Pongamia pinnata* Linn. revealed the presence of flavonoids, strelos and saponins and showed promising antiulcer activity. The ethanol, petroleum ether and aqueous extracts of leaves of *Pongamia pinnata* Linn. were screened for their antiulcer activity at different lethal doses screened against several experimental models of ulcer in rats. The ethanol and petroleum ether extracts showed significant reduction in the pylorus and aspirin induced ulcers in rats. These results emphasize on the need to diversify in to alternative therapeutic approaches pertaining to herbal medicine wherein a single easily available plant may provide answers to several therapeutic challenges as observed in the antiulcer activity shown by different extracts of *Pongamia pinnata* Linn.

Key Words: Antiulcer activity, *Pongamia pinnata* Linn., Aspirin.

INTRODUCTION

Peptic ulcer is an inflamed break in the lining of the stomach or the duedenum caused due to either increased acid production or damage to the mucus lining of the stomach. In most conditions the event of peptic ulser is due to imbalance taking place because of increased HCl acid secretion and decreased cytoprotective activity of the mucosal barrier[1]. Most synthetic drugs used to treat peptic ulcer act as a proton pump inhibitor or a H_2 receptor antagonist. Thus, they target the acid secretion mechanism of ulcer induction[2]. These synthetic drugs are not only highly complex, expensive and with side effects but, also act on a single cause of the disease. Hence, there exists a need to devote more research on finding a better preventive as well as curative agent that targets more than one if not all the intiating factors for peptic ulsers. Literature search revealed that herbs rich in flavonoids show several biological activities including

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antiulcerogenic activity[3]. One of the major drawbacks associated with the NASIDs in the treatment of post operative pain, edema, rheumatoid arthritis and fever is the gastric ulceration. These ulcerogenic agents may retard healing of gastric ulcer as observed in case of aspirin and indomethacin. Classical texts of herbal medicine provide a wide range of alternative therapeutic possibilities that have been used since ages, which are easily available and are much cheaper[4]. Moreover manifectation of side effects is minimum during the use of herbal therapies. This may be due to multiprotective functions of the different active components present in a single plant. Thus, a side effects caused due to a particular activity of a plant will be counteracted by its other active moieties resulting in to minimum or no tonic manifestation. In the light of these benefits, use of herbal extracts/formulations has recently gained more popularity as therapeutic tools. Thus, further work in recognizing plant based therapy of peptic ulcer is an important line of research. Considering the importance of plants as a source of medicine even today, we have selected a plant *Pongamia pinnata* Linn (family-Fabaceae) which is used for centuries for several diseases like ulcer, analgesic, diabetic,wounds, tapeworm infestation *etc.*[5]. But the plant has not been thoroughly investigated for its potential with the help of modern scientific methods of investigations of drugs. Hence an effort has been made to establish the various pharmacological activities of this plant. The main objective of this work was to study the antiulcer activity of various extracts of the leaves of *Pongonia pinnata* Linn. Earlier studies have indicated that *Pongania pinnata* Linn has antiviral[6], antifungal[7], antidiarrhoeal[8], antiparasitic[9], antiinflammatory[10], analgesic[11] and antiulcer[12] activities with various extracts of different parts of the plant.

EXPERIMENTAL

Leaves of *Pongania pinnata* Linn were collected in the month of October from the herbal garden of Karnataka College of Pharmacy and were identified by the botanist. Herbarium (TCL-15) was prepared and submitted to the museum of college.

Preparation of extracts: The leaves were shade-dried at room temperature. The dried leaves were subjected to size reduction to coarse powder by using dry grinder and passed through sieve no 40. The powder was packed in to soxhlet apparatus and extracted successively with petroleum ether (60-80ºC), ethanol and distilled water (yield 3.2, 7.5, 12.2), respectively.

All the extracts were dried at 45ºC in a hot air oven till solid to semi solid mass was obtained and were stored in air tight containers in a refrigerator below10ºC. The suspensions of ethanol, petroleum ether extracts were prepared by using 2 % gum acacia and the aqueous solution was prepared by using normal saline as solvents for the experiment.

Animal used: Wister albino rats (150-200 g) and wister albino mice (25-30 g) were procured from Indian Institute of Sciences, Bangalore, India. Before and during the experiment the rats were fed with standard diet (Gold mohr, Lipton India Ltd). After randomization in to various groups and before initiation of experiment, the rats were acclimatized for a period of 7 d under standard environmental conditions of temperature, relative humidity and dark/light cycle. Animals described as fasting were deprived of food and water for 16 h *ad libitum*. All the experiments were carried out under the guidance of ethical committee of Karnataka College of Pharmacy (Registration no 420/CPCSEA).

Toxicological studies: Preliminary oral LD_{50} doses of petroleum ether, ethanol and aqueous extracts of *Pongamia pinnata* Linn in mice were found to be 200, 100 and 200 mg/kg respectively.

Ulcer induction procedure: Gastric ulcers were induced in the experimental animals using three models, Pylorus ligation, aspirin-indused (500 mg) and swimming stress induced. All the animals were fasted for 24 h prior to dosing. The control animals were administered with the calculated dose of 1 mL of 2 % gum acacia. The positive control groups was dosed with the test extracts of different doses (200, 100, 200 mg/kg) at least 0.5 h prior to the procedures to be carried out for ulcer induction. Throughout the experiments water was provided *ad libitum* and food was withdrawn from the animals.

Stomachs from the rats were removed and cut along the greater curvature and washed in normal saline. The mucosal layer of the stomach was observed under a magnifying lens and was checked for ulcers, hemorrhagic areas or perforations. The ulcer index[13] and percentage protection was determined as described below percentage protection = (100-Ut)/Uc × 100 where Ut = Ulcer index of treated group, Uc = Ulcer index of control group.

After scoring the stomach, samples were preserved in 10 % formalin for histopathological studies. In order to calculate the difference between the control and the treated animals the results were subjected to students 't' test and Anova.

Aspirin-induced gastric ulcers: The rats were gavaged with aspirin suspended in 1 mL of 2 % gum acacia (500 mg/kg). The animals were then left as such for 6 h after which they were sacrificed, their intact stomach were removed, scored and preserved as above.

Swimming stress ulcer model: All the animals were deprived of food for 24 h and the test drugs *i.e.* ranitidine, ethanol extract, petroleum extract and aqueous extract were given 0.5 h before the stress. They are forced to swim inside the vertical trough (30 cm height and 15 cm diameter containing water which is maintained at 25ºC) after 4 h the stress were removed. Their intact stomachs were removed, scored and preserved as above.

Pylorus ligated model: The rats were subjected to pylorus ligation under ether anesthesia. They were sacrificed after 6 h post surgery and their intact stomach excised, observed, scored and preserved as mentioned above. Histo pathological examination was carried out for stomach tissue samples fixed in 10 % formalin for further studies.

Stastistical analysis: All the results are reported as mean ± SEM. Statistical significance was analyzed employing student test and one way Anova.

RESULTS AND DISCUSSION

Preliminary phytochemical screening of *Pongmia pinnata* Linn revealved the presence of sterols, flavonoids, tannins, glycosides, terpenes and alkaloids. The dose of the extracts determined by up and down stair-case method was found to be 100, 200 and 200 mg/kg body weight in mice. Preliminary investigations with ethanol extract of the leaves exhibited a significant ($p < 0.05$) ulcer index in all the models. In the groups of animals in which ulcer was induced using pylorus-ligation, ethanol showed significant activity in all the selected parameters (Table-1) with 72.11 % inhibition of ulcers and significant reduction in free acidity and total acidity and ulcer index ($p < 0.05$). pH of the gastric contents in this group was found to be 5.23 ± 0.01. The extracts significantly altered pH (4.1) and volume of gastric juice (6.1 mL), respectively. While significant effect ($p < 0.05$) on free acidity, total acidity and ulcer index was observed. The results of aspirin induced gastric ulcer in rats are summarized in Table-2. The data suggests that all the extracts showed significant reduction ($p < 0.05$) in ulcer index compared to control animals, but less reduction when compared to ranitidine treated animals. Among tested extracts ethanol showed a good per cent inhibition of ulcers (79.30 %). All the extracts showed significant activity ($p < 0.05$) in aspirin-induced ulcer model. The swimming-stress induced gastric ulcer results are summarized in Table-2. The data suggests that all the extracts showed significant reduction ($p < 0.05$) in ulcer index compared to control animals, but less reduction when compared to ranitidine- treated animals.

The antiulcerogenic activity of the extracts of leaves of *Pongania pinnata* Linn. was investigated. Gastric ulceration is related with integrity and mucosal layer and is mainly dependent on the arachidonic acid metabolism. The ulcerogens like NSAID's induce the effect by interfering with cyclooxygenase pathway. Alcohol can affect the mucosal blood flow, platelet thrombi[14] and can increase lipid peroxidation, which can damage the cell and cell membrane. While pylorus-ligation increases the presence of acid and pepsin in the stomach[14]. The extract of *Pongania pinnata* is reported to contain tannins, saponins, glycosides traces of alkaloids and

TABLE-1
EFFECT OF *Pongamia pinnata* Linn. ON PYLORUS-LIGATED GASTRIC ULCERS

Treatment	Dose	Gastric juice (mL\100 g)	pH	Free acidity (MEQ\L)	Total acidity (MEQ\L)	Mean ulcer index ± SEM	Protection (%)
Control	1 mL of 2 % Gum Acacia	6.28 ± 0.107	2.58 ± 0.15	90.17 ± 5.280	182.17 ± 0.22	7.18 ± 0.542	–
Ranitidine	30 mg/kg	3.37 ± 0.201	4.90 ± 3.12	40.17 ± 6.780	80 ± 15.09	1.0 ± 0.23	84.050
Ethanol	100 mg/kg	4.68 ± 0.310	5.01 ± 2.12	54.30 ± 7.870	107 ± 13.08	2. 1 ± 0.03	70.550
Petroleum ether	200 mg/kg	4.97 ± 0.230	3.22 ± 0.20	51.83 ± 6.840	111.67 ± 15.20	2.52 ± 0.732	62.020
Aqueous extract	200 mg/kg	6.14 ± 0.200	3.23 ± 1.32	29.50 ± 1.045	99.5 ± 5.67	4.06 ± 0.818	44.011

Significant at $p < 0.05$ *vs.* control (Student 't' test and one way Anova) each value is a mean ± SEM (n = 6).

TABLE-2
EFFECT OF *Pongamia pinnata* Linn. ON ASPIRIN AND SWIMMING STRESS INDUCED ULCER

Treatment	Aspirin induced ulcer aspirin (500 mg/kg, p.o.)			Swimming stress induced stress induced ulcer		
	Dose	Mean ulcer index ± SEM	Protection (%)	Dose	Mean ulcer index ± SEM	Protection (%)
Control	1 mL of 2 % Gum Acacia	7.010 ± 0.501	–	1 mL of 2 % Gum Acacia	4.01 ± 0.423	–
Ranitidine	30 mg/kg	0.647 ± 0.170	91.40	30 mg/kg	0.68 ± 0.150	82.24
Ethanol	100 mg/kg	1.400 ± 0.210	78.32	100 mg/kg	1.30 ± 0.240	65.74
Petroleum ether	200 mg/kg	2.810 ± 0.280	60.27	200 mg/kg	1.65 ± 0.320	55.25
Aqueous extract	200 mg/kg	4.620 ± 0.840	40.61	200 mg/kg	2.40 ± 0.261	38.74

Significant at $p < 0.05$ *vs.* control (Student 't' test and one way Anova) each value is a mean ± SEM (n = 6).

flavonoids like quercetin[5]. Phytopharmacological studies of flavonoids have opened new vistas in ulcer research. Quercetin is reported to have free radicals scavenging[16] and dose dependent antiulcer activity. It is reported to inhibit the mucosal content of platelet aggregating factor (PAF), suggesting the protective role of these substance to be mediated by endogenous PAF[17]. Further, the antiulcer activity of flavonoids is attributed to their free radical scavenging effect and their ability to inhibit the cyclooxygenase responsibility for synthesis of inflammatory prostaglandin. The pathophysiology of tissue necrosis has been attributed to the alkaline phosphate activity. Biochemical investigation are reported with increased alkaline phosphatase activity in gastro intestinal ulceration, liver and bone disease[18]. Moreover calcium ions are reported to maintain the integrity of cell membrane and regulate the cell adhesion. The animals treated with extracts have exhibited an increases in serum calcium level and decrease in alkaline phosphatase activity. Thus it can be concluded that the antiulcerogenic activity of the plant may be due to the cytoprotective and healing property of flavonoids. Determination of exact mode of action is subject of further research interest.

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