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(Research Article)

Anti-ulcer Activity of Siddha Drug *Panai Poo Chooranam* (Female Flower of *Borassus flabellifer*) in Rats

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ABSTRACT

The present study was undertaken to evaluate the protecting action of female flower of Borassus flabellifer on Aspirin induced and pylorus ligated induced model of gastro intestinal ulceration. Ranitidine 30 mg/kg served as reference drug. Two dose level of powder form of dried female flower of Borassus flabellifer (Panai Poo Chooranam – PPC) were studied in these models. Parameters include gastric volume, pH, free acidity, total acidity, ulcer index and percentage of inhibition of ulcer were analyzed. The results suggested that the both doses of PPC significantly ($p<0.01$) decreases the gastric volume, free acidity, total acidity and ulcer index and increases in pH when compared to the control. PPC 500 mg/kg has a more significant effect compared to PPC 250 mg/kg which indicated the dose dependent activity of Panai Poo Chooranam.

Key Words: Anti–ulcer, Aspirin–induced ulcers, *Borassus flabellifer*, *Panai Poo Chooranam*, Pyloric ligation.

INTRODUCTION

Peptic ulcer disease (PUD) remains a significant public health problem and has great influence over the people around the world[1.] PUD develops when the mucosa of the GI tract becomes susceptible to corrosive forces; the most important one is gastric acid. If the imbalance between the protective factors such as Prostaglandins, mucus secretion, and nitric oxide and destructive factors which include gastric acid, pepsin, and bile salts occurs, mucosal injury may result [2.]

The three main factors that contribute this imbalance are *H.pylori* infection, NSAID use, and smoking[3.] In the last few decades Peptic ulcer disease had a remarkable effect over morbidity and mortality rate. According to the latest WHO data published in April 2011, Peptic Ulcer Disease Deaths in India reached 108,392 or 1.20% of total deaths. PUD are very common in India, with 4 million individuals affected per year [4.] Greater than 20% of those with PUD experienced a decline in their functional status and over 30% complained of somatic and psychological symptoms [5.]

Though the treatment of this condition has a range of drugs available, most of them do not meet out the requirements and have side effects. Recently, the natural herbal medicines derived from traditional knowledge have emerged greatly and has effective results in treating many of the GI disorders.

The plant *Borassus flabellifer* Linn belongs to Arecaceae family. *Borassus flabellifer* is a very tall, erect, magnificent dioecious palm, found throughout tropical India, especially along the peninsula coast and in West Bengal and Bihar. Powder form of this dried female flower is used to treat acid peptic diseases in Siddha system of medicine[6]. It is also administered for its diuretic and laxative action [7].

Although the plant has tremendous therapeutic value based on classical literature and day today practice, till now the pharmacological studies has not been executed in the female flower of the plant for peptic ulcer. So the present study was undertaken to evaluate the protecting action of female flower of *Borassus flabellifer* on Aspirin induced and pylorus ligated induced model of gastro intestinal ulceration.

MATERIALS AND METHODS

Collection, Identification and Preparation of Stock Solution

The plant material used in this study was collected during the month of January - February (2012) from Soorapalli, Salem district, Tamilnadu, India and authenticated by Siddha experts of Gunapadam Department, Government Siddha medical college, Chennai and Botanist, Central Research Institute for Siddha, Chennai and a voucher specimen has been preserved in the department for future reference. The collected female flowers were dried, powdered and stored in air tight container. The powdered form of female flower of *Borassus flabellifer* was mixed uniformly in 2% CMC solution to achieve 100mg/ml as main stock solution and used in the pre-clinical study.

Chemicals and Reference Drug

All chemicals used in the present study were analytical grade and purchased from SD fine chemicals Ltd (Mumbai, India). Aspirin was obtained from BD Pharmaceutical Works and Ranitidine (Reference drug) was obtained from Ranbaxy Laboratories.

Animals

Healthy Swiss Albino rats of the Wister strain weighing 150-200 g were used for the study. The animals were used with the approval of the Institute animal ethics committee and obtained from Vels University, Chennai (Approval number:XIII/VELS/PCOL/11/2000/CPCSEA/IAEC/08.08.2012). They were fed with a balanced standard pellet diet and maintained under standard laboratory conditions, providing 24-28^{0}C temperature, standard light cycle (12 h light, 12 h dark) and water ad *libitum*.

Animals were kept in cages with raised floors of wide mesh to prevent coprophagy. Animal welfare guidelines were observed during the maintenance period and experimentation. The rats were randomly assigned to control and different treatment groups, six animals per group. The animals were acclimatized for one week under laboratory conditions.

Acute Toxicity Studies

Acute oral toxicity test was carried out as per OECD Guidelines 425 [8,9.] As with other sequential test designs, care was taken to ensure that animals are available in the appropriate size and age range for the entire study. The test substance is administered in a single dose by gavage using a stomach tube or a suitable intubation cannula. The fasted body weight of each animal is determined and the dose is calculated according to the body weight. After the substance has been administered, food was withheld for a further 2 hours in mice. Single animals are dosed in sequence usually at 48 h intervals.

However, the time interval between dosing is determined by the onset, duration, and severity of toxic signs. Treatment of an animal at the next dose was delayed until one is confident of survival of the previously dosed animal. The time interval was adjusted as appropriately in case of inconclusive response. The test is simpler to implement when a single time interval is used for making sequential dosing decisions.

Anti-ulcer Evaluation

Aspirin induced gastric ulcer:[10,11,12]

Animals were divided into four groups of six animals per group.

Group I – Control

Group II – Received 30mg/kg ranitidine as standard

Group III – Received 250mg/kg PPC 1

Group IV – Received 500mg/kg PPC 2

PPC (250, 500mg/kg) intra peritoneal and control vehicle were administered 30 min before the administration of aspirin (400mg/kg) per orally. The animals were sacrificed, after 6 hours following the administration of aspirin, stomach was removed and 2% formalin was injected into the stomach. The stomach was open along with greater curvature and immersed in 2% formalin solution. The length of each lesion was measured under the dissecting microscope. The sum of the length (mm) of all lesions for each rat was used in lesion index.

The ulcer score was determined by using a 10 × magnifying hand lens. The scoring of severity of ulceration was as follows:[12]

1 mm (pin point) = 1; 1-2 mm = 2; > 2 mm = 3; > 3 mm = 4.

The mean ulcer score was determined by dividing the total ulcer indices in a group by the total number of animals in that group.

Ulcer Score = Total ulcer index (UI) in a group/Total number of animals in that group.

Pylorus Ligation Induced Ulcer[13]

After 1 hr of treatment to different groups, the animals were anaesthetized using thiopentone sodium (35 mg/kg, i.p.), the abdomen was opened and pylorus ligation was done without causing any damage in its blood supply. After 4 hr their stomachs were dissected and its contents were collected into tubes for analysis of volume of gastric juice, pH, total and free acidity. The gastric juice was collected after 4 hr of Pylorus ligation induced ulcers and centrifuged for 5 min at 2000 rpm. The supernatant was collected and the volume of gastric juice was expressed as ml/100 g body weight.

Total acidity was determined in the supernatant by titrating against 0.1 N NaOH, using 2-3 drops of topfer's reagent as indicator until canary yellow color was observed. Volume of NaOH required was noted and this corresponds to free acidity. Further 2-3 drops of phenolphthalein was added and titrated with 0.1 N NaOH until pink color was restored and this gives total acidity.

Free acidity and total acidity is expressed in terms of 0.1 N HCL per 100 g of gastric contents and titrated with 0.1 N NaOH until pink color was restored and this gives total acidity.

Ulcer index was calculated as [14]:

$$\text{Ulcer index} = 10/x$$

Where x = Total mucosal area/Total ulcerated area.

The gastric ulcer score was recorded according to the method described by Aguwa and Ukwe (1997)[15.] Mean ulcer score in each group was calculated and was designated as ulcer index.[16,17]

% inhibition= (C-T/C) X 100 Where C= ulcer index in control group

T= Ulcer index in treated group

STATISTICAL ANALYSIS

The statistical analysis was carried out using one-way ANOVA followed by Dunnett's multiple comparison test. All the results obtained in the study were compared with the vehicle control group. P values <0.05 were considered statistically significant.

RESULTS AND DISCUSSION

The examination of acute toxicity carried out on mice indicated that PPC has no toxicity when administered orally (up to 5g/kg). The gastro protective effects of PPC were investigated against aspirin induced and pylorus ligation induced ulcer model in rats. It has shown gastro protective effects against these models.

Aspirin Induced Ulcer

Effect of PPC on ulcer index in Aspirin induced ulcer model in rats is represented in table-1. PPC has shown significant effect reduction in the ulcer index as compared to control group in aspirin-induced gastric ulcer model in both the doses.

Table-1: Effect of PPC on ulcer index in Aspirin induced ulcer model in rats

Groups	Ulcer index
CMC control	22.56 ± 0.28
Ranitidine (60mg/kg)	10.12 ± 0.14**
PPC 1(250mg/kg)	17.14 ± 0.21**
PPC 2 (500mg/kg)	12.36 ± 0.25**

**P values <0.05 as compared to control; Values are the mean ± S.E.M. of six rats/treatment. Significance *p <0.05, **p<0.01 Vs Control.*

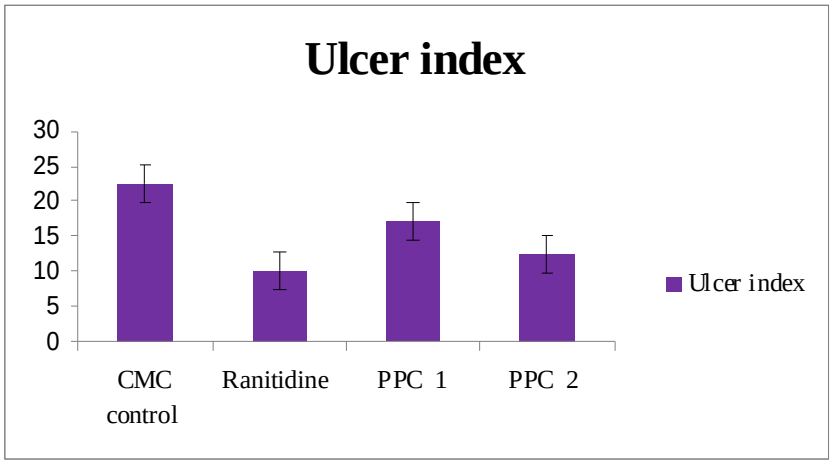

Fig-1: Effect of PPC on Ulcer index in Aspirin induced Ulcer

Pylorus Ligation Induced Ulcer

Effect of PPC against Pylorus ligation induced gastric ulcer in rats is represented in table-2. Standard drug ranitidine 30 mg/kg treatment has significantly reduced ulcer score (0.85±0.07), gastric volume (2.58± 0.15 ml),free acid (9.75±0.13mEq/L) and total acid (22.05±0.08mEq/L).

In pylorus ligation induced ulcer model both PPC 1 and PPC 2 have significantly reduced the ulcer score (2.95± 0.08 ,2.7±0.16 and the ulcer formation (71.15±0.07, 76.85±0.07) is significantly reduced. The anti ulcer activity of PPC in pylorus ligation model is evident from its significant reduction in gastric volume, total acidity, free acidity, ulcer index and increase in pH of gastric juice. Preliminary phytochemical studies of PPC revealed that the presence of tannins. So the possible mechanism of antiulcer action of PPC may be due to its tannin content.

Table-2: Effect of PPC against Pylorus ligation induced gastric ulcer in rats

Group	Treatment and Dose mg/Kg	Gastric volume (ml)	pH	Free acidity (mEq/l)	Total acidity (mEq/l)	Ulcer Index	% Inhibition of Ulcer
Group I	CMC Control	7.12±0.23	2.05±0.08	26.12±0.09	59.02±0.09	4.95±0.08	_
Group II	Standard ranitidine 30mg/kg	2.58± 0.15	5.05±0.08	9.75±0.13	22.05±0.08	0.85±0.07	82.83***
Group III	PPC 1 250mg/kg	4.77± 0.19	3.75±0.08	19.02±0.11	49.05±0.07	1.95± 0.08	60.61**
Group IV	PPC 2 500mg/kg	4.13±0.10	4.22±0.09	18.05±0.07	46.03±0.06	1.2±0.16	75.76***

**P values <0.05 as compared to control; Values are the mean ± S.E.M. of six rats/treatment. Significance *p <0.05, **p<0.01 Vs Control.*

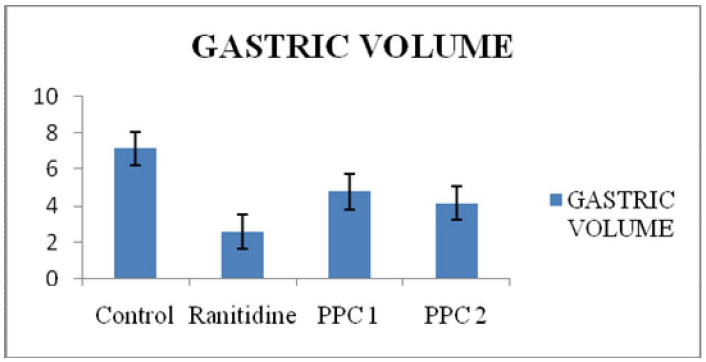

Fig-2: Effect of PPC on Gastric volume in Pylorus ligation induced ulcer

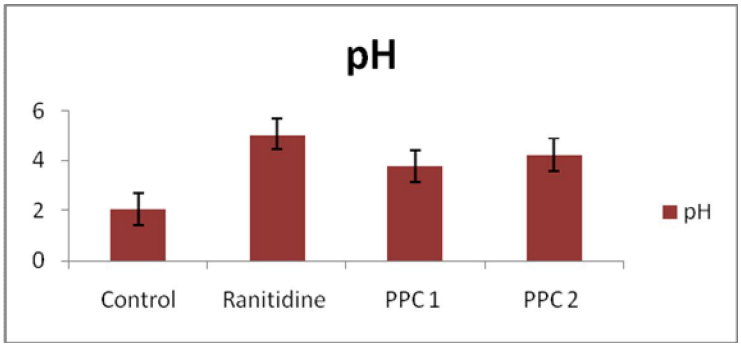

Fig-3: Effect of PPC on pH in Pylorus ligation induced ulcer

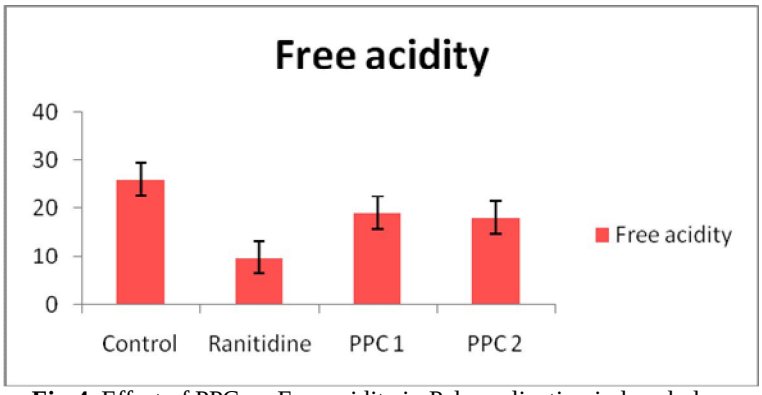

Fig-4: Effect of PPC on Free acidity in Pylorus ligation induced ulcer

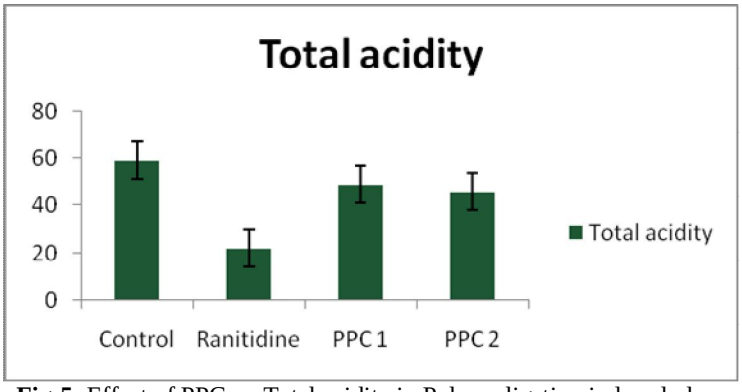

Fig-5: Effect of PPC on Total acidity in Pylorus ligation induced ulcer

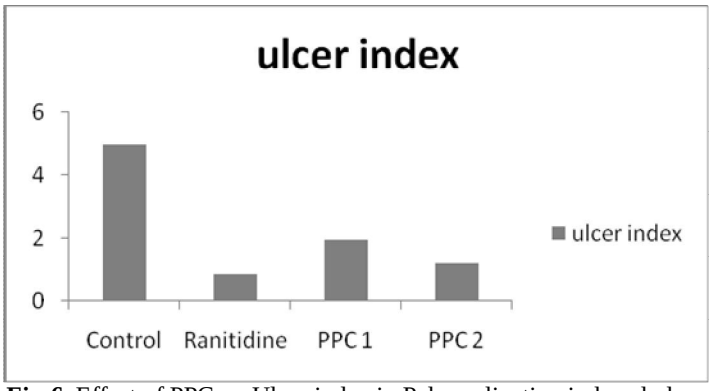

Fig-6: Effect of PPC on Ulcer index in Pylorus ligation induced ulcer

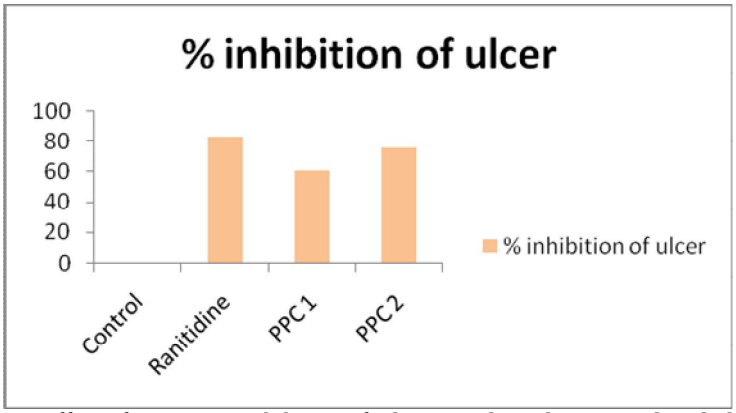

Fig-7: Effect of PPC on % inhibition of Ulcer in Pylorus ligation induced ulcer

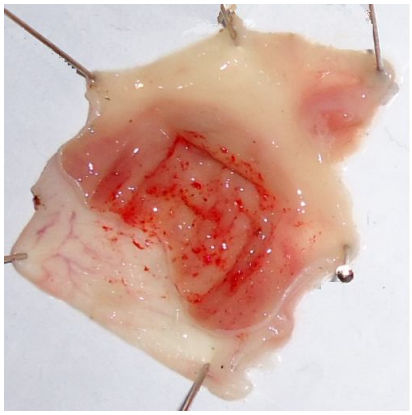

Fig 8a

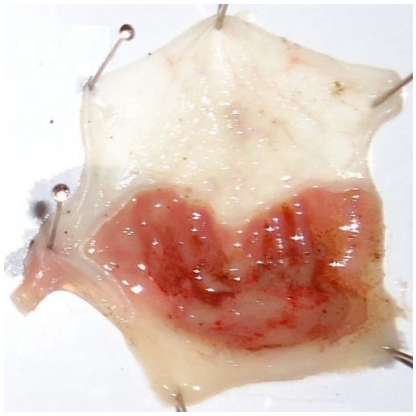

Fig 8b

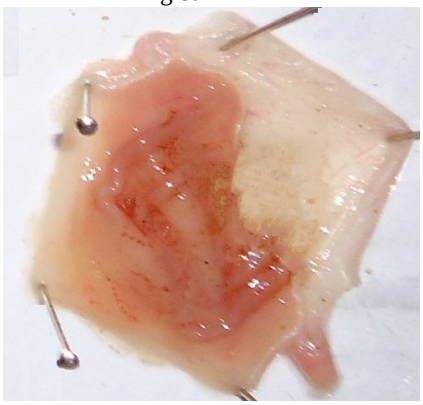

Fig 8c

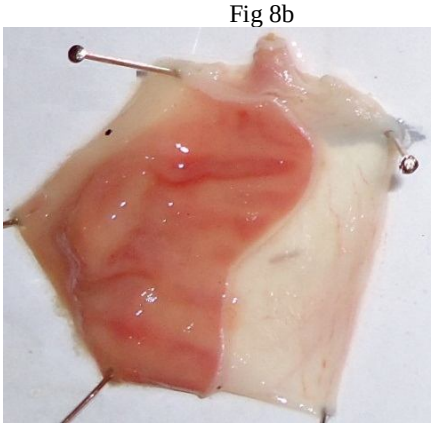

Fig 8d

Fig 8a - Stomach of control rat; **8b** - Effect of PPC 1 on Aspirin induced Ulcer in rat; **8c** - Effect of PPC 2 on Aspirin induced Ulcer in rat; **8d** - Standard drug treated

CONCLUSION

In conclusion, *Borassus flabellifer* significantly inhibits the occurrence of lesions in stomach. From this study, it is clear that *Borassus flabellifer* have significant anti-ulcer activity in animal models when compared with that of reference drug. The female flower of *Borassus flabellifer* is non-toxic even at relatively high concentrations. The anti-ulcer activity is probably due to the presence of active principles.

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