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Psidium guajava Linn confers gastro protective effects on rats

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Abstract. – *Background and Objectives:* **The best alternatives to synthetic medicines, available, for the treatment of gastric ulcer disorders, are the natural products found in plants. They are known to exhibit a variety of activities. The present study is aimed at the screening of *Psidium (P.) guajava Linn* for its gastro protective effect.**

Material and Methods: **The methanol extracts of the leaves of *P. guajava* were tested in three different ulcer models viz. aspirin (ASP), pyloric ligation (PL) and ethanol (EtoH) induced ulcer models in rats.**

Results and Discussion: **The treatment of *P. guajava* at varying doses (100 mg/kg and 200 mg/kg) significantly ($p < 0.001$) inhibited the gastric lesions induced by ASP (70.5%), PL (65.07%) and EtoH (70.4%) respectively and the potency was found to be equivalent as compared to the standard drug, omeprazole. Reduction in the gastric secretory volume, acid secretion and increased gastric pH were the factors observed in treated rats. The presence of volatile oil, flavonoids and saponins present in the extracts of *P. guajava* may be responsible for the anti-ulcer property exhibited.**

Conclusions: **The results further suggest that *P. guajava* possess gastro protective as well as ulcer healing properties which might also be due to its anti-secretory properties.**

Key Words:

Psidium guajava, Gastric acid, Antisecretory, Saponins, Pylorus.

Introduction

Peptic ulcer represents one of the major health problems, both in terms of morbidity and mortality[1]. Peptic ulcer is developed by increase in acid-pepsin secretion, decrease in mucosal resistance against gastric acid and prolonged use of NSAIDs. Infection with gram-negative bacteria such as *Helicobacter pylori* may also lead to peptic ulcer[2]. Even the normal rate of acid secretion may also lead to ulceration in the gastric mucosa in some cases when some of the gastro protective agents are reduced or lost[3]. Therapeutic approaches adopted to control the gastric ulceration include: (1) eradicating the *H. pylori* infection; (2) reducing the secretion of gastric acid with the use of H_2-receptor antagonist; and (3) providing agents that protect the gastric mucosa from damage[4].

Most of the antiulcerogenic drugs such as H_2 receptor blockers like Ranitidine and proton pump inhibitors such as Omeprazole are widely used to control acid secretion. However, there are reports of adverse effects and relapses which occur during prolonged use[5]. On the other hand, most of the herbal drugs reduce the ulcerogenic factors, proved to be safe and clinically effective[6]. So, the herbal extracts are one of the most attractive sources of new drugs and have been shown to produce a challenging result in the treatment of gastric ulcer.

Psidium guajava Linn commonly known as guajava is widely used in traditional medicine in India. Extracts of roots, bark, and leaves of this plant are widely used in the treatment of gastroenteritis, vomiting, diarrhoea, dysentery, wounds, ulcers, toothache, coughs, sore throat, inflamed gums, and a number of other conditions[7]. In the present study the leaf extracts of *P. guajava* are evaluated for gastro protective effects in various ulcer models.

Materials and Methods

Drugs and Chemicals

Aspirin and Omeprazole (Sigma Aldrich, Saint Louis, MO, USA) in methanol were used in this study. All substances were prepared just before use and the reagents used were all of analytical grade.

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Plant Materials

The leaves of *P. guajava* used in this study were collected from Krishnankoil, Srivilliputur (Virudhunagar dist, Tamil Nadu, India). The plant was authenticated by Dr. Stephen, Department of Botany, American College, Madurai, India.

Extract Preparation

P. guajava leaves were shade dried and coarsely powdered. The powdered materials were extracted with methanol. The last traces of the solvent were removed and concentrated to dryness under vacuum using a rotary evaporator. The dried extract was weighed and then kept at –4ºC until ready for use. The yield of the extract was 17.8 % (w/w). In each experiment, the extract was diluted with water to desired concentration.

Phytochemical Screening

A Preliminary phytochemical screening of *P. guajava* was conducted to determine the presence or absence of alkaloids, tannins, phenols, saponins, volatile oil, ascorbic acid, carbohydrates and glycosides by suitable methods[8].

Animals

Adult male Wistar albino rats weighing about 120-150 g were used in this study. They were maintained in clean, sterile, polypropylene cages and fed with commercial pellet rat chow (M/S Hindustan lever limited, Bangalore, India) and water ad libitum. The study was approved by the Institutional Ethical Committee, which follows the guidelines of Committee for the Purpose of Control and Supervision of Experimental Animals (CPSCEA).

Antiulcer Study

Pyloric Ligation (PL)-induced Ulcers

The animals were divided in to four groups, each group consisting of five rats.

Group I: Received normal saline orally for 8 days.
Group II: Received omeprazole at a dose of 20 mg/kg orally for 8 days.
Group III: Received PG at a dose of 100 mg/kg orally for 8 days.
Group IV: Received PG at a dose of 200 mg/kg orally for 8 days.

The animals were deprived of food for 24 hours before the commencement of the experiment. The ligation technique described by Shay *et al*. was used for the pylorus ligation[9]. *P. guajava* extract were administered at a dose of 100, 200 mg/kg by orally for 8 days. After one hour of the last dosing, pylorus ligation was made under ether anesthesia. The animals were returned to the observation chamber for 6h. After 6h, the animals were sacrificed by decapitation, the abdomen of each animal was opened and the stomach was isolated after suturing the lower esophageal end. The gastric juice was collected and the mucosal layer was washed with 1 ml distilled water. Ulcer scoring was performed in the stomach of each animal (Figure 1).

Titrations of Acid Concentrations

One ml of filtered gastric contents was pipetted into a small beaker, the washings and the gastric contents were centrifuged at 2000 rpm for 10 min. The supernatant fluid (1 ml) was diluted with 9ml of distilled water and 2-3 drops of Topfer's reagent was added and then titrated with 0.01N sodium hydroxide solution till the solution turns to orange color. Free acidity was calculated based on the volume of alkali added. The solution was further titrated with 0.01N sodium hydroxide solution till the solution regained pink color. The total acidity was calculated based on the total volume of alkali added. The appearance of yellow color after the addition of methyl orange indicates that no free acid is present. Each stomach was then examined carefully for scoring the severity of ulcers. The data obtained for pH, volume of acid, secretion of gastric juice and ulcer index were analyzed by Student's *t* test. The ulcers were graded as per following methods[8].

0 = Normal colored stomach;
0.5 = Red coloration;
1 = Spot ulcers;
1.5 = Hemorrhagic streaks;
2 = Ulcer $\geq$ 3 but $\leq$ 5;
3 = Ulcer > 5.

The mean ulcer scores of each animal were expressed as ulcer index.

Aspirin induced Ulceration

The animals were divided in to four groups, each group consisting of five rats.
Group I: Received aspirin+ normal saline orally for 7 days.
Group II: Received aspirin + omeprazole at the dose of 20 mg/kg orally for 7 days.
Group III: Received aspirin + PG at the dose of 100 mg/kg orally for 7 days.
Group IV: Received aspirin + PG at the dose of 200 mg/kg orally for 7 days.

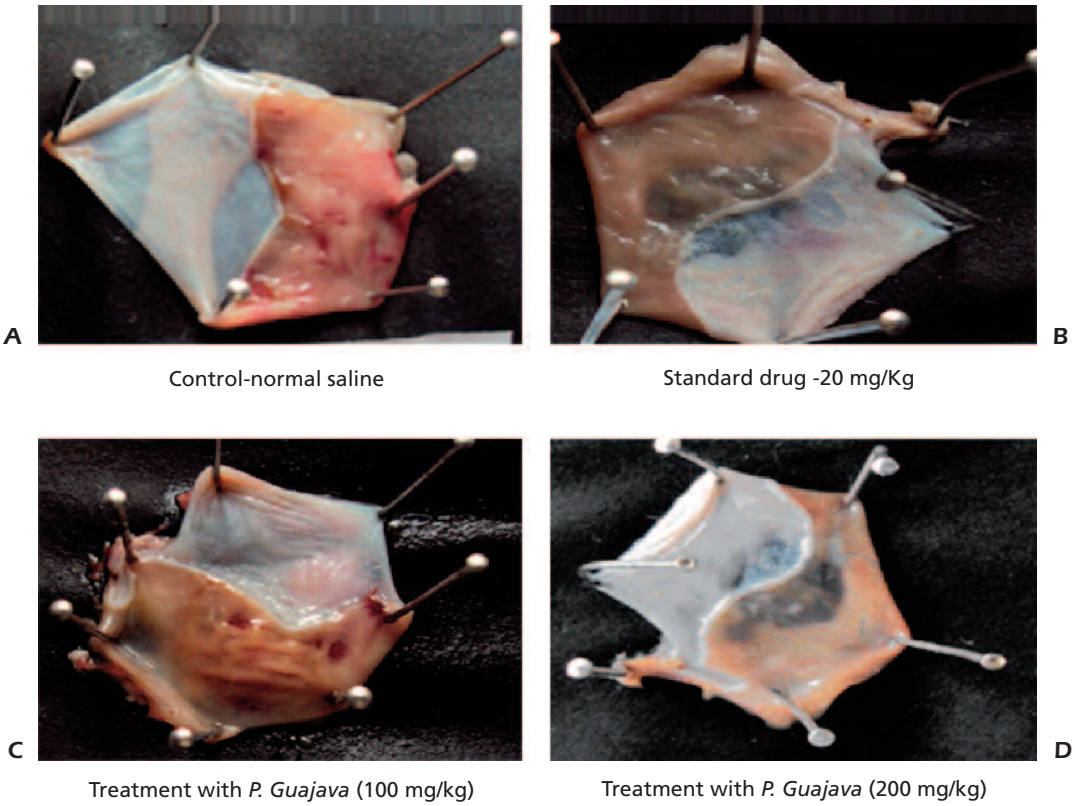

Figure 1. Effect of P. guajava on pylorus ligation induced ulcer. The rats were either untreated ***(A)*** or treated with standard drug ***(B)***, *P. guajava* leaf extracts at 100 mg/kg ***(C)*** and *P. guajava* leaf extracts at 200 mg/kg ***(D)*** 7 days after pylorus ligation

Aspirin at a dose of (0.06 ml) were administered every day by orally. After 4 hours of the aspirin administration, 100, 200 mg/kg of *P. guajava* were administered every day by orally and it was continued for up to 7 days. The animals were sacrificed after 18 hours of the last dose of drug on 7th day of experiment, to assess the ulcer size and healing.

Ulcer index (UI) was then calculated from the above scorings as follows.

$$\text{Ulcer Index} = 10/\times$$

× = Total mucosal area/total ulcerated area

The % inhibition was calculated by the following formula:

$$\% \text{ inhibition} = \frac{\text{UI control} - \text{UI treated}}{\text{UI control}} \times 100$$

Ethanol Induced Ulceration (Figure 2)

The animals were divided in to four groups, each group consisting of five rats.

Group I: Received normal saline orally for 3 days
Group II: Received omeprazole at the dose of 20 mg/kg orally for 3 days
Group III: Received PG at the dose of 100 mg/kg orally for 3 days
Group IV: Received PG at the dose of 200 mg/kg orally for 3 days

On day 3, after 30 minutes of extract and omeprazole treatment, EtoH (1 ml/200 g/kg) was administered to all the groups of rats. Animals were sacrificed after 1 hr and ulcer screening was done.

Statistical Analysis

The results are presented as mean ±SD. The data were analyzed by Students *t* test and $P < 0.05$ was considered to be significant.

Results

Preliminary phytochemical screening of the extracts of *P. guajava* revealed the presence of

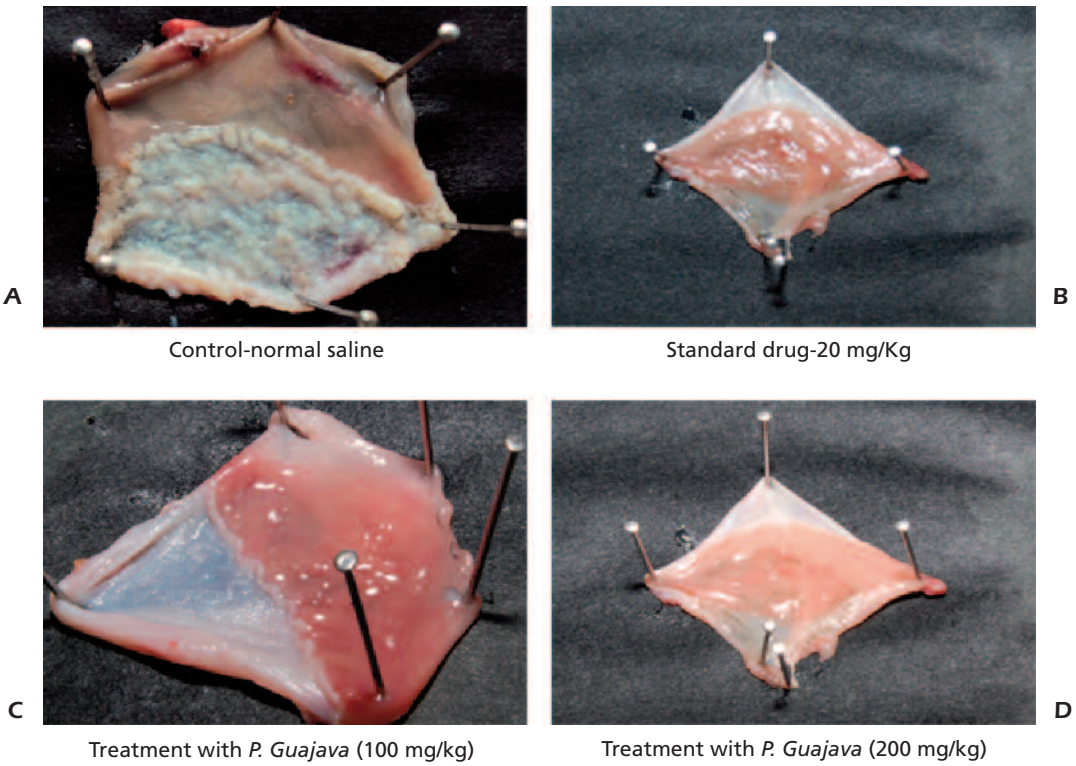

Figure 2. Effect of *P. guajava* on aspirin induced ulcer in wistar rats. The rats were either untreated ***(A)*** or treated with standard drug ***(B)***, *P. guajava* leaf extracts at 100 mg/kg ***(C)*** and *P. guajava* leaf extracts at 200 mg/kg ***(D)*** 7 days after aspirin treatment.

flavonoids, triterpenes, saponins, carotinoids, alkaloids, glycosides and carbohydrates (Table I). Acute toxicity studies of the alcoholic extract of the *P. guajava* did not exhibit any signs of toxicity up to 2 g/kg body weight. Since there was no mortality of the animals found at high dose. hence 100, 200 mg/kg dose of the extract was selected for evaluation of anti-ulcer activity.

Effect of P. guajava on Pylorus Ligation Induced Ulcer

The alcoholic extract of the *P. guajava* at a dose of 100 and 200 mg/kg produced a reduction in the ulcer index, gastric volume, free acidity, total acidity and raised gastric pH significantly ($p < 0.001$) in comparison to the control group. The reference drug omeprazole as expected produced a significant reduction in gastric ulcer and total acid output as compared to control group (Table II).

Effect of P. guajava on Aspirin Induced Ulcer

The results obtained in the experimental model of aspirin-induced gastric ulceration in rats are presented in Table III. The alcoholic extract was found to possess remarkable ulcer-protective properties at 100, 200 mg/kg. The maximum effect of ulcer protection (70.05%) was observed at 200 mg/kg of *P. guajava* fed animals whereas the standard drug omeprazole gave 82.03% of ulcer protection.

Ethanol Induced Ulcers

The results obtained in the experimental model of ethanol -induced gastric ulceration in rats is summarized in Table IV. The alcoholic extract was found to possess remarkable ulcer-protective properties at 100, 200 mg/kg. The maximum ef-

Table I. Phytochemical screening of the extracts of *Psidium guajava.*

S. No.	Test	*P. guajava*
1	Glycosides	+
2	Carbohydrates	+
3	Phytosterols	+
4	Flavanoids	+
5	Proteins	+
6	Alkaloids	–
7	Tannins	+
8	Saponins	+

Table II. Effect of Psidium guajava on Pylorus ligation induced ulcer in wistar rats

Treatment (dose)	Gastric juice volume (ml/4 h)	pH	Free acidity (mEq/L/100 g)	Total acidity (mEq/L/100 g)	Ulcer index	% inhibition of ulcer
Control group	5.02 ± 0.13	2.02 ± 0.07	87.53 ± 0.13	1.71 ± 1.77	5.83 ± 0.62	–
Psidium guajava 100 mg/kg	4.13 ± 0.13*	3.14 ± 0.19*	67.23 ± 3.12*	132.12 ± 3.23*	4.12 ± 0.42*	34.4*
Psidium guajava 200 mg/kg	2.71 ± 0.15**	4.61 ± .17**	64.13 ± 2.51**	86.68 ± 1.18**	2.13 ± 0.38**	63.5**
Omeprazole 20 mg/kg	1.82 ± 0.12**	5.23 ± 0.89**	52.00 ± 2.43**	68.42 ± 3.32**	1.21 ± 1.2**	79.6**

Results are expressed as mean ± SEM from five observation as compared to Control group by Student't test. *$p < 0.05$, **$p < 0.001$.

fect of ulcer protection (70.4%) was produced at 200 mg/kg and the standard drug (Omeprazole) gave 74.1% of ulcer protection.

Discussion

Peptic ulcer is developed due to the abnormal production of stomach acid or sub normal in gastric mucosal resistance. The anti-ulcer activity of the leaf of *Psidium guajava* was evaluated by employing aspirin, alcohol and pylorus ligation ulcer models. These models represent some of the most common causes of gastric ulcer in humans. The results of the present investigation indicate the protective effects of gastric ulcer models of *P. guajava.* The results reveals the fact that the animals treated with extracts of *P. guajava* exhibited a significant ($p < 0.001$) increase in gastric juice, pH and reduces the gastric volume, free acidity and total acidity in a dose-dependent manner when compared to control. The results were comparable to that of the standard reference drugs commercially available in the market.

Ulcers formed by pylorus-ligation are due to increased accumulation of acid-pepsin in the stomach. The accumulation of acid-pepsin in the stomach is because of pylorus obstruction and subsequent mucosal digestion[10]. Gastric acid is a crucial factor for the production of ulceration in pylorus -ligated rats[11]. The activation of the vagus-vagal reflux by stimulation of pressure receptors in the antral gastric mucosa in the hyper secretion model of pylorus ligature is understood to increase gastric acid secretion[12]. The present data clearly indicates a dose-dependent decrease in gastric acid secretion.

Aspirin induced ulcer is governed through tissue damaging free radicals[13], which are produced from the changing of hydroperoxyl to hydroxyl fatty acids, which leads to cell destruction. The hydroxyl peroxyl fatty acids are produced from the degeneration of mast cells and generalized lipid peroxidation accompanying cell damage[14]. *P. guajava* significantly reduced the ulcer index and significant protection against aspirin induced ulcer.

Ethanol induced gastric ulcer can arise as a result of direct damage to mucosal cells, development of free radicals and hyperoxidation of lipid[15]. But scavenging these free radicals can play a role in healing of these ulcers[16]. *P. guajava* significantly ($p < 0.001$) reduced the ulcer index and offered significant protection against ethanol induced ulcer.

Table III. Effect of *Psidium guajava* on aspirin induced ulcer in Wistar rats.

Treatment	Dose (mg/kg)	Aspirin induced ulcer	
		Ulcer index mean ± SEM	Percentage ulcer protection
Control	–	8.5 ± 0.50	–
Standard	20	1.50 ± 0.52**	82.3**
Psidium guajava	100	4.8 ± 0.27*	43.5*
Psidium guajava	200	2.52 ± 0.12**	70.5**

Results are expressed as mean ± SEM from five observations as compared to Control group Student't test. *$P < 0.05$, **$P < 0.001$.

Table IV. Effect of *Psidium guajava* on aspirin induced ulcer in Wistar rats.

Treatment	Dose (mg/kg)	EtoH induced ulcer	
		Ulcer index mean ± SEM	Percentage ulcer protection
Control	–	29.4 ± 1.45	–
Standard	20	7.6 ± 0.62*	74.1*
Psidium guajava	100	15.7 ± 0.57	46.5
Psidium guajava	200	8.7 ± 0.42*	70.4*

Results are expressed as mean ± SEM from five observations as compared to Control group Student't test. *$p < 0.001$.

The findings of the present study confirmed that the leaf extract of *P. guajava* possess gastro protective effects against experimentally induced gastric ulcer models. These data corroborate with the earlier observations on *Centella asiatica*[17,18] *Pongamia pinnata*[19] in reducing the experimentally induced gastric ulceration. Therefore, the anti-ulcer effects of the leaf extract of *P. guajava* may be due to its anti-secretory and anti- oxidant properties.

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