

Effect of methanolic extract of *Pongamia pinnata* Linn seed on gastro-duodenal ulceration and mucosal offensive and defensive factors in rats

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Pongamia pinnata has been advocated in Ayurveda for the treatment of various inflammatory conditions and dyspepsia. The present work includes initial phytochemical screening and study of ulcer protective and healing effects of methanolic extract of seeds of *P. pinnata* (PPSM) in rats. Phytochemical tests indicated the presence of flavonoids in PPSM. PPSM when administered orally (po) showed dose-dependent (12.5-50 mg/kg for 5 days) ulcer protective effects against gastric ulcer induced by 2 h cold restraint stress. Optimal effective dose of PPSM (25 mg/kg) showed antiulcerogenic activity against acute gastric ulcers (GU) induced by pylorus ligation and aspirin and duodenal ulcer induced by cysteamine but not against ethanol-induced GU. It healed chronic gastric ulcer induced by acetic acid when given for 5 and 10 days. Further, its effects were studied on various parameters of gastric offensive acid-pepsin secretion, lipid peroxidation (LPO) and nitric oxide (NO) and defensive mucosal factors like mucin secretion and mucosal cell shedding, glycoproteins, proliferation and antioxidants; catalase (CAT), superoxide dismutase (SOD) and glutathione (GSH) levels. PPSM tended to decrease acid output and increased mucin secretion and mucosal glycoproteins, while it decreased gastric mucosal cell shedding without any effect on cell proliferation. PPSM significantly reversed the increase in gastric mucosal LPO, NO and SOD levels caused by CRS near to the normal level while it tended to increase CAT and GSH level decreased by CRS and ethanol respectively. Thus, the ulcer protective effects of PPSM may be attributed to the presence of flavonoids and the actions may be due to its effects both on mucosal offensive and defensive factors.

Keywords: Acid secretion, Antioxidants, Free radicals, Gastro-duodenal ulcers, Mucosal defense, *Pongamia pinnata*

Pongamia pinnata (L) Pierre (Leguminosae, Papilionoidae, Syn. *P. glabra* Vent), commonly called Karanj in Hindi, and Karanja, Maktamala or Gaura in Sanskrit and an Indian beech in English, is a medium sized glabrous tree that grows on moist environment along rivers or sea coast all over India and further, distributed eastwards mainly in the littoral regions of South Eastern Asia and Australia¹. Different parts of the plant have been recommended in Ayurvedic literature as a remedy for various ailments. *P. pinnata* roots have been described as a useful remedy for foul ulcers, fistulous sores, gonorrhoea, urethritis etc². The seeds and seed oil have been used for treating various inflammatory and infectious diseases such as leucoderma, leprosy, lumbago and rheumatism³. The ulcer protective and healing effects and anti-

inflammatory activity of alcoholic extract of *P. pinnata* seeds⁴ and root⁵⁻⁷ have been reported. The ulcer protective effect in *P. pinnata* root seemed to be due to augmentation of defensive mucosal factors⁷. Protective role of ethanolic extract of *P. pinnata* root in ischemia-reperfusion injury and cerebrovascular insufficiency states provided additional evidence concerning the anti-oxidative stress and cognition-enhancing property of *P. pinnata*⁸. Flowers of the plant are rich in bioflavonoids and extensively used in various skin diseases, diabetes and renal disorders⁹. *P. pinnata* has been reported to contain a large number of furanoflavonoids, e.g. karanjin, pongapin, kanjone, pongamol and pongaglabrone, along with a number of simpler flavonoids and lipid like arachidonic acid^{10,11}.

Peptic ulcers occur when there is an imbalance between offensive and defensive mucosal factors¹². Ulceration in the mucosa can be because of either breakdown of mucosa with the development of surface defects or failure of restitution of mucosal

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integrity resulting in retardation or failure of healing of the ulcers. No apparent causal factor is sufficiently uniquely associated with peptic ulcers to warrant unequivocal implication in pathogenesis of the ulceration. The defense mechanism of the gastrointestinal mucosa against aggressive factors, such as hydrochloric acid, bile acid, free radicals, *Helicobacter pylori* colonization, non-steroidal anti-inflammatory drugs, etc., mainly consists of functional, humoral and neuronal factors. Mucus-alkaline secretion, mucosal microcirculation, cellular mucus, life span of mucosal cells and motility act as functional factors, while prostaglandins and nitric oxide act as humoral factors, and capsaicin sensitive sensory neurons act as neuronal factors. All the above factors are known to contribute to mucosal protection¹³. Our laboratory has been engaged in screening of various indigenous herbal drugs for their potential use in peptic ulcer diseases. Anti-ulcer and ulcer-healing properties of plantain banana (*Musa sapientum* var *paradisiaca*), *Tectona grandis*, *Azadirachta indica* and rasayana drugs like *Centella asiatica*, *Asparagus racemosus*, *Convolvulus pluricaulis*, *Emblica officinalis*, *Bacopa monniera* and *Withania somnifera*, etc have been reported¹⁴. These plants have shown antiulcer activity by their predominant action on mucosal defensive factors¹⁴. The present work is focused on studying the detailed effects of ethanolic extract of seeds of *P. pinnata* (PPSM) on different experimental ulcer models and attempts have also been made to elucidate the mechanism/s of its ulcer protective effects.

Materials and Methods

Collection and preparation of extract—Fresh seeds of *Pongamia pinnata* were collected locally during the month of November and were duly authenticated by Prof. V.K. Joshi, Dean, Faculty of Ayurveda and Department of Dravyaguna, IMS, BHU, Varanasi. The voucher specimen was preserved. The air-dried seeds were coarsely powdered (1 kg) and extracted with methanol (1 lit, 5 days) following the standard procedure and used for phytochemical screening. The percent yields of methanolic extract of seeds of *P. pinnata* (PPSM) was 11.8.

Animals—CF strain albino rats of either sex, weighing between 150-200 g, obtained from the central animal house of the Institute, were used. They were kept in colony cages in the departmental temperature controlled animal room ($26^{\circ} \pm 3^{\circ} \text{C}$) and

44-56% RH with 10:14 hr L: D cycle for at least 5 days before the treatment. The animals were provided with standard rodent pellet diet (Pashu Aahar Vihar, Ramnagar, Varanasi) and the food was withdrawn 18-24 h before experiment though water was allowed *ad libitum*. Ethical clearance was taken from Institute Animal Ethics Committee and Principles of Laboratory Animal Care' (NIH publication no.82-23, revised 1985) guidelines were followed.

Drug treatment—The anti-ulcer effects of orally administered, graded doses of methanolic extract of *P. pinnata* seeds (PPSM) were seen using doses of 12.5, 25.0 or 50 mg/kg, in two divided doses, daily for 4 days and the last full dose was given on 5th day of experiment 60 min before subjecting the 18 h fasted rats to 2 h cold restraint stress (CRS). For further studies, an optimal ulcer protective dose of 25 mg/kg/day was chosen. The animals received the drugs orally with the help of an orogastric tube in the volume of 1.0 ml/100 g body weight. For ulcer healing study, the animals received the drug either for 5 or 10 days. The antiulcer effect of PPSM including the possible mechanism of its ulcer protective actions were compared with standard ulcer protective drugs, omeprazole (OMZ, 2 mg/kg/day, a proton pump inhibitor)¹⁵ and Sucralfate (SFT, 500mg/kg/day, ulcer protective drug)⁷. The test and standard drugs were suspended in 1% carboxy methylcellulose (CMC) in distilled water and prepared fresh before use. Control group of animals received 1% CMC in the same volume as mentioned above.

Phytochemical study

Phytochemical screening—It was done in dichloromethane fraction. The dichloromethane soluble part was used for column chromatography over silica gel CC and eluted with hexane, acetone, ethyl acetate and finally with methanol¹¹. The TLC analysis (Silica gel G with 13% calcium sulphate as binder) of elutes obtained from hexane: acetone mixture showed positive color reaction to Libermann Burchard reagent, indicating presence of terpenoids and steroids. The ethyl acetate:methanol mixture showed positive colour reaction to ferric chloride reagent, indicating the presence of flavonoids.

HPTLC- evaluation of PPSM—Methanolic extract of seeds of *P. pinnata* (PPSM) was subjected to HPTLC (CAMAG TLC system) analysis for the detection of flavonoids. The extract was diluted in order to get a concentration of 1 $\mu\text{g}/\mu\text{l}$ and applied (5, 10 and 15 μl) on precoated silica gel 60 F₂₅₄ plate

(10×10, Merck, Germany) by using LINOMAT IV spotter. The plate was developed in CAMAG flat bottom chamber under conditions of partial or complete saturation of the tank atmosphere with solvent vapors. The solvent systems used were ethyl acetate:formic acid:glacial acetic acid:water (100:11:11:26). The plate was carefully dried and sprayed with natural product/poly ethylene glycol (NP/PEG) reagent for the detection of flavonoids¹⁶. The detection of spots was made by using Hg lamp at 366 nm and images were recorded by using CAMAG Reprostar 3. The plate was scanned by using CAMAG TLC scanner 3 with WinCATS evaluation software. The chromatograms of PPSM extract showed the presence of flavonoids.

Anti-ulcer study

Cold-restraint stress (CRS)-induced gastric ulcers (GU)—To 18 h fasted rats, cold restraint stress was given by strapping the rats on a wooden plank and keeping them for 2 h at 4°-6° C. The animals were then sacrificed by cervical dislocation¹⁷. Ulcer index was calculated by adding the total number of ulcers per stomach and the total severity of ulcers as one plus per stomach. The total severity of the ulcers was determined by recording the severity of each ulcer in pluses (+) by a person unaware of the experimental protocol. The ulcer index was calculated by adding the number of ulcer per stomach plus the severity of ulcer converted as 1 + (one plus) per stomach¹⁸.

Aspirin (ASP)-induced GU—ASP in dose of 200 mg/kg (20 mg/ml) was administered to the animals and ulcers were scored after 4 h¹⁹. The stomach was taken out and cut open along the greater curvature and ulcers were scored by a person unaware of the experimental protocol in the glandular portion of the stomach as described above.

Pylorus-ligated (PL)-induced GU—Drugs were administered for a period of 5 days as described above. On day six, the 18 h fasted rats were anaesthetized using pentobarbitone (35 mg/kg, ip), the abdomen was opened and pylorus ligation was done without causing any damage to its blood supply. The stomach was replaced carefully and the abdomen wall was closed in two layers with interrupted sutures. The animals were deprived of water during the post-operative period²⁰. After 4 h, the animals were killed with over dose of anaesthetic ether and the stomachs were dissected out and contents were collected into tubes for estimation of biochemical parameters. The ulcers were scored as described under CRS-induced ulcers.

Ethanol (EtOH)-induced GU—The gastric ulcers were induced in rats by administering EtOH (1 ml/200 g, 1 h)²¹ and the animals were sacrificed by cervical dislocation and stomach was incised along the greater curvature and examined for ulcers. The ulcer index was scored, based upon the product of length and width of the ulcers present in the glandular portion of the stomach (mm²/rat).

Cysteamine-induced duodenal ulcers—On 5th/10th day of drug treatment, the animals were kept on fast from 1000 hrs after giving the last dose of the test drug. Cysteamine (300 mg/kg, sc, 100 mg/ml) was administered at 1600 hrs and the fasting was then continued overnight and the fasted animals were then sacrificed at 1000 hrs on 6th/11th day of the experiment²². The abdomen was opened and the anterior and posterior wall of the duodenum near the pyloric end was examined for the presence or absence of ulcers. Statistical analysis was done by employing the Yates's modification of the Chi-square test.

Ulcer healing study

Acetic acid-induced chronic gastric ulcers—Rats were anaesthetized with pentobarbitone (35 mg/kg, ip). The abdomen was opened and the stomach was visualized. A cylindrical glass tube of 6 mm in diameter was tightly placed upon the anterior serosal surface of the glandular portion of stomach 1 cm away from the pyloric end. Acetic acid (50%; 0.06 ml/animal) was instilled into the tube and allowed to remain 60 sec on the gastric wall. After removal of the acid solution, the abdomen was closed in two layers and animals were caged and fed normally. The test drugs were given in their respective doses on day one, orally, twice daily, 4 hr after the application of acetic acid and continued either up to 5 or 10 days after induction of ulcer. The animals were then sacrificed after 18 h of the last dose of drug either on 6th day or 11th day of experiment to assess the ulcer size and healing. Ulcer index was calculated based upon the product of length and width (mm²/rat) of ulcers²³.

Gastric secretion study

Acid-pepsin and mucin secretions and cell shedding—Studies on offensive acid-pepsin and defensive mucin secretions and cell shedding were carried out in rat gastric secretion. The gastric juice was collected 4 h after PL 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. Acid concentration and output were determined by titrating with 0.01 N NaOH, using phenolphthalein as indicator and is expressed as $\mu\text{Eq/ml}$ and $\mu\text{Eq/4 hr}$ respectively. Peptic activity was determined using hemoglobin as substrate²⁴ and has been expressed as $\mu\text{Eq/ml}$ and $\mu\text{Eq/4 hr}$ for concentration and output respectively. Dissolved mucosubstances were estimated in the 90% alcoholic precipitate of the gastric juice. The precipitate, thus obtained was either dissolved in 1 ml of 0.1 N NaOH or 1 ml of 0.1 N H₂SO₄. The former was used for the estimation of protein, total hexoses, hexosamine and fucose while the latter was used for the estimation of sialic acid. The results are expressed in $\mu\text{g/ml}$ of gastric juice. The ratio of total carbohydrate (TC) (sum of total hexoses, hexosamine, fucose and sialic acid) to protein (P) has been taken as the index of mucin activity²⁵. DNA content was estimated and expressed as $\mu\text{g/ml}$ gastric juice²⁶.

Gastric mucosal study

Glycoproteins estimation—It was carried out in the gastric mucosa of 4 h PL rats. Mucosal scrapings of the glandular portion of rat stomach were homogenized in normal saline (20 mg/ml) and treated with 90% ethanol in the same manner as described for mucin estimation in the gastric juice. The precipitate thus obtained, was subjected for the estimation of carbohydrates and protein using the methods described above for gastric juice contents. The results were expressed as $\mu\text{g/100 mg}$ wet tissue and TC: P ratio has been taken as the index of glycoprotein activity²⁷.

Cell proliferation—Mucosal scrapings were homogenized in 2.5 ml of ice cold 0.6 N perchloric acid and DNA was then estimated²⁸. Protein content was estimated in 90% ethanolic precipitate of the mucosal homogenate (10 mg/ml) dissolved in 0.1 N NaOH²⁹. The concentration of DNA was expressed as $\mu\text{g DNA/mg protein}$ which is a reliable index of cell proliferation³⁰.

Estimation of free radical generation

Mucosal scrapings of the glandular portion (fundic part) of rat stomach were homogenized (5 %) in ice cold 0.9% saline with a Potter-Elvehjem glass homogenizer for 30 sec. The homogenate was then centrifuged at 800 g for 10 min followed by centrifugation of the supernatant at 12,000 g for 15 min and the obtained mitochondrial fraction was used for the following estimations³⁰.

Measurement of lipid peroxidation (LPO)—LPO product malondialdehyde (MDA) was estimated using 1,1,3,3-tetraethoxypropane as the standard and is expressed as n moles/g wet tissue³¹.

Estimation of nitric oxide (NO)—Mucosal scrapings of fundic part of stomach were homogenized in distilled water (50 mg/ml) for 30 sec. The 0.5 ml homogenate was then centrifuged with 0.5 ml of alcohol at 14,000 rpm for 30 min at 4° C to precipitate the protein. The supernatant was collected and the nitric oxide was estimated by Griess reaction method³².

Estimation of enzymatic antioxidant

Gastric mucosal scrap was homogenized (5%) in 0.25 M sucrose and 50 mM phosphate buffer (pH 7.2) and mitochondrial fraction was prepared as described above³⁰.

Superoxide dismutase (SOD)—It was estimated by following the procedure of Kakkar *et al*³¹. The inhibition of reduction of nitro blue tetrazolium (NBT) to blue colored formozan in presence of phenazine metha sulphate (PMS) and NADH was measured at 560 nm using n-butanol as blank. One unit of enzyme activity was defined as the amount of enzyme that inhibits rate of reaction by 50% in one min under the defined assay conditions and the results have been expressed as units (U) of SOD activity/g wet tissue.

Catalase (CAT)—Decomposition of H₂O₂ in presence of catalase was followed at 240 nm³⁴. One unit of (U) CAT was defined as the amount of enzyme required to decompose 1 μmol of H₂O₂ per min, at 25°C and pH 7.0. Results are expressed as units (U) of CAT activity/g wet tissue.

Glutathione (GSH)—A 400 mg sample of gastric mucosal scrap was homogenized in 8 ml of 0.02 M EDTA in an ice bath. The absorbance was read within 5 min of the addition of DTNB at 412 nm against a reagent blank with no homogenate³⁵. The results are expressed as $\mu\text{mol/g}$ wet tissue and calculated from the standard curve prepared by using standard glutathione.

Statistical analysis—Statistical analysis for amongst the control and treatment groups was done by One-Way Analysis of Variance (ANOVA) and for multiple comparisons versus control group by Dunnett's test. Yates's modification of Chi Square test was applied in DU model while comparing between two groups only. All statistical analyses were performed using SPSS statistical version 13.0

software package (SPSS® Inc., USA). *P* value <0.05 were considered statistically significant.

Results

Phytochemical study—Preliminary phytochemical screening of methanolic extracts of seeds of *P. pinnata* revealed the presence of steroids, flavonoids, saponins, alkaloids and glycosides in the methanolic extract. The presence of flavonoids was further confirmed by the HPTLC study (Fig. 1).

Anti-ulcer and ulcer healing studies—PPSM (12.5, 25 and 50 mg/kg in two divided doses daily) when given orally for 4 days plus the last full dose on 5th day, 1 hr before the experiment showed dose-

dependent ulcer protective activity against CRS-induced GU (41.9 to 64.3% protection; control Ulcer index- 25.8 ± 5.3) and PPSM (25 mg/kg) showing optimal ulcer protection was chosen for further study. The above selected dose of PPSM was found effective against CRS-, ASP- and PL-induced but not against ethanol-induced GU in rats. Sucralfate (SFT, 500 mg/kg in two divided doses daily) showed antiulcer effect against all the above GU models while omeprazole (OMZ, 2 mg/kg in two divided doses daily) was effective against all the GU models except ethanol induced GU. The antiulcer effect of PPSM seemed to be like OMZ (Table 1). PPSM significantly decreased the incidence of duodenal ulcers induced by

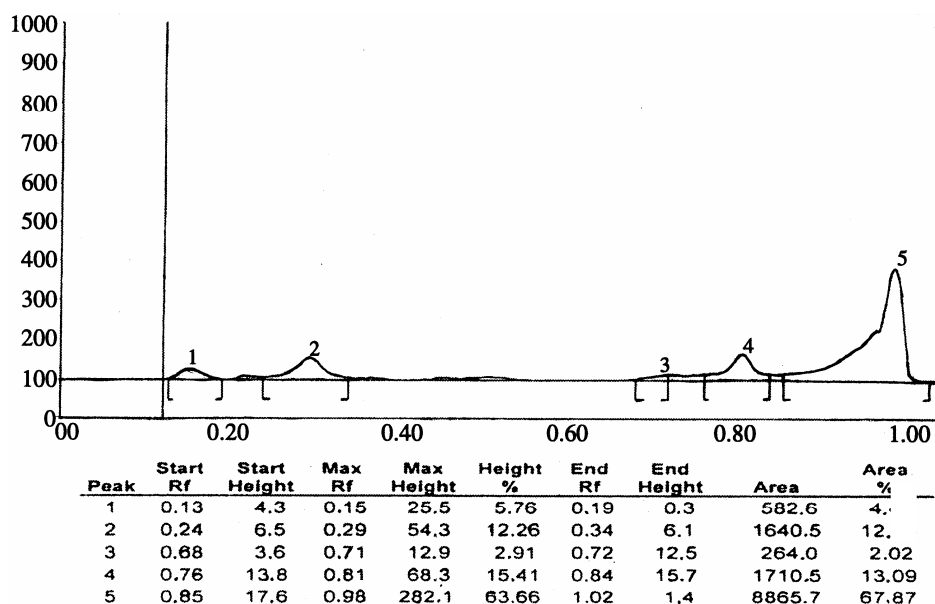


Fig. 1—HPTLC-Chromatogram of PPSM showing the presence of flavonoids (CAMAG TLC Scanner 3 at wavelength 366 showed presence of flavonoids in PPSM)

Table 1—Effect of PPSM, SFT and OMZ on acute gastro-duodenal ulcers induced by physical [4 hr pyloric ligation (PL) and 2 hr cold restraint stress (CRS)] and chemical [aspirin (ASP, 200 mg/kg, po, 4 hr), ethanol (EtOH, 100%, 1 ml/200 g, po, 1 hr)] and duodenal ulcer (DU) induced by cysteamine (CYT, 300 mg/kg, sc, 18 hr) in rats

[Values are mean ± SE of 8 rats in each group. Results in parenthesis indicate % protection compared to respective control group]

Oral treatment (mg/kg, od × 5 days)	Gastric ulcer [#]				Duodenal ulcer [§]
	CRS	PL	ASP	EtOH	CYT
			Ulcer index		Presence of ulcer/rat
Control (1% CMC)	25.8 ± 5.3 (-)	10.0 ± 0.87 (-)	25.6 ± 3.9 (-)	18.3 ± 3.2 (-)	9/10
PPSM 25	9.8 ± 2.7* (62.0)	4.9 ± 1.2* (51.0)	11.5 ± 1.8* (55.2)	16.2 ± 3.3 (11.5)	4/10*
SFT 500	8.3 ± 1.7* (68.0)	2.1 ± 0.51* (79.0)	7.3 ± 3.6* (71.5)	5.7 ± 1.6* (68.9)	3/10*
OMZ 2	10.6 ± 1.4* (58.8)	3.4* ± 0.71 (66.2)	9.8* ± 2.1 (61.9)	14.8 ± 2.2 (18.7)	3/10*

**P* value: < 0.05 compared to control group ([#] ANOVA followed by Dunnett's test, [§] Chi square test)

cysteamine (Table 1) showed significant ulcer healing both after 5 and 10 days treatments (Table 2). The ulcer healing effect of PPSM was comparable with both SFT and OMZ (Table 2).

Acid-pepsin and mucin secretions and cell shedding—PPSM tended to decrease acid and pepsin secretion and significantly decreased the DNA content of the gastric juice indicating increased life span of the mucosal cells (Table 3). PPSM either tended to increase or significantly increased individual carbohydrate and TC content without any effect on protein content thus, leading to increase in TC: P ratio (which is a reliable marker for mucin secretion) (Table 3). SFT decreased pepsin concentration and increased mucin secretion while OMZ decreased both acid and pepsin secretion without any effect on mucin secretion though both decreased the DNA content of the gastric juice (Table 3).

Mucosal glycoproteins and cell proliferation—PPSM either tended to increase individual carbohydrates or total carbohydrates and decrease protein content thus, leading to significant increase in TC: P ratio and glycoprotein content. SFT either tended to increase or increased the individual and total carbohydrates and TC:P ratio indicating increased mucosal glycoprotein while OMZ showed little or no effect on the above parameters (Table 4). A little or no change was observed with PPSM, SFT and OMZ on μg DNA/mg protein (a good marker for cell proliferation) content of the gastric mucosa indicating no effect on cell proliferation (Table 4).

Estimation of free radical generation and enzymatic antioxidants—CRS significantly increased the LPO, NO and SOD and decreased CAT levels in the rat gastric mucosal homogenate when compared with unstressed rats. However, treatment with PPSM not only showed ulcer protection in CRS rats, but also

Table 2—Effect of PPSM (25 mg/kg, od \times 5 days), SFT (500 mg/kg, od \times 5 days) and OMZ (2 mg/kg, od \times 5 days) on chronic gastric ulcer induced by 50% acetic acid in rats
[Values are mean \pm SE of 8 rats in each group]

Oral treatment	5 days treatment			10 days treatment		
	Ulcer index	Protection (%)	Incidence perforation (%)	Ulcer index	Protection (%)	Incidence Perforation (%)
Control	13.7 \pm 1.52	-	37.5	7.7 \pm 1.01	-	0
PPSM	9.5 \pm 0.89*	30.7	25.0	2.1 \pm 0.70*	73.4	0
SFT	6.1 \pm 0.59*	55.4	12.5	1.7 \pm 0.67*	78.2	0
OMZ	8.3 \pm 0.74*	39.4	25.0	2.3 \pm 0.56*	70.1	0

*P value: < 0.05 compared to control group (One way ANOVA followed by Dunnett's test)

Table 3—Effect of PPSM, SFT and OMZ on gastric juice volume, acid, pepsin and DNA content in 4 hr PL rats.
[Values are mean \pm SE of 8 rats in each group]

Gastric juice	Control	PPSM	SFT	OMZ
		<i>Acid-pepsin secretion</i>		
Volume (ml/100 g)	1.32 \pm 0.12	1.16 \pm 0.10	1.33 \pm 0.12	1.24 \pm 0.10
Acid conc. ($\mu\text{Eq}/\text{ml}$)	121.8 \pm 8.9	106.1 \pm 5.4	116.0 \pm 12.9	81.3 \pm 8.1*
Acid output ($\mu\text{Eq}/4$ hr)	160.0 \pm 18.1	123.1 \pm 13.0	146.9 \pm 13.6	100.4 \pm 13.5*
Pepsin conc. ($\mu\text{mol}/\text{ml}$)	242.4 \pm 16.2	251.0 \pm 13.0	185.8 \pm 14.1*	179.7 \pm 8.5*
Pepsin output ($\mu\text{mol}/4$ hr)	312.3 \pm 25.5	282.9 \pm 13.3	240.8 \pm 18.9	220.4 \pm 16.4*
		<i>Mucin secretion ($\mu\text{g}/\text{ml}$)</i>		
Total hexoses (A)	384.8 \pm 37.9	438.1 \pm 28.3	384.2 \pm 22.9	338.3 \pm 22.8
Hexosamine (B)	191.6 \pm 20.2	227.8 \pm 19.1	226.8 \pm 12.4	191.5 \pm 18.7
Fucose (C)	83.5 \pm 6.6	111.0 \pm 7.0*	92.9 \pm 4.4	72.9 \pm 5.8
Sialic acid (D)	14.5 \pm 2.0	15.7 \pm 0.96	21.7 \pm 2.1*	12.3 \pm 1.2
Total carbohydrates (TC) (A+B+C+D)	674.4 \pm 51.4	792.6 \pm 34.1	725.5 \pm 31.0	615.0 \pm 30.3
Protein (P)	524.0 \pm 30.7	502.4 \pm 41.3	440.8 \pm 33.0	580.5 \pm 41.3
TC : P	1.29 \pm 0.09	1.65 \pm 0.14	1.69 \pm 0.10*	1.15 \pm 0.11
		<i>Mucosal cell shedding (μg DNA/ml)</i>		
	243.0 \pm 11.7	162.0 \pm 8.0*	142.0 \pm 10.2*	395.4 \pm 40.1*

*P value: < 0.05 compared to control group (One way ANOVA followed by Dunnett's test)

are different. Non-steroidal anti-inflammatory drugs like aspirin, phenylbutazone etc have been well documented to induce gastric mucosal damage¹². These drugs are widely used for various inflammatory conditions and for prolonged period. Attempts have always been made to screen anti-inflammatory drugs, which are either devoid of gastric toxicity or have gastric protective effects. Synthetic NSAIDs like aspirin cause mucosal damage by interfering with PGs synthesis, increasing acid secretion and back diffusion of H⁺ ions and thus leading to breaking up of mucosal barrier. Stress plays an important role in aetiopathology of gastro-duodenal ulceration. Ulcers due to stress are both due to physiological and psychological factors. Stress induced ulcers are also caused by a number of other factors like increase in gastric motility, vagal over activity, mast cell degranulation, decreased gastric mucosal blood flow and decreased PG synthesis. Pyloric ligation -induced ulcers are thought to be due to autodigestion of mucosa by gastric juice leading to breakdown of mucosal barrier¹².

Ulcerations caused by ethanol are due to perturbations of superficial mucosal cells, notably the mucosal mast cell leading to release of vasoactive mediators like histamine and leukotriene C₄/D₄ (LTC₄/D₄) etc. causing damage to gastric mucosa³⁸ therefore it is possible that PPSM may not be affecting the release of histamine or LTC₄/D₄ and is not effective against ethanol-induced GU. Acetic acid-induced ulcer better resembles clinical ulcers in location, chronicity and severity and serves as the most reliable model to study healing process. Chronic ulcers by acetic acid are due to increase in volume of acid output leading to subsequent pyloric obstruction and mucosal necrosis³⁹.

As aetiopathogenesis of these ulcers models are different, mechanism of ulcer protective activity should then include number of predisposing factors. Although the mechanism of ulcerogenesis may be different, the net result of these factors is disturbance of balance between offensive and defensive pre-epithelial, epithelial and sub-epithelial factors. To ascertain the possible mechanism involved in the ulcer protection by PPSM, its effects were evaluated on the gastric mucosal offensive factors like acid-pepsin secretion and defensive factors like mucin secretion, glycoproteins, cell shedding and cell proliferation in 4 h pylorus ligated rats. Further, its effect on lipid peroxidation (LPO) and nitric oxide

(NO) and antioxidants like superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) was studied *per se* as well as in GU rats.

PPSM showed a tendency to decrease in acid and pepsin secretion and output in PL rats. Increases in these offensive factors have been reported to be essential for many experimental and clinical gastro-duodenal ulcers¹². The ulcer protective effects of extracts of both seeds and roots of *P. pinnata*^{4,7} and the ethanolic extract of the roots of this plant that tended to decrease acid-pepsin and increase mucin secretion have been reported⁷. The present work using PPSM do confirm our previous reported findings of protective effects of extracts of seeds of *P. pinnata*⁴.

PPSM tended to increase both gastric mucin secretion and mucosal glycoproteins, quantified in terms of TC: P ratio, which is taken as a reliable marker for both mucin secretion and mucosal glycoproteins^{18,19}. Increase in mucin secretion could be due to an increase in the dissolved mucopolysaccharides while, decrease in protein content may be due to decrease in leakage of plasma proteins into the lumen indicating increased mucosal resistance²⁷. Mucus is endowed with an array of mucosal protective properties and also acts as a first line of defense. Mucus is secreted by the mucus neck cells and covers the gastric mucosa thereby preventing physical damage and back diffusion of hydrogen ions. Mucin is viscous glycoprotein with physiochemical properties producing relatively resistant acid barrier. It makes up the major part of the mucus, an important pre-epithelial factor that acts as a first line of defense against various ulcerogens. The main components of gastric mucous are the acidic glycoprotein sialic acid and neutral mucopolysaccharides like total hexoses, hexosamine and fucose. Increase in mucin was due to significant increase in individual mucopolysaccharide leading to significant increase in total carbohydrates¹². Further, strengthening of the mucosal barrier lead to decrease mucosal cell exfoliation as observed from decrease in DNA content in the gastric juice, which is taken as a reliable marker for cell shedding²⁶.

The strengthening of the mucosal barrier led to decrease in mucosal cell exfoliation as evidenced from decrease in DNA content in the gastric juice, which is taken as a reliable marker for cell shedding²⁶. The DNA content of gastric juice, which comes from the exfoliation of gastric mucosal cells, indicates the life span of gastric mucosal cells and the mucosal

resistance. Any noxious stimuli will lead to increase exfoliation, increasing the content of gastric juice DNA while, factors affecting enhancement of defensive factors will lead to decrease in it. Decrease in DNA content of gastric juice by PPSM indicates increase in life span of mucosal cells.

Repair of gastric mucosa is both by the process of restitution and cell proliferation. Cell proliferation was estimated in term of μg DNA/mg of protein in the gastric mucosal homogenate of rats. PPSM did not show any effect on cell proliferation as evidenced by little or no change in μg DNA/mg of protein content of the gastric mucosa. Thus, the prophylactic and healing effects of these drugs may not be dependent on cell proliferation which is however, reported to be increased during mucosal damage. It is possible that the enhanced healing could be due to their predominant effect on cell shedding, which was decreased. Further, an increase in mucus secretion is also important for the repair of superficial damage to the mucosa and it acts by forming a protective coat and this mucoid cap provides a suitable microenvironment for repair by process of restitution, a process of repair involving migration of viable surface mucosal cells to cover the damaged mucosa¹².

OMZ, a proton pump inhibitor showed ulcer protective effects by virtue of its predominant action on offensive acid-pepsin secretion while SFT showed it by promoting mucosal defensive factors as evidenced by an increase in mucin secretion, mucosal glycoproteins and life span of mucosal cells.

Stress induced ulceration involves damage by ROS apart from acid and pepsin related factors⁴⁰. In the present study, during cold restraint stress LPO, NO and SOD were increased and CAT level was decreased significantly. The increase in SOD may be due to increase in ROS generation during mucosal damage. This led to increased generation of H_2O_2^- and its accumulation due to decreased CAT level. Inactivation of gastric peroxides during stress⁴¹ may also aggravate the mucosal damage. This evidently caused increased lipid peroxidation and mucosal damage as seen from the increase in ulcer index in comparison to the control group. However, PPSM treatment effectively alleviated stress-induced ulcers with marked decrease in LPO and NO suggesting decrease in oxidative damage. This was mostly due to increased balance between SOD and CAT levels, effectively counteracting the free radicals generated by cascade of reactions as described earlier. Thus the

anti-ulcerogenic activity of PPSM may also be due its gastric anti-oxidant effects. Alcoholic extracts of *P. pinnata* flowers have been reported to possess significant antioxidant properties⁴².

Further, ethanol ingestion was found to decrease the level of GSH. The decrease in GSH level in rats by ethanol ingestion however, was tended to increase by PPSM treatment. Thiols such as GSH are able to bind reactive free radicals and may influence the physical properties of mucus, since its subunits are joined by disulfide bridges. Diethyl malate, an agent that markedly depletes gastric glutathione, causes severe gastric ulceration, suggesting a possible modulatory role for glutathione in ulcerogenesis⁴³. Tendency to Increase in GSH levels by PPSM in the gastric mucosa of ethanol ingested rats indicated that their anti-ulcerogenic activity could be due to their effects on non-protein sulfhydryls.

Both OMZ and SFT failed to show any noticeable effects on free radical generation and antioxidant activity though they were effective ulcer protective drugs.

PPSM thus, showed both antisecretory (OMZ like) and ulcer protective (SFT like) activities as evidenced by tendency to decrease in acid-pepsin secretion and increase in mucin secretion, mucosal glycoproteins and life span of mucosal cells besides its free radical scavenging and antioxidant effects which play an important role in ulcer healing⁴⁴. Further studies on other factors like *H. pylori* and cAMP, which play important role in ulcerogenesis, may provide more insights on the antiulcerogenic activity of *P. pinnata* seeds.

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