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Effect of *Piper longum* Linn, *Zingiber officianalis* Linn and *Ferula species* on gastric ulceration and secretion in rats

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Use of *Dipaniya Mahakasaya,* a group consisting of 10 herbal drugs, has been suggested in Charaka Samhita to improve digestion. Out of these 10 plants, three, viz. *P. longum* (water decoction), *Z. officianalis* (water decoction) and *Ferula* species (colloidal solution) were studied for their antiulcer and mechanism of antiulcer effects in rats. All the drugs in the dose of 50 mg/kg, po, 60 min prior to experiment, showed significant protection against gastric ulcers induced by 2 hr cold restraint stress, aspirin (200 mg/kg, 4 hr) and 4 hr pylorus ligation. The antiulcerogenic effect seemed to be due to the augmentation of mucin secretion and decreased cell shedding rather than offensive acid and pepsin secretion which however, were found to be increased by them.

Pippali (*Piper longum* Linn), sringvera (*Zingiber officianalis* Linn) and hingu (*Ferula* species) are three plant drugs of the *Dipanya mahakasaya* group, which is one of the 50 great extracts used in Ayurveda. Drugs which increase the digestive fire are called as Dipanya[1]. Fruits of pippali have been used as thermogenic, stomachic, aphrodisiac, carminative, expectorant, laxative, digestive, emmolient, antigiardiasis, antiamoebic and antiseptic[2-5]. Rhizomes of sringvera are useful in anorexia, dyspepsia, abdominal discomfort and as appetiser, laxative, stomachic, rubefacient, anticancer, antiemetic and carminative[6-9]. The oleoresin of hingu is bitter and acrid, carminative, expectorant, anthelmintic, digestive, sedative and is used in flautulent colic, dyspepsia and constipation[10,11].

Ulcers are thought to be due to imbalances in gastric offensive and defensive mucosal factors. While, acid and pepsin make up the offensive factors, the defensive factors include mucin secretion, mucosal glycoprotein, cell shedding, cell proliferation, prostaglandins (PGs), the urogastrone/ epidermal healing factor (URO/EHF)[12] etc. Inspite of numerous drugs available, peptic ulcer is still a major cause of morbidity. Attempt has been made in our laboratory to screen some Ayurvedic drugs for their potentional antiulcerogenic properties.

As only sparse reports are available on the possible anti-ulcer effects of the above preparations, the present work has been carried out to study the effect of three constituents of Dipanya mahakasaya in experimentally induced gastric ulcers in rats and their posssible mechanisms of action by studying their effects on various mucosal offensive acid-pepsin and defensive factors like mucin secretion, mucosal cell shedding and glycoproteins.

Materials and Methods

Albino rats (CF strains) of either sex weighing between 100-160 g were procured from the central animal house of the institute. The animals were housed in well ventilated colony cages and kept in the departmental animal house for a week for acclimatization. The animals were provided with standard rodent pellet diet (Hind liver) and the food was withdrawn 18-24 hr before the experiment though water was allowed *ad libitum*.

Pippali, sringvera and hingu were purchased as crude drugs from local market (Varanasi) and were pharmacognistically identified in the department of Dravyaguna I.M.S, B.H.U. Hot water decoctions of fruits of pippali and rhizomes of sringvera and colloidal solution of hingu[13] were prepared and were used for the study. The preparations were given orally with help of an orogastric tube (1 ml/100g body weight), 60 min before the experimentation in 18 hr fasted rats[14]. The preparations were initially screened using doses of 10, 50 and 100 mg/kg body weight against 2 hr cold restraint stress-induced ulcers in rats[15] (% protection afforted by three test drugs at 10

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mg, 25.6 -39.4 %, $P < 0.1$ - < 0.05; 50 mg, 61.2 - 79.4, $P < 0.05$ - < 0.01 and 100 mg, 67.4 -81.2 %, $P < 0.05$ - < 0.001) and the dose of 50 mg/kg body weight was found to be the optimal effective dose and hence was chosen for further experiments.

Anti-ulcer studies—The antiulcer activity of the test drugs were screened in the dose of 50 mg/kg body weight administered orally, given 60 min before subjecting the animals to the following experimental ulcer models.

a) Cold-restraint stress (CRS)—induced ulcers[15] were produced by immobilising the rats on a wooden plank and subjecting them to stress at 4°-6°C for 2 hr.
b) Aspirin (ASP)-induced gastric ulcers[16] were produced by oral administration of 200 mg/kg of aspirin suspenstion in water (20 mg/ml) on the day of the treatment and the animals were sacrificed after 4 hr of ASP administration.
c) Pyloric ligation (PL)-induced gastric ulcers[17] were produced by ligating the pyloric end of the stomach for 4 hr.

The stomach was taken out and cut along the greater curvature and ulcers were scored in the glandular portion of the stomach by a person unaware of the experimental protocol. The number of ulcers per stomach was noted and the severity of the ulcers was scored after histological confirmation as follows : 0, no ulcer; +, changes limited to superficial layers of mucosa and no congestion; ++, half of the mucosal thickness showed necrotic changes; +++, more than two-thirds of the mucosal thickness destroyed with marked necrosis and congestion, muscularis remaining unaffected; ++++, complete destruction of the mucosa with necrosis and haemorrhage, muscularis still remaining unaffected. The pooled group ulcer score was then calculated[18]. Statistical analysis was done by using Willcoxon Sum Rank test[19].

Gastric secretion study—The gastric juice was collected 4 h after PL[17], centrifuged for 5 min at 2000 rpm and the volume of the supernatant was expressed as ml/100g body weight. Acid concentration and total acid output were determined by titrating with 0.01 *N* NaOH, using phenolphthalein as indicator, and were expressed as μEq/ml and μEq/4 hr respectively. Similarly peptic concentration and output were determined by using hemoglobin as the substrate[20] and expressed as μmoles/ml and μmole/4 hr for concentration and output respectively. Mucin activity was estimated in the mucosubstances precipitated by treating the gastric juice with 90% ethanol in a 1:9 ratio. The precipitate, thus obtained was either dissolved in 1 ml of 0.1 *N* NaOH or 1 ml of 0.1 *N* H_2SO_4. The former was used for the estimation of protein[21], total hexoses[22], hexosamine[23] and fucose[24], while the latter was used for the estimation of sialic acid[25]. The results are expressed in μg/ml. The ratio of total carbohydrates (TC) (sum of total hexoses, hexosamine, fucose and sialic acid) to protein (P) has been taken as the index of mucin activity[26]. DNA content was estimated and expressed as μg/ml gastric juice/100g weight of rat[27].

Gastric mucosal studies —Rat gastric mucosal scraping in normal saline were homogenised in distilled water and treated with 90% ethanol and different fractions of glycoproteins were estimated following the methods as described above[21-26] for the mucin secretion and expressed as TC : P ratio. Statistical analysis of data was done by using unpaired Student's *t* test method.

Results

Pippali, sringvera and hingu were found to have significant antiulcer activities in all the three models of gastric ulcers except pippali which tended to decrease ulcer index against ASP- induced ulcers as observed from the reduction of ulcer index compared to the control group (Table 1). All the three test drugs either tended to increase or increased significantly the gastric juice volume, acid-pepsin concentration and output compared to that of the control group. However, they showed a significant reduction of DNA content of the gastric juice (Table 2).

All the three test substances either tended to decrease or decreased the protein content with little effect either on individual carbohydrates or total carbohydrate contents, but the TC:P ratio was significantly increased (Table 3). However, in gastric mucosa none of the drugs produced any significant change either in the protein, individual carbohydrates, total carbohydrates or T :P ratio indicating no change in the glycoprotein contents of the mucosa in the treated groups compared to the control group (Table 4).

Discussion

The results demonstrate the antiulcerogenic activity of pippali, sringvera and hingu, which are some of the constituents of Dipanya Mahakasayas

Table 1—Effect of various *Dipania Mahakasaya* drugs (50 mg/kg, po, 60 min before) on cold-restraint stress (CRS)-, aspirin (ASP)- and pylorus ligation (PL)- induced gastric ulcers in rats
[Values are mean ± SE of 6 animals in each group]

Oral treatment	Ulcer per stomach (a)	Severity per stomach (b)	Ulcer index (a + b)
	CRS		
Control	8.0 ± 1.4	8.0 ± 1.4	16.0 ± 2.8
Pippali	1.6 ± 0.4[b]	1.6 ± 0.4[b]	3.3 ± 0.7[b]
Sringvera	2.2 ± 1.1[b]	2.2 ± 1.1[b]	4.4 ± 2.2[b]
Hingu	3.1 ± 1.6[a]	3.1 ± 1.6[a]	6.2 ± 3.1[a]
	ASP		
Control	9.7 ± 1.4	12.9 ± 3.1	22.6 ± 4.5
Pippali	6.0 ± 1.0	6.3 ± 1.3	12.3 ± 2.3
Sringvera	5.0 ± 1.7	5.0 ± 1.7[a]	10.0 ± 3.3[a]
Hingu	4.5 ± 1.9	4.5 ± 2.0[a]	9.0 ± 3.9[a]
	PL		
Control	6.8 ± 1.4	6.7 ± 3.1	13.5 ± 2.7
Pippali	1.4 ± 1.0	1.4 ± 1.4	2.8 ± 2.8[a]
Sringvera	1.6 ± 1.2[a]	1.6 ± 1.2[a]	3.2 ± 2.4[a]
Hingu	1.3 ± 0.9[b]	1.4 ± 1.0[b]	2.7 ± 1.9[b]

P values : [a]<0.05, [b]< 0.01

against CRS-, PL- and ASP- induced gastric ulcers. The drugs either tended to increase or increased acid-pepsin secretion indicating enhancement of offensive acid and pepsin secretion. However, they augmented the defensive factors like enhancement in gastric juice mucin secretion and decrease in cell shedding.

The essential basis which determines the status of mucosal defense against the offensive gastric and pepsin secretion is the quality and quantity of mucin secretion[28]. These drugs were found to augment the mucin secretion as they were found to increase the TC : P ratio significantly which is taken as a reliable index for mucin secretion[26,29]. Most of the Ayurvedic herbal or metallic drugs showed antiulcerogenic activity by virtue of their predominant action on mucin secretion, which is one of the important defensive mucosal factors[12,18,30]. DNA content of the gastric juice is one of the important marker of gastric mucosal damage or cell shedding, which is augmented by ulcerogenic agents and reduced by ulcer protective agents[12,27]. The decrease in DNA content of gastric juice by the three drugs indicates the cytoprotective effect of these drugs. It has also

Table 2—Effect of various *Dipanaya mahakasaya* drugs (50 mg/kg, po, 60 min before) on volume, acid, pepsin and DNA content of gastric juice in PL- rats
[Values are mean ± SE of 6 animals in each group]

Treatment	Volume	Acid		Pepsin		DNA
		Concentration	Output	Concentration	Output	(μg/ml/100g rat)
	(ml/100 g body wt)	(μEq/ml)	(μEq/4 hr)	(μmol/ml)	(μmol/4 hr)	
Control	1.10 ± 0.23	200.0 ± 40.4	274.4 ± 42.8	161.9 ± 31.5	228.0 ± 86.0	249 ± 31
Pippali	1.72 ± 0.18	315.0 ± 56.6	568.0 ± 125.0[a]	250.8 ± 44.9	382.8 ± 61.8	169 ± 15[a]
Sringvera	1.44 ± 0.34	338.3 ± 33.6[a]	501.0 ± 86.0[a]	362.8 ± 82.3[a]	446.3 ± 109.0	149 ± 23[a]
Hingu	2.36 ± 0.47[a]	223.6 ± 29.0	527.7 ± 106.0[a]	148.2 ± 16.7	349.8 ± 98.7	162 ± 19[a]

P values: [a] <0.05; [b] < 0.01

Table 3—Effect of various *Dipanaya mahakasaya* drugs (50 mg/kg, po, 60 min before) on mucin secretion in PL- rats
[Values are mean ± SE of 6 animals in each group]

Mucoprotein (μg/ml)	Control	Pippali	Sringvera	Hingu
Protein (P)	372.5 ± 34.8	270.2 ± 15.4[a]	307.8 ± 53.3	210.0 ± 15.5[a]
Total hexoses	289.6 ± 15.7	324.9 ± 19.6	371.0 ± 42.1	277.9 ± 23.3
Hexosamine	246.9 ± 27.7	283.1 ± 27.7	280.0 ± 33.3	198.8 ± 23.6
Fucose	95.0 ± 7.3	85.5 ± 8.90	93.7 ± 9.4	84.3 ± 8.2
Sialic acid	53.9 ± 6.5	64.3 ± 3.30	48.0 ± 6.4	57.9 ± 6.8
Total carbohydrates (TC)	685.4 ± 39.4	757.8 ± 53.9	792.7 ± 55.2	618.9 ± 43.5
TC : P	1.84 ± 0.20	2.80 ± 0.11[b]	2.57 ± 0.10[a]	2.95 ± 0.29[a]

P values : [a]< 0.05; [b]< 0.01

Table 4—Effect of various *Dipanaya mahakasaya* drugs (50 mg/kg, po, 60 min before) on gastric mucosal glycoproteins (μg/100 mg wet tissue) in PL- rats

[Values are mean ± SE of 6 animals in each group]

	Control	Pippali	Sringvera	Hingu
Protein (P)	5437 ± 614	4942 ± 536	4874 ± 410	5312 ± 467
Total hexoses	3097 ± 293	2918 ± 151	2785 ± 142	3184 ± 271
Hexosamine	2001 ± 191	1790 ± 180	1657 ± 160	2037 ± 243
Fucose	234 ± 18	233 ± 19	271 ± 19	290 ± 32
Sialic acid	146 ± 19	100 ± 12	141 ± 10	182 ± 23
Total carbohydrates (TC)	5478 ± 418	5041 ± 407	4854 ± 347	5698 ± 465
TC : P	1.01 ± 0.11	1.02 ± 0.09	1.00 ± 0.11	1.07 ± 0.13

been observed that these drugs did not produce any change in the glycoprotein content of the gastric mucosa. As the drugs were given acutely i.e. only 60 min before subjecting the animals to experiments, the observed effect may not be discernible so early and possibly a prolonged treatment with these drugs for 3 to 5 days may show a significant change in this important defensive factors.

Thus, the present study, underlines the usefulness of pippali, sringvera and hingu, the three important constituents of Dipanaya Mahakasayas in gastric ulceration. Further studies on various other factors of mucosal defensive factors like prostaglandins release, mucosal blood flow, cell proliferation and antioxidant effects would provide more insight knowledge on the antiulcerogenic activity of these Ayurvedic drugs.

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