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EXPERIMENTAL EVALUATION OF *EMBLICA OFFICINALIS* FRUIT ON GASTRIC ULCERS AND MUCOSAL OFFENSIVE AND DEFENSIVE FACTORS IN RATS

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Emblica officinalis Gaertn has been mentioned in the Ayurveda as a Maharasayana. The rasayanas are drugs which enhance body resistance and are similar to adaptogens in modern medicine and are advocated for use in rejuvenation therapy. Emblica officinalis is reported to protect the individual against biological, physical and chemical stress manifestations. Stress is considered to be one of the important aetiopathogenic factors for peptic ulceration and hence juice of fresh fruits of Emblica officinalis Gaertn (EOJ) was evaluated for its anti-ulcerogenic effect against various rat gastric ulcers induced by cold restraint stress, pylorus ligation, ethanol and aspirin. A preliminary study done with fresh EOJ (240, 480 or 960 mg.kg^{-1}) administered perorally, twice daily for five days, showed a dose-dependent anti-ulcerogenic effect. A dose of 480 mg.kg^{-1} was then selected for studying the mechanism of anti-ulcer effect on aggressive acid-pepsin secretion and defensive mucosal factors like mucin secretion, cell shedding and mucosal glycoproteins in 4h pylorus ligated rats. EOJ showed a decrease in aggressive acid-pepsin concentration and output and increased the defensive mucin secretion and glycoprotein content. It decreased cell shedding indicating a decrease in exfoliation and an increase in life span of cells. Hence anti-ulcerogenic effect of EOJ could be due to its effects both on offensive and defensive mucosal factors.

Key words: *Amla; Gastric ulcer; Mucin; Cell shedding*

Introduction

Emblica officinalis Gaertn is a plant described in Ayurveda, the Indian traditional system of medicine, to promote health and life-span by increasing defense against disease, arresting the aging process and revitalizing the body in debilitated conditions (1). The clinical efficacy of the fruits of *E. officinalis,* commonly known as Indian gooseberry (amla), is held in high esteem in Ayurveda and amla is refereed to as a *Maharasayana* (2) and used in hypercho-lesterolaemia (3). The fruits form the major constituent of Chayavanprash awaleha, a polyherbal Ayurvedic rasayana preparation which has been described in Charaka Samhita (4). Experimental investigations on *E. officinalis* have indicated its antihypercholesterolaemic (5), antimutagenic (6), antimicrobial (7), antifungal (8), anticancer (9) and anti-inflammatory (10) activities and also its use in acute pancreatitis (11). It was reported to protect the individual against biological, physical and chemical stress manifestations (12).

Gastric ulceration is known to be due to the involvement of many aetiopathogenic factors like inheritance, diet, stress, drugs, alcohol, smoking, refluxed bile, and *H. pylori* infection. Recently, *E. officinalis* has been reported to be protective against biological, physical and chemical stress manifestations (12). Stress plays an impor-tant role in gastroduodenal damage which is due to the result of imbalance between the aggressive acid-pepsin secretion versus impaired mucosal defensive factors (13). The present investigation incorporates the effect of fresh juice of *E. officinalis* fruit on various rat gastric ulcer models and to delineate the probable mechanism of action by studying both the aggressive acid-pepsin secretion and mucosal defensive factors like mucin secretion, mucosal cell shedding and glycoprotein content in rats.

Materials and methods

Animals: The study was conducted with inbred Charles-Foster (CF) albino rats (130-180 g) of either sex, obtained from the central animal house of the

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Institute of Medical Sciences, Banaras Hindu University. They were kept in the departmental animal house at 26 ± 2 ^{0}C with a relative humidity of 44-56 % (light and dark cycles of 10 and 14 h respectively) for 1 week before and during the experiments. Animals were provided with standard rodent pellet diet (Hind liver) and the food was withdrawn 18-24 h before the experiment though water was allowed *ad libitum*.

Drug treatment: *E.officinalis* fruits were purchased from the local drug market (Varanasi) between November-January. Botanical identification was performed at the department of Dravyaguna, Institute of Medical Sciences, Banaras Hindu University. Fresh juice was obtained from 1kg of seedless fruits by a juicer and filtered and the yield was 500 ml and the dry weight in terms of solid content was 4.8 %. The juice was stored in a refrigerator at –20^0 C and was used within seven days. *E. officinalis* juice (EOJ) in doses of 0.5, 1.0 and 2.0 ml. (240, 480 and 960 mg.kg^{-1} in terms of dry weight) and sucralfate (SFT) in the dose of 250 mg.kg^{-1} were administered perorally twice daily at 10 am and 4 pm respectively for five days and the experiments were conducted on the 6th day, using 18 h fasted rats. Control group animals received distilled water (1 ml.kg^{-1}).

Experimental

The following methods were used:

a) Drug induced gastric ulcers: The gastric ulcers were induced in rats by administering ethanol (1ml.200 g^{-1}, 1 h) (13) and aspirin (ASP, 200 mg. kg^{-1}. 4 h) (14), perorally with the help of an oro-gastric tube. EtOH and ASP were administered on day 6 after 18 h of fasting and the animals were sacrificed by cervical dislocation and stomachs were incised along the greater curvature and examined for ulcers.

b) Cold-restraint stress (CRS)-induced ulcers (15): On day six, 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 ^{0}C. The animals were then sacrificed by cervical dislocation and ulcers were scored on the dissected stomachs.

c) Pylorus-ligated (PL)- rats (16): Drugs were administered for a period of 5 days. On day six, to 18 h fasted rats, pyloric ligation was done. Animals were anaesthetized using pentobarbitone (35 mg. kg^{-1}, ip), the abdomen was cut open and pylorus ligation was done without causing any damage to its blood supply. The stomach was replaced carefully and the abdomen wall was closed with interrupted sutures. The animals were deprived of water during the post-operative period.

After 4 h, the stomach was dissected out and cut open along the greater curvature and contents were collected into tubes. Ulcers in the glandular portion of the stomach were scored by an observer unaware of the experimental protocol. The number of ulcers per stomach were noted and the severities of the ulcers were scored after histological confirmation as follows: 0, no ulcer; +, pin point ulcer and histology-cal changes limited to superficial layers of mucosa with no congestion; ++, ulcer size less than 1 mm and half of the mucosal thickness showed necrotic changes; +++, ulcer size 1-2 mm with more than two-thirds of the mucosal thickness destroyed with marked necrosis and congestion, muscularis remaining unaffected; ++++, ulcer either more than 2 mm in size or perforated with complete destruction of the mucosa with necrosis and haemorrhage, muscularis still remaining unaffected. The pooled group ulcer score was then calculated (17). Statistical analysis was done by using Wilcoxon Sum Rank test (18). In case of ethanol, the ulcer index was scored, based upon the product of length and width of the ulcers present in the glandular portion of the stomach (mm^2/rat). Statistical analysis of data were done by using the unpaired Student's *t* test.

Gastric secretion studies: The gastric juice was collected 4 h after PL and centrifuged for 5 min at 2000 rpm and the volume of the supernatant was expressed as ml/100g body weight. Total acid output was determined by titrating with 0.01 N NaOH, using phenolphthalein as the indicator and expressed as μEqml^{-1} for concentration and μEq/4h for output. Peptic activity was determined using haemoglobin as the substrate and was expressed as μmol.ml^{-1} and μmol/4h of tyrosine for concentration and output respectively (19). 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 H_2S0_4.The former was used for the estimation of protein (20), total hexoses (21), hexosamine (22) and fucose (23), while the latter was used for the estimation of sialic acid (24). The results were expressed as μg.ml^{-1}. 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 activity (25, 26). DNA contents were estimated and expressed as μg.ml^{-1} gastric juice/100g weight of rat (27).

Gastric mucosal studies: Samples of gastric mucosal scraping were homogenized in distilled water and treated with 90% ethanol and were subjected to estimations of carbohydrates and proteins using the methods described above for gastric juice contents. Statistical analysis of data were done by using the unpaired Student's *t* test.

Results

The antiulcerogenic activity of EOJ on various gastric ulcer models are presented in table 1. Dose dependent antiulcer effect was observed with doses of 240, 480 and 960 $mg.kg^{-1}$ twice daily for five days against different experimental gastric ulcerations in rats. The incidence of severity of ulcerations were significantly reduced and percent protection ranged from 17.7 to 80.0%, SFT at the dose of 250 $mg.kg^{-1}$

Table 1-Effect of juice of *E. officinalis* fruit (EOJ) on ethanol (EtOH, 100%, 1 ml/200 g, po, 1 h)-, aspirin (ASP, 200 mg/kg, po, 4 h)-, 2 h cold restraint stress (CRS)-, and 4 h pylorus ligated (PL)- induced gastric ulcers in rats

Treatment (mg/kg, bd x 5 days)		Ulcer Index	% Protection
		EtOH-INDUCED ULCERS (mm^2/rat)	
Control	-	22.0 ± 5.3	-
EOJ	240	18.1 ± 5.2	17.7 %
	480	8.8 ± 2.1[a]	60.0 %
	960	5.2 ± 1.7[a]	76.4 %
SFT	250	4.5 ± 2.3[a]	79.5 %
		ASP- INDUCED ULCERS	
Control	-	17.5 ± 2.5	-
EOJ	240	10.8 ± 3.3	61.7 %
	480	6.8 ± 3.2[a]	61.2 %
	960	5.2 ± 2.3[b]	70.3 %
SFT	250	7.0 ± 2.1[b]	60.0 %
		CRS- INDUCED ULCERS	
Control	-	29.2 ± 4.8	-
EOJ	240	28.2 ± 5.6	34.0 %
	480	14.5 ± 4.1[a]	50.3 %
	960	6.0 ± 2.7[b]	79.5 %
SFT	250	6.8 ± 3.0[b]	76.7 %
		PL- INDUCED ULCERS	
Control	-	22.0 ± 4.7	-
EOJ	240	11.6 ± 3.8	47.3 %
	480	7.6 ± 4.2[a]	65.5 %
	960	4.4 ± 2.6[b]	80.0 %
SFT	250	4.3 ± 2.8[b]	80.5 %

Values are mean ± SEM

Significance : a = $P < 0.05$; b = $P < 0.01$; as compared to their control, n = 6

Table 2-Effect of EOJ on gastric juice volume, acid, peptic, and DNA contents in 4 h PL- rats

Treatment (mg/kg, bd x 5 days)	Volume (ml/100g)	Acid Concentration (μEq/ml)	Acid Output (μEq/4h)	Pepsin Concentration (μmol/ml)	Pepsin Output (μmol 4 h)	DNA (μg/ml 100 g)
Control	3.25 ± 0.71	57.5 ± 3.2	185.8 ± 39.3	577.2 ± 44.0	1875.9 ± 319.2	116.2 ± 9.0
EOJ 480	1.40 ± 0.27[a]	37.5 ± 2.5[c]	52.0 ± 10.6[b]	245.0 ± 66.8[c]	342.7 ± 70.2[c]	81.8 ± 9.0[a]
SFT 250	2.62 ± 0.52	42.6 ± 6.4	111.6 ± 29.3	232.9 ± 89.2[b]	610.2 ± 139.2[b]	72.1 ± 12.4[a]

Values are mean ± SEM
Significance : [a] = P < 0.05; [b] = P < 0.01; [c] = P < 0.001 as compared to their respective control, n = 6

TABLE 3-Effect of EOJ on gastric juice mucoprotein and glycoprotein in 4 h PL rats.

Treatment (mg/kg, bd x 5 days)	Protein	Total hexoses	Hexosamine	Fucose	Sialic acid	TC	TC : P
Mucoprotein (μg/ml)							
Control	562.5± 30.9	270.8 ± 17.2	120.3 ± 15.0	68.3 ± 8.1	29.0 ± 4.0	488.4 ± 28.0	0.89 ± 0.08
EOJ 480	389.4± 28.1[b]	439.9 ± 35.8[b]	189.6 ± 14.2[b]	90.9 ± 9.1	40.5 ± 3.5[a]	740.9 ± 48.0[c]	1.98 ± 0.12[b]
SFT 250	410.0± 36.6[b]	367.7 ± 36.0[a]	156.2 ± 12.0	89.7 ± 9.7	43.0 ± 3.9[a]	656.6 ± 56.0[a]	1.63 ± 0.21[b]
Glycoprotein (μg/100 mg wet tissue)							
Control	4427 ± 526	2912 ± 312	1798 ± 256	125.0 ± 12	91 ± 12	4926 ± 407	1.11 ± 0.13
EOJ 480	3742 ± 370	4548 ± 356[b]	2664 ± 203[a]	147.4 ± 15	138 ± 14[a]	7694 ± 476[b]	2.04 ± 0.19[b]
SFT 250	3801 ± 289	3802 ± 242[a]	2190 ± 178	154.0 ± 10	160 ± 15[b]	6306 ± 393[a]	1.69 ± 0.17[a]

Values are mean ± SEM Significance : a = P < 0.05; b = P < 0.01; c = P < 0.001 as compared to their respective control
n = 6

significantly decreased the ulcer index and percent protection ranged from 60.0 to 80.5%. For subsequent studies the dose of 480 $mg.kg^{-1}$ was selected to study the effect on various parameters of gastric secretion and mucosa in 4hr PL rats.

EOJ in the dose of 480$mg.kg^{-1}$ caused a significant decrease in volume, acid-pepsin concentration and output and DNA contents of gastric juice indicating decrease in offensive acid-pepsin secretion and cell shedding (indicating increase in life span of mucosal cells) while SFT showed decreases in pepsin concentration and output and DNA content of the gastric juice (Table 2).

EOJ in the above dose increased the carbohydrates and total carbohydrates, while it decreased protein contents leading to a significant increase in TC:P ratio, an indicator of mucin secretion. The above effect of EOJ seems to be similar to that of SFT. On mucosal glycoproteins both EOJ and SFT showed similar effects on individual total carbohydrates and TC:P ratio indicating enhancement of glyco-protein content in gastric mucosa and overall mucosal defence (Table 3).

Discussion

Investigations with EOJ indicated protection of rat gastric mucosa dose dependently against various experimentally induced gastric ulcers in this work. Studies on rats have shown that oxygen derived free radicals were directly implicated in EtOH (28), ASP (29), CRS (30), and PL (31) induced gastric ulcers. EOJ significantly inhibited the lipid peroxidation in biological membranes (32). Hence the effective protection afforded by EOJ in various gastric ulcer models was comparable to the reference drug SFT. Ulcers are believed to develop because of excess acid and pepsin for a given degree of mucosal defense. EOJ was found to decrease the volume, acid-pepsin concentration and output which may be considered as highly desirable properties of anti-ulcerogenic drugs. SFT was reported to be clinically effective in healing gastric ulcers and peptic ulcer recurrence with a significant antipeptic activity (33, 34).

The mucus layer is the first line of defense in gastric ulcer protection. Mucus is a protective complex mixture, which is disturbed by stress and various drugs like aspirin by inhibiting the synthesis of mucus glycoprotein through impairment of glycosylation. The status of mucin and mucosal glycoprotein was studied by esti-mating the different fractions of mucosubs-tances *viz.* the total hexoses, hexosamine, fucose, sialic acid and proteins of the gast-ric juice and mucosa. The ratio between the total carbohydrates (sum of individual carbohydrates) to protein reflects the functional integrity of the gastric mucosal barrier and serves as a reliable index of mucosal resistance (35, 36). EOJ and SFT increased the TC: P ratio which may be due to their predominant action on mucin and glycoprotein, which confirms the role of defensive mucosal factors in the antiulcerogenic activity. Sialic acid present as "sialic acid containing glycoprotein" or "sialylated glycoprotein" was significantly increased by EOJ and SFT. DNA content was measured in gastric juice, and quantified to be an important marker of gastric mucosal damage or cell shedding (37). The observed decrease in DNA content with an augment increase in TC: P ratio indicates the gastric cytoprotective role of EOJ. Thus, the present investigation establishes the antiulcerogenic and cytoprotective activities of the juice of *Emblica officinalis* through its effect both on offensive and defensive mucosal factors.

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