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EXPERIMENTAL EVALUATION OF *BOCOPA MONNIERA* ON RAT GASTRIC ULCERATION AND SECRETION

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Abstract : The anti-ulcerogenic effect of fresh juice from the whole plant of *Bocapa monniera* Wettst. (BMJ) commonly known as Brahmi in Hindi was examined using gastric ulcer models induced by ethanol, aspirin, 2 h cold restraint stress and 4 h pylorus ligation. *Bocapa monniera* juice (BMJ) at doses of 100 and 300 mg/kg and sucralfate at a dose of 250 mg/kg were given orally, twice daily for 5 days. BMJ 100–300 mg/kg produced significant antiulcer activity in all the experimental gastric ulcer models except in case of ethanol-induced ulcers where 100 mg/kg was not found to decrease it significantly. BMJ (100–300 mg/kg) was found to have little or no effect on the offensive acid-pepsin secretion, while cell shedding (µg DNA/mg of protein) and mucin secretion in terms of total carbohydrates : protein ration (TC : P), the two important parameters of defensive factors were significantly decreased and increased respectively indicating enhancement of protective mucosal factors. Both BMJ (300 mg/kg) and SF showed tendency to increase the mucosal glycoproteins in terms of TC : P, though individual carbohydrates and total carbohydrates were either increased or showed a tendency to increase. Thus, ulcer protective effect of BMJ may be due to its effect on mucosal defensive factors like enhanced mucin secretion, mucosal glycoprotein and decreased cell shedding rather than on offensive factors such as acid and pepsin.

Key words : *Bacopa monniera* antiulcer mucin cell shedding

INTRODUCTION

Bocapa monniera Wettst. (syn. Herpestis monniera L : Scrophulariaceae), commonly known as 'Brahmi' is an annual creeping found throughout the Indian subcontinent in wet, damp and marshy areas. The plant is mentioned in Ayurvedic materia medica and finds place in several ancient texts including the Caraka Samhita (6th century A. D.) and the *Bhavprakash Varg-Prakarana* (16th century A. D.). It is classified as *Medhyarasayana* drugs which promote mental healing, improve intellect and augment memory. In the above compendia, *Bacopa monniera* finds use in clinical mental disorders akin to modern classification of anxiety disorders, including generalized anxiety syndrome, obsessive compulsive disorders, panic attacks, hysteria, epilepsy and insomnia (1). Recently 50% ethanolic extract of *Bocopa*

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monniera has been shown to have significant anxiolytic activity, with an advantage over the widely used benzodiazipine anxiolytics, that it promotes cognition unlike the amnesic action of the latter (2). Several anxiolytics drugs are used in treatment of stress-induced ulcers (3). BM contains active chemicals such as bacoside A, which was reported to be responsible for facilitation of memory and bacoside B its optical isomer, which co-occurs with it (4). Bacoside A_3, a triterpine saponin has been reported and its activity is unknown (5). Also bacosoponins A, B, C (6) and D (7) have been isolated and characterised. Peptic ulcers may be produced due to the imbalances in aggressive luminal factors like acid and pepsin and defensive factors like much secretion and cell shedding (8). Now it is well known that offensive acid-pepsin and defensive factors are crucial to the health of gastroduodenal mucosa.

In light of above observations the present study was undertaken to evaluate the juice of whole plant of *Bacopa monniera* on experimental gastric ulceration and gastric secretary changes in rats. Sucralfate a nonabsorbable aluminium salt of sucrose octasulfate, was used as the reference compound. The drug sucralfate is also clinically effective in preventing stress ulceration in critically ill patients (9).

METHODS

Animals: The antiulcerogenic effects of *Bacopa monniera* were studied on inbred Charles-Foster (CF) albino rats (130–180 g), of either sex, obtained from the central animal house of Institute of Medical Sciences, Banaras Hindu University, Varanasi. They were kept in the department animal house at 26 ± 2^0 C and relative humidity 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 (Hindi liver) and the food was withdrawn 18–24 h before the experiment though water was allowed *ad libitum*.

Drug treatment: Whole plant of cultivited variety of *Bacopa monniera* (Ayurvedic Gardens, Banaras Hindu University) were collected in the month of march and was identified with the standard sample preserved in the department of Dravyaguna, Institute of Medical Sciences, Varanasi.

The fresh whole plant of *Bacopa monniera* was size reduced and juice was extracted by a juicer and filtered. One kg of whole plant yielded 650 ml of juice and dry weight in terms of solid content was 2%. The juice was stored in a refrigerator at –20°C and was used with in seven days. In an initial pilot study against cold restraint induced ulcers, juice of *Bacopa monniera* (BMJ) in doses of 50, 100, 300 and 450 mg/kg (in terms of dry weight) and sucralfate (SFT) in the dose of 250 mg/kg were administered orally twice daily at 10 AM and 4 PM respectively for five days in 18 h fasted rats showed ulcer protection from 31.9% to 86.5% ($P < 0.02 - < 0.01$). Control group of animals received water (10 ml/kg). For further studies the effective doses of 100 and 300 mg/kg of BMJ was selected.

Experimental studies

The following methods were used

a) Drug induced gastric ulcers : The gastric ulcers were induced in rats by administering ethanol (EtOH, 100%, 1 ml/ 200 g, 1 h) (10) and aspirin (ASP, 200 mg/ kg, 4 h) (11). EtOH and ASP were administered on day 6 after 18 h fasting and the animals were sacrificed by cervical dislocation and stomach was incised along the greater curvature and examined for ulcers.

b) Cold-restraint stress (CRS)-induced ulcers : 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 4^0–6^0 C (12).

c) Pylorus-ligated (PL)-ulcers : Drugs were administered for a period of 5 days After the last dose, the rats were kept for 18 h fasting and care was taken to avoid coprophagy. On day six, the animals were anaesthetized with pentobarbitone (35 mg/ kg, ip), the abdomen was opened and pylorus ligation was done without causing any damage to any 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 period. After 4 h, stomachs were dissected out and contents were collected into tubes. The stomach was opened along the greater curvature and examined for ulcers (13).

Ulcers were scored by an observer unaware of the experimental protocol, on the basis of number and severity of ulcers (+ to + + + +) per stomach and ulcer index was calculated (14). Statistical analysis was carried by using Wilcoxan Sum Rank test method (15).

Gastric secretion study : 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/100 g body weight. Total acid output was determined by titrating with 0.01 N NaOH, using phenolphthalein as indicator and is expressed as µEq/ml concentration or µEq/ 4h as output. Peptic activity was determined using haemoglobin as substrate and was been expressed as µmol/ml as concentration or µmol/4 h as output (16). Dissolved mucosubstances were estimated in the 90% alcoholic precipitate of the gastric juice. The precipitate, thus obtained was either dissolved in i 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 (17), total hexoses (18), hexosamine (19) and fucose (20), while the latter was used for the estimation of sialic acid (21). The results are expressed in µg/ ml. 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 (22). DNA content were estimated and expressed as µg/ ml gastric juice/100g weight of rat (23).

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

RESULTS

BMJ was found to posses anti ulcerogenic with doses of 100 and 300 mg/

kg against different experimental gastric ulcerations in rats and was comparable to the reference drug SFT. The range of percent protection of BMJ for different models were, EtOH– 68.3% – 99.4% (P<0.2 – < 0.05), ASP – 60.7% – 73.6% (P<0.05 – < 0.01), CRS – 53.6 – 85.5% (P<0.05 – < 0.01) and PL – 78.5 % – 83.1% (P<0.05). The percent protection of SFT ranged from 66.8% to 87.6% (P < 0.01) in various ulcer models (Table I).

In PL-induced gastric secretion, BMJ at 300 mg/kg significantly increased the volume (P<0.05) and showed a tendency to increase acid-pepsin output, while, SFT significantly decreased concentration (P<0.01) and output (P<0.05) of pepsin. The DNA concentration of gastric juice, which is considered to be an important marker of gastric mucosal damage or cell shedding (DNA µg/ml) was significantly decreased with both BMJ (P<0.05 – <0.01) and SFT (P<0.01) treated groups (Table II)

The mucin content of the gastric juice calculated in terms of total carbohydrates : protein ratio (TC : P) was significantly increased both with higher dose of BMJ (P<0.05) and SFT (P<0.05). The individual carbohydrates such as total hexoses, fucose and sialic acid showed a tendency to increase, although the increase was insignificant in both the doses (Table III).

TABLE I : Effect of Bocapa monniera juice (BMJ) 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 ligation (PL)- induced ulcers in rats

Treatment (mg/kg, bd x 3 days)		*Ulcer Index*	*% Protection*
		EtOH-INDUCED ULCERS (mm^2 /rat)	
Control	–	16.1±4.9	–
BMJ	100	5.1±2.4	68.3 %
	300	0.1±0.1[b]	99.4 %
SFT	250	2.0±0.9[a]	87.6 %
		ASP-INDUCED ULCERS	
Control	–	24.2±4.5	–
BMJ	100	9.5±3.6[a]	60.7 %
	300	6.4±3.1[b]	73.6 %
SFT	250	7.5±3.8[b]	69.0 %
		CRS-INDUCES ULCERS	
Control	–	30.4±5.3	–
BMJ	100	14.1±3.0a	53.6 %
	300	4.4±2.1b	85.5 %
SFT	250	10.1±4.7a	66.8 %
		PL-INDUCED ULCERS	
Control	–	13.0±3.9	–
BMJ	100	2.8±2.0[a]	78.5 %
	300	2.2±1.6[a]	83.1 %
SFT	250	2.5±1.3[a]	80.1 %

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

TABLE II : Effect of BMJ on gastric juice volume, acid, pepsin and DNA contents in 4 h PL rats.

Treatment		*Volume*	*Acid*		*Pepsin*		*DNA*
(mg/kg, bd x 5 days)		*(ml/100 g)*	*Concentration* (µEq/ml)	*Output* (µEq/4h)	*Concentration* (µmol/ml)	*Output* (µmolq/4h)	(µg/ml/100 g)
Control		1.48±0.25	85.0±25.6	125.8± 19.3	527.3±40.8	780.4±89.1	118.2±9.0
BMJ	100	1.93±0.16	65.0±5.0	125.5± 10.3	531.5±31.5	1025.8±201.3	81.8±9.0[a]
	300	2.40±0.30[a]	60.0±5.0	144.0± 10.8	491.5±45.9	1179.6±243.2	63.6±9.1[b]
SFT	250	1.42±0.17	80.0±4.1	113.6± 12.9	304.8±42.7[b]	432.8±74.2[a]	72.1±8.8[b]

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

TABLE III : Effect of BMJ on gastric juice mucoprotein and mucosal glycoprotein in 4 h PL rats.

Treatment *(mg/kg bd×5 days)*		*Protein (P)*	*Total hexoses (A)*	*Hexosamine (B)*	*Fucose (C)*	*Sialic acid (D)*	*TC (A+B+C+D)*	*TC : P*
				Mucoprotein (µg/ml)				
Control		525.0±42.0	392.0±39.8	169.5±25.4	66.8±4.3	58.5±5.3	686.8±41.0	1.31±0.20
BMJ	100	468.8±56.3	398.2±39.7	286.0±33.2[a]	72.8±4.7	72.5±5.5	829.0±53.5	1.77±0.13
	300	403.8±48.9	404.1±29.5	302.0±32.6[b]	83.0±8.1	61.8±4.9	850.9±56.1[a]	2.11±0.27[a]
SFT	250	375.0±56.6	457.7±36.0	186.2±25.0	69.7±6.7	63.0±4.1	776.6±36.0	2.07±0.21[a]
				Glycoprotein (µg/100 mg wet tissue)				
Control		4236±404	2522±246	1261±123	135±13	161±15	4079±307	0.96±0.08
BMJ	100	4046±483	2641±229	1324±114	151±14	169±10	4285±389	1.06±0.10
	300	4169±462	3243±261	1621±131	166±10	234±19[a]	5263±403[a]	1.26±0.12
SFT	250	4552±284	3302±142[a]	1290±103	154±15	298±35[b]	5044±298[a]	1.11±0.09

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

A significant increase in total carbohydrates was observed with 300 mg/kg of BMJ. In gastric mucosal glycoproteins, although most of the carbohydrates showed a tendency to increase they were insignificant except for significant increases in sialic acid and total carbohydrates (P<0.05) with 300 mg/kg treatment of BMJ. SFT significantly increased the individual carbohydrates such as total hexoses (P<0.05) and sialic acid (P<0.05) and total coabohydrates (<0.05) (Table III)

DISCUSSION

The present study clearly indicates the ability of BMJ to significantly protect the formation of gastric ulcers in various experimental models in rats. Significant protection was also observed with SFT. SFT is reported to be clinically effective in healing of gastric ulcers and preventing the recurrence of peptic ulcers (24).

On gastric secretion, significant changes in acid and pepsin were not observed with

BMJ in contrast to SFT which had significant anti peptic activity as reported earlier (24). BMJ, however increased the volume of acid secretion significantly, but the increase in acid pepsin output was not significant. However, BMJ showed significant protection in all the experimental models, which indicated that the protective effect of BMJ could be due to its predominant effect on mucosal defensive factors rather than on the offensive acid-pepsin secretion. Thus is further evidenced by decrease in DNA content of gastric juice (one of the important marker to quantify gastric mucosal damage or cell shedding), which is augmented by ulcerogenic agents and reduced by ulcer protective agents (23). Hence, decrease in DNA content of gastric juice showed that BMJ significantly protected the integrity of the gastric mucosa by decreasing cell shedding. These beneficial changes may be due to augmentation of defensive factors as BMJ produced qualitative (individual carbohydrates) and quantitative (total carbohydrates : protein) changes in dissolved mucin and glycoprotein contents. Biochemical constituents of the gastric secretion have been considered to play an important role in the physiopathological state of the gastric mucosa (8). BMJ and SFT increased the total carbohydrates (sum of total hexoses, hexosamine, fucose and sialic acid) to protein ratio (TC:P), which reflects the functional integrity of the gastric mucosal barrier and serves as a reliable index of mucosal resistance. BMJ at the higher dose significantly increased sialic acid and total carbohydrates in the gastric mucosa. SFT increased sialic acid, total hexoses and total carbohydrates there by increasing the contents of glycoproteins and mucosal resistance.

Thus, the present study exemplifies the use of BMJ as a potential antiulcer drug, and the protection afforded was mostly due to the augmentation of mucosal defensive factors.

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