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Prophylactic and curative effects of *Bacopa monniera* in gastric ulcer models

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Summary

***Bacopa monniera* Wettst. (BM, syn. Herpestis monniera L; Scrophulariaceae), is an Ayurvedic drug used as a rasayana. Its fresh juice was earlier reported to have significant antiulcerogenic activity. In continuation, methanolic extract of BM (BME) standardized to bacoside-A content (percentage-38.0 ± 0.9), when given in the dose of 10–50 mg/kg, twice daily for 5 days, showed dose-dependent anti-ulcerogenic on various gastric ulcer models induced by ethanol, aspirin, 2 h cold restraint stress and 4 h pylorus ligation. BME in the dose of 20 mg/kg, given for 10 days, twice daily showed healing effects against 50% acetic acid-induced gastric ulcers. Further work was done to investigate the possible mechanisms of its action by studying its effect on various mucosal offensive acid-pepsin secretion and defensive factors like mucin secretion, mucosal cell shedding, cell proliferation and antioxidant activity in rats. BME 20 mg/kg showed no effect on acid-pepsin secretion, increased mucin secretion, while it decreased cell shedding with no effect on cell proliferation. BME showed significant antioxidant effect *per se* and in stressed animals. Thus, the gastric prophylactic and curative effects of BME may be due to its predominant effect on mucosal defensive factors.**

Key words: *Bacopa monniera*, bacosides, anti-ulcer models, gastric mucosal offensive and defensive factors

Introduction

Bacopa monniera Wettst. (syn. Herpestis monniera L; Scrophulariaceae), commonly known as 'Brahmi' is an annual creeping plant found throughout the Indian subcontinent in wet, damp and marshy areas. BM is classified in Ayurveda, an ancient system of Indian medicine, as a rasayana, which is believed to arrest aging and induce rejuvenation. Further it is sub classed as a Medhya rasayana, a class of plant drugs used to promote mental health and improve memory and intellect (Udupa and Singh, 1995). In accordance, BM has been reported to posses memory enhancing effects (Dhavan and Singh, 1996). BM has also been reported to posses anxiolytic (Bhattacharya and Ghosal, 1998) and antioxidant effects (Bhattacharya et al., 2000; Tripathi et al., 1996). Both of these activities can have beneficial effects in treatment of gastric ulceration as psychological factors (Miller, 1987) and free radicals (Itoh and Guth, 1985) have been implicated in genesis of gastric ulcers. BM contains many glycosides such as bacoside A and its optical isomer, bacoside B (Garai et al., 1996a, b). Bacoside A has been reported to be responsible for facilitation of memory (Rastogi et al., 1994) and an extract standardized to bacoside A, has been reported to have anxiolytic activity (Bhattacharya and Ghosal, 1998). In view of these reports, the methanolic extract of fresh plants of BM was standardized to bacoside A content and studied for ulcer protective and healing effects. We had earlier reported the anti-ulcerogenic effect of fresh juice of BM and the activity was mostly due to the augmentation of mucosal

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defensive factors rather than on the offensive factors (Rao et al., 2000). It is well documented that ulcers are caused due to imbalances between offensive and defensive factors (Goel and Bhattacharya, 1991).

Thus, the present study pertains to evaluation of prophylactic and curative effects of standardized extract of BME in various gastric ulcer models and its effect on offensive acid-pepsin secretion, defensive factors like mucin secretion and cell shedding in gastric juice and, cell proliferation and anti-oxidant effects in the gastric mucosa. Sucralfate, a non-absorbable aluminum salt of sucrose octasulfate, was used as the reference compound (McGraw and Caldwell, 1981).

Materials and Methods

Animals

The antiulcerogenic effect of *Bacopa monniera* was 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 departmental animal house at 26 ± 2 °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 (Hind liver) and the food was withdrawn 18–24 h before the experiment though water was allowed *ad libitum*. 'Principles of laboratory animal care' (NIH publication no. 82–23, revised 1985) guidelines were followed.

Drug treatment

Whole plant of cultivated variety of *Bacopa monniera* (Ayurvedic Gardens, Banaras Hindu University) was 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 plants of *Bacopa monniera* were size reduced and were macerated with methanol for 7 days. The extract was filtered, vacuum dried and stored in a refrigerator until further use. The yield was 1.2%. The methanolic extract was subjected to HPTLC (CAMAG TLC system;evaluation software; CATS 3.16/ Scanner II v 3.14) as described earlier (Chatterjee et al., 1965; Bhattacharya and Ghosal, 1998) for estimation of bacoside A. The solvent systems used were n-butanol-acetic acid-water (4:1:1) and ethyl acetate-acetic acid-formic acid-water (100:11:11:27); mode of detection, quenching at λ 260 nm; staining reagent-.2,4-dinitrophenyl-hydrazine; reflectance spectra: λ max 278 nm. The percentage of bacoside A was 38.0 ± 0.9. The doses were fixed based on our earlier studies on the fresh juice of BM (Rao et al., 2000). BME (suspended in 1% carboxymethyl cellulose in distilled water) in doses of 10, 20 and 50 mg/kg 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 for ulcer protective studies. Further the effective dose of 20 mg/kg for 5 days was used for secretion and mucosal studies, and upto 10 days for ulcer healing study. Control group of animals received suspension of 1% carboxymethyl cellulose in distilled water.

Experimental methods

Anti-ulcer study

The following experimental models were used.

• *Ethanol (EtOH)-induced ulcers:* The gastric ulcers were induced in rats by administering EtOH (1 ml/ 200 g, 1 h) (Hollander et al., 1985) 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 (mm^2/ rat). Statistical analysis of data was done by using unpaired Student's *t* test.

• *Aspirin (ASP)-induced ulcers:* ASP in dose of 200 mg/kg (20 mg/ml) was administered to the animals and ulcers were scored after 4 h (Goel et al., 1985). 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. Ulcer index has been calculated by adding the total number of ulcers per stomach and the total severity of ulcers 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. Severity of the ulcers was scored after histological confirmation (Sanyal et al., 1982). Statistical analysis was done by using Wilcoxon Sum Rank test (Padmanabha Pillai et al., 1982)

• *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 4–6 °C. The animals were then sacrificed by cervical dislocation and ulcers were scored on the dissected stomachs (Gupta et al., 1985) as described above.

• *Pylorus-ligated (PL)-induced ulcers:* Drugs were administered for a period of 5 days as described above. On day six after the last dose, the rats were kept for 18 h fasting and care was taken to avoid coprophagy. Animals 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 period (Sanyal et al., 1971). After 4 h, stomachs were dissected out and contents were collected into tubes for estimation of biochemical parameters. The ulcers were scored as described under ASP-induced ulcers.

• *Acetic acid-induced ulcers:* The rats were anaesthetized with pentobarbitone (35 mg/kg, i.p.). 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. 50% acetic acid (0.06 ml/animal) was instilled into the tube and allowed to remain 60 seconds on the gastric wall. After removal of the acid solution, the abdomen was closed in two layers and animals were caged and fed normally. BME was given in the dose of 20 mg/kg 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 hr of the last dose of drug either on 6^{th} day or 11^{th} day of experiment to assess the ulcer size and healing. Ulcer index was calculated based upon the product of length and width (mm^2/rat) of ulcers (Okabe et al., 1971). Statistical significance was calculated using unpaired Student's *t* test.

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/100g body weight. Total acid output was determined by titrating with 0.01 N NaOH, using phenolphthalein as indicator and is expressed as µEq/ml as concentration or µEq/4 h as output. Peptic activity was determined using haemoglobin as substrate and was been expressed as µmol of tyrosine/ml as concentration or µmol of tyrosine/4 h as output (Debnath et al., 1974). 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_2SO_4. The former was used for the estimation of protein (Lowry et al., 1951), total hexoses, hexosamine and fucose, while the latter was used for the estimation of sialic acid (Sanyal et al., 1983). 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 (Sanyal et al., 1983). DNA content was estimated and expressed as µg/ml gastric juice/100 g weight of rats (Mukhopadhyay et al., 1987).

Gastric mucosal study

Estimation of cell proliferation

• *Estimation of DNA in gastric mucosa*: Mucosal scraping was homogenized in 2.5 ml of ice cooled 0.6 N perchloric acid (PCA) (Schneider, 1957). DNA (Goel et al., 1986) and protein (Lowry et al., 1951), were then estimated. The concentration of DNA is expressed as µg DNA/ mg protein.

• *Measurement of glandular weights of stomach*: The weights of the whole stomach (rumen and glandular portion) and rumen were taken and the weight of the glandular portion was then calculated. The weights of the glandular portions are expressed in mg/100 g body weight of animals. Statistical analysis was done by Student's *t* test

Estimation of free radical generation

BME in the dose of 20 mg/kg was given orally, daily for 5 days and on day 6 of experiment, 1 h prior to subjecting the animals to CRS. The animals were then sacrificed and the ulcer index was calculated as described earlier. The fundic part of the stomach was homogenized (5%) in ice cold 0.9% saline with a Potter-Elvehjem glass homogeniser 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 estimations (Das and Banerjee, 1993).

• *Measurement of lipid peroxidation* (LPO): LPO product malondialdehyde (MDA) was estimated using 1,1,3,3- tetraethoxypropane as the standard and is expressed as nmoles/mg protein (Okhawa, 1979)

• *Enzymatic antioxidants:* The fundic stomach was homogenized (5%) in 0.25 M sucrose and 50 mM phosphate buffer (pH 7.2) and mitochondrial fraction was prepared as described above (Das and Banerjee, 1993).

• *Superoxide dismutase (SOD)*: SOD was estimated by following the procedure of (Kakkar et al., 1984). 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/mg protein.

• *Catalase* (CAT): Decomposition of H_2O_2 in presence of catalase was followed at 240 nm (Aebi, 1974). One unit of (U) CAT was defined as the amount of enzyme required to decompose 1 µmol of H_2O_2 per min, at 25 °C and pH 7.0. Results are expressed as units (U) of CAT activity/mg protein.

Statistical analysis was done by Student's *t* test.

Table 1. Effect of graded doses of methanolic extract of *Bacopa monniera* (BME) 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 (data are mean ± SEM, n = 8 in each group).

Treatment (mg/kg, bd × 5 days)	EtOH (mm^2/rat)	ASP	CRS	PL
Control	15.5 ± 3.9	21.4 ± 4.5	23.9 ± 3.8	12.5 ± 3.0
BME 10	8.7 ± 2.9 (43.9)	13.1 ± 2.9 (38.8)	17.3 ± 1.3 (27.6)	6.9 ± 2.3 (44.0)
20	5.3 ± 2.7[a] (65.8)	8.5 ± 3.1[a] (60.3)	12.1 ± 2.5[a] (50.6)	4.3 ± 2.2[a] (65.6)
50	2.0 ± 0.8[b] (87.1)	4.5 ± 2.8[b] (79.0)	5.4 ± 2.6[b] (77.4)	2.0 ± 1.9[a] (84.0)
SFT 250	2.1 ± 0.9[b] (86.6)	6.5 ± 3.7[a] (69.2)	7.9 ± 3.7[b] (66.7)	2.6 ± 1.2[b] (79.2)

numbers in parathesis indicacte percentage protection.
[a] $P < 0.05$; [b] $P < 0.01$ compared to control.

Table 2. Effect of BME on 50 % acetic acid induced healing model of gastric ulcer (data are mean ± SEM, n = 8 in each group).

Treatment (mg/kg, po,bd)	Acetic acid induced ulcers (healing) 5 days		10 days	
	Ulcer index	% incidence of perforations	Ulcer index	% incidence of perforations
Control	20.4 ± 1.8	75.0%	10.4 ± 2.7	37.5%
BME 20	16.0 ± 2.2	37.5%	0.5 ± 0.3[c]	0.0%

[c] $P < 0.001$ compared to control.

Results

Ulcer protective and healing effects

BME, 10–50 mg/kg, twice daily given for 5 days showed dose-dependant ulcer protective effect, though significant effect was seen with of 20 and 50 mg/kg, against various acute gastric ulcers induced by ethanol, aspirin, cold restraint stress and pyloric ligation. The effect was comparable to the ulcer protective drug sucralfate. The percentage protection ranged from 27.6% to 87.1% in all the models (Table 1). In chronic ulcers induced by 50% acetic acid, BME in the dose of 20 mg/kg reduced ulcer index after five days ($P < 0.2$), but significant ulcer healing ($P < 0.001$) with decreased perforations was observed after 10 days treatment (Table 2).

Effect on gastric juice parameters

BME in the dose of 20 mg/kg showed a tendency to decrease the volume, concentration and output of acid, and pepsin but it however, significantly decreased the DNA content ($P < 0.05$) (Table 3). BME showed a tendency to increase the individual mucopolysaccharides namely total hexoses, hexosamine, fucose and sialic acid. Although these changes were statistically insignificant, the changes were significant enough to increase the TC:P ratio ($P < 0.05$) (Table 4).

Effects on gastric mucosal parameters

BME did not significantly alter the DNA content of the gastric mucosa calculated in terms of µg DNA/mg of protein/100 mg of wet tissue compared to the control groups. Similarly significant changes were also not observed on the weight of glandular portion of the stomach (Table 5).

The anti-oxidant effect of BME was evaluated by studying its effect on lipid peroxidation and anti-oxidant enzymes SOD and CAT both *per se* and in CRS model. BME in the dose of 10 and 20 mg/kg *per se* significantly decreased LPO ($P < 0.01$) and in the dose of 20 mg/kg increased SOD ($P < 0.05$) and CAT ($P < 0.01$) levels (Table 6). Stress significantly increased the ulcer index with concomitant increase in LPO and SOD levels and decrease in CAT level, compared to the control group. BME in the dose of 20 mg/kg significantly reversed these changes as observed from the reduction in ulcer index, LPO and SOD, and increase in CAT levels in comparison to the stress group (Table 7).

Table 3. Effect of BME (mg/kg, bd × 5 days) on gastric secretion in 4 h PL rats : effect on volume, acid, pepsin and DNA contents (data are mean ± SEM, n = 8 in each group).

Treatment	Volume (ml/100g)	Acid		Peptic		DNA (µg/ml/100g)
		Concentration (µEq/ml)	Output (µEq/4 h)	Concentration (µmol/ml)	Output (µmol/4 h)	
Control	2.47 ± 0.17	98.1 ± 15.5	248.4 ± 38.9	295.3 ± 31.5	729.4 ± 89.3	254. ± 32.4
BME 20	2.04 ± 0.28	90.6 ± 11.9	184.0 ± 30.7	286.1 ± 33.6	583.6 ± 79.3	130.8 ± 30.8[a]
SFT 250	2.11 ± 0.19	86.0 ± 8.1	181.5 ± 29.3	204.8 ± 29.6	453.2 ± 69.7[a]	149.3 ± 25.6[a]

[a] $P < 0.05$, compared to control.

Table 4. Effect of BME (mg/kg, bd × 5 days) on gastric juice mucoproteins ((g/ml) in 4 h PL- rats (data are mean ± SEM, n = 8 in each group).

Treatment	Total hexoses (A)	Hexosamine (B)	Fucose (C)	Sialic acid (D)	TC (A+B+C+D)	Protein (P)	TC : P
Control	260.8 ± 39.3	170.6 ± 17.8	62.3 ± 4.4	26.2 ± 3.8	519.9 ± 53.6	534.4 ± 51.5	1.05 ± 0.11
BME 20	343.3 ± 29.5	184.7 ± 14.2	70.3 ± 5.9	35.2 ± 2.3	633.5 ± 49.3	450.1 ± 41.3	1.48 ± 0.15[a]
SFT 250	304.3 ± 24.1	180.9 ± 17.1	69.8 ± 6.8	36.0 ± 2.3[a]	591.0 ± 36.3	391.3 ± 46.7	1.53 ± 0.16[a]

[a] $P < 0.05$; [b] $P < 0.01$ compared to control.

Table 5. Effect of BME (mg/kg, bd × 5 days) on cell proliferation and weight of glandular portion of stomach in pylorus-ligated rats (data are mean ± SEM, n = 8 in each group).

Treatment	Wt of Glandular portion of stomach	Cell proliferation		
	(mg/100 g body weight)	Protein (µg/100 mg wet tissue)	DNA	µg DNA/mg protein
Control	442.7 ± 43.9	5746 ± 265	653 ± 21	113.6 ± 16.1
BME 20	521.6 ± 27.2	5421 ± 331	587 ± 42	108.2 ± 6.2

Table 6. *per se* effect of BME (mg/kg, bd × 5 days) on LPO (MDA, nmoles/mg of protein), SOD and CAT (Units/mg of protein) activities in rat gastric mucosa (data are mean ± SEM, n = 8 in each group).

Treatment	LPO	SOD	CAT
Control	0.40 ± 0.02	111.1 ± 9.1	24.1 ± 1.0
BME 10	0.28 ± 0.02[b]	126.3 ± 3.9	26.6 ± 0.6
BME 20	0.21 ± 0.01[c]	143.0 ± 3.4[a]	33.2 ± 1.3[c]

[a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$ when compared to control.

Table 7. Effect of BME (mg/kg, bd × 5 days) on LPO (MDA, moles/mg of protein), SODand CAT (Units/mg of protein) activities in rat gastric mucosa against CRS (data are mean ± SEM, n = 8 in each group).

Oral treatment	Ulcer Index	LPO	SOD	CAT
Control	0.0 ± 0.0	0.40 ± 0.02	101.2 ± 10.7	31.5 ± 2.0
Stress	35.8 ± 2.5[***]	0.58 ± 0.03[***]	247.6 ± 6.4[***]	19.2 ± 1.2[***]
Stress+ BME 20	4.0 ± 1.2[c]	0.25 ± 0.02[c]	199.0 ± 5.4[c]	27.4 ± 2.9[a]

[***] $P < 0.001$ when compared to control group and [a] $P < 0.05$; [c] $P < 0.001$ when compared to stress group.

Discussion

BME showed significant dose dependent anti-ulcerogenic in different models of acute gastric ulcers induced by ethanol, aspirin, cold restraint stress and pyloric ligation and, chronic gastric ulcers induced by acetic acid. Ulcerations by ethanol are caused due to perturbations of superficial mucosal cells, notably the mucosal mast cell leading to release of vasoactive mediators including histamine, thus causing damage to gastric mucosa (Miller and Henagan, 1984). Synthetic NSAIDs like aspirin cause mucosal damage by interfering with PG synthesis, increasing acid secretion and back diffusion of H^+ ions and thus leading to breaking up of mucosal barrier (Vane, 1971). Stress plays an important role in aetiopathology of gastro-duodenal ulceration. Apart from psychological factors, stress induced ulcers are also caused by a number of other factors like increase in gastric motility, vagal over activity (Cho et al., 1976), mast cell degranulation (Cho and Ogle, 1979), decreased gastric mucosal blood flow (Hase and Moss, 1973) and decreased PG synthesis (Miller, 1987). Pyloric ligation-induced ulcers are thought to be due to autodigestion of mucosa by gastric juice leading to breakdown of mucosal barrier (Goel and Bhattacharya, 1991). Gastric ulcer is often a chronic disease and it may persist for 10 to 20 years characterized by repeated episodes of healing and re-exacerbations. Acetic acid-induced ulcer better resembles clinical ulcers in location, chronicity and severity and servers as the most reliable model to study healing process (Okabe and Pfeiffer, 1972). BME in the dose of 20 mg/kg significantly healed penetrating ulcers induced by acetic acid after 10 days, although significant changes were not observed after 5 days treatment. Although specific mechanisms remain controversial, increase gastric secretion due to increase in volume of acid output and subsequent pyloric obstruction may be the cause for ulceration due to acetic acid. These observations were similar to those observed with fresh juice of BM (Rao et al., 2000). As aetiopathogenesis of these ulcers models are different, mechanism of BME should then include number of predisposing factors. Although the mechanism of ulcerogeneis may be different, the net result of these factors is disturbance of balance between offensive factors and defensive pre-epithelial, epithelial and sub- epithelial factors. To ascertain the possible role of BME on the above factors, studies on offensive acid-pepsin and defensive mucin secretion and cell shedding were conducted.

BME in the dose of 20 mg/kg reduced acid and pepsin output, but the decreases were mild and insignificant. BME in the above dose significantly increased dissolved mucus as seen from the increase in TC:P ratio , which is taken as reliable marker for mucin secretion (Goel et al., 1985). Surface mucus cells and mucus neck cells secrete mucus by exocytosis. The main components of gastric mucous are the acidic glycoprotein sialic acid and neutral mucopolysaccarides like total hexoses, hexosamine and fucose. Mucus is endowed with a array of mucosal protective properties and also acts as a first line of defense (Zalewsky and Moody, 1979). Thus significant increase in defensive factors may account for a major part in the activity of BME. This finding was consistent with our earlier reports on juice of BM. 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 shedding (Mukhopadhyay et al., 1987). BME showed no significant effect on cell proliferation as evidenced by little or no changes in DNA content of the gastric mucosa (Goel et al., 1986). This is further supported by the observation on mucosal glandular weight, which was not significantly altered in comparison with the control. Thus, the prophylactic and healing effect of BME may not be dependent on cell proliferation, which is however reported to be increased during mucosal damage (Wright, 1984). It is possible that the activity is due to its predominant effect on cell shedding, which was decreased. Decreased cell shedding is an indicator of increase in life span of cells. Further, increase in mucus provides a suitable environment for the restitution, a process of repair involving migration of viable surface mucosal cells to cover the damaged mucosa (Svanes et al., 1983).

The pathogenesis of lesions during stress is still unclear. The role of acid secretion is questionable and diminution of gastric mucus formation is also reported (Guth et al., 1971). Hence the pathogenesis of lesions may be due to alteration in one or more factors apart from the offensive and defensive factors. The role of free radicals in gastric ulcerations is well documented (Cochran et al., 1983). BME significantly reduced lipid peroxidation in rat gastric mucosa. BM has been reported to possess significant antioxidant properties in rat brain regions (Bhattacharya et al., 2000). This may be due to increase in free radical scavenging enzymes SOD and CAT in the gastric mucosa. SOD scavenges the super oxide radical O_2^-, one of the reactive oxygen species (ROS) responsible for lipid peroxidation (Fridovich, 1986). This reaction leads to increase in generation of peroxyl radical $H_2O_2^-$, which is also capable of producing more oxidative damage (Das et al., 1997). CAT and other peroxidases further reduce $H_2O_2^-$. Hence, the antioxidant activity in gastric mucosal homogenates observed from decrease in LPO may be due to increase in SOD and CAT levels. Stress induced ulceration involves damage by ROS apart from acid and pepsin related factors (Miller, 1987). During stress LPO

and SOD were significantly increased and CAT level was significantly decreased. The increase in SOD was due to increased 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 peroximes during stress (Boyd et al., 1981) 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. BME effectively alleviated stress-induced ulcers with marked decrease in LPO, suggesting decrease in oxidative damage. This was mostly due to maintenance of balance between SOD and CAT levels, effectively counteracting the free radicals generated by cascade of reactions as described earlier. Thus the antiulcerogenic activity of BME may also be due its anti-oxidant effects.

Thus, the present study does indicate the anti-ulcerogenic activity of BME. The gastric prophylactic and curative effects of BME may be predominantly due to its activity on defensive mucosal factors with no discernible effect on cell proliferation. The inherent antioxidant activity of BME may be one of the important factors contributing towards its activity. Further studies on other factors like *H. pylori*, PGs, nitric oxide and cAMP, which play important role in ulcerogenisis may provide more insights on the antiulcerogenic activity of BME.

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