Role of gastric antioxidant and anti-*Helicobacter pylori* activities in antiulcerogenic activity of plantain banana (*Musa sapientum var. paradisiaca*)

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Studies with plantain banana (*Musa sapientum var. paradisiaca*) have indicated its ulcer protective and healing activities through its predominant effect on various mucosal defensive factors [Sanyal et al., Arch Int Pharmacodyn., 149 (1964) 393; 155 (1965) 244]. Oxidative stress and *Helicobacter pylori* colonization are considered to be important factors in the pathogenesis of gastric ulcers. In the present study methanolic extract of plantain banana pulp (BE) was evaluated for its (i) antulcer and antioxidant activities in 2 hr cold restraint stress and (ii) anti-*H. pylori* activity in *vitro*. The extract (BE, 50 mg/kg, twice daily for 5 days) showed significant antulcer effect and antioxidant activity in gastric mucosal homogenates, where it reversed the increase in ulcer index, lipid peroxidation and super oxide dismutase values induced by stress. However it did not produce any change in catalase values, which was significantly decreased by stress. Further, in the *in vitro* study, BE (0.32 – 1000 µg/ml) did not show any anti-*H. pylori* activity. The results suggest absence of anti-*H. pylori* activity of methanolic extract of banana in *vitro* and its antioxidant activity may be involved in its ulcerprotective activity.

Banana, a commonly used folklore medicine has been extensively investigated for its antulcer effects. The dried powder of plantain banana pulp is reported to have anti ulcerogenic activity against various models of gastric ulcer induced by phenylbutazone, prednisolone and restraint stress in rats, and histamine induced gastric and duodenal ulcers in guinea pigs. The maximum activity was found in unripe, green plantain banana obtained from the *Musa sapientum* Linn. var. *paradisiaca* collected between September to March. Activity was found only in fruits collected from the northern gangetic belt and those from the southern belt of the Indian subcontinent were comparatively less effective.

Ulcers are caused due to imbalances between mucosal offensive and defensive factors of the gastric mucosa. The antulcerogenic activity is reported due to increase in mucosal defensive factors rather than decrease in the offensive factors. Banana strengthened the mucosal defenses by increasing mucus secretion, mucosal glycoprotein content and cell proliferation. Ethanolic extract of banana was also reported to increase accumulation of eicosanoids by both human gastric and colonic mucosal incubates. Oxidative stress is considered to be one of the important etiological factors in various diseases including gastric ulcers. *Helicobacter pylori* after being first isolated in human biopsies in 1983, is now considered to be the major cause of peptic ulcers, duodenal ulcers and gastritis. *H. pylori* infection is also reported to be one of the important cause for relapse of ulcers. Long-term infection may also lead to B-cell lymphoma and adenocarcinoma of stomach. Multi drug therapy including an anti *H. pylori* drug is often prescribed along with standard antulcer agents.

The present study evaluates the anti-oxidant effect of methanolic extract of dried pulp powder of plantain banana (BE) in cold restraint stress induced ulcers in rats and in *vitro* evaluation of anti *H. pylori* activity.

Animals—Inbred Charles-Foster (CF) albino rats (120-160 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 44-56% RH, 10: 14 hr L: D cycle for one week for acclimatization. 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*.

Drug treatment—Unripe, green plantain banana fruits (*Musa sapientum* Linn. var. *paradisiaca*) were purchased locally during January and the pulp was...
dried in shade. The dried pulp (DRBP) was then powdered and methanolic extract was prepared by adding 1 liter of methanol twice, at an interval of two days. The methanol containing extract so obtained each time was mixed and later dried in vacuum drier. The percentage yield of the extract was 2%. It was stored in deep freezer at -20°C until further use. BE was given in the doses of 10 and 50 mg/kg po, twice daily for 5 days before induction of ulcers, the last dose was given on day 5, 18 hr before subjecting the animals to stress. Control group of animals received distilled water (10 ml/kg).

**Experimental studies**

**Anti-ulcer study**

2 hr cold restraint stress (CRS) -induced ulcers— Stress was induced in 18 hr fasted rats on day 6 of experiment by stretching and strapping them to a wooden plank with adhesive tape after stretching each limb to the plank individually. The animals were then kept for 2 hr at 4-6°C and were sacrificed by decapitation. 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 earlier. Briefly, the 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 (+ - +++) after histological confirmation. Statistical analysis was done by using Wilcoxon Sum Rank test.  

Estimation of free radical generation—The fundic mucosal part of the stomach was 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 following centrifugation of the supernatant at 12,000 g for 15 min and the obtained mitochondrial fraction was used for the following estimations.  

Measurement of lipid peroxidation—Lipid peroxidation (LPO) product malondialdehyde (MDA) was estimated according to the method of Ohkawa et al. Briefly 1 ml sample was mixed with 0.2 ml 4% (w/v) sodium dodecyl sulfate, 1.5 ml 20% acetic acid in 0.27 M hydrochloric acid (pH 3.5) and 1.5 ml of 0.8% thiobarbituric acid (TBA, pH 7.4). The mixture was heated in a hot water bath at 85°C for 1 hr. The intensity of the pink colour developed was read against a reagent blank at 532 nm following centrifugation at 1200 g for 10 min. Tetraethoxypropane was used as a standard.

**Estimation of preventive 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.  

Superoxide dismutase (SOD)—SOD was estimated as per Kakkar et al. Assay mixture contained sodium pyrophosphate buffer (0.052 M, pH 8.3), phenazine methasulfate (PMS, 6.2 M), nitroblue tetrazolium (NBT, 30 M), potassium cyanide (KCN, 10 μm, pH 7) and 0.2 ml of sample fraction. Samples were preincubated for 5 min at 36°C prior to the addition of reduced nicotinamide adenine dinucleotide (NADH, 52 μm). Mixure was further incubated for 120 sec at 37°C in a water bath and the reaction was stopped by adding 1 ml glacial acetic acid (17.4 M). The violet color developed was extracted in 4.0 ml of n-butanol reagent blank. The activity was measured at 560 nm and the results have been expressed as units (U) of SOD activity/mg protein. One unit of enzyme activity was defined as the enzyme concentration required to inhibit the chromogen production by 50% in 1 min under the defined assay conditions.

Catalase (CAT)—Decomposition of H2O2 in presence of catalase was followed at 240 nm (Aebi). A 50 μm sample was added to buffered substrate (50 mM phosphate buffer, pH 7 containing 10 mM H2O2) to make total volume 3 ml and decrease in the absorbance was monitored at 37°C for 2.5 min at an interval of 15 sec. The activity was calculated using extinction coefficient of H2O2, 0.041/μmole/cm2 at 240 nm. Results are expressed as units (U) of CAT activity/mg protein. Statistical analysis was done by Student’s t test.

**Evaluation of H. pylori activity**

The strain of *Helicobacter pylori* NCTC 12822 (National Collection of Type Cultures, Public Health Laboratory Service, 61 Colindale Avenue, London) was cultured on brain heart infusion agar supplemented with 5% horse blood and incubated at 37°C under microaerophillic conditions (5% O2, 10% CO2, 80% N2).  

Preparation of test solutions—BE 50 mg was dissolved in 5 ml (10 mg/ml) distilled water and was sterilized by passing through Minisart N filter. Bismuth subcitrate (BSC, Brocades House, Surrey) was prepared in the dilution of 10 mg/ml and was
sterilized by autoclaving (MIC 5μg/ml). Using sterile water, dilutions of 0.32, 1.6, 8, 40, 200, 1000 μg/ml of BE and BSC were prepared and used for the experiments.

Detection of inhibitory activity—The experiment was done using a 96 well plate technique. To each test well 30 μl of BE or BSC, 250 μl growth medium and 20 μl H. pylori suspension were added in order. H. pylori was reconstituted aseptically introducing a loopful of the organism in 100 ml sterile Ringer’s solution, while 100% growth controls contained 30 μl solvent, 250 μl growth medium and 20 μl H. pylori suspension, 0% control contained 30 μl solvent, 250 μl growth medium and 20 μl Ringer solution. The inner wells were alone used for the experiment with 12 replicates for each group. The plates were then incubated in microaerophilic atmosphere at 37°C for 72 hr, and examined for growth on surface of each well.

Cold restraint stress (CRS) produced significant increase in ulcer index with increase in lipid peroxidation as observed from increase in MDA levels with a concomitant increase in SOD and decrease in CAT levels (Table 1). Pretreatment with BE (50 mg/kg) showed significant decrease in ulcer index, LPO and SOD levels thus, reversing the changes induced by stress except in case of CAT, which was however not affected (Table 1).

Evaluation of BE for its in vitro action on H. pylori growth showed that there was no significant inhibitory activity with all the concentrations used (0.32 to 1000 μg/ml), while bismuth subcitrate (1000 μg/ml) showed significant anti-H. pylori activity (Table 2).

BE showed significant antiulcerogenic effect in CRS-induced gastric ulcers in rats. This is in consonant with our earlier study on restraint stress-induced ulcers. The process of ulcerogenesis by stress may involve apart from the damaging effect of acid and pepsin, the damage by reactive oxygen species (ROS), primarily caused by the alteration of antioxidant enzymes. This increase in ROS leads to decreased activity of prostaglandin synthetase and loss of gastric mucosal cytoprotection. Banana is considered to be a cytoprotective drug as it augments mucosal defenses with little or no effect on acid secretion and as in cold restraint stress there is loss of cytoprotection, it is therefore a suitable model for evaluation of oxidative status of gastric ulcers and its modification by BE. In stress induced gastric damage, LPO and SOD levels were significantly increased with concomitant decrease in CAT concentration as reported earlier. Increase in LPO levels indicates increase of reactive oxygen species (ROS), the major radicals being superoxide anion (O2·−), H2O2 and hydroxyl radical (OH·). These induce cell degeneration by increasing peroxidation of cell membrane lipids causing loss of structural and functional integrity of cell membranes. Increase in SOD level is in response to increased tissue O2·− speeding up their dismutation and converting it immediately into H2O2 (Ref. 21). Accumulation of H2O2 occurs in the mitochondria and cytosol, if not scavenged by CAT (Ref. 22) and thus leads to increased generation of OH· radical. As CAT levels are decreased, H2O2 is not effectively scavenged, resulting in increased lipid peroxidation.

Treatment of the rats with BE for 5 days in the stressed animals in the dose of 50 mg/kg, orally,

Table 1—Effect of DRBP extract (BE, mg/kg, bd x 5 days) on LPO (MDA, nmol/mg of protein), SOD (Units/mg of protein) and CAT activities in rat gastric mucosal homogenates

<table>
<thead>
<tr>
<th>Oral treatment</th>
<th>Ulcer Index</th>
<th>LPO</th>
<th>SOD</th>
<th>CAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.0±0.0</td>
<td>0.27±0.01</td>
<td>115.6±10.9</td>
<td>30.7±2.0</td>
</tr>
<tr>
<td>Stress</td>
<td>33.8±6.5^a</td>
<td>0.40±0.03^b</td>
<td>282.8±28.8^a</td>
<td>18.7±1.6^a</td>
</tr>
<tr>
<td>BE-10 + Stress</td>
<td>27.4±3.1</td>
<td>0.34±0.03</td>
<td>290.4±36.1</td>
<td>17.4±1.4</td>
</tr>
<tr>
<td>BE-50 + Stress</td>
<td>4.4±1.7</td>
<td>0.30±0.02</td>
<td>184.2±19.5</td>
<td>14.9±1.1</td>
</tr>
</tbody>
</table>

P values: ^a<0.05, ^b<0.01 compared to respective control group and ^c<0.05 compared to respective stress group.

Table 2—Effect of BE and bismuth subcitrate (BSC) on in vitro growth of H. pylori

<table>
<thead>
<tr>
<th>Test substance (μg/ml)</th>
<th>Wells showing inhibitory activity Number</th>
<th>Inhibition (%)</th>
<th>P values (Chi-square test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSC</td>
<td>0/12</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>200</td>
<td>3/12</td>
<td>20</td>
<td>NS</td>
</tr>
<tr>
<td>1000</td>
<td>6/12</td>
<td>50</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>BSC</td>
<td>0/12</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>200</td>
<td>0/12</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>1000</td>
<td>0/12</td>
<td>0</td>
<td>NS</td>
</tr>
</tbody>
</table>
reversed, the stress-induced changes in LPO and SOD, while the change in CAT activities was insignificant. BE reduced LPO levels suggesting decreased lipid peroxidation and free radicals induced damage. This naturally led to a decrease in SOD levels in response to decreased generation of \( \text{O}_2^- \). This led to decrease in \( \text{H}_2\text{O}_2 \) and OH accumulation and decreased gastric damage. Although increase in CAT is necessary for effective antioxidant activity, the changes in CAT levels were insignificant. It is possible that this is due to low activity of CAT in the gastric mucosa and may be due to complimentary increase in the more active peroxidases that scavenge \( \text{OH}^- \) radicals. Hence the antioxidant activity of BE may be one of the important defensive factors in addition to various other effects on mucosal defensive factors like increase in mucus secretion, glycoprotein content, PG synthesis, cell proliferation as reported earlier. BE showed no significant inhibition of \( H_pylori \) in vitro. Hence anti-\( H_pylori \) activity may not be involved in the ulcer protective effects of BE.

The present study thus, indicates the absence of anti-\( H_pylori \) activity of methanolic extract of banana in vitro but its antioxidant activity appears to be involved in its ulcer protective effect.

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References
2. Sanyal A K, Banerjee C R & Das P K, Studies on peptic ulceration Part II- Role of banana in restraint and prednisolone induced ulcer in Albino rats, Arch Int Pharmacodyn, 155 (1965) 244.