EVALUATION OF A PROBIOTIC DAIRY PRODUCT FOR ANTIULCER ACTIVITY IN RATS

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ABSTRACT: A marketed probiotic dairy product (curd) containing Bifidobacterium lactis, Lactobacillus acidophilus and Lactobacillus casei each at a count of 10^6-10^8 cfu/g was evaluated for antiulcer activity in different models of gastric ulceration in rats. Gastric ulcers were induced by pylorus ligation method, stress-induced, ibuprofen-induced and ethanol-induced models. Probiotic dairy product was administered in the dose of 5 g/animal for 30 days prior to ulcer induction. Mean ulcer number, size and index were determined. Volume of gastric juice, total and free acidity were estimated in the pylorus ligated rats. Ranitidine was used as a reference drug. The probiotic dairy product showed significant antiulcer activity in pylorus ligation model. However, the antiulcer activity was less than that of ranitidine.

KEY WORDS: Antiulcer activity, Probiotic dairy product, Ranitidine, Ulcer index

INTRODUCTION

Probiotics are defined as selected, viable microbial dietary supplements that, when introduced in sufficient quantities beneficially affect host through their effects in the intestinal tract (Zubillaga et al., 2001, Holzapfel and Schillinger, 2002). Also FAO/WHO has adopted the definition of probiotics as “live organisms which when administered in adequate amounts confer a health benefit on the host” (FAO/WHO, 2002).

Probiotics have varied systemic and physiological functions. The most promising targets for them are the gastrointestinal functions, including effect on the transit time, bowel habits, mucosal motility, modulating epithelial cell proliferation, ulcerative colitis, antibiotic-induced diarrhea, gastrointestinal immune activity and hormonal activity of gastrointestinal system (Schiffin et al., 1995, Clydesdale, 1997).

Helicobacter pylori are a highly prevalent pathogen which is considered as a major risk factor for gastro duodenal ulcers, gastric adenocarcinoma and lymphoma in humans. Current data suggests the efficacy of probiotics present in a variety of functional foods especially yogurts and curd in eradicating H. Pylori infection in animals and also in humans (Gotteland et al., 2006). The present study is an attempt at evaluating the antiulcer activity of a locally marketed probiotic dairy product (PDP).

MATERIALS AND METHODS

Materials: The PDP (curd) used in the present study has a labeled composition of Bifidobacterium lactis (BB 12), Lactobacillus acidophilus (LA 05) and Lactobacillus casei (LC 01) (each 10^6 - 10^8 cfu/g). The biopreparation (curd) was procured daily from the local market and stored at 40 C till it is administered to animals. Absolute ethanol was procured from Changshu yangyuan chemicals, China, anesthetic ether from T.K.M. Pharma, India and all other chemicals used were from S.D. Fine Ltd., India.

Animals: Male albino rats of Wistar strain weighing between 150-200 g were used for the present study. They were fed with commercial rat feed pellets (Gold Mohur, Lipton, India) along with water given ad libitum and maintained on standard animal facilities according to CPCSEA guidelines. Institutional animal ethical committee approved the experimental protocol and the procedures.

Treatment schedule: The antiulcer activity was assessed by pylorus ligation (Shay rat), stress-induced, ibuprofen-induced...
and ethanol-induced ulcer models. In each model, the animals were randomly divided into four groups of eight each. Group I, II and III animals received normal saline (2 ml/animal), probiotic curd (5 g/animal) and normal curd with out probiotic supplementation (5 g/animal) respectively by gastric intubation for 30 days. Group IV animals were given standard drug ranitidine (38 mg/kg, orally) one hour prior to the induction of ulcers.

In pylorus ligation method (Shay et al., 1945), after treatment with PDP, the animals were kept on fast for 48 hours with free access to drinking water. Pylorus of the rat was ligated under light ether anesthesia. Five hours later, rats were sacrificed with an over dose of ether and their stomachs were dissected out after ligating the cardiac end. Each stomach was cut open along the greater curvature and the contents were collected. The mucosa was washed under running tap water and the extent of ulceration was scored (Rao et al., 1990). The gastric juice collected from each stomach was centrifuged and its volume was measured. Free and total acidity were estimated titrimetrically with 0.01N NaOH using phenolphthalein and Toepfer’s reagents as indicators. The acid present was expressed as meq/L (hour) 100 g body weight.

In ibuprofen-induced ulcer model (Parmar and Desai, 1993), after treatment with PDP, the animals were kept on fast for 48 hours with free access to drinking water. Ibuprofen at a dose of 300 mg/kg was orally administered twice at 15 hours intervals and they were sacrificed 6 hours after the second dose of ibuprofen with excess of ether and thus ulceration was scored (Rao et al., 1990).

In stress-induced ulcer model (Takagi et al., 1964), after treatment with PDP, the animals were kept on fast for 48 hours with free access to drinking water. They were put vertically for one hour in individual restraint cages containing water at 22°C for one hour. Evans blue (30 mg/kg i.v) was injected through the tail vein and ten minutes later they were sacrificed in ether anesthesia and their stomachs were excised after ligating both pylorus and cardiac ends. Formal saline (2% V/V) was then injected into totally ligated stomachs and stored over night. The next day each stomach was cut open along the greater curvature, the mucosa was washed under running tap water and the extent of ulceration was scored (Rao et al., 1990).

In ethanol-induced mucosal damage model (Parmar et al., 1988), after treatment with PDP, the animals were kept on fast for 18 hours with free access to drinking water. Absolute ethanol (1 ml/animal) was orally administered to each rat. After 1 hour, the rats were euthanized with excess of anesthetic ether and their stomachs were cut open along the greater curvature, cleaned with normal saline and the inner surface was examined for ulceration (Rao et al., 1990).

Statistical analysis: The results were expressed as mean ± S.E.M. and analyzed by Student’s t-test.

RESULTS

In pylorus ligation (Shay) model, the groups treated with PDP showed a significant (P<0.001) decrease in mean ulcer number (MUN), mean ulcer size (MUS) and mean ulcer index (MUI) when compared to the normal control animals. The normal curd was not exhibit any significant decrease in these parameters and the values were similar as observed in normal animals. However, in the animals treated with standard drug ranitidine, there was a significant (P<0.001) decrease in ulcers (Table 1). The animals treated with PDP and ranitidine also showed a significant (P<0.001) decrease in the volume and acidity of gastric juice when compared to normal animals and the animals treated with normal curd (Table 2).

**TABLE 1. Effect of the Probiotic dairy product on ulcers produced by pylorus ligation model**

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Treatment</th>
<th>MUN</th>
<th>MUS</th>
<th>MUI</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal</td>
<td>10.0±1.97</td>
<td>6.6±1.09</td>
<td>59.3±9.93</td>
</tr>
<tr>
<td>II</td>
<td>Probiotic curd</td>
<td>3.5±1.5±*</td>
<td>2.1±0.96±*</td>
<td>11.9±6.6±*</td>
</tr>
<tr>
<td>III</td>
<td>Normal curd</td>
<td>9.8±1.8</td>
<td>6.3±1.02</td>
<td>52.3±7.2</td>
</tr>
<tr>
<td>IV</td>
<td>Ranitidine</td>
<td>2.9±0.48±*</td>
<td>1.4±0.15±*</td>
<td>10.5±5.2±*</td>
</tr>
</tbody>
</table>

MUN - Mean Ulcer Number
MUS – Mean Ulcer Size
MUI – Mean Ulcer Index

**Table 2. Effect of the Probiotic dairy product on gastric volume and acidity in pylorus ligation model**

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Treatment</th>
<th>Volume of gastric juice ml/100 g bw</th>
<th>Free acidity - - meq/ lit / hr/100 g bw - -</th>
<th>Total acidity - - meq/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal</td>
<td>5.4±0.52</td>
<td>18.5±1.51</td>
<td>22.1±1.92</td>
</tr>
<tr>
<td>II</td>
<td>Probiotic curd</td>
<td>3.5±0.23 a</td>
<td>14.1±0.92 a</td>
<td>10.5±1.2 a</td>
</tr>
<tr>
<td>III</td>
<td>Normal curd</td>
<td>4.9 ±0.32</td>
<td>18.3±1.20</td>
<td>20.6±1.43</td>
</tr>
<tr>
<td>IV</td>
<td>Ranitidine</td>
<td>3.4 ±0.33 a</td>
<td>12.4±0.63 a</td>
<td>10.5±1.3 a</td>
</tr>
</tbody>
</table>

*P< 0.05, aP< 0.01, *cP < 0.001 compared to normal group; bw: Body weight; meq: Milli equivalent

In stress-induced, ibuprofen-induced and ethanol-induced ulcer models, the PDP did not exhibit a significant difference in MUS, MUN, and MUI parameters when compared to the normal animals.

DISCUSSION

Results of the present study indicate that the probiotic dairy product possesses anti ulcer activity in pylorus ligation model. It has shown a significant reduction in gastric lesions, volume of gastric juice, total and free acidity. Normal curd did not show significant effect on the parameters studied indicating the anti ulcer activity is due to supplementation of curd with probiotic microorganisms. It has been suggested that the bacteria H. pylori may contribute to the pathogenesis of gastric ulceration by increasing gastric acid secretion (El-Omar et al., 1995; Cover and Blaser, 1996). Lactobacilli and Bifidobacteria have previously been shown to prevent infection of the stomach of gnobiotic mice with H. pylori (Kabir et al., 1997). The ability of the probiotics such as Lactobacilli to reduce injury in the gastrointestinal tract and inhibit the growth of potentially pathogenic bacteria has been attributed to a number of possible mechanisms, including competition for adhesion receptors, competition for nutrients and production of antimicrobial substances and stimulation of immunity (Fuller, 1991; Fabia et al., 1993).
Ibuprofen was used in the present study to induce ulcers. Ibuprofen is a non-selective cyclo-oxygenase inhibitor that causes more gastric damage compared to other nonsteroidal anti-inflammatory agents (Feldman and Memahon, 2000). Ibuprofen was shown to reduce synthesis of gastro protective PGE2, which is necessary to maintain blood flow to gastric mucosa and plays an important role in mucus production (Kataoka et al., 2000). In the present study, the lack of protection in nonsteroidal anti-inflammatory drug-induced gastric ulcers by probiotic dairy product may be due to the fact that probiotic curd did not have any effect on prostaglandin inhibition, thus unable to promote mucus production.

Ethanol-induced gastric lesions occur probably due to stasis in gastric blood flow produced because of vascular congestion and mucosal capillary necrosis, which contribute to development of hemorrhagic and necrotic aspect to tissue injury (Oladejo et al., 2003). It is also reported to produce pathogenicity by direct damage to gastric mucosa, alterations in permeability, gastric mucus depletion and free radical production (Salim, 1990). Lactobacilli were reported to stabilize the mucosal barrier by increasing mucin, stimulating mucosal immunity and synthesizing antioxidants (Gotteland et al., 2001). PDP did not exhibit any significant protection against ethanol-induced gastric lesions. This might be due to its inability to stabilize surface epithelial cells and to increase mucosal resistance that is considered as important factor to boost mucosal defense mechanisms (Maity et al., 1998; Pal and Nag Chowdary, 1991).

Stress can produce consistent disturbances in acid secretion, bile and pancreatic juice reflux, which are important factors leading to ulcer formation (Richardson, 1990). Emotional stress was reported to decrease both quality and quantity of mucus by affecting translation, acylation and glycosylation of the ribosomal peptides which cause loss of integrity of the mucosal membrane (Tsukada et al., 1998). In the present study PDP was not effective in stress-induced ulcers, which might be due to its lack of effect in maintaining integrity of mucosal membrane.

In conclusion, the present study shows antiulcer effect of the probiotic dairy product and its mechanism of action may be due to inhibition of *H. pylori*. Further studies using other probiotic strains at different concentrations (cfu), increasing the duration of treatment and studying the effect of combination of probiotics with standard antiulcer drugs may also help in suggesting a better therapy for peptic ulcer.

REFERENCES


