EFFECT OF PROBIOTIC DAIRY PRODUCT, PREBIOTIC HONEY AND THEIR COMBINATION ON DIFFERENT TYPES OF ULCERS IN ALBINO RATS

M. Sushma, D. Sujatha, K. Spandana, K. Bharathi and K.V.S.R.G. Prasad*

Institute of Pharmaceutical Technology, Sri Padmavati Mahila Visvavidyalayam (Women's University), Tirupati- 517 502, India

Summary

Lifestyle and eating habits are partly responsible for overall health status. The latest and most exciting stage in the evolution of intestinal-health products is the synergy between probiotics and prebiotics i.e., synbiotics which represents an interesting area of research. The present study verifies whether probiotics, prebiotics and synbiotics offer a better therapeutic alternative for treating different ulcer conditions like gastric ulcers induced by pylorus ligation model, duodenal ulcers induced by cysteamine and ulcerative colitis induced by 8% acetic acid. In gastric and duodenal ulcer models, the parameters monitored were acid volume, gastric pH, total acidity, free acidity, ulcer index and the *in vivo* antioxidant parameters lipid peroxidation, reduced glutathione, catalase and nitrite. In ulcerative colitis model, diarrhea, wet weight of the colon, activity score, gross mucosal inflammation, gross morphological disease score, crypt abscesses were studied. Myeloperoxidase activity of the mucosal scrapings of the duodenum and colon was estimated in duodenal and ulcerative colitis models. The results of the present study clearly show that probiotic dairy product, prebiotic honey and combination treatments offered a significant protection against both gastric and duodenal ulcers. The combination was more effective in reducing the severity of colitis when compared to probiotic dairy product and prebiotic honey alone. Whereas, probiotic dairy product offered more benefit against gastric and duodenal ulcers than prebiotic honey and combination.

KEY WORDS: acetic acid induced colitis, cysteamine induced duodenal ulcer, prebiotic honey, probiotic dairy product, pylorus ligation, ulcerative colitis

* Address correspondence to: K.V.S.R.G. Prasad at the above address
(E-mail: kvsrgprasad@yahoo.co.in)
Phone: +91-0877-2241625

Introduction

Consumer interest in foods that may enhance health beyond their nutritional value is at an all time high.[1] Many so called functional foods (designer foods some times considered as nutraceuticals) are being developed and marketed to deliver specific health benefits to consumers. Among the functional components, probiotics, prebiotics, soluble fibers, omega-3-poly unsaturated fatty acids, conjugated linoleic acid, plant antioxidants, vitamins, minerals, some proteins, peptides, amino acids as well as phospholipids are mentioned frequently.[2] The use of probiotics, prebiotics and synbiotics is a promising area for the development of functional foods.[3] Promising targets for the functional foods are gastrointestinal functions that are associated with a balanced colonic microbiota, control of nutrient bioavailability (ions in particular) that modify the gastrointestinal immune activity or that are mediated by the endocrine activity of the gastrointestinal system.[4] The combination of probiotics and prebiotics is called a synbiotic which might improve the survival of the bacteria crossing the upper part of the gastrointestinal tract, there by enhancing their effects in the large bowel and in addition their effects might be additive or even synergistic.[4]

Recently, there has been a rapid progress in the understanding of the pathogenesis of peptic ulcer. Most of the studies focus on newer and better drug therapy. These have been made possible largely by the availability of the proton pump inhibitors, histamine-2 receptor blockers, drugs affecting mucosal barrier and prostaglandin analogs.[5] However, these drugs showed development of tolerance and incidence of relapses and side effects that make their efficacy arguable. This has been the rationale for the development of new antiulcer drugs, which includes functional foods.

Earlier studies carried out in our laboratory with a locally marketed probiotic dairy product (PDP) were found to be beneficial against peptic ulcer induced in experimental animals.[6] Hence, the present study intends to verify the potential of a probiotic dairy product (PDP), prebiotic honey (PH) and their combination in different types of ulcers like gastric ulcer (GU), duodenal ulcer (DU) and ulcerative colitis (UC) in experimental animals.

Materials and methods

Experimental animals

Wistar strain adult albino rats of either sex weighing between 150-200 g were used in the present study. The animals were housed in polypropylene cages in a well ventilated room under hygienic conditions and were exposed to 12 h day and night cycle. The animals were fed with commercial rat pellet feed (Gold Mohur, Ltd., India) and were given water *ad libitum*. All the experimental protocol and procedures were approved by Institutional animal ethical committee.

Materials

The marketed PDP has a labelled composition of *Bifidobacterium lactis* (BB 12), *Lactobacillus acidophilus* (LA 05) and *Lactobacillus casei* (LC 01), with a count of 10^6-10^8 CFU/g. Fresh samples were procured daily from local market and stored at 8°C before administration to the animals. Prebiotic used in the present study was honey (Dabur India Ltd.), cysteamine hydrochloride was procured from Sigma chemicals and all other chemicals used in the study were procured from S.D. Fine Ltd., India.

Pharmacological studies

Pylorus ligation model

Gastric ulcers were induced by pylorus ligation model.[7] Animals were divided into six groups (n= 8). Group I receives no treatment, serves as normal animals. Group II received vehicle (distilled water 2 ml/animal) that serves as vehicle control. Group III received standard drug ranitidine (38 mg/kg body weight, per oral) one hour prior to pylorus ligation. Group IV, V and VI received PDP (2.5 ml/animal, per oral), PH (1ml/ animal, per oral) and PDP+PH (2.5 ml and 1ml/animal, per oral) respectively for 30 days.

At the end of the treatment schedule, the animals of all groups were starved for 48 h with free access to drinking water. Under light ether anesthesia, the pylorus of rat was ligated, 19 h later the ligated rats were sacrificed by decapitation. The abdomen was opened; stomach was removed after ligating the cardiac end and opened along the greater curvature. The contents were drained into a centrifuge tube and centrifuged at 2000 rpm, 3 min (REMI R8C Laboratory Centrifuge) for assessing parameters like acid volume, gastric pH, total acidity and free acidity.

The stomach was thoroughly washed under running tap water and pinned onto a cork plate. The no of ulcers and severity was scored as per Rao *et al.,* 1990.[8] The stomachs from all the groups were simultaneously assessed for antioxidant parameters like lipid peroxidation, reduced glutathione, catalase and nitrite.

Cysteamine induced ulcers

Duodenal ulcers were induced by cysteamine.[9] Animals were divided into six groups (n=8). Group I receives no treatment, serves as normal animals. Group II received vehicle (distilled water 2 ml/animal) that serves as vehicle control. Group III received standard drug ranitidine (38 mg/kg body weight, per oral) one hour prior to administration of cysteamine. Group IV, V and VI received PDP (2.5 ml/animal, per oral), PH (1ml/animal, per oral) and PDP+PH (2.5 ml and 1ml/animal, per oral) respectively for 30 days.

After completion of the treatment schedule, the animals of all groups were deprived of food 24 h prior to the induction of ulcers but had free access to drinking water. Cysteamine hydrochloride was administered at a dose of 60 mg/100 g body wt (in normal saline). The rats were sacrificed 4 h post cysteamine challenge. The stomach and duodenum were excised and exposed for scoring the ulcers as mentioned earlier. The parameters assessed in this model were acid volume and ulcer index. Antioxidant parameters and myeloperoxidase activity were assessed in mucosal scrapings of duodenum including Group I.

Acetic acid induced ulcerative colitis

In this model 8 % of acetic acid (in normal saline) was used to induce ulcerative colitis.[10] Animals were divided into six groups (n=8). Group I receives no treatment, serves as normal animals. Group II received vehicle (distilled water 2 ml/animal) that serves as vehicle control. Group III received standard drug sulfasalazine (100 mg/kg body weight, per oral), 30 minutes prior to the induction of colitis. Group IV, V and VI received PDP (2.5 ml/animal, per oral), PH (1ml/ animal, per oral) and PDP+PH (2.5 ml and 1ml/animal, per oral) respectively for 30 days.

At the end of the treatment schedule, animals of all groups were fasted for 24 h. Each rat was lightly anesthetized with ether and a polyethylene cannula (6 mm diameter)

was inserted into the lumen of the colon via the anus. The cannula was advanced so that the tip was 8 cm proximal to the anus. Initially, each rat received a 1 ml normal saline flush followed by manual palpitation of the abdomen to remove any fecal matter. Later 1 ml of 8% acetic acid was slowly infused into the distal colon and the rat was maintained in a head-down position for 30 seconds to limit the expulsion of the solution. Finally each rat received 1 ml of colonic wash containing phosphate buffered saline (pH 7.4). Control (I) animals were treated identically except that instead of 8% acetic acid, they received 1 ml normal saline infusion. Control (I) animals and the colitic rats were studied 24 h post saline instillation.[(11)]

Parameters assessed in this model were diarrhea, wet weight of the colon (3 cm long), activity score [(12)], gross mucosal inflammation [(11)], crypt abscesses [(13)], gross morphological disease score [(14)]. Myeloperoxidase[(17)] activity was assessed on the mucosal scrapings of the colon of all the groups I to VI.

ESTIMATION OF ANTIOXIDANT PARAMETERS

Stomach in pylorus ligation model, duodenum in cysteamine induced ulcer and colon in acetic acid induced colitis was perfused with ice cold saline and made into pieces. The stomach and duodenum were homogenized in phosphate buffer pH 7.4 and the homogenates were centrifuged at 800 rpm for 5 min at 4ºC (REMI CM-12) to separate the molecular debris. The supernatant so obtained was centrifuged at 10,500 rpm for 20 min at 4ºC to get the post mitochondrial supernatant (PMS) which was used to assay antioxidant parameters [(15)] like catalase, reduced glutathione and lipid peroxidation.

Catalase was estimated in PMS by the method of Claiborne.[(16)] Catalase activity was assayed spectrophotometrically following a decrease in absorbance of H_2O_2 at 240 nm and the specific activity was expressed as micro moles per gram of protein.

Glutathione content was estimated in PMS according to the method of Jollow *et al.,* 1974. [(17)] The measurement was based on reduction with 5, 5^1-dithiobis-(2-nitro-benzoic acid) and the optical density was measured at 412 nm. The results were expressed as micro moles of total sulfhydryl groups (TSH) per gram of protein.

The concentrations of malondialdehyde (MDA) in the PMS sample were determined to obtain a quantitative estimation of the membrane lipid oxidative damage.

MDA was assayed in terms of thiobarbituric acid reacting substrates (TBARS) expressed as micromoles per minute per gram of protein.[18]

Nitrite estimation in PMS sample was done by the method of Green *et al.,* 1982. [19] Briefly, the homogenates were incubated with Griess reagent for 10 min at room temperature and the absorbance was read at 546 nm. The standard curve was prepared using sodium nitrite and results are expressed as micromoles of nitrite per gram of protein.

The mucosal scrapings of the perfused colon/duodenum were used for the assay of MPO activity. It was homogenized in 3 ml of 0.05M phosphate buffer (pH 6.0) containing CTAB (cetyl trimethyl ammonium bromide) 0.5% w/v using a homogenizer. The homogenates were centrifuged at 1700 rpm for 20 min at 4ºC (REMI CM-12). The supernatant was diluted five fold with potassium phosphate buffer. To 0.05 ml of the diluted sample 1.4 ml of 0.00107 % H_2O_2 diluted with potassium phosphate buffer was added. To this mixture 0.05 ml of 0.03 M O-dianisidine solution was added and the tissue MPO activity was determined from the increment of absorbance at 450 nm for 60 seconds.[20]

Statistical Analysis

Results were expressed as mean ± SEM. The statistical significance of any difference in each parameter among the groups was evaluated by Student's t-test.

Results

In the gastric ulcers induced by pylorus ligation, a significant decrease in mean ulcer number, mean ulcer score was observed in all the groups receiving PDP (IV), PH (V) and PDP+PH (VI) when compared to the control (II) animals. Similarly ulcer index, total acidity and free acidity also showed a significant decrease in groups receiving PDP (IV), PH (V) and PDP+PH (VI) on comparison with the control (II) group. The effect of PDP+PH (VI) was not significant when compared to PDP (IV) and PH (V) treatment alone. The effect of PH (V) and PDP+PH (VI) was peculiar on gastric pH with significant decrease, whereas significant increase was observed with standard and PDP (IV) treatment when compared to the control group (I) (Table 1, 2).

TABLE 1. **E**FFECT OF **P**ROBIOTICS, **P**REBIOTICS AND **P**DP+**P**H ON **P**YLORUS **L**IGATION **I**NDUCED **G**ASTRIC ULCER.

Group	**Acid volume (ml/100 g b wt)**	**pH**	**Total-acidity (mEq/L)**	**Free-acidity (mEq/L)**
II- Control	5.68±0.94	2.5±0.87	139.8±4.33	75±1.96
III-Standard	3.4±0.18	5.5±0.65 [a]	42±2.94[c]	13±3.42[c]
IV-PDP	18.3±0.34[c]	6.0±0.41 [a]	39.5±3.30[c]	18±1.63[c]
V-Prebiotic	17.13±0.76[c]	1.0±0	95±15.55 [a]	41.25±6.65 [b]
VI-PDP+PH	13.3±1.54 [b]	1.75±0.48	125.5±5.64	65±3.54

Values are expressed as mean ± SEM of 6 observations.
Statistical comparisons are made between Group III, IV, V and VI vs Group II.
Values with superscripts a-c are not statistically significant at the given levels ([a] $P<0.05$, [b] $P<0.01$, [c] $P<0.001$).

On pylorus ligation, a significant decrease in catalase, reduced glutathione and nitrite along with a significant increase in lipid peroxidation levels was observed in the control (II) group. Treatment with PDP (IV) and PH (V) showed significant increase in the levels of catalase, reduced glutathione and nitrite along with a significant decrease in lipid peroxidation levels when compared to the control (II) (Table 3).

PH (V) showed a significant decrease ($P<0.001$) of lipid peroxidation levels when compared to the other treatment groups. Whereas, the effect of PDP+PH (VI) on reduced glutathione levels and PDP (IV) on catalase ($p<0.01$) and nitrite levels ($p<0.001$) were more significant over other treatment groups. The group receiving standard drug ranitidine (III) offered good protection against gastric ulcers induced by pylorus ligation in terms of reduced mean ulcer number, mean ulcer score, ulcer index, total acidity, free acidity and restoration of antioxidant parameters to the normal levels.

TABLE 2. EFFECT OF PROBIOTICS, PREBIOTICS AND PDP+PH ON PYLORUS LIGATION INDUCED GASTRIC ULCER.

Group	MUN	MUS	UI
II- Control	4.0±0.53	1.66±0.13	15.66
III-Standard	0.66±0.66[b]	0.16±0.16 [c]	7.4
IV-PDP	0.33±0.33[c]	0.5±0.3 [b]	4.13
V-Prebiotic	0.66±0.33[c]	0.5±0.3 [b]	7.76
VI-PDP+PH	0.66±0.33 [c]	0.66±0.33 [a]	11.66

Values are expressed as mean ± SEM of 6 observations.
Statistical comparisons are made between Group III, IV, V and VI vs Group II.
Values with superscripts a-c are not statistically significant at the given levels ([a] $P<0.05$, [b] $P<0.01$, [c] $P<0.001$).

In the cysteamine induced duodenal ulcers, a significant increase in acid volume was observed in the groups treated with PDP (IV), PH (V) and PDP+PH (VI). Whereas, a significant decrease in acid volume was observed in animals treated with standard drug (ranitidine 38 mg/kg) when compared with the control group (II). Treatment with PH (V) offered no significant protection against cysteamine induced ulceration, but the groups receiving ranitidine (III), PDP (IV) and PDP+PH (VI) showed significant reduction in UI against cysteamine induced ulcers (Table 4).

Similarly, a significant decrease in catalase and reduced glutathione along with a significant increase in lipid peroxidation and myeloperoxidase levels were observed in the control (II) group indicating the severity of the damage induced by cysteamine. The group receiving PDP (IV) was able to show significant increase in reduced glutathione ($p<0.01$) and catalase levels ($p<0.001$) with a significant decrease ($P<0.01$) in myeloperoxidase activity when compared to the control (II). The group receiving PH (V) was not effective against any of the antioxidant parameters. Where as, the group

receiving PDP+PH (VI) has shown a significant increase ($P<0.01$) of reduced glutathione levels only (Table 5, 8).

TABLE 3: **E**FFECT OF **P**ROBIOTICS, **P**REBIOTICS AND **P**DP+**P**H ON *IN VIVO* **A**NTIOXIDANT **P**ARAMETERS ON **P**YLORUS **L**IGATION **I**NDUCED **G**ASTRIC **U**LCER.

Group	**LPO (µM/g tissue)**	**Catalase (µM /g tissue)**	**Reduced glutathione (µM/g tissue)**	**Nitrite (µM/g tissue)**
I- Normal	0.0328±0.0002	5.50±0.13	0.25±0.005	54.34 ±0.93
II- Control	0.045±0.0003 [a]	3.05±0.15 [a]	0.12±0.006[a]	31.67±0.50 [a]
III-Standard	0.028±0.0004 [d]	4.6±0.18 [d]	0.14±0.003 [b]	56.94±1.38 [d]
IV-PDP	0.027±0.0010 [d]	4.78±0.30[c]	0.16±0.006 [c]	58.44±2.05 [d]
V-Prebiotic	0.014±0.0014 [d]	1.84±0.085	0.17±0.003 [d]	42.85±1.02 [d]
VI-PDP+PH	0.022±0.0007 [d]	1.54±0.057	0.19±0.003 [d]	25.15±0.58

Values are expressed as mean ± SEM of 6 observations.
Statistical comparisons are made between Group III, IV, V and VI vs Group II.
Values with superscripts a-c are not statistically significant at the given levels ([a] $P<0.001$, [b] $P<0.05$, [c] $P<0.01$, [d] $P<0.001$).

TABLE 4: **E**FFECT OF **P**ROBIOTICS, **P**REBIOTICS AND **P**DP+PH ON **C**YSTEAMINE **I**NDUCED **D**UODENAL ULCER.

Group	**Acid volume (ml/100 g b wt)**	**UI**
II- Control	4.58±0.05	15
III-Standard	3.45±0.18 [b]	7.4
IV-PDP	6.65±0.59[a]	8.56
V-Prebiotic	5.9±0.33[b]	13.6
VI-PDP+PH	6.65±0.73[a]	8.92

Values are expressed as mean ± SEM of 6 observations.
Statistical comparisons are made between Group III, IV, V and VI vs Group II.
Values with superscripts a-c are not statistically significant at the given levels ([a] $P<0.05$, [b] $P<0.01$).

TABLE 5. **E**FFECT OF **P**ROBIOTICS, **P**REBIOTICS AND **P**DP+**P**H ON *IN VIVO* **A**NTIOXIDANT **P**ARAMETERS ON **C**YSTEAMINE **I**NDUCED **D**UODENAL **U**LCER.

Group	**LPO (µM/g tissue)**	**Catalase (µM /g tissue)**	**Reduced glutathione (µM/g tissue)**
I- Normal	0.0104±0.001	3.45 ±0.11	0.172±0.001
II- Control	0.0143±0.002[a]	1.91±0.035[a]	0.081±0.002[a]
III-Standard	0.0109±0.001	2.98±0.13 [b]	0.102±0.002[b]
IV-PDP	0.0107±0.0002	3.69±0.12[c]	0.106±0.004 [b]
V-Prebiotic	0.0148±0.0003	0.53±0.017	0.129±0.004 [c]
VI-PDP+PH	0.0111±0.001	0.39±0.021	0.131±0.003 [c]

Values are expressed as mean ± SEM of 6 observations.
Statistical comparisons are made between Group III, IV, V and VI vs Group II.
Values with superscripts a-c are not statistically significant at the given levels ([a] $P<0.05$, [b] $P<0.01$, [c] $P<0.001$).

TABLE 6. EFFECT OF PROBIOTICS, PREBIOTICS AND PDP+PH ON ACETIC ACID INDUCED ULCERATIVE COLITIS.

Group	Activity score	Gross mucosal inflammation	Crypt abscess	Wet weight
II- Control	2.75±0.25	6.0±0	2±0	250±0
III-Standard	3.0±0	2.0±0	0±0	255±2.89
IV-PDP	1.75±0.48	2.0±0.41	1±0	250±0
V-Prebiotic	3.0±0	3.0±0	1±0	317.5±23.23
VI-PDP+PH	3.0±0	1.25±0.25	1±0	255±15.55

Values are expressed as mean ± SEM of 6 observations.

TABLE 7. GROSS MUCOSAL DISEASE SCORE IN DIFFERENT TREATMENT GROUPS-ULCERATIVE COLITIS

Group	No of rats	None	Mild	Moderate	Severe
II- Control	4	0	0	0	4
III-Standard	4	3	1	0	0
IV-PDP	6	3	1	2	0
V-Prebiotic	6	1	1	3	1
VI-PDP+PH	8	4	4	0	0

TABLE 8. **E**FFECT OF **P**ROBIOTICS, **P**REBIOTICS AND **P**DP+**P**H ON **M**PO **A**CTIVITY

Group	**MPO activity (Δ/min/g tissue)**	
	Cysteamine induced duodenal ulcer model	**Acetic acid induced UC**
I- Normal	0.59±0.13	0.693±0.12
II- Control	0.74±0.041 [a]	0.835±0.019 [b]
III-Standard	0.49±0.022 [c]	0.32±0.18 [e]
IV-PDP	0.42±0.012 [d]	0.53±0.27 [e]
V-Prebiotic	0.66±0.009	0.635±0.039 [d]
VI-PDP+PH	0.68±0.022	0.32±0.036 [e]

Values are expressed as mean ± SEM of 6 observations.
Statistical comparisons are made between Group III, IV, V and VI vs Group II.
Values with superscripts a-e are not statistically significant at the given levels ([a] $P<0.01$, [b] $P<0.001$, [c] $P<0.05$, [d] $P<0.01$, [e] $P<0.001$).

In ulcerative colitis induced by 8% acetic acid, the gross mucosal disease score in various groups receiving PDP (IV), PH (V) and PDP+ PH (VI) indicated decrease in the severity of colitis when compared to the control group (II) (Table 7). The group fed with PDP+PH (VI) was more effective in reducing the severity of colitis when compared to groups receiving PDP (IV) and PH (V) alone.

Wet weight (3cm) of the colon increased in the groups fed with PH (V) and PDP+PH (VI) when compared to the control (II) group with no significant change in the group receiving PDP (IV) alone. Activity score of animals did not show any significant

changes after treatment with sulfasalazine (III), PDP (IV), PH (V) and PDP+PH (VI) when compared to the control group (II). Gross mucosal inflammation significantly decreased in all the groups fed with PDP (IV), PH (V) and PDP+PH (VI) as compared with the control (II) group. Among all groups PDP+PH (VI) was observed to be much protective against mucosal inflammation, comparable with that of group receiving sulfasalazine (III). Crypt abscess decreased in all the treatment groups when compared to control group (II) and the group receiving sulfasalazine (III) showed no crypt abscess. The effect of PDP+PH group (VI) was comparable with that of sulfasalazine (III) (Table 7). The myeloperoxidase activity showed a significant increase in the control group (II) on colitis formation. Treatment with PDP (IV), PH (V), PDP+PH (VI) and sulfasalazine (III) showed a significant decrease ($P<0.001$) in the myeloperoxidase levels of mucosal scrapings of colon indicating protection against acetic acid induced colitis (Table 8).

Discussion

Ulcer is a major global problem affecting day to day life in humans and the causative factors being unavoidable such as stress, use of non steroidal anti inflammatory drugs (NSAID's) etc. Ulcers are also associated with the development of upper gastrointestinal damage including lesions, life threatening perforations and hemorrhage. Keeping in view the side effects of available antiulcer drugs, alternatives that are safer, but effective in ulcer prevention must be envisaged. Etiopathology of gastric ulcer is not known in most cases [(21)], but generally accepted that it results from an imbalance between aggressive factors and defensive factors. Recently, phytomedicines and nutraceuticals have become attractive sources of new and natural drugs.[(22)]

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.[(23)] Probiotics would fortify the resident microbiota that forms an integral part of the mucosal barrier and offers resistance against pathogens.[(24)]

Prebiotic carbohydrates can change the composition of the microbial flora significantly of the human large bowel mucosa as reported by Langlands *et al.,* [25]. Hence, the present study aims to investigate the potential of highly interested functional foods like probiotics, prebiotics and their combination in different ulcer conditions.

Pylorus ligation model is considered as a potential tool to evaluate efficacy of new drugs against gastric ulcers. Pylorus ligation–induced ulcers are produced due to autodigestion of the gastric mucosa by gastric acid and breakdown of the gastric mucosal barrier.[26] Acid secretion remains with in the normal range in 40-70% of cases of duodenal ulcers and normal or below normal in gastric ulcer patients. [27] Thus, decreased mucosal resistance could be the dominant factor in the pathogenesis of peptic ulcers. The gastrointestinal epithelium is covered by a protective mucosal gel composed predominantly of mucin glycol-proteins that were synthesized and secreted by goblet cells. Intestinal microbes may affect goblet cell dynamics as well as secretion of mucus, directly via the local release of bioactive factors or indirectly via activation of host immune cells. [28]

Many reports are available suggesting the role of probiotics in maintaining the mucosal resistance. Halper *et al.,* discovered that metabolites of lactobacilli culture induce angiogenesis and proteoglycans deposition which is crucial for tissue remodeling.[29] *L. rhamnosus* GG enhances angiogenesis and reduced cell apoptosis.[30] These findings provide evidence to support the notion that probiotics could indeed heal mucosal lesions and ulcer. In addition, Resta-Lenert and Barrett, demonstrated that both the live probiotics and their metabolites increase trans-epithelial resistance, a parameter measuring the integrity of intestinal epithelium.[31]

Treatment with PDP, PH and PDP+PH showed a significant decrease in free acidity, total acidity, ulcer index, mean ulcer number and mean ulcer score instead of a significant increase in acid volume. This clarifies that the functional foods used in the present study mainly targeted mucosal integrity rather than acid secretion.

The ulcerogenic effect of cysteamine is both rapid and constant, thus providing a reliable model for investigating the drugs effective against duodenal ulcers.[32, 33] The exact mechanism of pathogenesis in the cysteamine induced duodenal ulcer model is not

known but hypersecretion of gastric acid, deterioration in mucosal resistance and promoting gastric emptying are among the possible mechanisms. [34, 35]

It is now well established that peptic ulcer disease can be prevented by strengthening the defensive mechanisms of gastric and duodenal mucosa rather than attenuating the factors of aggression causing ulceration.[36] The results of the present study in cysteamine induced duodenal ulcer model, clearly show that though there was no decrease in acid secretion, still PDP and PDP+PH treatments offered a significant protection against duodenal ulcers as shown by a significant decrease in ulcer index. It was reported that probiotic strains such as bifidobacteria and lactobacilli increase intestinal mucus production, antagonize the adhesion and colonization of pathogenic bacteria to the intestinal mucosa and thus accelerate ulcer healing.[37, 38, 39] The mucus layer plays an important role in preventing damage to the epithelial cells by gastric acid produced in the stomach and by foreign substances such as chemicals. Phospholipids also play an important role in the preservation of gastrointestinal homeostasis and mucosal integrity.[40] The hydrophobicity of the mucosal lining is attributed to a surfactant like phospholipids monolayer that defends gastric mucosa against damage induced by strong acids [41] and other barrier breaking agents.[42, 43] Probiotics favour the synthesis of phospholipids as indicated by the earlier studies.[44] Thus, the mechanism of action of functional foods in the present study may involve the reinforcement of mucosal barrier function, together with their ability to enhance levels of phospholipids.

Another common form of ulceration in intestinal mucosa is ulcerative colitis. It is a chronic inflammatory bowel disease of unknown origin. Oxidative stress has been implicated in the pathogenesis of ulcerative colitis in both experimental animals [45] and also in humans.[46] Luminal bacteria could play a major role in the initiation and perpetuation of chronic ulcerative colitis.[47, 48] Analysis of the luminal enteric flora however has revealed differences in the composition of this flora when compared to healthy human controls. In ulcerative colitis, concentrations of bacteroides, eubacteria, peptostreptococci and facultative anaerobic bacteria are increased, whereas the number of bifidobacteria was significantly reduced. Manipulation of the colonic bacteria with probiotics proved to be more effective and tolerable than immunosuppressants. [49, 50]

Induction of colitis by acetic acid in rats is one of the standardized methods to produce an experimental model of irritable bowel syndrome (IBS). Several major causative factors in the initiation of human colitis such as enhanced vasopermeability, prolonged neutrophils infiltration and increased production of inflammatory mediators are also seen involved in this animal model of IBS. [51] In the present study, moderate but persistent ulceration was observed in the control animals supporting acetic acid induced ulcerative colitis as one of the suitable model for screening of drugs against ulcerative colitis.

Administration of PDP, PH and PDP+PH alleviated the diarrhea, wet weight of the colon, gross mucosal inflammation, crypt abscesses and gross morphological disease score caused by acetic acid treatment which can be attributed to some extent to their ability to preserve mucosal integrity. This decrease in colitis observed in experimental animals may also be due to the antiinflammatory properties of probiotics like upregulation of immunoglobulin IgA, [52, 53] inhibition of inflammatory cytokines like IL-8, IL-4, IL-5, tissue necrosis factor and interferons.[54, 55] The production of butyrate, which was previously reported to have an anti-inflammatory effect, [56] is increased in the intestine following administration of both probiotics and prebiotics. [57, 58] n- Butyrate is one of the most important beneficial short chain fatty acids and is the primary energy source for colonic epithelial cells, necessary for the healthy metabolism of the colonic mucosa and have been shown to have protective effects against colorectal cancers. Butyrate affects cell proliferation and differentiation, increases mucus secretion and decreases inflammation.[59] Although it is conceivable that PDP and PH produced antiinflammatory substance(s), as well as butyrate, that exert protection against colitis induced by acetic acid, the underlying mechanisms are yet to be elucidated.

Myeloperoxidase is an important enzyme of neutrophils, related to oxidant burst for bacterial killing. The colonic myeloperoxidase activity, an index of neutrophil activation and inflammation was increased in both cysteamine and acetic acid treated animals. Activated neutrophils pass out of the circulation and enter the inflamed mucosa and submucosa of the large intestine during acute inflammation, leading to over production of reactive oxygen and nitrogen species, proteases, lactoferin and lipid mediators that can contribute to intestinal injury.([60, 61, 62] This increase in

myeloperoxidase activity was substantially reduced in rats treated with a PDP and combination of PDP+PH.

Reactive oxygen species (ROS) are generated through numerous normal metabolic processes that are needed for normal functioning of the organism. Various antioxidant enzymes like catalase, reduced glutathione control their accumulation.[63] Any imbalance in the ability of these enzymes normally leads to faulty disposal of free radicals and its accumulation. These ROS are responsible for the oxidation of tissues leading to lipid peroxidation and tissue damage. Oxidative damage is considered to be an important factor in the pathogenesis of ulcer as evidenced in different experimental and clinical models.[64]

In our present work, we also observed an increase in the oxidative free radicals, lipid peroxides and nitric oxide leading to oxidative damage in pylorus ligation and cysteamine induced duodenal ulcer. The principal free radical in tissues is superoxide anion (O_2^-). Superoxide anion (O_2^-) can be produced by both endothelial cells through xanthine oxidase and activated neutrophils through NADPH oxidase, which reduces molecular oxygen to the O_2^- radical and through the enzyme myeloperoxidase. Superoxide ion (O_2^-) if not scavenged by the catalase causes lipid peroxidation by an increase in the generation of hydroxyl free radicals resulting in tissue damage.[65] The above effect could be further aggravated by the decreased activity of catalase and reduced glutathione during pylorus ligation and cysteamine induced duodenal ulcer.

Results reveal improvement in catalase, reduced glutathione and nitrite levels in stomach after treating with PDP, PH and PDP+PH in the pylorus ligation model. This treatment might have restored the balance between free radical scavenging enzymes catalase, reduced glutathione and nitrite in the gastric mucosa counteracting the free radicals, generated by the cascade of free radical generation and thus decreasing lipid peroxidation levels in stomach homogenate. Free radical generation in cysteamine induced duodenal ulcer model was observed to be more severe than pylorus ligation model. Treatment with PDP, PH and PDP+PH was unable to restore catalase and reduced glutathione to normal levels. Hence, lipid peroxidation was not reduced significantly. So, this indicates that functional foods (PDP, PH and PDP+PH) are unable to combat severe oxidative stress. Prebiotics significantly enhance survival and prolong the retention

period of probiotic inocula *in vivo*. [66] Results show that PDP was beneficial in all kinds of ulcers, whereas PH alone offered benefit only in pylorus ligation model. But, PH was observed to enhance the activity of PDP, more prominently in ulcerative colitis rather than in other ulcers. The rationale could be the production of short chain fatty acids by prebiotics and they may be probably playing major role in remission of ulcerative colitis and may not be much significant in pathogenesis of other forms of ulcers.

To conclude, PDP, PH and PDP+PH used in the present study were observed to strengthen defensive factors like mucosal resistance and increase protective antioxidant enzymes rather than suppressing offensive factors. Further investigations on how they regulate the phospholipids, influence intestinal permeability and increase n-butyrate levels are warranted. The results of the present study clearly exemplify that functional foods possess cytoprotection rather than antisecretory effect.

References

1. Witwer RS. Marketing bioactive ingredients in. food products. Food Technol 1999; 53:50–53.
2. Wlodzimierz G. Probiotics, Prebiotics and Antioxidants as functional foods. Acta Biochemica Polonica 2005; 52: 665-671.
3. Tannock GW. Probiotics. In: A critical review. (Norfolk, ed.) Horizon Scientific Press England, 1999.
4. Marcel BR. Prebiotics and Probiotics: are they functional foods? Am J of Clin Nutr 2000; 71:1682-1687.
5. Manonmani S, Viswanathan VP, Subramanian S, Govindasamy S. Biochemical studies on the antiulcerogenic activity of cauvery 100, an ayurvedic formulation in experimental ulcers. Indian J Pharmacol 1995; 27: 101-105.
6. Neelima M, Sujatha D, Bharathi K, Kumar APA, Prasad KVSRG. Evaluation of a Probiotic Diary Product for Antiulcer activity in Rats. Int J of Probiotics and Prebiotics 2007; 2: 137-140.

7. Shay M, Komarov SA, Fels D, Meranze D, Gruenstein H, Siplet H. A simple method for the uniform production of gastric ulceration in the rat. Gastroenterol 1945; 5:43-61.
8. Rao CM, Ramesh KV, Biary KL, Kulkarni DR. Zinc complexes of NSAIDs abolish gastric ulceration propensity of parent drugs. Indian drugs 1990; 28: 64-67.
9. Selye H, Szabo S. Experimental model for production of perforating duodenal ulcers by cysteamine in the rat. Nature 1973; 244:458-459.
10. Fabia RW, Ar'Rajab R, Andersson A, Ahren R, Bengmark BS. Acetic acid-induced colitis in the rat: a reproducible experimental model for acute ulcerative colitis. Eur Surg Res 1992; 24: 211-225.
11. Brain SM, John SM, Daniel TD, Henry PP, Rebecca MT, James P. Acute experimental colitis decreases colonic circular smooth muscle contractility in rats. Am J Physiol Gastrointest Liver Physiol 1997; 273: 928-936.
12. Zhou JS, Shu Q, Rutherfurd KJ, Prasad J, Gopal PK, Gill HS. Acute oral toxicity and bacterial translocation studies on potentially probiotic strains of lactic acid bacteria. Food Chem Toxicol 2000; 38: 153-161.
13. Noselove V, Bobek P, Cerne S, Galbavd L, Stvrtina S. Effects of pleuran (ß-Glucan isolated from *Pleurotus ostreatus*) on experimental colitis in rats. Physiol Res 2001; 50: 575-581.
14. Dasgupta A, Kesari KV, Ramaswamy KK, Amenta PS, Das KM. Oral administration of unmodified colonic but not small intestinal antigens protects rats from hapten-induced colitis. Clin Expl Immunol 2001; 125: 41-47.
15. Naveen T, Sangeeta P, Anurag K and Kanwaljit Chopra. Hesperidine, a citrus bioflavonoid, decreases the oxidative stress produced by carbon tetra chloride in rat liver and kidney. BMC Pharmacol 2005; 471:221-230.
16. Claiborne A. In Handbook of methods for oxygen radical research. (Boca Raton, ed.) CRC press, F.L., 1985 pp. 283-284.
17. Jollow D, Mitchell L, Zampaglione N and Gillete J. Bromobenzene induced liver necrosis: protective role of glutathione and evidence for 3, 4-bromobenzenoxide as the hepatotoxic intermediate. Pharmacol 1974; 11: 151-169.

18. Niehaus WGJr and Samuelsson B. Formation of malondialdehyde from phospholipids arachidonate during microsomal lipid peroxidation. Eur J Biochem 1968; 6: 126-30.
19. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite and [15N] nitrate in biological fluids. Anal Biochem 1982; 126: 131-138.
20. Yasushi H, Mikie S, Nobuo K. Thromboxane A2 Up-Regulates Neutrophil Elastase Release in Syrian Hamsters with Trinitrobenzene Sulfonic Acid-Induced Colitis. J Pharmacol Sci 2005; 98: 430-438.
21. Goyal Rk, Bhattacharya SK. Gastroduodenal mucosal defense and mucosal protective agents. Indian J Exp Biol 1991; 29: 701-705.
22. Srikanta BM, Siddaraju MN, Dharmesh SM. A novel phenol-bound peptic polysaccharide from *Decalepsis hamiltonii* with multi-step ulcer preventive activity. World J Gastroenterol 2007; 13: 5196-5207.
23. Fuller R. Probiotics in human medicine. Gut 1981; 32: 439-442.
24. Sarah L, Tine LAV, Monica PV, Jos V, Sigrid CJDK. Impact of environmental and genetic factors on biofilm formation by the probiotic strain *Lactobacillus rhamnosus* GG. Appl Environ Microbiol 2007; 73: 6768-6775.
25. Langlands SJ, Hopkins, Coleman N, Cummings JH. Prebiotic carbohydrates modify the mucosa associated microflora of the human large bowel. Gut 2004; 53: 1610-1616.
26. Sairam K, Rao ChV, Dora babu M, Agarwal VK, Goel RK. Antiulcerogenic activity of methanolic extract of *Emblica officianalis*. Jour of Ethnopharmacol 2002; 82: 1-9.
27. Gupta JP, Rai Y, Debnath PK, Sanyal AK. A comparative study on the gastric secretion of histamine and pentagastrine on the same Indian subjects. Asian Med Jl 1980; 23: 553-565.
28. Bart D, Rex HG. Microbial modulation of innate defense: goblet cells and the intestinal mucus layer. Am J of Clin Nutr 2001; 73: 1131-1141.

29. Halper J, Leshin LS, Lewis SJ, Li WI. Wound healing and angiogenic properties of supernatants from Lactobacillus cultures. Exp Biol Med (Maywood) 2003; 228: 1329-1337.
30. Emily KYL, L Yu, Helen PSW, William KKW, Vivian YS, Emily KKT, et al. Probiotic *Lactobacillus rhamnosus* GG enhances gastric ulcer healing in rats. Eur J of Pharmacol 2007; 565:171-179.
31. Resta-Lenert S, Barrett KE. Live probiotics protect intestinal epithelial cells from the effects of infection with enteroinvasive *Escherichia coli* (EIEC). Gut 2003; 52:988-997.
32. Szabo S, Reynolds ES, Lichtenberger LM, Haith LR, Dzau VJ. Pathogenesis of duodenal ulcer, gastric hyperacidity caused by propionitrile and cysteamine in rats. Res Commun pathol pharmacol 1997; 16: 311-323.
33. Pascaus XB, Chovet M, SoulardP, Chevalier E, Roze C, Junien JL. Effects of a new ligand, JO 17847, on cysteamine ulcers and duodenal alkaline secretion in rats. Gastroenterol 1993; 104: 427-434.
34. Lichtenberger LM, Szabo S, Reynolds ER. Gastric emptying in the rat is inhibited by the duodenal ulcerogens, cysteamine and propionitrile. Gastroenterol 1977; 73: 1072-1076.
35. Briden S, Flemstrom G, Kivilaakso E. Cysteamine and propionitrile inhibit the rise of duodenal mucosal alkaline secretion in response to luminal acid in rats. Gastroenterol 1985; 88: 295-302.
36. Mitra SK, Gopumadhavan S, Hemavathi TS, Muralidhar TS, Venkataranganna MV. Protective effect of UL-409, a herbal formulation against physical and chemical factor induced gastric and duodenal ulcers in experimental animals. J of Ethnopharmacol 1996; 52: 165-169.
37. Elliott SN, Buret A, Mc Knight W, Miller MJ, Wallace JL. Bacteria rapidly colonize and modulate healing of gastric ulcers in rats. Am J Physiol 1998; 275: 425-432.
38. Oro HS, Kolsto AB, Wenneras C, Svennerholm AM. Identification of asialo GMI as a binding structure for *Escherichia coli* colonization factor antigens. FEMS Microbiol Lett 1990; 60:289-292.

39. Servin AL, Coconnier MH. Adhesion of probiotic strains to the intestinal mucosa and interaction with pathogens. Best Pract Res Clin Gastroenterol 2003; 17: 741-754.
40. Sturm A, Dignass AU. Modulation of gastrointestinal wound repair and inflammation by phospholipids. Biochim Biophys Acta 2002; 1582: 282-288.
41. Lichtenberger LM, Graziani LA, Dial EJ, Butler BD, Hills BA. Role of surface-active phospholipids in gastric cytoprotection. Science 1983; 219: 1327-1329.
42. Szelenyi I, Engler H. Cytoprotective role of gastric surfactant in the ethanol-produced gastric mucosal injury of the rat. Pharmacol 1986; 33: 199-205.
43. Swarm RA, Ashley SW, Soybel DI, Ordway FS, Cheung LY. Protective effect of exogenous phospholipids on aspirin-induced gastric mucosal injury. Am J Surg 1987; 153: 48-53.
44. Brudnak MA. Peripheral Neuropathy: The Role of Probiotics and Other Nutrients in Neurodegenerative Disease. Townsend Letter for Doctors and Patients 2003; 241: 58-59
45. Kitohora T, Suzuki K, Asakura H, Yoshida T, Suematsu M, Watanabe M, et al. Active oxygen species generated by monocytes and polymorphonuclear cells in patients in crohn’s disease. Dig Dis Sci 1998; 33: 951-955.
46. Keshavarzian A, Morgan G, Sedghi S, Gordon JH, Doria M. Role of reactive oxygen metabolites in experimental colitis. Gut 1990; 31: 786-790.
47. Campieri M, Gionchetti P. Bacteria as the cause of ulcerative colitis. Gut 2001; 48: 132-135.
48. Karban A, Eliakim R, Brank SR. Genetics of inflammatory bowel disease. Isr Med Assoc J 2002; 4: 798-802.
49. Linskns RK, Huijsbens XV, Savelkoul PH, Wanden Broucke-Grauls CM, Meuwissen SG. The bacterial flora in inflammatory bowel disease: Current insight in pathogenesis and the influence of the antibiotics and probiotics. Scand J of Gastroenterol 2001; 234: 29-40.
50. Guarner F, Malagelada JR. Gut flora in health and disease. Lancet 2003; 361: 512-519.

51. Elson CO, Sartor RB, Tennyson GS, Riddell RH. Experimental models of inflammatory bowel disease. Gastroenterol 1995; 109: 1344-1367.
52. Malin M, Suomalainen H, Saxelin M, Isolauri E. Promotion of IgA immune response in patients with Crohn's disease by oral bacteriotherapy with *Lactobacillus GG*. Ann Nutr Metab 1996; 40:137-45.
53. Shanahan F. Inflammatory bowel disease: immunodiagnostics, immunotherapeutics and ecotherapeutics. Gastroenterol 2001; 120: 622-635.
54. Ulisse S, Gionchetti P, D'Alo S, Russo FP, Pesce I, Ricci G, et al. Expression of cytokines, inducible nitric oxide synthase, and matrix metalloproteinases in pouchitis: effects of probiotic treatment. Am J Gastroenterol 2001; 96: 2691-9.
55. Pathmakanthan S, Li CK, Cowie J, Hawkey CJ. *Lactobacillus plantarum* 299: beneficial in vitro immunomodulation in cells extracted from inflamed human colon. J Gastroenterol Hepatol 2004; 19: 166-173.
56. Ohkawara S, Furuya H, Nagashima K, Asanuma N, Hino T. Oral administration of butyrivibrio fibrisolvens, a butyrate-producing bacterium, decreases the formation of aberrant crypt foci in the colon and rectum of mice. J Nutr 2005; 135: 2878-2883.
57. Olivares M, Di´az-Ropero MP, Go´meza N, Lara-Villoslada F, Sierra S, Maldonado JA, et al. Oral administration of two probiotic strains, *Lactobacillus gasseri* CECT5714 and *Lactobacillus coryniformis* CECT5711, enhances the intestinal function of healthy adults. Int J of Food Microbiol; 2006:107 :104 – 111
58. Liong M. Roles of Probiotics and Prebiotics in Colon Cancer Prevention:Postulated Mechanisms and In-vivo Evidence. Int J Mol Sci 2008; 9: 854-863.
59. Brauns F, Kettlitz B, Arrigoni E. Resistant starch and the butyrate revolution. Trends Food Sci Technol 2002; 3: 251-261.
60. Bobin-Dubigeon X, Collin N, Grimaud JM, Robert G, Le Baut L, Petit JY. Effects of tumor necrosis factor-synthesis inhibitors on rat trinitrobenzene sulphonic acid-induced chronic colitis. Eur J Pharmacol 2001; 42: 103-110.
61. Abreu MT. The pathogenesis of inflammatory bowel disease: transplantation implications for clinicians. Curr Gastroenterol Rep 2002; 4: 481-489

62. Kruidenier L, Verspaget HW. Oxidative stress as a pathogenic factor in inflammatory bowel disease-radicals or ridiculous? Aliment Pharmacol Ther 2002; 16: 1997-2015.
63. Fridovich I. Biological effects of the superoxide radicals. Arch Biochem Biophys 1986; 247: 1-11.
64. Joshi MC, Dorababu M, Prabha T, Kumar MM, Goel RK. Effects of Pterocarpus marsupium on NIDDM-induced rat gastric ulceration and mucosal offensive and defensive factors. Int J of Pharmacol 2004; 36: 5: 296-302.
65. Das D, Bandyopadhyay D, Bhattacharya M, Banarjee RK. Hydroxyl radical is the major causative factor in stress-induced gastric ulceration. Free Radic Biol Med 1997; 23: 8-18.
66. Henriksson A, Su P, Mitchell H. Prebiotics enhance survival and prolong the retention period of specific probiotic inocula in an in vivo murine model. J of Applied Microbiol 2007; 103: 2392-2400.