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ORIGINAL ARTICLE

Efficacy of barley based probiotic food mixture in treatment of pathogenic *E.coli* induced diarrhoea in mice

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Abstract An indigenous food mixture was developed by mixing barley flour (raw and germinated), whey powder and tomato pulp in the ratio of 2:1:1:1 (w/w). The developed food (100 g) was mixed with water (500 ml) and autoclaved at 1.5 kg/cm^2 for 15 min. It was then cooled and inoculated with *Lactobacillus acidophilus* curd (5%) and incubated at 37°C for 12 h containing 10^6 cfu/ml broth. Fermented food mixture formulated from germinated barley flour maintained adequate cell viability (8.88 cfu/ml) as compared to non-germinated food mixture. To study the therapeutic effect of the food mixture, diarrhoea was induced in mice using 0.5 ml of aqueous suspension orally with the help of sterilized syringe to each of the overnight fasted mice and the mice were examined till onset of diarrhoea. The aqueous suspension was prepared by using 10 ml of six h old culture of *E. coli* cells (5×10^{11} cfu/ml) and 6 ml alkaline solution (powdered chalk (40%), colloidal kaolin (43%) and magnesium trisilicate (17%) and both were mixed in 10:6 (v/v) proportions. After induction of diarrhoea, the mice were divided in two groups, control and experimental. The control group was fed on unfermented food mixture whereas experimental group was fed on fermented food mixture. Faecal ash, nitrogen, moisture and coliform count increased while faecal lactobacilli count decreased in mice having diarrhoea. In the experimental group, which was fed on fermented food mixture, normal values were reached within 7 days of feeding but no such changes were observed in control group which was fed on unfermented food mixture. Liver and kidney showed lesions due to *E. coli* infection were significantly alleviated on feeding of probiotic food mixture.

Keywords Probiotic food mixture · *E. coli* induced diarrhoea · Faecal composition · Histopathological changes

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Introduction

Probiotics are defined as 'live microbial feed supplements' which beneficially affect the host animal by improving its microbial balance (Fuller 1992). Probiotics has long been suggested to have role in the management of diarrhoeal diseases. Intestinal diseases caused by pathogenic micro-organisms including *Shigella, Vibrio cholerae*, pathogenic *Escherichia coli* and rotavirus are the main causes of death in the developing countries (Nomoto 2005). Moreover, overuse of antibiotics has allowed the spread of nosocomial infections with antibiotic-resistant bacteria, particularly multidrug-resistant bacteria, as adverse effects (Reid et al. 2003, Reid 2005). Hence, some useful bacteria found in yoghurt, *lactobacillary* beverages and other fermented foods have been recognized as probiotic and such probiotic foods seem to have a promising future in the cure of various health problems such as lactose intolerance, hypercholesterolemia, diarrhoeal diseases etc. (Reid 2005; Gill 2006). There is enough evidence that probiotic organisms have a role in the management of antibiotic associated and eneteropathogenic *Escherichia coli* induced diarrhoea (Nomoto 2005; Sharma and Ghosh 2006). In the prophylaxis of infection, the probiotic organism may occupy the place of pathogen in the gut and alter the microenvironment, which may inhibit the growth of pathogens and thus, inhibits its deleterious effects (Sindhu and Khetarpaul 2001). If benefit with probiotics could be

demonstrated, it would be significant clinical advance as acute infectious diarrhoea is one of the most common illnesses in the world and causes up to 4 million deaths annually in preschool aged children, mostly in the developing countries (Peter 1996; Henryk et al. 2006). In the market, pure cultures of probiotic organisms viz *Saccharomyces boulardii, Lactobacillus acidophilus* are being sold by some pharmaceutical organizations at a quite high cost. These products provide only the microorganisms and no nutrients. If pure cultures of such probiotic organisms are used in the fermentation of cereal-legume blends, then such foods may not only have improved digestibility and availability of nutrients but may also have therapeutic advantages. Development of freeze dried fermented powders of coarse cereals, millets-whey powder-fruit containing probiotic organism can be encapsulated or commercialized as instant mixtures for the promotion of general health of normal infants and children and also those suffering from GIT disturbances. But very little work has been done on development and therapeutic role of cereal based probiotic fermented food mixtures. Hence, keeping these facts in mind, present paper will focus on potential beneficial effects of probiotic fermented food mixture (containing sprouted barley flour, whey powder and tomato pulp) in treatment of *E. coli* induced diarrhoea in mice.

Methods and materials

Procurement of material Barley seeds were procured from Department of Plant Breeding, Bikaner (Rajasthan), India. Whey powder was provided by Mahaan Proteins Ltd., New Delhi, India. Tomatoes were purchased from the local market in a single lot. Seedless tomato pulp was obtained by mashing and sieving the blanched tomatoes in a thick strainer. Skim milk was obtained from the Department of Animal Products Technology, CCS Haryana Agricultural University, Hisar, India.

Microbial culture The culture of probiotic micro-organism *Lactobacillus acidophilus* (NCDC-16) was purchased from the Microbial Culture Collection Centre, NDRI, Karnal, India. The stock culture of *Lactobacillus acidophilus* was added to 100 ml sterilized milk to obtain 10^6 cfu/ml, incubated at 37°C for 12 h and this inoculum (5%) was used for preparation of probiotic curd which was used further for probiotic fermentation of food mixtures.

Development of probiotic curd Fresh skimmed milk (100 ml) was autoclaved at 121°C, at 1.5 kg/cm^2 for 15 min and cooled to 40°C. Then 5 ml inoculum of *L. acidophillus* containing 10^6 cfu/ml was used in sterilized milk and fermentation was carried out at 37°C for 6 h for preparation of probiotic curd.

Preparation of raw and germinated barley flour Barley grains were cleaned thoroughly and half of the raw seeds were ground in an electric grinding mill using 1.5 mm sieve size and rest of the seeds were soaked in distilled water for 12 h at room temperature. A seed to water ratio of 1:5 (w/v) was used. The unabsorbed water was discarded. The soaked seeds were germinated in sterile petri dishes lined with wet filter paper for 24 h at 37°C with frequent spraying of water. After 24 h, the sprouts were rinsed in distilled water and then dried at 55-60°C. The dried samples of germinated seeds were ground to fine powder in an electric grinder and then stored in plastic containers for further use.

Development of food mixtures and their probiotic fermentation Freshly ground raw and germinated barley flours were mixed with tomato pulp and whey powder in the ratio 2:1:1, w/w. Addition of tomato pulp and whey powder in food mixtures provided better media for growth of organism and enhance the nutritional quality of food mixtures.

The food mixtures (100 g) were mixed with distilled water (500 ml) to obtain homogenous slurry, autoclaved at 1.5 kg/cm^2 for 15 min at 121°C, cooled and inoculated with 5% (v/v) probiotic curd, to provide 10^6 cfu/ml to the slurry incubated at 37°C for 12 h. At the end of the fermentation, fermented as well as unfermented food mixtures were used for determining pH, titratable acidity (Amerine et al. 1967)) and *L. acidophilus* count.

Animals Germ free weanling female mice were obtained from Disease Free Small Animal House, CCS Haryana Agricultural University, Hisar, India after the approval by Institutional Animal Ethics Committee (IAEC) held on 5th Oct, 2006. The mice were randomly divided in two groups. These were housed individually in cages kept in room maintained at 21±1°C with 12 h light and dark cycle.

Preparation of aqueous suspension containing E. coli for inducing diarrhea The composition of aqueous suspension containing the pathogenic micro-organisms namely 10 ml of six h old culture of *E. coli* in nutrient broth (5×10^{11} cfu/ml), 6 ml alkaline solution containing—powdered chalk (40%), colloidal kaolin (43%) and magnesium trisilicate (17%). *E. coli* suspension and the alkaline solution were mixed in 10:6 (v/v) proportions.

Induction of diarrhea To induce diarrhoea, 0.5 ml of above mentioned aqueous suspension containing *E. coli* cells and alkaline solution was given orally with the help of sterilized syringe to each of the overnight fasted mice and the mice were examined for the onset of diarrhoea till 72 h.

Feeding trial Mice having diarrhoea were divided into two groups, control and experimental, each group containing

Table 1 Effect of probiotic fermentation on pH, titratable acidity (g lactic acid/100 ml) and *Lactobacilli* count (log cfu/ml), of barley based food mixtures

Processing treatments	pH	Titratable Acidity	*Lactobacilli* count
Food mixtures			
(A) Non-germinated			
Raw mixture (control)	6.02±0.21	1.69±0.05	–
Autoclaved mixture	5.82±0.12	1.78±0.02	–
Autoclaved and fermented mixture	4.23±0.17	2.60±0.19	7.75±0.05
(B) Germinated			–
Germinated raw mixture (control)	5.14±0.52	2.00±0.13	–
Germinated autoclaved mixture	4.90±0.08	2.38±0.01	
Germinated, autoclaved and fermented mixture	3.90±0.11	3.10±0.11	8.88±0.05
CD (A)	0.44	0.24	–
CD (B)	0.55	0.32	–
CD (A x B)	0.78	0.45	3.28*

Values are mean±SE of three independent determinations

ten mice. aqueous suspension Mice in experimental group were fed with probiotic fermented barley based food mixture while the mice in control group were fed on unfermented food mixture. Feeding trial was carried out for 7 days. Food and water were given ad libitum. During this period, faecal samples of mice were collected.

Collection and analysis of faeces Faecal samples of all the mice in both control and experimental group were collected separately before onset of diarrhoea and after induction diarrhoea i.e. on 1st, 3rd, 5th and 7th day of feeding trial. The collected samples of faeces were analyzed for moisture, nitrogen and ash by employing standard methods of analysis (AOAC 1995). For estimation of sodium and potassium in faeces, the samples were wet acid-digested using a nitric acid and perchloric acid mixture [HNO_3: $HClO_4$, 5:1 (v/v)]. The amount of sodium and potassium in the digested samples were determined by flame photometer (Lindsey and Norwell 1969). The fresh faecal samples of control and experimental group were analyzed for *L. acidophilus* count using MRS medium by pour plate method.

Histopathological examination of organs At the end of the experiment, all control and experimental mice were sacrificed. Histopathological examinations were conducted and gross changes if any were recorded. A portion of liver and kidney was collected in 10% formalin for histopathological examinations. The formalin fixed tissues were

Table 2 Effect of feeding probiotic barley based food mixture on moisture, ash and nitrogen (%, dry matter basis) in faeces of mice having *E. Coli* induced diarrhoea

Days of faecal collection	Faecal moisture		't' value	Faecal ash		't' value	Faecal nitrogen		't' value
	Control	Experimental		Control	Experimental		Control	Experimental	
Before induction of diarrhea	18.21±0.14	18.30±0.21	0.37	6.7±0.07	6.60±0.17	0.14	1.38±0.02	1.37±0.03	0.53
After induction of diarrhea	33.48±0.46	33.99±0.77	0.58	9.30±0.10	9.32±0.13	0.70	1.56±0.022	1.58±0.02	1.00
Feeding trial									
Day 1	34.12±0.21	34.22±0.18	0.37	9.90±0.07	9.01±0.04	5.01	1.68±0.01	1.67±0.02	0.48
Day 3	33.47±0.28	30.25±1.44	2.19	9.76±0.18	8.43±0.09	0.35	1.68±0.03	1.56±0.02	3.25
Day 5	33.05±0.76	21.92±1.90	4.30	9.81±0.14	8.08±0.09	4.09	1.60±0.03	1.42±0.05	3.31
Day 7	33.18±0.74	18.36±1.04	7.33	9.90±0.12	7.10±0.17	3.90	1.50±0.04	1.39±0.02	2.64
CD (P<0.05)	NS	4.32		0.44	0.35		0.09	0.09	
Pooled CD (P<0.05)	3.23	3.37		0.37	0.38		0.08	0.09	

Values are mean±SD of ten Swiss mice NS = Non-significant

Table 3 Effect of feeding probiotic barley based food mixtures on sodium and potassium (mg/100 g) in faeces of mice having *E. coli* induced diarrhoea

Days of faecal collection	Faecal sodium		't' value	Faecal potassium		't' value
	Control	Experimental		Control	Experimental	
Before induction of diarrhea	86.52±1.28	88.61±1.08	0.82	281.56±1.69	281.78±5.64	0.36
After induction of diarrhoea	90.67±0.78	90.97±1.32	0.47	292.72±2.48	292.53±3.15	0.72
Feeding trial						
Day 1	90.43±1.37	90.64±0.65	0.14	292.96±3.50	292.06±0.85	0.25
Day 3	94.43±0.74	69.22±1.35	16.37	298.42±2.98	281.73±3.32	4.82
Day 5	106.00±2.51	58.72±1.78	15.35	300.12±2.56	256.42±4.60	8.30
Day 7	111.46±2.85	51.31±0.73	20.43	304.25±1.96	255.13±3.16	13.19
Pooled CD ($P<0.05$)	5.56	3.79		7.27	11.70	

Values are±SD of ten Swiss mice

thoroughly washed in running tap water for 2 h, dehydrated in ethanol, cleaned in benzene and embedded in paraffin wax (melting point 60–62°C). Sections, 3 to 5 μm in thickness, were cut and stained with haematoxyline and eosin method (Luna 1968) to observe pathological changes.

Statistical analysis The data were subjected to statistical analysis for analysis of variance in a completely randomized design according to standard methods (Panse and Sukhatme 1961).

Results and discussion

pH and titratable acidity of indigenously developed food mixtures The pH and titratable acidity of raw (non-germinated) food mixtures were 6.02 and 1.69 g lactic acid/100 ml, respectively (Table 1). After autoclaving, a significant decline in pH i.e. 5.82 was observed. Fermentation of autoclaved mixtures caused further significant decrease (4.23) in pH with a corresponding increase 2.63 g lactic acid/100 ml in titratable acidity. However, in case of germinated samples, pH again dropped significantly on germination, autoclaving and fermentation as compared to non-germinated mixtures whereas titratable acidity was simultaneously increased in all cases. The reduction in pH may be due to hydrolysis of starch into sugars during germination, which is readily utilized by the organisms and converted to lactic acid. Autoclaving of germinated mixtures caused further decrease in pH with simultaneous increase in titratable acidity. A similar trend in pH and titratable acidity in germinated mixtures and fermented mixtures was also observed by various workers in cereal legume based food mixtures (Antony et al. 1996; Sripriya et al. 1997). In all the processed food mixtures, pH had a significantly ($P<0.05$) negative correlation with titratable acidity. During fermentation, probiotic organisms convert glucose to lactic acid which is responsible for the decline in pH of the developed product. A rapid drop in pH with corresponding increase in titratable acidity has been reported in cereals and legumes (Antony et al. 1996; Sripriya et al. 1997).

Cell count of fermented food mixtures At the end of fermentation period, the growth of *L. acidophilus* was observed in fermented food mixtures. It was found that germinated + autoclaved + fermented food mixture had higher growth of lactobacilli (8.88 log cfu/ml of wet faeces) as compared to raw fermented food mixture (Table 1). This may be due to hydrolysis of germinated flours which provided better media for growth. The growth of this bacteria in the fermented samples was due to decrease in pH and increase in acidity. On the basis of higher *L. acidophilus* count, germinated + fermented barley based food mixture

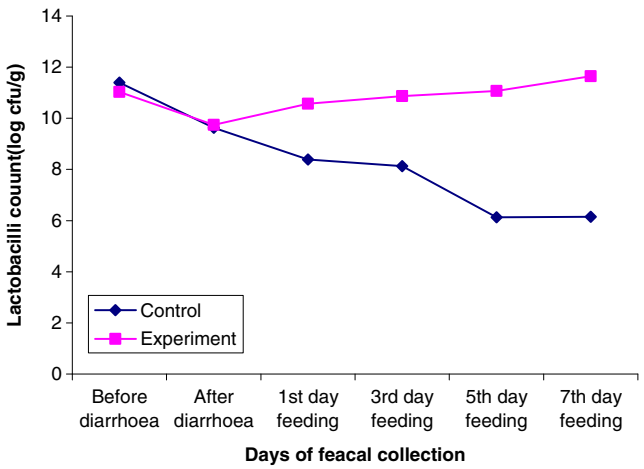

Fig. 1 Effect of feeding of probiotic based food mixture on faecal *Lactobacilli* count of mice having E. coli induced diarrhea

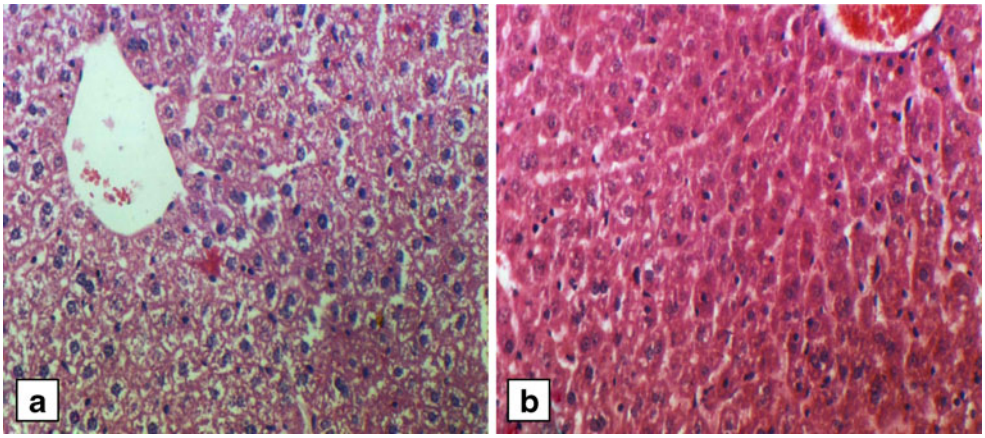

Fig. 2 **a** Liver section from experimental mice fed on fermented food mixture, showing normal liver. **b** Control mice fed on unfermented food mixture showing accumulation of leucocytes in liver (Haematoxylin and Eosin; x 250)

was used for feeding trial. Similarly, other workers also reported 8.98 to 9.34 cfu/ml cell count in food mixtures containing cereal-pulse along with tomato pulp and banana paste blends (Agte et al. 1997; Chahal 1999).

Feeding trial

Faecal moisture content Before induction of diarrhoea, faecal moisture content in both the groups was within normal range (Table 2). The faecal moisture content of both the groups of mice varied from 18.21 to 33.99%, respectively. After the induction of diarrhoea, a significant ($P<0.05$) increase in moisture content in both control and experimental group was found. Control group was fed on unfermented food mixture which had no probiotics, hence, such mixture could not check diarrhoea and the faecal moisture content did not decrease as observed during the feeding trial of 7 days. However, in experimental group fed on fermented food slurry, a declining trend was observed in faecal moisture content till the 7th day of feeding. After 7 days of feeding trial, moisture content of mice in experimental group reached almost the same level as observed prior to induction of diarrhoea.

Faecal ash content Before induction of diarrhoea, the ash content in faeces of rats was 6% and it increased significantly ($P<0.05$) by 38.81% after induction of diarrhea (Table 2). In the control group, no significant difference was observed in the faecal ash content. Ash content in the faeces of control group mice was almost same on 7th day of feeding trial as it was after induction of diarrhoea. However, in experimental group of mice fed on fermented diet, the ash content was lowered down significantly. There was a sharp decline in faecal ash content i.e. from 9.3 to 9.01, 8.43, 8.08 and 7.10 on 1st, 3rd, 5th and 7th day of the feeding trial, respectively.

Faecal nitrogen content Initial content of faecal nitrogen in control and experimental group was 1.38 and 1.37%, respectively (Table 2). After induction of diarrhoea, faecal nitrogen content increased significantly to 9.4–11.68% in control and experimental groups, respectively. This increase in faecal nitrogen content was continued upto 5th day of feeding trial in control group. However, in experimental group, a significant reduction was observed in faecal nitrogen content after 3rd day of feeding trial and it reduced even further until the 7th day after feeding. The raised level of nitrogen, ash and moisture contents in faeces might be because of the presence of mucosal lining in faeces as

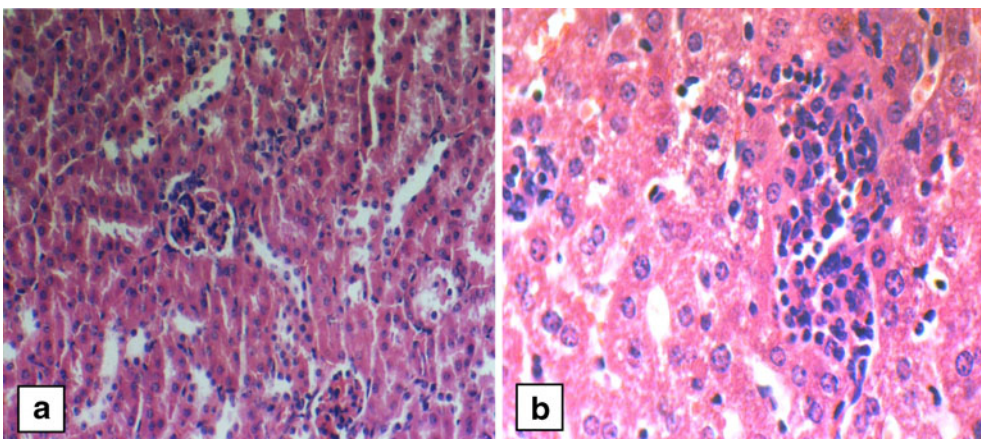

Fig. 3 **a** Kidney section from experimental mice fed on fermented food mixture, showing normal kidney. **b** Control mice fed on unfermented food mixture showing vascular degeneration and congestion of the renal tubules (Haematoxylin and Eosin; x 200)

mucosal walls are damaged during pathogen infection (Chaudhary 1998). On feeding of probiotic food mixture to mice, counts of *E. coli* decreased significantly as reported in this study and by other workers (Chaudhary 1998; Chahal 1999) thereby the *E. coli* induced diarrhoea is also checked resulting in normal excretion of moisture, nitrogen and ash contents.

Faecal sodium and potassium content Faecal sodium and potassium content were 86.52 and 281.56 mg/100 g in control and 88.61 and 281.78 mg/100 g in experimental groups of mice before the induction of diarrhoea. A significant ($P<0.05$) increase in faecal sodium and potassium content was observed in both the groups after the induction of diarrhoea. In control group, faecal excretion of sodium and potassium continued to increase significantly up to 7th day of feeding. However, in experimental group of mice who were fed on fermented diet, a significant ($P<0.05$) decrease was observed in faecal excretion of sodium and potassium (Table 3). Thus, feeding of probiotic food mixture has resulted in reduced excretion of sodium and potassium in the faeces.

Faecal microflora Faecal lactobacilli count of mice before the induction of diarrhoea was 11.4 and 11.03 log viable counts per ml of faeces in control and experimental groups, respectively (Fig. 1). After the induction of diarrhoea, faecal lactobacilli count decreased significantly in both the groups. In the control group, this declining trend continued and lactobacilli count had reached to a value of 6.16 log cfu/ml wet faeces after 7th of feeding trial. However, when mice in experimental group were fed with *L. acidophilus* fermented food slurry, log viable counts of lactobacilli increased significantly ($P<0.05$). The number of lactobacilli in the faecal matter of mice belonging to this group increased from 9.74 (after induction of diarrhoea) to 11.65 (after 7 days of feeding) log cfu/ml wet faeces. Increase in the faecal lactobacilli counts as observed in the present study may be attributed to the function of probiotics. Several studies have also reported a decrease in coliform and increase in lactobacilli count with feeding of cereal-legume-skim milk powder and banana paste blends containing lactobacilli (Gorbach 1996; Chahal 1999; Rani and Khetarpaul 1999; Mishra and Prasad 2001). The probiotic bacteria produce organic acid and hydrogen peroxide which creates unfavorable environment for the growth, survival and multiplication of pathogenic *E. coli*. It may involve secretion of substances toxic to the pathogen that are either directly inhibitory or alter the local chemical milieu. These possible mechanisms would be dependent on the ability of the probiotic to survive and colonize the gut (Blomberg et al. 1993; Henryk et al. 2006).

Histopathological examinations of organs The liver and kidney sections of mice fed on fermented and unfermented food mixtures were examined for histopathological comparisons. Liver section of mice from control group showed swelling of hepatic cells with increased granularity. The hepatic cords were disrupted with advanced degree of cloudy and fatty changes in perilobular area. Whereas in case of mice fed on fermented food mixture, it was observed that the degenerative changes in the liver initiated by *E. coli* induced diarrhoea were significantly reversed. With regard to kidney, in control group, kidney showed shrinkage of glomerular tuft resulting in increase in Bowman's space. It also resulted in vascular degeneration and congestion of the renal tubules. Whereas kidney section from the mice fed on fermented food mixture showed no vascular degeneration that might be a regeneration of the kidney parenchyma during administration of probiotic food mixture (Figs. 2 and 3). These results are in line with those reported earlier (Chaudhary 1998; Chahal 1999; Santosa et al. 2006). They also reported no abnormalities in mice after 7 days of feeding of single and sequential culture fermented cereal-legume based mixture.

Conclusion

Fermentation of food mixtures, mainly based upon barley, whey powder and tomato pulp resulted in a nutritionally superior, organoleptically and microbiologically safe product. Therefore, feeding of such fermented mixture containing live cells of probiotic organism i.e. *L. acidophilus* was found to be beneficial in controlling *E. coli* induced diarrhoea in mice. Further understanding the role of the human microflora in diarrhoeal diseases needed as well as insights in to the mechanism whereby probiotics may have beneficial effect. This may allow better selection of probiotic organism. Well conducted and controlled clinical trials may then establish the usefulness of probiotics in diarrhoeal diseases.

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References

Agte VV, Gokhale MK, Chiplonkar SA (1997) Effect of natural fermentation on in *vitro* zinc bioavailability in cereal legume mixtures. Int J Food Sci Technol 32:29–32

Amerine MA, Berg HW, Cruess WV (1967) The technology of wine making. AVI, Westport, p 692

Antony U, Sripriya G, Chandra TS (1996) Effect of fermentation on the primary nutrients in finger millet (*Eleusine coracana*). J Food Sci 44:2616–2618

AOAC (1995) Official methods of analysis of the association of official agricultural chemists. Association of Analytical Chemists, Washington DC, pp 125–129

Blomberg L, Henriksson A, Conway PL (1993) Inhibition of *Escherichia coli* K-88 to piglet ileal mucus by *Lactobacillus* spp. App Environ Microbiol 59:34–39

Chahal S (1999) Development of probiotic fermented indigenous food blends: Their nutrient composition and their therapeutic use. Ph. D. thesis. CCS Haryana Agricultural University Hisar India

Chaudhary P (1998) Probiotic fermentation of blends containing locally available food stuffs: Their nutrient composition and role in control of diarrhea. Ph.D. thesis. Haryana Agricultural University Hisar India.

Fuller R (1992) History and development of probiotics. In: Fuller R (ed) Probiotics. London, Chapman and Hall

Gill HS (2006) Probiotics to enhance anti effective defenses in gastrointestinal tract. Best Pract Res Clin Gastroenterol 17:833–848

Gorbach SL (1996) Efficacy of Lactobacilli in treatment of acute diarrhea. Nutr Today 31:195–235

Henryk S, Agnieszka C, Magdalena S, Jerzy P (2006) Colonization of the gastrointestinal tract by probiotic *L. rhamnosus* strain in acute diarrhoea in children. Diges Liver Dis 38:38–40

Lindsey WL, Norwell MA (1969) A new DPTA—TEA soil test for zinc and iron. Agro Abstr 61:61–84

Luna LG (1968) Manual of histological staining methods of armed forces institute of pathology. McGraw Hill Book Company, New York

Mishra V, Prasad DN (2001) Prebiotic foods and their applications in health management. Ind Food Ind 20:59–61

Nomoto K (2005) Review: prevention of Infections by probiotics. J Biosci Bioeng 100:583–592

Panse YG, Sukhatme PV (1961) Statistical methods of agricultural coworkers. New Delhi, Indian Council of Agricultural Research

Peter HK (1996) Probiotic control of diarrhoeal disease. Asia Pac J Clin Nutr 5:39–43

Rani B, Khetarpaul N (1999) Probiotic fermented food mixture: possible application in clinical and anti-diarrhoea usage. Nutr Health 12:97–105

Reid G (2005) The importance of guidelines in the development and application of probiotics. Curr Pharma Des 11:11–16

Reid G, Jass J, Sebulsky MT, McCormick JK (2003) Potential uses of probiotics in clinical practice. Clin Microbiol Rev 16:658–672

Sindhu SC, Khetarpaul N (2001) Probiotics: nutritional and health benefits. Life Sci Reporter 3:1–10

Santosa S, Farnworth E, Jones P (2006) Probiotics and their potential health claims. Nutr Rev 64:265–274

Sharma G, Ghosh BC (2006) Probiotic dairy foods and probiotics for health benefits. Ind Food Ind 25:68–73

Sripriya G, Usha A, Chandra TC (1997) Changes in carbohydrates, free amino acid, organic acid, phytate and HCl-extractability of minerals during germination and fermentation of finger millet (*Eleusine coracana*). Food Chem 58:345–350