A COMPARATIVE EVALUATION OF FAECAL B-GLUCURONIDASE ACTIVITY OF ADULT ALBINO MICE FED WITH PROBIOTIC (*BIFIDOBACTERIUM ANIMALIS* SUBSP. *LACTIS* B420), PREBIOTIC (INULIN) AND THEIR COMBINATION (SYNBIOTIC) *

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Abstract

A study was conducted to assess the effect of Bifidobacterium animalis subsp. lactis B420, inulin or their combinations on the activity of β- glucuronidase, a procarcinogenic enzyme, in the faeces of adult albino mice. Compared to control group, a statistically significant reduction (P<0.05) in the faecal β-glucuronidase activity was observed in all the three treatment groups after 10 days of administration of feed supplements and this effect was persistent throughout the treatment period. In view of their ability to reduce the faecal β-glucuronidase activity, it is suggested that administration of Bifidobacterium animalis subsp. lactis B420, inulin or their combinations can be potential options in reducing colon cancer risk.

Keywords: *Probiotics, prebiotics, synbiotics, β-glucuronidase activity.*

The gut microflora and their metabolism have a strong influence on the etiology of colorectal cancer -one of the major health problems in the world. As scientific evidences are found to link the occurrence of colon cancer to the imbalance of intestinal microbiota, dietary options like probiotics, prebiotics or synbiotics which modulate intestinal microbiota can be effective options, reducing the risk of colon cancer. Studies show that bacterial enzymes like β -glucuronidase, β-glucosidase, azoreductase and nitroreductase play an important role in cancer development as they hydrolyse pre-carcinogenic compounds to carcinogens. Of these β -glucuronidase, a commonly considered marker for procarcinogenic activity (Gadelle et al., 1985) is important in initiating colon cancer due to its wide substrate specificity and ability to hydrolyse different glucuronides. The present study was carried out to evaluate the effect of probiotic organism (*Bifidobacterium animalis subsp. lactis B420*), prebiotic substrate (inulin) and a synbiotic combination (i.e. inulin in combination with *Bifidobacterium animalis subsp. lactis B420*) on the faecal β -glucuronidase activity in adult albino mice.

Materials and Methods

Prebiotic and Probiotic

The prebiotic inulin was procured from Orafti, Belgium. The probiotic culture, *Bifidobacterium animalis* subsp. lactis B420 (B-420, Danisco, Germany) was maintained in

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modified MRS broth (MRS broth supplemented with 0.05% L-cysteine hydrochloride: mMRS, Arroyo *et al*., 1994). Subculturing was done weekly. In between transfers, cultures were maintained under refrigeration. For feeding trials, the culture (2 %; v/v) was inoculated into mMRS and incubated at 37°C for 48 h under anaerobic conditions The cells were harvested by centrifugation at 12000 rpm for 10 min at 4 °C and mixed with the basal diet in order to get a count of 10^8 live cells of bifidobacteria/g feed

Feeding trial

The feeding trial was conducted in 6-7 weeks old adult male albino mice with an average body weight of 31g. The animals obtained from Small Animal House, National Dairy Research Institute, Karnal, Haryana, India were kept in plastic cages under conventional conditions and was given basal diet (crushed wheat-15%, crushed bengal grams-57.6%, crushed groundnut cake - 4%, skim milk powder- 5%, casein-4%, salts mixture=4%, vitamins-0.2%, choline chloride mixture-0.2%) and water *ad libitum*. The study was conducted with the approval of institutional ethics committee.

After assigning the mice to four groups of seven each, group 2, 3 and 4 were given probiotics, prebiotics and synbiotics respectively. Group 1 served as the control. The study period of 30 days consisted of 5 days of pre-treatment period, 15 days of treatment period and 10 days of post-treatment period. The pre-treatment period was intended to acclimatize the animals to the study conditions and the post-treatment period to assess the sustainability of favourable changes, if any. The feeding regime followed in the study is detailed in table. Faecal samples were collected by gently squeezing the rectal area of the mice and analyzed for β-glucuronidase activity at 10 days intervals.

Determination of β-Glucuronidase Activity

The substrate used for the assay was p-nitrophenyl-β-D-glucuronide. The release of p-nitrophenol from the substrate was assessed to determine the faecal β-glucuronidase activity. For this fresh faecal samples were suspended in 10 ml buffer (0.1 mol Na_2HPO_4/L, 0.15 mol NaCl/L, pH 7.4) and sonicated in an ice bath. This solution was then filtered through Whatman No.1 filter paper and the filtrate was used for β-glucuronidase(Marteau *et al*., 1990) and protein determination (Lowry *et al*., 1951). Two ml of the appropriately diluted sample was then combined with 1 ml of 0.13 mol/L of the substrate p-nitrophenyl β-D-glucuronide (Sigma-Aldrich, USA) prepared in the above mentioned buffer. The reaction which was allowed to proceed at 37°C was stopped after 15 min by the addition of 1 ml of cold 1 mol Na_2CO_3/L solution. After centrifugation for 10 min at 4000 X g and 4°C, the absorbance of the supernatant was read at 405 nm. The blank was prepared in the same way as the sample except that it contained 1 ml of buffer instead of the substrate solution. The amount of p-nitrophenol released was determined from the standard p-nitrophenol curve. The enzyme activity was expressed as specific activity (µmol/mg protein per 30 min).

Data were statistically analysed using ANOVA according to the General Linear Models procedure of Systat Version 6.0.1 (1996, SPSS Inc.). When significant (1 and 5% levels) differences were observed, individual values were compared by Fisher's Least Significant difference test.

Results and Discussion

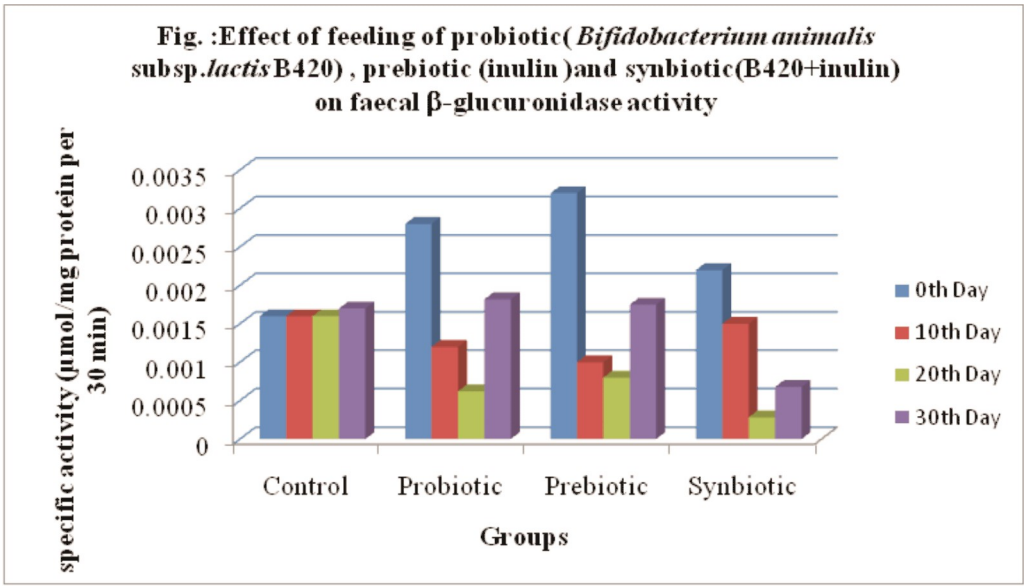
In this study, when compared to the control a significant reduction in the faecal

Table: Overview of the substrates administered per day during the 30 days study period in the different groups

Group	Pre - Treatment period (5days)	Treatment period(15days)	Post-treatment period(10days)
Control	Basal diet	Basal diet	Basal diet
Probiotic	Basal diet	Basal diet +10^8 live cells of B-420 /g feed	Basal diet
Prebiotic	Basal diet	Basal diet + inulin (5% w/w feed)	Basal diet
Synbiotic	Basal diet	Basal diet + 10^8 live cells of B- 420 /g feed + inulin (5% w/w feed)	Basal diet

β-glucuronidase activity was evident in all the three treatment groups after 10 days of administration of feed supplements and this effect was persistent throughout the treatment period (P<0.05) as seen in figure. In control group, the β-glucuronidase activity remained almost in the same level throughout the study. No significant difference was observed among probiotic, prebiotic and synbiotic treatments in terms of their ability to reduce the faecal β-glucuronidase activity (P<0.05). Despite the random assignment of mice, variation was observed in the baseline values of faecal β-glucuronidase activity of the different groups. On noticing this variation, the faecal β-glucuronidase activity of all the groups were reassessed on the 5th day of experiment period, i.e. the last day of pre-feeding period and then also the same trend was observed. Such variations in baseline values (0th day value) of β-glucuronidase activity are difficult to be explained, but have been reported in other studies also (Goossens *et al*., 2003, deMoreno de LeBlank and Perdigon 2005, De Preter *et al*., 2008). Regardless of the variation observed in the baseline values, the faecal β-glucuronidase activity of all the treatment groups were lower than their corresponding baseline values during the feeding period. During the post-treatment period also, the β-glucuronidase activity of all the three treatment groups remained below their corresponding baseline values, though there was a tendency to revert to the initial values.

β -glucuronidase is an inducible enzyme involved in the release of a number of dietary carcinogens from their conjugated form in the colon i.e. conversion of pro-carcinogens to carcinogens in the colon. Among the intestinal microflora the highest activity of β–glucuronidase is shown by *Escherichia coli* and species belonging to the genera *Clostridium, Bacteroides, Ruminococcus, Peptostreptococcus, Staphylococcus* and *Eubacterium (*deMoreno de LeBlank and Perdigon 2005). Presence of bacterial strains with high β–glucuronidase activity in the large intestine may be a risk factor leading to the formation of tumor (De Preter *et al*., 2008). In the present study, a significant reduction in β–glucuronidase activity could be observed upon administration of the probiotic *Bifidobacterium animalis* subsp. lactis B420, the prebiotic-inulin and their combination. These findings are in line with those of De Preter *et al*., (2008) and Hijova *et a*l., (2011) who also reported reduced β–glucuronidase activity upon intake of bifidobacteria or prebiotics. Alterations in the metabolic activities of intestinal bacteria, increase in the number of bacteria having low β–glucuronidase activity and reduction in the number of bacteria involved in pro-carcinogenic and mutagenic pathways are proposed as the possible mechanisms for these food related modifications in host enzymes (Fotiadis *et al*., 2008, Uccello *et al*., 2012). Findings of the current study are supportive of this proposal as the decrease in β–glucuronidase activity observed



in this study was concurrent with an increase in bifidobacterial population and a decrease in coliform and clostridial counts (data not shown). So it can be inferred that the reduction in faecal β–glucuronidase upon administration of *Bifidobacterium animalis* subsp. lactis B420, and/ or inulin could be due to the increase in the population of *Bifidobacterium* species which possesses comparatively low β–glucuronidase activity than other major anaerobes in the gut (Mital and Garg, 1995, Saito *et al*., 1992). *Bifidobacterium animalis* subsp.lactis B420 has been reported to impart anticarcinogenic effects through counteracting the genotoxic potential of faecal water, a factor considered to play a potential role in induction of colorectal cancer (Burns and Rowland, 2004). The genotoxic potency of faecal water could be attributed to the presence of carcinogens generated by faecal enzymes. So it is presumed that reduction in the β–glucuronidase activity might be one of the reasons behind the antigenotoxic potential exhibited by *Bifidobacterium animalis* subsp. lactis B420.

Another important observation made in the present study was that no significant difference was observed between the treatments, probiotic, prebiotic and synbiotic in their ability to reduce the faecal β -glucuronidase activity. These findings are in line with those of De Preter *et al*., (2008) who also could not observe any extra beneficial effect on combined administration of the probiotic and prebiotic compared to individual administration. So it is concluded that the administration of prebiotic alone was sufficient to elicit a similar effect as that of probiotic. This option is much more feasible from a technological point of view as it takes care of a number of problems pertaining to administration of probiotic cultures in viable form and in sufficient dose. In this study it was also observed that the faecal β -glucuronidase activity remained lower than the baseline values even after the cessation of treatment. Thus it could be postulated that the feed supplements administered in the present study were capable of sustaining their effect on β -glucuronidase activity.

In conclusion, the probiotic *Bifidobacterium animalis* subsp. lactis B420 was able to significantly reduce the faecal β -glucuronidase activity of adult albino mice. Considering the species as well as strain specificity associated with probiotic attributes, the ability of *Bifidobacterium animalis* subsp. lactis B420 to reduce the activity of the procarcinogenic enzyme β -glucuronidase calls for further research to elucidate the anticarcinogenic potential of this probiotic strain. Inulin, the prebiotic used in this study was also able to exhibit similar effects as that of the probiotic used, suggesting the possibility of exploiting the potential of this prebiotic to suppress the activity of carcinogen-metabolizing enzymes as a means of reducing the risk of colon cancer. Synbiotics being synergistic combinations of both probiotics and prebiotics are expected to provide improved effects than either of its components. However contrary to this expectation,the synbiotic combination used in our study did not appear to be more beneficial than either compound alone.

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