

## EXPERIMENTAL STUDY

## Prebiotic foodstuffs and their health benefits in experiment

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**Abstract:** *Objectives:* The aim of the present experimental study was to evaluate the effect of prebiotic on chemically induced carcinogenesis in rats fed with high fat diet (HF).

*Methods:* Wistar albino rats were divided into control and experimental groups. In experimental group rats were treated with prebiotic at the dose of 2 % of HF diet. N,N-dimethylhydrazine (DMH) injections were applied in dose 20 mg/kg b.w., two times at week interval. In the end of eight weeks experimental period blood samples and feces were taken from the rats and used for laboratory analysis.

*Results:* Treatment with prebiotic inulin significantly ( $p < 0.001$ ) decreased enzymatic activity of bacterial enzymes in feces. Similar tendency was noticed in concentration of bile acids and lipid parameters. Prebiotic undergo fermentation in the colon and enhanced short chain fatty acid production.

*Conclusion:* Prebiotics may have potential health implications for protection against colon cancer (Tab. 2, Fig. 1, Ref. 14). Full Text (Free, PDF) [www.bmj.sk](http://www.bmj.sk).

**Key words:** colon cancer, prebiotics, bacterial enzymes, bile acids, lipid parameters.

Colon cancer is one of the most common forms of malignant tumours in humans, and its incidence is increasing. Cancer deaths will continue to rise with an estimated 9 million people dying from cancer in 2015, and 11.4 million dying in 2030 according to World Health Statistics 2008 (World Health Statistics 2008). Diet makes an important contribution to colon cancer risk, which implies the risks of colon cancer are potentially reducible. Prebiotics are generally defined as nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth or activity of health-promoting lactobacilli and bifidobacteria. Prebiotics can complement probiotic functions and synbiotics as combination of prebiotic and probiotic might represent a novel therapeutic or preventive agents (Rafter et al, 2007; Trafalska and Grzybowski, 2006; Geier et al, 2006).

This experimental work was designed to investigate the efficacy of prebiotic inulin on the activity of bacterial enzymes, concentration of bile acids, short chain fatty acids (SCFA), total cholesterol and triacylglycerols in rats with dimethylhydrazine induced colon cancer, also taking into consideration high intake of dietary fat as risk factor.

**Material and methods**

*Animals.* Wistar albino rats ( $n=24$ , (Central vivarium, Medical Faculty, P.J. Šafárik University, Košice, Slovak Republic, six months old with mean body weight  $387.50 \pm 69.90$  g in control group and with mean body weight  $350.83 \pm 32.64$  g in experimental group were housed in plastic cages with wire tops and maintained at 22 °C, on a 12 light/dark cycle, according to the principles provided in the Law No. 289/2003 and 489/2003 of Slovak Republic for the Care and Use of Laboratory Animals. Animals were fed with high fat diet (HF) containing 10 % of fat (Biofer, SR) as the diet of some western populations at risk for colon cancer supplied with drinking water respectively, *ad libitum*. Food and drinking consumption were monitored daily.

*Treatments.* In experimental group rats were treated with prebiotic BeneoSynergy 1 (ORAFIT, Tienen, Belgium) at a dose of 2 % of HF diet. BeneoSynergy 1 (PRE) is an oligofructose-enriched inulin preparation. It is a commercialized food ingredient composed of a mixture of long chain inulin and short chain oligofructose. Inulin is a natural food ingredient that is extracted from the chicory root with hot water. It is a linear  $\beta(2-1)$ -linked fructan with a degree of polymerization (DP) ranging from 3 to 65. Inulin chains with a DP of 2–8 (average DP:4) are oligofructoses, which are highly soluble in water (>80 %, by wt) and are rapidly fermented. The chains with a DP>12 (average DP: 25) are hardly soluble in water (<5 % in water at room temperature) and are slowly fermented. Both fractions are produced on a commercial scale as food ingredients worldwide. It was shown that a mixture of the 2 fractions is physiologically more efficacious than are the individual compounds (Van Loo 2004). The product contains 95 % fructan chains and 5 % monosaccharides and dis-

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**Tab. 1. Changes activity of bacterial enzymes in rats.**

Bacterial enzymes	Control group	Experimental group
$\alpha$ -GAL	2.00 $\pm$ 0.26	1.32 $\pm$ 0.42
$\beta$ -GAL	3.92 $\pm$ 0.80	1.92 $\pm$ 0.37 ***
$\beta$ -GLUCUR	4.25 $\pm$ 0.52	0.83 $\pm$ 0.52 ***
$\alpha$ -GLU	3.83 $\pm$ 0.68	1.83 $\pm$ 0.26 ***
$\beta$ -GLU	2.08 $\pm$ 0.80	1.83 $\pm$ 0.26

Data are expressed as means $\pm$ SD. Significant differences calculated to control group are designated as \*\*\*  $p$ <0.001

accharides (fructose, glucose, and sucrose). Besides carbohydrates, the product contains 5 % humidity.

Two weeks after beginning the diets, rats were treated with N,N dimethylhydrazine (DMH, Merck, DE), at a dose of 20 mg/kg s.c., two times at week interval, dietary treatments were continued for the entire experiment. In the end of eight weeks experimental period rats were anaesthetized (Ketamin 100 mg/kg + Xylazin 15 mg/kg b.w., i.p.) and blood samples were taken from heart by puncture.

**Laboratory analysis.** Samples were centrifuged at 2500 G for 15 min and serum specimens were used for determination of bile acids concentration with commercial kit (Trinity Biotech, Ireland), and lipid parameters with commercial kits Biolatest (CZ). The measurement was carried out on an automatic spectrophotometric analyser Cobas Mira S (Roche, Switzerland). Freshly collected faeces samples were examined for enzymatic activity of bacterial enzymes- $\alpha$ -galactosidase ( $\alpha$ -GAL),  $\beta$ -galactosidase ( $\beta$ -GAL),  $\beta$ -glucuronidase ( $\beta$ -GLUCUR),  $\alpha$ -glucosidase ( $\alpha$ -GLU),  $\beta$ -glucosidase ( $\beta$ -GLU) using an API-ZYM kit (Biomérieux, France). Activities were determined according to the manufacturer's instructions and expressed on scale of 0 (negative reaction) to 5 (maximum activity). The SCFA were analyzed in the colon contents using gas chromatography Hewlett Packard (USA). The colonic pH was measured using pH meter kit with pH electrode SP 1DT (Merck, DE).

**Statistical analysis.** Statistical analysis was performed by Student's t-test and analysis of variance (ANOVA) to determine significance. Statistical significance was accepted at  $p$ <0.05.

## Results

During experimental period the mean body weight of rats in the control group (CG) was increased by 2.1 % (395.83 $\pm$ 74.40 g) and by 2.8 % (360.83 $\pm$ 47.19 g) in the experimental group (EG). Food consumption was changed in relationship to body weight of rats. All rats were killed six weeks after the first DMH injection. The control group represented group with the highest risk for development of colon cancer (cumulative effect of HF diet and DMH) as well as control group against group with applied prebiotic. Changes in activity of bacterial enzymes are summarized in Table 1. Inulin treatment significantly decreased ( $p$ <0.001) activity of bacterial enzymes  $\beta$ -GAL,  $\beta$ -GLUCUR, and  $\alpha$ -GLU as compared to the control group. The colonic pH in experimental group against control group was significantly decreased (pH

**Tab. 2. Changes of bile acids and SCFA concentration during experiment.**

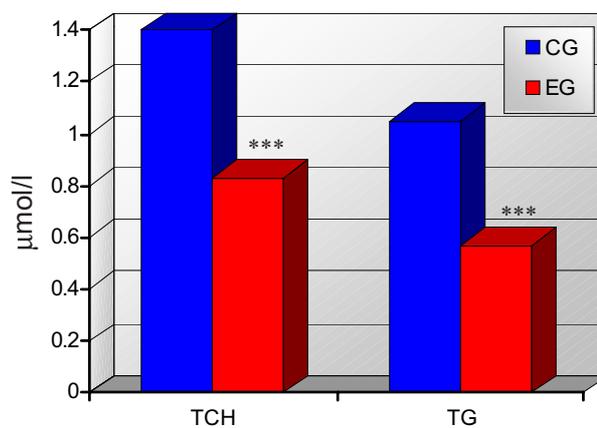
Parameters	Control group	Experimental group
Bile acids ( $\mu$ mol/l)	16.84 $\pm$ 6.33	11.72 $\pm$ 4.22 **
Acetic acid (mmol/100 ml)	11.45 $\pm$ 1.98	11.76 $\pm$ 1.67
Propionic acid (mmol/100 ml)	2.77 $\pm$ 0.35	2.76 $\pm$ 0.57
Butyric acid (mmol/100 ml)	2.41 $\pm$ 0.46	2.91 $\pm$ 0.62 **

Data are expressed as means $\pm$ SD. Significant differences calculated to control group are designated as \*\*  $p$ <0.01

(CG)=6.29 $\pm$ 0.14 vs pH (EG)=6.04 $\pm$ 0.11;  $p$ <0.001). Similar tendency decreasing was in concentrations of total cholesterol and triacylglycerols (Fig. 1). The concentration of short chain fatty acids (butyric, acetic, and propionic acids) and bile acids are shown in Table 2.

## Discussion

Prebiotics is a term coined by Professor Gibson and Dr. Roberfroid in 1995. The word is generally defined as consisting of nondigestible food fibers which stimulate the growth and activity of certain bacteria in the intestines. Therefore, in order to be effective, a prebiotic must escape digestion in the upper gastrointestinal tract so that it can be released in the lower tract and used by beneficial microorganisms in the colon, mainly bifidobacteria and lactobacilli. Unlike probiotic bacteria, prebiotic carbohydrates are not destroyed when cooked. Prebiotic carbohydrates are found naturally in such fruit and vegetables as bananas, berries, asparagus, garlic, wheat, oatmeal, barley (and other whole grains), flaxseed, tomatoes, Jerusalem artichoke, onions and chicory, greens (especially dandelion greens, spinach, kale, mustard greens, and other), and legumes (lentils, kidney beans, white beans, black beans, peas). The various oligosaccharides



**Fig. 1. The effect of inulin on lipid parameters. Data are expressed as means $\pm$ SD. Significant differences calculated to control group are designated as: \*\*\*  $p$ <0.001, CG – control group, EG – experimental group.**

classified as prebiotic and added to processed foods and supplements include Fiber gums, Fructo-oligosaccharides (FOS), Inulins, Isomalto-oligosaccharides, Lactilol, Lactosucrose, Lactulose, Oligofructose, Pyrodextrins, Soy oligosaccharides, Transgalacto-oligosaccharides (TOS), and Xylo-oligosaccharides.

Inulins are a group of non-digestible oligosaccharides belonging to a class of carbohydrates known as fructans. Inulin-producing plant species are found in chicory, onions, leeks, garlic, bananas, asparagus and artichokes. However, only chicory (*Cichorium intybus*) and Jerusalem artichokes (*Helianthus tuberosus*) are used to produce Inulin commercially. Oligofructose is a sweet product derived from native inulin, consists mainly of fructose and is also available as a mixture with inulin. Unlike inulin, oligofructose has the ability to brown, making it a valuable addition to baked products.

Dietary carbohydrates escaping digestion/absorption in the small bowel and prebiotics undergo fermentation in the colon and enhance short chain fatty acids production (Hijova and Chmelarova, 2007). Of these, butyrate and propionate inhibit growth of colon tumour cells and histone deacetylases. Butyrate also causes apoptosis, reduces metastasis in colon cell lines, and protects from genotoxic carcinogens. The elevated butyric acid and acetic acid concentrations, and decreased concentrations of total cholesterol and triacylglycerol during experimental period are in accordance with research in experimental animal models which revealed that inulin-type fructans have anticarcinogenic properties (Pool-Zobel and Sauer, 2007), hypolipidaemic effect (Beylot 2005), and anti-atherogenic effects (Rault-Nania et al, 2006). The human intervention study (SYNCAN project) provided experimental evidence that inulin modulates parameters of colon cancer risks in humans colon cells (Van-Loo et al, 2005).

Although epidemiological and experimental studies indicate an association of elevated fecal levels of secondary bile acids as well as total bile acids with high risk of colon cancer development, the cellular mechanism for the actions of bile acids is not clear (Cheng and Raufman, 2005; Hagiwara 2006). The bile acids concentration in the control group was  $16.84 \pm 6.33 \mu\text{mol/l}$ . Supplementation with prebiotic significantly ( $p < 0.01$ ) decreased the bile acids concentration to  $11.72 \pm 4.22 \mu\text{mol/l}$ .

Elevated activity of bacterial enzymes is associated with an increased risk for various cancer. The enzymes are produced by colonic microflora and involved in phase II liver detoxification during which toxins are conjugated with glucuronic acid in the liver by glucuronidation and excreted through the enterohepatic circulation allowing the toxin to be more easily excreted from the body. The activity of these enzymes with toxicological importance could be altered by the diet, ultimately results in potentially decreasing the risk of carcinogenesis (Nalini et al, 2004; Manju and Nalini, 2006). Supplementary ingestion of prebiotic inulin significantly ( $p < 0.001$ ) decreased the enzymatic activity of bacterial enzymes  $\beta$ -GAL,  $\beta$ -GLUCUR, and  $\alpha$ -GLU, probably resulted in increasing excretion of conjugated xenobiotic compounds and decreasing activity of harmful substances that are the most active in their deconjugated state. The activities of  $\alpha$ -GAL and  $\beta$ -GLU were decreased nonsignificantly.

## Conclusion

Dietary habits have been associated with aetiology and prevention of civilizing diseases that represent the most serious health, economic, and social problem. For these reasons, worldwide, the interest in using the ecological methods of prevention and therapy using the substances of biotechnological and natural origin has been increasing. Among potentially protective foods, growing attention should be devoted to prebiotics which have health benefits. We could find that prebiotic substances in many foods including yogurts, cereals, breads, biscuits, milk dessert, nutrition bars, ice-creams, drinks, water and infant foods when will be adopted by the commercial industry.

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