# Effect of horse chestnut and inulin as single supplements or in combination on chemically induced colon cancer in rats

V. Szabadosova, E. Hijova, L. Strojny, R. Salaj, A. Bomba, D. Cokasova, B. Pramukova, J. Zofcakova, I. Bertkova, A. Supukova, A. Soltesova, A. Brandeburova, A. Chmelarova

Pavol Jozef Safarik University in Kosice, Kosice, Slovak Republic

**ABSTRACT**: Natural bioactive supplements have been extensively studied as preventive agents. The aim of this study was to investigate the efficacy of inulin enriched with oligofructose and *Hyppocastani extractum siccum* as preventive agents of experimentally induced colon carcinogenesis in rats. Colon carcinogenesis was induced by N,N dimethylhydrazine (DMH) in a dose of 21mg/kg body weight *s.c.*, five times at weekly intervals. Sprague Dawley rats ($n$ = 45) were divided into a control group without DMH; control group with injected DMH; group receiving inulin and injected DMH; group receiving *Hyppocastani extractum siccum* and injected DMH; group receiving inulin and *Hyppocastani extractum siccum* and injected DMH. The beneficial effects of natural compounds were determined by analysis of caecal parameters such as pH, composition of microflora, activity of bacterial glycolytic enzymes and production of short-chain fatty acids (SCFA). The counts of coliforms were decreased in the groups receiving inulin enriched with oligofructose ($P < 0.01$), *Hyppocastani extractum siccum* ($P < 0.001$) and the combination of these supplements ($P < 0.001$). The counts of lactobacilli were significantly increased in all experimental groups receiving natural compounds ($P < 0.01$; $P < 0.001$). Experimental groups receiving natural compound alone and in combination resulted in a significant decrease in the activity of β-glucuronidase ($P < 0.01$; $P < 0.001$). Administration of inulin and *Hyppocastani extractum siccum* separately significantly increased the concentration of SCFA compared to the control group with DMH. The achieved results indicate the beneficial effect of prebiotics and plant extracts on metabolic processes in the colon and suggest that they could exert a preventive effect on colon carcinogenesis induced by DMH.

**Keywords**: carcinogenesis; prebiotics; plant extract; prevention

**List of abbreviations**

DMH = N, N dimethylhydrazine; SCFA = short chain fatty acid; CRC = colorectal cancer; α-GAL = α-galactosidase; β-GAL = β-galactosidase; β-GLUCUR = β-glucuronidase; α-GLU - α-glucosidase; β-GLU - β-glucosidase

Globally, colorectal cancer (CRC) is the fourth most common cancer in men and the third most common cancer in women. CRC is a disease with a high incidence and mortality rate and has increasing prevalence worldwide (Ferlay et al. 2007). Therefore, it is very important to investigate various prevention strategies. Colon cancer is a well-studied cancer, but the progress in the field of preventing or curing this disease has not been significant. While there are chemotherapeutic drugs available for the treatment of this disease the majority of patients do not respond to these drugs and side effects remain problematic. Therefore, emphasis has been focused on a variety of clinical and basic studies of

Partially supported by the Agency of the Slovak Ministry of Education for the Structural Funds of the EU, Project ITMS: No. 26220220104 (60%) and by the Scientific Grant Agency of the Ministry of Education of Slovak Republic and the Academy of Sciences, Grant VEGA No. 1/0372/10 (40%).

chemoprevention using naturally occurring dietary substances, since they might provide useful strategies to inhibit colon cancer with minimal toxicity.

Herbal-based dietary supplements contain a large array of phytochemicals with polyphenolic constituents (flavonoids) which might mediate physiological functions related to cancer suppression. Although evidence from epidemiological and animal studies suggests that increased consumption of a plant-based diet can reduce the risk of colon cancer, bioactive cancer preventives remain to be identified (Steinmetz and Potter 1991; Caragay 1992). Horse chestnut (*Aesculus hippocastanum*) extract is widely used in the pharmaceutical and cosmetic industries. Aescin is the medical constituent of *Aesculus hippocastanum* seeds, a mixture of triterpenoid saponin glycosides (Sirtori 2001). A number of other products have been isolated from chestnut seeds, i.e., coumarin derivates (aesculin, fraxin, scopolin), flavonoids, essential oils (oleic acid, linoleic acid) and tanins (leucocyanidine, proanthocyanidin A2) (Bombardelli et al. 1996). The extract of horse chestnut shows beneficial effects in the treatment of chronic venous insufficiency, oedema and haemorrhoids and may possess anti-inflammatory, anti-hyaluronidase, anti-histamine, chemopreventive, anti-proliferative, anti-angiogenic and apoptotic effects (Patlolla et al. 2006; Niu et al. 2008; Mojzisova et al. 2012).

Prebiotics are food ingredients that selectively stimulate the growth and activity of specific species of bacteria in the gut, usually bifidobacteria and lactobacilli, with benefits to health (Gibson and Roberfroid 1995). For a dietary substrate to be classified as a prebiotic, the substrate must not be hydrolysed or absorbed in the stomach or small intestine, but must be selectively fermented by beneficial commensal bacteria in the colon such as bifidobacteria. The fermentation of the substrate should induce beneficial luminal/systemic effects within the host (Manning and Gibson 2004). Well established prebiotic compounds include inulin and oligofructose or fructooligosaccharides, galactooligosaccharides and lactulose. Prebiotics have been postulated to be protective against the development of colon cancer through a variety of mechanisms. These include promoting a favourable colonic microflora by modifying its metabolic activities and composition; increasing the production of short chain fatty acids; dilution and adsorption of potential faecal carcinogens; decreasing the exposure period of colonic epithelial cells to carcinogens, co-carcinogens or promoters through reduction of contact time between intraluminal contents and the colonic mucosa; modification in bile acid metabolism by binding potential carcinogens like bile acids (Limburg and Kolars 2004; Grau et al. 2006; Hersenyi et al. 2008; Pearson 2009).

The colonic microflora has been suggested to have a critical role in setting the tone for a healthy bowel including the risk of developing colorectal cancer (O'Keefe 2008). Key physiological functions that might be related to cancer risk include control of epithelial cell proliferation and differentiation, production of essential nutrients and/or bioactive food components, prevention of overgrowth of pathogenic organisms, and stimulation of intestinal immunity (Tappenden and Deutsch 2007). Specific strains of bacteria have been implicated in the pathogenesis of cancer, including *Bacteroides, Clostridia* and *Helicobacter pylori* (Nakamura et al. 2002; Peek and Blaser 2002). Conversely, some strains of bacteria, including *Lactobacillus acidophilus* and *Bifidobacterium longum*, have been shown to inhibit experimentally induced colon tumour development (Rowland et al. 1996; McCintosh et al. 1999). Therefore it is important to maintain the balance between harmful and beneficial bacteria and thus to influence carcinogen bioactivation and reduce cancer risk.

## MATERIAL AND METHODS

**Animals and diets**. Male and female four month old Sprague Dawley rats ($n$ = 45), (Central vivarium, Medical Faculty, P.J. Safarik University, Kosice, Slovak Republic), with a mean body weight of 335.80 ± 15.20 g were housed in plastic cages with wire tops and maintained at 22 °C ± 1–2 °C, on a 12:12 h light-dark schedule according to the principles stipulated by Law No. 23/2009 of the Slovak Republic for the Care and Use of Laboratory Animals. The experiment was carried out from May to October over six months. The rats were randomly divided into experimental groups. Nine rats were included in each experimental group, five males and four females.

Group 1 (G1) = control group without application of DMH; Group 2 (G2) = control group with application of DMH; Group 3 (G3) = experimental group receiving prebiotics, application of DMH; Group 4 (G4) = experimental group receiving plant extract, application of DMH; Group 5 (G5) = ex-

Table 1. Composition of conventional diet

| Ingredients | Amount of ingredients |
|---|---|
| Dry matter | 880 g/kg |
| Crude fat | 3.5% |
| Nitrogenous-compounds | 180 g/kg |
| Lysine | 6 g/kg |
| Calcium | 15 g/kg |
| Phosphorus | 8 g/kg |
| Sodium | 2 g/kg |
| Vitamin A | 8000 IU/kg |
| Vitamin B1 | 4 mg/kg |
| Vitamin B2 | 4 mg/kg |
| Vitamin E | 65 mg/kg |

perimental group receiving prebiotics and plant extract, application of DMH.

All animals were fed a conventional laboratory MP diet (Table 1) (Biofer, Slovak Republic). Experimental diets and water were available *ad libitum*. Food intake and body weight was monitored weekly. BeneoSynergy 1 was used as a prebiotic in a dose 80 g/kg of feed. BeneoSynergy 1 is an oligofructose-enriched inulin preparation. It is a commercialized food ingredient composed of a mixture of long-chain inulin and short-chain oligofructose. The product contains 95% fructan chains and 5% monosaccharide and disaccharide (fructose, glucose and sucrose). Besides carbohydrates, the product consists of 5% humidity. *Hyppocastani extractum siccum* (Calendula, Slovak republic) was used as a nutritional plant supplement in a dose 40 g/kg of feed. Most of the beneficial effects of *Aesculus hippocastanum* L., (Hippocastanaceae) commonly known as horse chestnut are attributed to its main component aescin.

Two weeks after we had started feeding animals the experimental diet, experimental groups of rats (G2, G3, G4, G5) were treated with N,N dimethylhydrazine (DMH, Merck Germany) in a dose of 21mg/kg b.w. *s.c.*, five times at weekly intervals and always at the same time of 3 p.m. Six months after the beginning of the experiment rats were anaesthetized *i.p.* using ketamine 100 mg/kg and xylazine 15 mg/kg b.w.

**Bacterial enumeration**. Fresh samples of digesta from cecum were collected; pH was measured and then placed into sterile polyethylene Stomacher Lab Blender bags with sterile diluents of Ringer's solution (9 ml) and homogenised in a Stomacher 400 Bag Mixer (France). Each digesta homogenate in Ringer's solution was serially diluted from $10^{-2}$ to $10^{-8}$. Dilutions were subsequently plated on selective agar media for enumeration of target bacterial groups. Coliforms were enumerated using McConkey agar (Merck, Germany) and *Lactobacillus* spp. using Rogosa agar (Biocar Diagnostics, France). Plates for lactobacilli were then incubated at 37 °C for 48 h anaerobically. Anaerobic incubation was achieved using a Gas pack system (Gas Pack, USA). Plates for coliforms were incubated aerobically at 37 °C for 24 h. Colonies were counted and results were expressed as the log 10 of colony-forming units per gram of caecal digesta (CFU/g).

**Microbial glycolytic activity**. Microbial glycolitic activities of α-galactosidase (α-GAL), β-galactosidase (β-GAL), β-glucuronidase (β-GLUCUR), α-glucosidase (α-GLU) and β-glucosidase (β-GLU) were determined in the caecal digesta homogenate supernatants (10 000 × g for 15 min at 4 °C), through the rate of release of p-nitrophenol from the respective *p*-nitrophenylglucoside substrates (5mM), namely α-galactoside, β-galactoside, β-glucuronide, α-glucoside and β-glucoside, via absorbance measurement at 400 nm as described by Juskiewicz et al. (2002). Enzymatic activity was expressed as μmol product formed per min (IU) per g digesta in the sample.

**Short chain fatty acid concentration**. The levels of acetic, propionic and butyric acids were determined from caecal digesta using capillary isotachophoresis (ITP) as described by Kastel et al. (2007). $10^{-2}$ mol/l HCl + $2.2 \times 10^{-2}$ mol/l ε-aminocapronic acid + 0.1 methylhydroxyethylcellulose acid (MHEC) was used as the leading electrolyte and $5 \times 10^{-3}$ mol/l capronic acid as the terminating electrolyte.

**Statistical analysis**. The experimental data were analysed using a two-tailed independent sample *t*-test and multifactor analysis of variance (ANOVA). The data are expressed as means ± SD.

## RESULTS

The mean body weight of the rats at the beginning of the experiment was 335.80 ± 15.20 g and at the end of the experiment this increased to 395.30 ± 26.61 g. The tendency of changes in the body weight of the rats was similar in all experimental groups and did not depend on the administered diet.

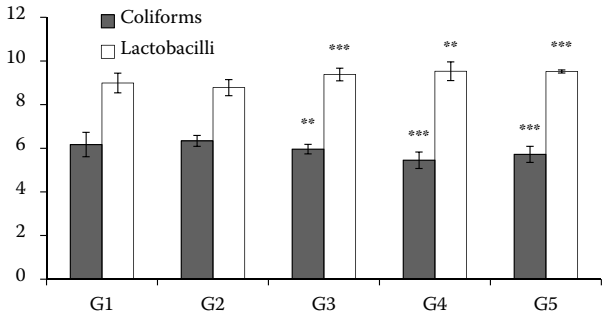


Figure 1. Differences in the counts (mean ± SD) of lactobacilli and coliforms between control and experimental groups expressed as the log 10 of CFU/g of faeces

G1 = control group without application of DMH
G2 = control group with application of DMH
G3 = experimental group receiving prebiotics, application of DMH
G4 = experimental group receiving plant extract, application of DMH
G5 = experimental group receiving prebiotics and plant extract, application of DMH
Statistical significance: G1 and G2: not significant; G2 and G3, G4, G5: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$

Conventional microbiological techniques using selective agar media were used to analyse counts of coliforms and lactobacilli in caecal digesta samples. Counts of individual bacterial populations found in experimental and control animals are presented in Figure 1. No significant differences were observed in counts of coliforms in G1 (6.17 ± 0.56 $\log_{10}$ CFU/g) and G2 (6.34 ± 0.25 $\log_{10}$ CFU/g). In the caecal digesta of animals receiving oligofructose-enriched inulin (5.96 ± 0.22 $\log_{10}$ CFU/g, $P < 0.01$), the plant extract of *Aesculus hippocastanum L.* (5.45 ± 0.38 $\log_{10}$ CFU/g, $P < 0.001$) and in the group receiving a combination of these compounds (5.72 ± 0.37 $\log_{10}$ CFU/g, $P < 0.001$), counts of coliforms were significantly decreased compared to the control. In contrast to the counts of coliforms, the counts of lactobacilli in experimental groups significantly increased relative to the control groups. Control groups G1 and G2 did not differ significantly in counts of lactobacilli. On the other hand, significantly higher counts of lactobacilli were determined in groups receiving prebiotics (9.38 ± 0.29 $\log_{10}$ CFU/g, $P < 0.01$), plant extract (9.53 ± 0.43 $\log_{10}$ CFU/g, $P < 0.01$) and a combination of these compounds (9.52 ± 0.07 $\log_{10}$ CFU/g, $P < 0.001$) compared to the G2.

Colonic pH (Figure 2) in control group G2 was higher (6.53 ± 0.13, $P < 0.05$) than in control group G1 (6.36 ± 0.16). The lowest pH value in caecal digesta was observed in group G3 (6.27 ± 0.29, $P < 0.05$). Compared to the control group G2, the pH value was significantly increased in group G4 (6.80 ± 0.19, $P < 0.01$) and G5 (6.74 ± 0.9, $P < 0.001$).

The modulatory effect of dietary supplements on the gut microflora may be revealed by the analysis of bacterial glycolytic enzyme activities in the colon. Changes in microbial glycolytic enzyme activity are summarised in Table 2. Administration of DMH in G2 resulted in a significant decrease in activity of enzymes α-GAL ($P < 0.01$) and β-GLU ($P < 0.01$). On the other hand, enzyme activity of β-GLUCUR (non-significantly) and α-GLU ($P < 0.01$) increased in G2 compared to G1. In group G3 significantly increased α-GAL activity ($P < 0.01$) and decreased β-GLUCUR ($P < 0.01$), β-GAL, α- and β-GLU activity (non-significant) were observed. Administration of plant extract significantly decreased the activity

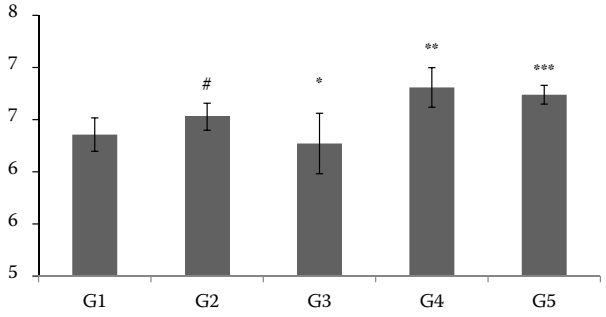


Figure 2. Differences in pH value (mean ± SD) of cecal digesta of rats between control and experimental groups
For explanation see Figure 1
Statistical significance: G1 and G2: $^{\#}P < 0.05$; G2 and G3, G4, G5: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$

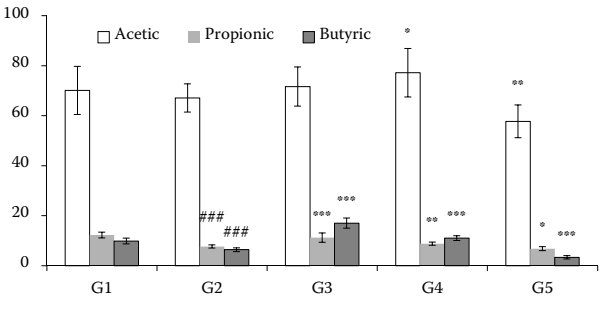


Figure 3. Differences in concentration (mean ± SD) of short chain fatty acids (mmol/l) in cecal digesta of rats between control and experimental groups
For explanation see Figure 1
Statistical significance: G1 and G2: $^{\#\#\#}P < 0.001$; G2 and G3, G4, G5: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$

Table 2. Activity of bacterial glycolytic enzymes (**α**-, β-galactosidase, β-glucuronidase, **α**-, β-glucosidase) in cecal digesta of rats

| | α-GAL | β-GAL | β-GLUCUR | α-GLU | β-GLU |
|---|---|---|---|---|---|
| G1 | 0.21 ± 0.07 | 0.07 ± 0.02 | 0.28 ± 0.11 | 0.08 ± 0.04 | 0.08 ± 0.02 |
| G2 | 0.12 ± 0.07$^{\#\#}$ | 0.07 ± 0.04 | 0.38 ± 0.16 | 0.15 ± 0.06$^{\#\#}$ | 0.04 ± 0.02$^{\#\#}$ |
| G3 | 0.31 ± 0.13** | 0.08 ± 0.03 | 0.18 ± 0.09** | 0.11 ± 0.06 | 0.03 ± 0.02 |
| G4 | 0.03 ± 0.02** | 0.04 ± 0.01 | 0.02 ± 0.01*** | 0.03 ± 0.02*** | 0.05 ± 0.03 |
| G5 | 0.05 ± 0.03* | 0.03 ± 0.01** | 0.03 ± 0.01*** | 0.03 ± 0.01*** | 0.02 ± 0.01** |

G1 = control group without application of DMH; G2 = control group with application of DMH; G3 = experimental group receiving prebiotics, application of DMH; G4 = experimental group receiving plant extract, application of DMH; G5 = experimental group receiving prebiotics and plant extract, application of DMH
α-, β-GAL = α-, β-galactosidase; β-GLUCUR = β-glucuronidase; α-, β-GLU = α-, β-glucosidase
Statistical significance: G1 and G2: $^{\#\#}$ $P < 0.01$; G2 and G3, G4, G5: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

of α-GAL ($P < 0.01$), β-GLUCUR ($P < 0.001$) and α-GLU ($P < 0.001$). No significant differences were observed in the activities of β-GAL and β-GLU. Also, the activity of bacterial glycolytic enzymes in the group receiving the mixture of prebiotic and plant extract was lower than in control groups.

The concentration of SCFA (Figure 3) was lower in G2 compared to G1. Administration of prebiotics and plant extract separately significantly increased the concentration of SCFA but the combination of substances resulted in a significant decrease in SCFA compared to the G2. The highest concentration of acetic acid was measured in the group G4 ($P < 0.05$) and the lowest in G5 ($P < 0.01$). The concentration of propionic acid was highest in group G3 ($P < 0.001$) and lowest in G5 ($P < 0.05$); the concentration of butyric acid was highest in G3 ($P < 0.001$) and lowest in G5 ($P < 0.001$).

## DISCUSSION

An important focus in the field of nutritional sciences is dietary modulation of the human gut. The human gut is intensively populated by an array of bacterial species, which develop important metabolic and immune functions. The presence and metabolic activities of this specific bacterial community play an important role in maintaining the host´s overall health and well-being and have a critical role in setting the tone for a healthy bowel and in determining the risk of developing colorectal cancer. Prebiotics have been postulated to be protective against the development of colon cancer through the production of protective metabolites and enhancing the saccharolytic activity in the colon, while reducing proteolysis. The prebiotic concept is based on the selective stimulation of the host´s own beneficial microflora by providing specific substrates for their growth and metabolism. The effect is measured by using bifidobacteria or lactobacilli as markers. Lactobacilli are believed to maintain and restore normal intestinal balance and play an important role in retarding colon carcinogenesis by increasing the acidity of the gastrointestinal environment, destroying toxic substances and producing antimicrobial compounds (Brady et al. 2000; Pietro Femia et al. 2002). Several dietary components, other than complex carbohydrates, may also modulate the microflora of the gastrointestinal tract. Phytochemicals and their derived products can also affect the intestinal ecology as a significant part of them are not fully absorbed and are metabolised in the liver, excreted through the bile as glucuronides and then accumulate in the ileal and colorectal lumen (Bazzocco et al. 2008). It was shown that several polyphenols, such as caffeic acid, catechin, epicatechin, coumaric acid, phloridzin, rutin, naringenin, daidzein, genistein and quercetin, inhibit the growth and adhesion of bacterial pathogens to human Caco-2 cells and enhance the proliferation and adhesion of probiotic bacteria *L. rhamnosus* (Parkar et al. 2008). The consumption of flavonol-rich foods has been shown to modify the composition of the gut microbiota, exerting prebiotic-like effects (Tzonuis et al. 2008). Tea phenolics have shown to inhibit the growth of *Bacteroides* spp., *Clostridium* spp. (*C. perfringens* and *C. difficile*), *E. coli* and *Salmonella typhimurium* (Lee et al. 2006). Our results confirm the above-mentioned findings in that oligofructose-enriched inulin and horse chestnut extract were

able to enhance the growth of lactobacilli and inhibit the growth of coliforms.

The activity of α-glucosidase contributes to starch fermentation in the gut (Bielecka et al. 2002). The activity of β-glucosidase contributes to the fermentation of NSP-glucose of cellulose and β-glucan origin. An increase in β-glucosidase activity could potentially be regarded as beneficial to health due to the hydrolysis products of plant glycosides, some of which have more antimutagenic, antioxidative, anticarcinogenic and immune stimulatory properties than the respective glycosides (Pool-Zobel et al. 2002). However, it is also possible for β-glucosidase to be involved in the formation of toxic aglycons from plant glycosides. Therefore, its relevance for animal and human health will also depend upon the nature of dietary plant glycosides (Mountzouris et al. 2006). α-Galactosidase contributes to the hydrolysis of dietary α-galactosides such as rafinose, stachyose and other oligosaccharide components of feedstuffs. β-Galactosidase contributes to the hydrolysis of β-galactosides as in the case of some prebiotics and lactose (Djouzi and Andrieux 1997). Bacterial α- and β-galactosidase are mainly produced by bifidobacteria and lactobacilli (Lay et al. 2004). β-Glucuronidase is an inducible enzyme produced by anaerobic *E. coli*, *Peptostreptococcus*, *Bacteroides* and *Clostridia*. Increased activity of this enzyme has been implicated in increased enterohepatic recirculation of toxins, drugs and carcinogens, and also β-glucuronidase hydrolyses glucuronic acid conjugates of heterocyclic amines and forms reactive metabolites, which can damage the colonic mucosal cells (Humbolt et al. 2004). In our results, experimentally induced carcinogenesis decreased the activity of galactosidases and glucosidases, and increased the activity of β-glucuronidase, which led to higher amounts of toxic compounds. Supplementary ingestion of prebiotic inulin increased the activity of α- and β-galactosidase, which could be attributed to the increased levels of *Lactobacillus* spp. and decreased levels of coliforms. Also, prebiotic supplementation significantly decreased the faecal activity of β-glucuronidase, a carcinogen metabolising enzyme, compared to control animals. The same results were described by (Nalini et al. 2004; Manju and Nalini 2006; De Preter et al. 2008). It has been shown that some polyphenols may also influence bacterial metabolising enzymes and thus influence overall cancer risk. For example, administration of resveratrol at a dose of 8 mg/kg body weight/day intragastrically significantly reduced the activities of faecal and host colonic mucosal enzymes, such as β-glucuronidase, β-glucosidase, β-galactosidase, mucinase and nitroreductase compared to control animals (Sengottuvelan and Nalini 2006). In this work administration of plant extract significantly decreased the activity of the enzymes α-galactosidase, β-glucuronidase and β-glucosidase. The same results were obtained in a study, in which rats were fed with grapefruit polyphenols: the activities of bacterial β-glucosidase and β- and α-galactosidases were observed to be lowest among all dietary treatments (Zdunczyk et al. 2006). The mechanisms underlying plant extract-dependent alterations in bacterial enzymes are not currently known.

The protective effects of inulin-type fructans may stem from higher levels of other faecal components that are more related to cancer prevention, such as SCFA. Short-chain fatty acids are organic acids produced within the intestinal lumen by bacterial fermentation of mainly undigested dietary carbohydrates, but also in a minor part by dietary and endogenous proteins, such as mucous, and sloughed epithelial cells. Three main short chain fatty acids have been determined to present in the caecal digesta – acetic, propionic and butyric acid. The molar ratios of acetate to propionate to butyrate varied between 48 : 29 : 23 and 70 : 15 : 15, respectively, with mean values of approximately 60 : 20 : 20 (Topping and Clifton 2001). One of the proposed beneficial effects of butyrate on human intestinal health is the prevention and inhibition of colon carcinogenesis. The exact underlying mechanisms of action have not yet been clarified, but it is considered to be because of its regulation of gene expression, which is often attributed to its inhibition of histone deacetylases (Gibson et al. 1999; Daly and Shirazi-Beechey 2006). However, butyrate has other intracellular activities, including hyper-acetylation of non-histone proteins, alteration of DNA methylation, selective inhibition of histone phosphorylation and modulation of intracellular kinase signalling (Daly and Shirazi-Beechey 2006). These effects may underlie the ability of butyrate to modulate gene expression and have an impact on key regulators of apoptosis and cell cycle (Hinnebusch et al. 2002). Propionic acid is mainly investigated in the context of ruminant physiology in general, and for its role in liver physiology and metabolism in particular. In contrast to butyrate, the majority of the propionic acid produced in the colon is absorbed, passes through the colonocytes and the viscera, and drains into the portal vein.

Around 90% of the propionic acid is metabolised by the liver and the rest is transported into the peripheral blood (Wong et al. 2006). Among the specific effects of SCFAs is the lowering of caecal/colonic pH, a change that is thought to protect against undesirable changes through reducing the bioavailability of toxic amines (Topping and Clifton 2001). In our study, increased concentrations of SCFA were observed in the group fed with prebiotics and plant extract alone. The diet with prebiotics decreased caecal pH which is the result of increased production of SCFA from carbohydrate fermentation. The treatment with prebiotics caused the highest increase in butyrate, a compound which has been demonstrated to have anti-tumour effects (Bhatnagar et al. 2009). The diet with plant extract alone and in combination with prebiotics increased the caecal pH compared to the control group. This was also shown in an experiment, in which supplementation with grapefruit polyphenols significantly increased the pH of caecal digesta (Zdunczyk et al. 2006). Increased levels of SCFA were observed in the group with plant extract alone, but the combination of prebiotics with plant extract had the opposite effect. The experiments reported by Zdunczyk et al. (2006) suggested a negligible influence of grapefruit polyphenols on the caecal SCFA in the case of simultaneous dietary inulin addition. However, addition of grapefruit extract to a diet containing 5% inulin significantly decreased the butyrate concentration.

This study demonstrates that beneficial aspects of the consumption of prebiotics and plant extract lie in improving colonic ecology by decreasing faecal pH, increasing the relative proportions of lactobacilli, and reducing the proportion of coliforms in caecal digesta. However, confirmatory studies in humans should be carried out. Furthermore, it needs to be established whether these changes confer a health benefit on the host.

## REFERENCES

Bazzocco S, Mattila I, Guyot S, Renard CM, Aura AM (2008): Factors affecting the conversion of apple polyphenols to phenolic acids and fruit matrix to short-chain fatty acids by human faecal microbiota in vitro. European Journal of Nutrition 47, 442–452.

Bhatnagar N, Li X, Chen Y, Zhou X, Garrett SH, Guo B (2009): 3,3'-Diindolylmethane enhances the efficacy of butyrate in colon cancer prevention through down-regulation of survivin. Cancer Prevention Research (Philadelphia) 2, 581–589.

Bielecka M, Biedrzycka E, Majkowska A, Juskiewicz J, Wroblewska M (2002): Effect of non-digestible oligosaccharides on gut microecosystem in rats. Food Reviews International 35, 139–144.

Bombardelli E, Morazzoni P, Griffini A (1996): Aesculus hippocastanum L. Fitoterapia 21, 437–447.

Brady LJ, Gallaher DD, Busta FK (2000): The role of probiotic cultures in the prevention of colon cancer. Journal of Nutrition 13, 410–414.

Caragay AB (1992): Cancer-preventive food and ingredients. Food Technology 46, 65–68.

Daly K, Shirazi-Beechey SP (2006): Microarray analysis of butyrate regulated genes in colonic epithelial cells. DNA and Cell Biology 25, 49–62.

De Preter V, Raemen H, Cloetens L, Houben E, Rutgeerts P, Verbeke K (2008): Effect of dietary intervention with different pre- and probiotics on intestinal bacterial enzyme activities. European Journal of Clinical Nutrition 62, 225–231.

Djouzi Z, Andrieux C (1997): Compared effects of three oligosaccharides on metabolism of intestinal microflora in rats inoculated with a human faecal flora. British Journal of Nutrition 78, 313–324.

Ferlay J, Autier P, Boniol M, Heanue M, Colombet M, Boyle P (2007): Estimates of the cancer incidence and mortality in Europe in 2006. Annals of Oncology 18, 581–592.

Gibson, GR, Roberfroid, MB (1995): Dietary modulation of human colonic microbiota: introducing the concept of prebiotics. Journal of Nutrition 125, 1401–1412.

Gibson PR, Rosella O, Wilson AJ, Mariadason JM, Rickard K, Byron K, Barkla DH (1999): Colonic epithelial cell activation and the paradoxical effects of butyrate. Carcinogenesis 20, 539–544.

Grau MV, Rees JR, Baron JA (2006): Chemoprevention in gastrointestinal cancers: current status. Basic Clinical Pharmacology and Toxicology 98, 281–287.

Hersenyi L, Farinati F, Miheller P, Tulassay Z (2008): Chemoprevention of colorectal cancer: feasibility in everyday practice? European Journal of Cancer Prevention 17, 502–514.

Hinnebusch BF, Meng S, Wu JT, Archer SY, Hodin RA (2002): The effect of short-chain fatty acids on human colon cancer cell phenotype are associated with histone hyperacetylation. Journal of Nutrition 132, 1012–1017.

Humbolt C, Lhoste E, Knasmuller S, Gloux K, Bruneau A, Bensaada M, Duaro J, Rabot S, Andrieux C, Kassie F (2004): Protective effect of Brussels sprouts, oligosaccharides and fermented milk towards 2-amino-3-methylimidazol[4,5-f]quinolone (IQ)-induced

genotoxicity in the human flora associated F344 rat: role of xenobiotic metabolizing enzymes and intestinal microflora. Journal of Chromatography B – Analytical Technologies in the Biomedical Life Sciences 802, 231–237.

Juskiewicz J, Zdunczyk Z, Wroblewska M, Oszmianski J, Hernandez T (2002): The response of rats to feeding with diets containing grapefruit flavonoid extract. Food Reviews International 35, 201–205.

Kastel R, Bomba A, Vasko L, Trebunova A, Mach P (2007): The effect of probiotics potentiated with polyunsaturated fatty acids on the digestive tract of germfree piglets. Veterinarni Medicina 52, 63–68.

Lay C, Sutren M, Lepercq P, Juste C, Rigottier-Gois L, Lhoste E, Lemee R, Le Ruyet P, Dore J, Andrieux C (2004): Influence of Camembert consumption on the composition and metabolism of intestinal microbiota: A study in human microbiota-associated rats. British Journal of Nutrition 92, 429–438.

Lee HC, Jenner AM, Low CS, Lee YK (2006): Effect of tea phenolics and their aromatic fecal bacterial metabolites on intestinal microbiota. Research in Microbiology 157, 876–884.

Limburg PJ, Kolars JC (2004): Colorectal cancer chemoprevention. Chinese Journal of Digestive Disorders 5, 7–11.

Manju V, Nalini N (2006): Effect of ginger on bacterial enzymes in 1,2-dimethylhydrazine induced experimental colon carcinogenesis. European Journal of Cancer Prevention 15, 377–383.

Manning TS, Gibson GR (2004): Microbial-gut interactions in health and disease. Prebiotics. Best Practice and Research in Clinical Gastroenterology 18, 287–298.

McCintosh GH, Royale PJ, Playne MJ (1999): A probiotic strain of L. acidophilus reduces DMH-induced large intestinal tumors in male Sprague-Dawley rats. Nutrition and Cancer 35, 153–159.

Mojzisova G, Mojzis J, Pilatova M, Varinska L, Ivanova L, Strojny L, Richnavsky J (2012): Antiproliferative and antiangiogenic properties of Horse Chestnut extract. Phytotherapy Research 27, 159–165.

Mountzouris KC, Balaskas Ch, Fava F, Tuohy KM, Gibson GR, Fegeros K (2006): Profiling of composition and metabolic activities of the colonic microflora of growing pigs fed diets supplemented with prebiotic oligosaccharides. Anaerobe 12, 178–185.

Nakamura J, Kubota Y, Miyaoka M, Saitoh T, Mizuno F, Benno Y (2002): Comparison of four microbial enzymes in Clostridia and Bacteroides isolated from human feces. Microbiology and Immunology 46, 487–490.

Nalini N, Manju V, Menon VP (2004): Effect of coconut cake on the bacterial enzymes activity in 1,2 dimethylhydrazine induced colon cancer. Clinica Chimica Acta 342, 203–210.

Niu YP, Li LD, Wu LM (2008): Beta-aescin: A potent natural inhibitor of proliferation and inducer of apoptosis in human chronic myeloid leukemia K562 cells in vitro. Leukemia and Lymphoma 29, 1–8.

O'Keefe SJ (2008): Nutrition and colonic health: the critical role of the microbiota. Current Opinion in Gastroenterology 24, 51–58.

Parkar SG, Stevenson DE, Skinner MA (2008): The potential influence of fruit polyphenols on colonic microflora and human gut health. International Journal of Food Microbiology 124, 295–298.

Patlolla JM, Raju J, Swamy MV, Rao CV (2006): Beta-escin inhibits colonic aberrant crypt foci formation in rats and regulates the cell cycle growth growth by inducing p21(waf/cip1) in colon cancer cell. Molecular Cancer Therapeutics 5, 1459–1466.

Pearson JR, Gill CI, Rowland IR (2009): Diet, fecal water, and colon cancer – development of a biomarker. Nutrition Reviews 67, 509–526.

Peek RM, Blaser MJ (2002): Helicobacter pylori and gastrointestinal tract adenocarcinomas. Nature Reviews Cancer 2, 28–37.

Pietro Femia A, Luceri C, Dolara P, Giannini A, Biggeri A, Salvadori M, Clune Y, Collins KJ, Paglierani M, Cardeni G (2002): Antitumorigenic activity of the prebiotic inulin enriched with oligofructose in combination with the probiotics Lactobacillus rhamnosus and Bifidobacterium lactis on azoxymethane induced colon carcinogenesis in rats. Carcinogenesis 23, 1953–1960.

Pool-Zobel B, Van Loo J, Rowland I, Roberfroid MB (2002): Experimental evidences on the potential of prebiotic fructansto reduce the risk of colon cancer. British Journal of Nutrition 87, 273–281.

Rowland IR, Bearne CA, Fischer R, Pool-Zobel BL (1996): The effect oflactulose on DNA damage induced by DMH in the colon of human flora-associated rats. Nutrition and Cancer 26, 37–47.

Sengottuvelan M, Nalini N (2006): Dietary supplementation of resveratrol suppresses colonic tumour incidence in 1,2-dimethylhydrazine-treated rats by modulating biotransforming enzymes and aberrant crypt foci development. British Journal of Nutrition 96, 145–153.

Sirtori CR (2001): Aescin: Pharmacology, pharmacokinetics and therapeutic profile. Pharmacological Research 44, 183–193.

Steinmetz KA, Potter JD (1991): Vegetables, fruit, and cancer. II Mechanisms. Cancer Causes and Control 2, 427–442.

Tappenden KA, Deutsch AS (2007): The physiological relevance of the intestinal microbiota-contributions

to human health. Journal of American College of Nutrition 26, 679–683.

Topping DL, Clifton PM (2001): Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. Physiological Reviews 81, 1031–1064.

Tzonuis X, Vulevic J, Kuhnle GG, George T, Leonczak J, Gibson GR, Kwik-Uribe C, Spencer J P (2008): Flavanol monomer-induced changes to the human faecal microflora. British Journal of Nutrition 99, 782–792.

Wong JM, de Souza R, Kendall CW, Emam A, Jenkins DJ (2006): Colonic health: fermentation and short chain fatty acids. Journal of Clinical Gastroenterology 40, 235–243.

Zdunczyk Z, Juskiewicz J, Estrella I (2006): Cecal parameters of rats fed diets containing grapefruit polyphenols and inulin as single supplements or in combination. Nutrition, 22, 898–904.

Received: 2012–04–11
Accepted after corrections: 2013–09–20

Corresponding Author:

Viktoria Szabadosova, Pavol Jozef Safarik University in Kosice, Institute of Experimental medicine, road SNP 1, 040 11 Kosice, Slovak Republic

E-mail: viktoria.szabados@gmail.com