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Effectiveness of Bioactive Food Components in Experimental Colon Carcinogenesis

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Abstract

The aim of the present study was the evaluation of possible protective effects of selected bioactive food components in experimental N,N-dimethylhydrazine (DMH)-induced colon carcinogenesis.

Wistar albino rats (n = 92) were fed a high fat diet or conventional laboratory diet. Two weeks after the beginning of the trial, DMH injections were given to six groups of rats at the dose of 20 mg/kg b.w. twice weekly. The activity of bacterial enzymes in faeces and serum bile acid concentrations were determined.

High fat diet, DMH injections, and their combination significantly increased the activies of ß-galactosidase, ß-glucuronidase, and α-glucosidase ($p < 0.001$) compared to the control group of rats. Treatment with the prebiotic inulin, Hyppocastani extractum siccum and Lini oleum virginale significantly decreased the activity of ß-galactosidase, ß-glucuronidase, and α-glucosidase ($p < 0.001$), as well as the bile acid concentration compared to the group at the highest risk.

The protective effects of selected bioactive food components in experimentally induced colon carcinogenesis allow for their possible use in cancer prevention or treatment.

Colon cancer, prevention, prebiotic, Horse chestnut, flaxseed oil, rats

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 the World Health Statistics (2008).

Current treatments of colon cancer including chemotherapy, radiotherapy, and surgery are all asociated with a high risk of complications and are not always successful, highlighting the need to develop new treatment strategies. Many factors have been found to be associated with development of colon cancer, such as environmental pollution and life style (low physical activity, smoking, alcohol consumption, low consumption of fruits and vegetables and high meat consumption). Diet and nutrition play a major role in the aetiology and prevention of chronic civilization diseases. Prebiotics, probiotics, synbiotics as their combination, and potentiated probiotics as biopreparations containing production strains of microorganisms and synergistically acting components of biotechnological and natural origin may be a feasible chemoprevention of colon cancer in humans and present a novel therapeutic or preventive option (Rafter et al. 2007; Bomba et al. 2006; Trafalska and Grzybowski 2006; Geier et al. 2006).

This experimental work was designed to investigate the efficacy of prebiotics, Hyppocastani extractum siccum as a nutritional plant extract, and Lini oleum virginale on the activity of bacterial enzymes and bile acid concentration in rats with colon cancer induced by dimethylhydrazine. In addition to chemically induced colon cancer as one of the risk factors for the development of colon cancer and other diseases of civilization we recognized high intake of dietary fat.

Materials and Methods

Male and female Wistar albino rats (n = 92), (Central vivarium, Medical Faculty, P.J. Šafárik University, Košice, Slovak Republic), six months old with a mean body weight of 376.51 ± 18.61 g were housed in plastic

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cages with wire tops and maintained at 22 ºC ± 1-2 ºC, on a 12:12 h light-dark schedule according to the principles stipulated by the Law No. 289/2003 and 489/2003 of the Slovak Republic on the Care and Use of Laboratory Animals. The experiment was carried out from February to April over 8 weeks. The rats were randomly divided into experimental groups presented in Table 1.

Table 1. Experimental groups and treatment

Group	N	Diet	DMH	Treatment
1	8	Conventional diet	-	-
2	12	High fat diet	-	-
3	12	Conventional diet	+	-
4	12	High fat diet	+	-
5	12	High fat diet	+	PRE + HES
6	12	High fat diet	+	PRE
7	12	High fat diet	+	HES
8	12	High fat diet	+	Lini oleum virginale

PRE – prebiotic, HES - Hypocastani extractum siccum

Animals were fed a conventional laboratory MP diet with 2.5% of fat, (Top Dovo, Dobrá voda, Slovak Republic), and high fat diet (HF) containing 10% of fat (Biofer, Slovak Republic) as does the diet of some western populations at risk for colon cancer. Drinking water was provided *ad libitum*. Food and water intake was monitored daily.

The treatment consisted of prebiotics: Hyppocastani extractum siccum and Lini oleum virginale were administered separately or in combination. We administered the prebiotic BeneoSynergy 1 (ORAFTI, 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 extracted with hot water from the chicory root. It is a linear ß(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, highly soluble in water (> 80%, by wt) and 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 two fractions is physiologically more efficacious than the individual compounds (Van Loo 2004). The product contains 95% fructan chains and 5% monosaccharides and disaccharides (fructose, glucose, and sucrose). Besides carbohydrates, the product contains 5% humidity.

Hyppocastani extractum siccum (Calendula, Slovak Republic) as nutritional plant supplement was administered at a dose of 1% of HF diet. Most of the beneficial effects of the extract of *Aesculus hippocastanum* L., (Hippocastanaceae) commonly known as Horse chestnut are attributed to its principal component beta-escin or aescin. Lini oleum virginale (Dr. Kulich Pharma, Czech Republic) at a dose of 2% of HF diet is obtained from flaxseed *Linum usitatissimum* L. containing a high amount of polyunsatured fatty acids (PUFA).

Two weeks after we started feeding the diets, the rats were treated with N,N dimethylhydrazine (DMH, Merck, Germany), at a dose of 20 mg/kg s.c., at a two times a week interval, dietary treatments were continued during the entire experiment. At the end of the eighth week the rats were anaesthetized (Ketamine 100 mg/kg + Xylazine 15 mg/kg b.w., i.p.) and blood samples were taken from the heart by puncture. Samples were centrifuged at 2,500 *g* for 15 min and serum specimens were used for determination of bile acid concentration with a commercial kit (Trinity Biotech, Ireland). The measurement was carried out on an automatic spectrophotometric analyser Cobas Mira S (Roche, Schwitzerland). Freshly collected faecal samples were examined for enzymatic activity of bacterial enzymes – α-galactosidase (α-GAL), ß-galactosidase (ß-GAL), ß-glucuronidase (ß-GLUCUR), α-glucosidase (α-GLU), ß-glucosidase (ß-GLU) using an API-ZYM kit (Biomérieux, France). Faecal samples (0.1 g) were dissolved in 2 ml saline. This solution was incubated (90 µl to each cup) on an API ZYM strip. Activities were determined according to the manufacturer's instructions and expressed on the scale of 0 (negative reaction) to 5 (maximum activity).

Statistical analysis was performed by Student's *t*-test and analysis of variance (ANOVA).

Results

The mean body weight of the rats at the beginning of the experiment was 376.51 ± 18.61 g and in the end of the experiment decreased to 372.08 ± 12.27 g. The tendency of changes in body weight of the rats was similar in all experimental groups and did not depend on the administered diet (HF, conventional diet or their mixture). In the first week of the experimental period, the body weight of the rats increased ($p < 0.001$). After administration of DMH in the second and third weeks a decrease in body weight was observed after the first injection ($p < 0.02$) and after the second injection ($p < 0.001$) calculated from those in the first week. All rats were killed six weeks after the first DMH injection.

Table 2. Changes in activity of bacterial enzymes

Group	α-GAL	ß-GAL	ß-GLUCUR	α-GLU	ß-GLU
1	1.62 ± 0.32	1.88 ± 0.20	2.17 ± 0.26	1.75 ± 0.27	1.57 ± 0.28
2	1.83 ± 0.26	2.60 ± 0.25	2.92 ± 0.20	2.38 ± 0.31	1.67 ± 0.26
3	1.80 ± 0.24	2.72 ± 0.40	3.50 ± 0.32	2.75 ± 0.27	1.75 ± 0.27
4	2.00 ± 0.26	3.92 ± 0.80	4.25 ± 0.52	3.83 ± 0.68	2.08 ± 0.80
5	1.67 ± 0.26	2.00 ± 0.32***	0.72 ± 0.25***	1.92 ± 0.20***	1.52 ± 0.16
6	1.32 ± 0.42	1.92 ± 0.37***	0.83 ± 0.52***	1.83 ± 0.26***	1.83 ± 0.26
7	1.33 ± 0.46	2.05 ± 0.39***	0.58 ± 0.20***	1.25 ± 0.52***	1.58 ± 0.38
8	0.75 ± 0.27*	1.88 ± 0.38***	0.47 ± 0.15***	1.37 ± 0.71***	1.25 ± 0.38

α-GAL (α-galactosidase), ß-GAL (ß-galactosidase), ß-GLUCUR (ß-glucuronidase), α-GLU (α-glucosidase), ß-GLU (ß-glucosidase), data are expressed as means ± SD. Significant differences calculated from group 4 are designated as: *$p < 0.05$; ***$p < 0.001$

Changes in activity of bacterial enzymes are summarized in Table 2. High fat diet, DMH injections, respectively, and DMH in combination with HF diet (group 4) significantly elevated ($p < 0.001$) the activities of ß-GAL, ß-GLUCUR, and α-GLU as compared to the control rats (group 1). Supplementation of prebiotic-inulin (group 6), Hyppocastani extractum siccum (group 7), and Lini oleum virginale (group 8) and their combination (group 5) against DMH with HF-treated rats significantly decreased ($p < 0.001$) the activity of ß-GAL, ß-GLUCUR, and α-GLU compared to group 4 with the highest risk (DMH+HF diet) for development of colon cancer as well as the control group. The activities of α-GAL and ß-GLU were non-significantly decreased.

Bile acid concentration in the group 4 was 16.58 ± 3.99 µmol/l. Supplementation of selected products significantly decreased the bile acid concentration (group 5, 13.56 ± 6.07 µmol/l), (group 6, 11.72 ± 4.22 µmol/l, $p < 0.01$), (group 7, 12.85 ± 3.67 µmol/l, $p < 0.05$), (group 8, 12.05 ± 2.77 µmol/l, $p < 0.01$) compared to group 4 (Fig. 1).

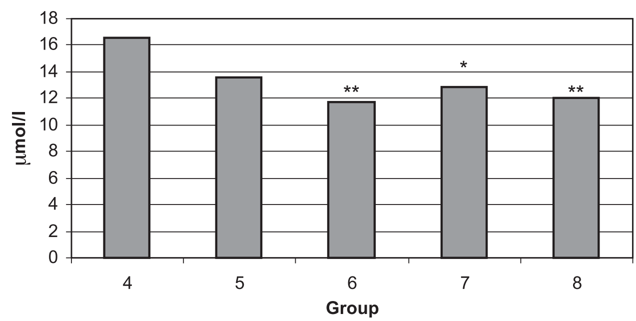

Fig. 1. Changes in bile acid concentration
Significant differences calculated from group 4 are designated as: *$p < 0,05$; **$p < 0.01$

Discussion

Globally, colon cancer is the leading cause of mortality due to malignant diseases. Dietary habits have been associated with aethiology and prevention of diseases of civilization that represent a most serious health, economic, and social problem. Synthetic therapeutics have many side effects unfavourably influencing human health and at the same time inducing new ones. For these reasons, the interest in ecological methods of prevention and therapy using the substances of biotechnological and natural origin has been increasing worldwide (Bengmark 1998). Regarding potentially protective foods, increasing attention has been paid to prebiotics (non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth or activity of one or a limited number of resident colonic bacteria), probiotics (live microbial food ingredients that may be beneficial to health such as lactobacilli or bifidobacteria), plants and their extracts and polyunsaturated fatty acids (Brown and Valiere 2004; Sanders 2003; Wollowski et al. 2001; Sanders 2000).

The interest in the role of probiotics for human health dates back to the beginning of the 20th century (1908) when a Nobel Prize winner, Russian scientist Elie Metchnikoff linked the long, healthy life of Bulgarian peasants to their high intake of fermented milk products containing lactic acid-producing microorganisms. He theorized that the lactic acid bacteria in fermented milk displace bacteria normally present in the intestine, resulting in a healthier life. The mechanism by which lactic acid bacteria may have a protective effect from colon carcinogenesis is unknown. Some of the possible mechanisms that may be involved include enhancement of the host's gut immune response, suppression of harmful intestinal bacteria, reduction of pH concentration in the colon, reduction of bacterial enzymes converting procarcinogens to carcinogens, production of antimutagenic compounds or sequestration of potential mutagens.

Elevated activity of bacterial enzymes is associated with an increased risk for various types of 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, thus ultimately potentially decreasing the risk of carcinogenesis. Supplementary ingestion of prebiotic-inulin, Hyppocastani extractum siccum, Lini oleum virginale in combination with HF diet in DMH-treated rats decreased the activity of bacterial enzymes during experiment, probably resulted in increasing excretion of conjugated xenobiotic compounds and decreasing activity of harmful substances that are the most active in their deconjugated state. Our results revealed that the diet rich in fat as well as DMH injections increased ß-glucuronidase activity, which led to a higher amount of toxic compounds in the colon. The same results were recorded by Eriyamremu and Adamson (1995), Nalini et al. (2004), and Manju and Nalini (2006).

Inulin-type fructans extracted from chicory roots are prebiotic food ingredients that are fermented to lactic acid and short-chain fatty acids in the gut lumen (Wong et al. 2006). 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. Research in experimental animal models 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), and they decrease the activity of bacterial enzymes and bile acid concentration in our experiment. The human intervention study (SYNCAN project) provided experimental evidence that inulin modulates indicators of colon cancer risks in human colon cells (Van Loo et al. 2005).

Most of the beneficial effects of the *Aesculus hippocastanum* (Horse chestnut) seed are attributed to its component beta-aescin or aescin; it also contains flavonoids, namely glycosides of quercetin and kaempferol. Beta-aescin, a natural triterpenoid saponin is known to generate a wide variety of biochemical and pharmacological effects used in nutraceutical, cosmetic, and food supplement industries. Recent studies suggest that beta-aescin may be used in the treatment of chronic venous insufficiency, oedema, haemorrhoids, and may possess anti-inflammatory, anti-hyaluronidase, anti-histamine, chemopreventive, anti-proliferative, apoptotic and anti-obesity efficacy (Patlolla et al. 2006; Niu et al. 2008; Hu et al. 2008). This novel feature in our experimental study – the changes in bacterial enzymes and bile acids confirmed the opinion that Hyppocastani extractum siccum may be a useful candidate agent for colon cancer chemoprevention and treatment.

Fatty acid composition of dietary fat plays a vital role in colon tumour development in animal models. Fats containing omega-6 fatty acids (e.g., corn oil) enhanced and omega-3 fatty acids (e.g., flaxseed oil) reduced chemically induced colon tumour development in rats. Dietary flaxseed is high in the lignan content. Lignans are phytoestrogens, good sources of dietary fibre, protein, antioxidant, and other nutritional elements and have a preventive role in development of colon cancer tumour in experimental animals and humans (Bommareddy et al. 2006; Theodoratou et al. 2007). Lini oleum virginale (Dr. Kulich Pharma, Czech Republic) administered at a dose of 2% of HF diet had an anti-tumour effect in our experimental animals. Dupertuis et al. (2007) suggest that n-3 PUFA may play an important role not only in cancer prevention but also in cancer management. It may act synergistically with radio/chemotherapy to kill tumour cells by increasing oxidative stress while reducing angiogenesis, inflammation, and metastasis induction.

Although epidemiological and experimental studies indicate an association of elevated faecal levels of secondary bile acids as well as total bile acids with a high risk of colon cancer development, the cellular mechanism for the actions of bile acids is not clear (Debruyne et al. 2002; Cheng and Raufman 2005; Hagiwara 2006). Elevated concentration of bile acids in the highest risk was significantly reduced by administration of selected nutritional products.

The results of this experiment show that the diet plays a very important role in prevention of diseases. Regular intake of bioactive food components as an everyday part of nutrition is safe and can be recommended for humans, but considerable clinical studies on humans are still needed to confirm this finding. Future research should be aimed at the enhancement of the effectiveness of cancer disease prevention using nutritional supplements. It will be important to search for a way to improve the efficacy of bioactive food components by their adequate combination.

Účinok bioaktívnych potravinových komponentov v experimentálnej karcinogenéze kolónu

Cieľom predloženej štúdie bolo sledovanie protektívneho účinku vybraných bioaktívnych potravinových komponentov počas experimentálne N,N-dimethylhydrazínom (DMH) vyvolanom karcinóme kolónu. Potkany kmeňa Wistar albino boli krmené vysokotukovou alebo konvenčnou laboratórnou diétou. Dva týždne po začatí diéty bol 6 skupinám aplikovaný DMH v dávke 20 mg/kg. ž.hm., dvakrát s týždenným intervalom. Aktivita bakteriálnych enzýmov v truse a koncentrácia žlčových kyselín v sére boli stanovované. Vysokotuková diéta, DMH aplikované individuálne, alebo v kombinácii signifikantne zvýšili aktivitu ß-galaktozidázy, ß-glukuronidázy a α-glukozidázy ($p < 0{,}001$) v porovnaní s kontrolnou skupinou zvierat. Liečba prebiotikom, suchým extraktom pagaštanu a ľanovým olejom signifikantne znížila aktivitu ß-galaktozidázy, ß-glukuronidázy, α-glukozidázy ($p < 0{,}001$), ako aj koncentráciu žlčových kyselín v porovnaní s najrizikovejšou skupinou.

Protektívne účinky vybraných bioaktívnych potravinových komponentov v experimentáne vyvolanej karcinogenéze kolónu poukázali na ich možné využitie v prevencii alebo liečbe rakoviny.

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