

The effect of probiotic microorganisms and bioactive compounds on chemically induced carcinogenesis in rats

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Diet interventions and natural bioactive supplements have now been extensively studied to reduce risks of colon cancer, which is one of the major public health problem throughout the world. The objective of our investigation was to study the effects of probiotic, prebiotic, nutritional plant extract, and plant oil on selected biochemical and immunological parameters in rats with colon cancer induced by N,N dimethylhydrazine (DMH). Male and female Wistar albino rats were fed by a high-fat (HF) diet (10% fat in the diet) and were divided into 9 groups: Control group; PRO group – HF diet supplemented with probiotic *Lactobacillus plantarum* to provide 3×10^9 c.f.u. of strain/1 ml of medium; PRE group – HF diet supplemented with inulin enriched with oligofructose (2% of HF diet); HES group – HF diet supplemented with plant extract of *Aesculus hippocastanum* L. (1% of HF diet); OIL group – HF diet comprised Lini oleum virginale (2% of HF diet); and combination of probiotic microorganisms and bioactive compounds in the groups - PRO-PRE, PRO-HES, PRO-OIL, PRE-OIL. Carcinogenesis was initiated with subcutaneous injection of DMH (20 mg/kg) two times at week interval and dietary treatments were continued for the six weeks. Application of probiotic microorganisms and bioactive compounds in all treated groups significantly decreased the activities of bacterial enzymes ($p < 0.001$), the fecal bile acids concentration ($p < 0.01$; $p < 0.001$) and significantly increased serum TNF α level ($p < 0.001$) in comparison to the control rats. The number of coliforms was reduced in PRO, PRO-PRE, PRO-OIL and PRE-OIL groups and significantly higher count of lactobacilli ($p < 0.05$) was observed in PRO-PRE, PRO-OIL and PRE-OIL groups in compare with the controls. In conclusion, the results of this study indicate that probiotic microorganisms and bioactive compounds could exert a preventive effect on colon carcinogenesis induced by DMH.

Key words: probiotics, prebiotics, Horse chestnut, flaxseed oil, colon cancer

The epidemiological studies and animal experiments suggest that several foods and dietary constituents have been associated with a reduced risk of a variety of diseases, including cardiovascular diseases [1], cancer [2] and other chronic conditions. Food consists of a complex mixture of a wide variety of components, many of which are biologically active. The term “bioactive food component” refers to nonessential biomolecules that are present in foods and exhibit the capacity to modulate one or more metabolic processes, which results in the promotion of health. In general, it is thought that bioactive food components represented a wide spectrum of plant extracts derived from whole grains, fruit, vegetables, herbs or other edible plants very quickly for bioactivity and to isolate and characterise the main bioactive component in the extract. However, probiotics, conjugated linolenic acid, polyunsaturated fatty acids, and bioactive peptides are the most

commonly found in animal products such as milk, fermented milk products and cold-water fish.

Among potentially protective foods, growing attention has been assigned to prebiotics such as oligosaccharides, probiotic microorganisms such as *Lactobacilli*, plants and their extracts and poly-unsaturated fatty acids. Some studies suggest that their consumption may decrease experimentally induced colon cancer in animals [3, 4]. Prebiotics are defined as non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of colon bacteria [5]. Probiotics are biopreparations containing living cells or metabolites of stabilised autochthonous microorganisms that activate the mucosal immune system and prevent pathogen colonization and translocation by strengthening the mucosal barrier, interfering with pathogen colonization, and in some instances,

producing secretory antibacterial substances. Plant extract of *Aesculus hippocastanum* L., (Hippocastanaceae) is commonly known as Horse chestnut. The beneficial effects are attributed to its principal component β -escin or aescin. Many experimental and clinical studies focused on the influence of diet with high content of ω -3 polyunsaturated fatty acids (PUFA) on occurrence of tumors have been carried out. Fats containing ω -6 PUFA (e.g., corn oil) enhanced and ω -3 PUFA (e.g., flaxseed oil) reduced chemically induced colon tumor development in rats [6]. In addition to chemically induced colon cancer as one of the risk factors for the development of colon cancer and other civilizing diseases we recognized high intake of dietary fat.

The aim of this study was to determine the effects of probiotic (*Lactobacillus plantarum*), prebiotic (inulin), nutritional plant extract (*Hyppocastani extractum siccum*), and plant oil (*Lini oleum virginale*) - which were applied separately and in combination, on selected biochemical and immunological parameters in rats with colon cancer induced by dimethylhydrazine.

Materials and methods

Animals. We used six-month-old male and female Wistar albino rats (n=108) (Central vivarium, Medical Faculty, P.J. Šafárik University, Košice, Slovak Republic), with a mean body weight of 363.98g (range 287.5 - 482.5g). The animals were maintained in a room with 12 hr light/dark cycle, room temperature $22\pm 2^\circ\text{C}$ and housed in plastic cages with wire tops, 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 a 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 for each cage were monitored daily and body weights were recorded weekly.

Treatment. Rats were randomly divided into following nine experimental groups of 12 rats each: The untreated controls were fed a HF diet. For the treatment of the animals in the PRO group was HF diet supplemented with probiotic *Lactobacillus plantarum* to provide 3×10^9 c.f.u. of strain/1 ml of medium, prepared as a night cultures at 37°C aerobically. Before used, 4 ml of *L. plantarum* cultures were mixed with 36 ml of skim milk. The milk (temperature was $22 - 25^\circ\text{C}$) was filled into screw-capped bottles and was administered every day. In the PRE group was applied the prebiotic BeneoSynergy 1 (ORAFIT, Tienen, Belgium) at a dose of 2% of HF diet. 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. 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 [7]. The product contains 95% fructan chains and 5% monosaccharides and disaccharides (fructose, glucose, and sucrose). Besides carbohydrates, the product contains 5% humidity. The rats in the HES group were fed HF diet supplemented with nutritional plant - *Hyppocastani extractum siccum* - HES (*Calendula*, SR) at a dose of 1% of HF diet. This plant extract was selected on the basis *in vitro* testing, which was aimed at study its antiproliferative effect on cancer cell lines. To understand the growth inhibitory effects, carcinoma cell lines (Jurkat, CEM, HeLa, MCF-7 and CaCo 2) were treated with various concentrations of HES. The cell proliferation was evaluated by using colorimetric microculture assay with the MTT end-point. The rats in the OIL group were fed HF diet comprised 2% *Lini oleum virginale* (Dr. Kulich Pharma, CR), gained from flaxseed *Linum usitatissimum* L., containing high levels of polyunsaturated fatty acids. In the next three groups rats were fed by combined HF diet of probiotic microorganisms plus bioactive compounds. The PRO-PRE group : rats were fed by the same diet as the PRE group supplemented with *L. plantarum* to provide 3×10^9 c.f.u. of strain/1 ml of medium. The PRO-HES group : rats were fed by the HF diet supplemented with nutritional plant and probiotic (as the PRO and the HES group). Treatment consisted of probiotic and plant oil was applied in the PRO-OIL group. The PRE-OIL group : rats were HF fed by diet supplemented with prebiotic and plant oil. Two weeks after beginning feeding by the experimental diets, rats were administered 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 100mg/kg + Xylazin 15mg/kg b.w., i.p.) and blood samples were taken from heart by puncture. Samples were centrifuged for 15 minutes at 2500 G, the serum was separated, aliquoted and kept frozen at -80°C until further analysis.

Biochemical and immunological analysis. The specimens were used for determination of chosen biochemical and immunological parameters. The bile acids concentration was detected in blood serum with commercial kit (Trinity Biotech, Ireland). The measurement was carried out on an automatic spectrophotometric analyser Cobas Mira S (Roche, Switzerland). Serum levels of tumor necrosis factor- α (TNF α) and interleukin-6 (IL-6) were performed by enzyme-linked immunosorbent assay (ELISA) using commercial kits (Ray Biotech, Inc., USA and Thermo Scientific, USA) following the manufacturer's instructions. Freshly collected faeces samples were examined for enzymatic activity of bacterial enzymes - α -galactosidase (α -GAL), β -galactosidase (β -GAL),

β -glucuronidase (β -GLUCUR), α -glucosidase (α -GLU), β -glucosidase (β -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 short-chain fatty acids (SCFA) were analysed in the colon content using gas chromatography Hewlett Packard (USA). The colonic pH was measured using pH meter kit with pH electrode SP 1DT (Merck, DE).

Bacteriological examination. The microbial analyses of the faecal samples of the rats were carried out after the completion of the experiment, there involved the enumeration of total lactobacilli and coliforms. 1g of faeces was placed in sterile polyethylene Stomacher Lab Blender bag with 9 ml of sterile 0.9% NaCl diluent. Series of 10-fold dilutions (10^{-2} to 10^{-8}) were made in the same sterile diluent. From appropriate dilution, 0.1 ml aliquots were spread onto two selective Mc Conkey agar (MERC-Germany) for coliforms and Rogosa agar (Biocar diagnostics-France) for lactobacilli. The plates (were made into anaerobic (Gas Pak Plus BBL). Plates for the enumeration of aerobic bacteria were incubated for 2 days at 37° C. Colonies were counted and bacteria were Gram stained and visualized under the microscope for morphological characterization. The viable counts are expressed as the log 10 of colony-forming units (CFU/g) of faeces.

Statistical analysis. The mean values of the various parameters studied were calculated and compared between groups using a two-tailed independent sample t-test or ANOVA as appropriate. The data are expressed as mean \pm SD.

Results

Weight gain of the rats. The mean body weight of the rats at the beginning of the experiment was 363.98 \pm 58.19g and in the end of the experiment increased to 384.35 \pm 74.85g. The tendency of changes in body weight of the rats was similar in

experimental groups PRE, HES and OIL, however in PRO, PRO-PRE, PRO-OIL and PRE-OIL group was marked an increase of body weight of the rats, in comparison to the control group. In the first week of experimental period body weight of the rats in the PRE, HES and OIL groups was increased ($p < 0.001$). After application of DMH in the second and third weeks of experimental period 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.

Activity of microbial enzymes, the bile acid concentrations. Changes in activity of bacterial enzymes are summarized in Table 1. Application of probiotic microorganisms and bioactive compounds in all treated groups significantly decreased ($p < 0.001$) the activities of β -GAL, β -GLUCUR, and α -GLU as compared to the control rats. The activities of β -GLU and α -GAL were in all experimental groups nonsignificantly decreased (only in OIL group activity of α -GAL decreased significantly, $p < 0.01$). The bile acids concentration in the control group was 16.84 \pm 6.33 μ mol/l. Supplementation of selected products significantly decreased the bile acids concentration in treated groups as is showed in Fig 1.

Concentration of short-chain fatty acids in colon contents. The three main SCFA were determined in the colon contents at the end of experiment – acetic, propionic and butyric acid. The concentrations of these three SCFA were lower in groups PRO, HES, OIL, PRO-PRE, PRO-HES, PRO-OIL and PRE-OIL than in control group (Fig 2). Only in the PRE group, concentration of acetic acid and butyric acid was higher in compare to the controls ($p < 0.01$).

pH value. The colonic pH in the group PRO-PRE was lower than in control group (6.18 \pm 0.11 vs. 6.29 \pm 0.14). The lowest pH value in the colon was observed in PRO-OIL group (5.66 \pm 0.14, $p < 0.001$) and PRE group (6.04 \pm 0.11, $p < 0.01$). Compared to the control group, the pH in the treated group

Table 1. Activity of bacterial enzymes in colon contents of rats.

Groups	α -GAL	β -GAL	β -GLUCUR	α -GLU	β -GLU
Control	2.00 \pm 0.26	3.92 \pm 0.80	4.25 \pm 0.52	3.83 \pm 0.68	2.08 \pm 0.80
PRO	1.96 \pm 0.17	2.25 \pm 0.27***	2.67 \pm 0.28***	2.44 \pm 0.27***	1.96 \pm 0.17
PRE	1.32 \pm 0.42	1.92 \pm 0.37***	0.83 \pm 0.52***	1.83 \pm 0.26***	1.83 \pm 0.26
HES	1.33 \pm 0.46	2.05 \pm 0.39***	0.58 \pm 0.20***	1.25 \pm 0.52***	1.58 \pm 0.38
OIL	0.75 \pm 0.27**	1.88 \pm 0.38***	0.47 \pm 0.15***	1.37 \pm 0.71***	1.25 \pm 0.38
PRO-PRE	1.86 \pm 1.43	2.03 \pm 0.44***	1.83 \pm 0.22***	2.06 \pm 0.39***	1.85 \pm 0.44
PRO-HES	1.79 \pm 0.42	1.47 \pm 0.58***	1.96 \pm 0.33***	1.47 \pm 0.50***	1.57 \pm 0.51
PRO-OIL	1.20 \pm 0.42	1.15 \pm 0.53***	1.66 \pm 0.50***	1.41 \pm 0.18***	1.31 \pm 0.43
PRE-OIL	1.17 \pm 0.37	1.70 \pm 0.23***	2.17 \pm 1.33***	1.22 \pm 0.30***	1.35 \pm 0.39

Data presented as mean \pm SD. Different from the control group, ** $p < 0.01$; *** $p < 0.001$

Control group – high fat diet without any bioactive substances; PRO – high fat diet supplemented with probiotic *Lactobacillus plantarum*; PRE – high fat diet supplemented with inulin enriched with oligofructose; HES – high fat diet supplemented with plant extract of *Aesculus hippocastanum* L.; OIL – high fat diet comprised Lini oleum virginale (ω -3 polyunsaturated fatty acids); PRO-PRE – high fat diet supplemented with probiotic *L. plantarum* and inulin; PRO-HES – high fat diet supplemented with probiotic *L. plantarum* and plant extract of *Aesculus hippocastanum* L.; PRO-OIL – high fat diet supplemented with probiotic *L. plantarum* and Lini oleum virginale; PRE-OIL – high fat diet supplemented with inulin and Lini oleum virginale. All rats were administered with N,N dimethylhydrazine.

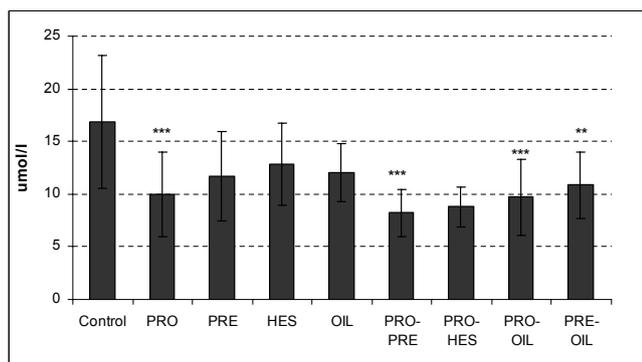


Fig.1. Concentration of bile acids in blood serum of rats .

Data presented as mean ± SD. Different from the control group, ** p < 0.01; *** p < 0.001

Control group – high fat diet without any bioactive substances; PRO – high fat diet supplemented with probiotic *Lactobacillus plantarum*; PRE – high fat diet supplemented with inulin enriched with oligofructose; HES – high fat diet supplemented with plant extract of *Aesculus hippocastanum* L.; OIL – high fat diet comprised *Lini oleum virginale* (ω-3 polyunsaturated fatty acids); PRO-PRE – high fat diet supplemented with probiotic *L. plantarum* and inulin; PRO-HES – high fat diet supplemented with probiotic *L. plantarum* and plant extract of *Aesculus hippocastanum* L.; PRO-OIL – high fat diet supplemented with probiotic *L. plantarum* and *Lini oleum virginale*; PRE-OIL – high fat diet supplemented with inulin and *Lini oleum virginale*. All rats were administered with N,N dimethylhydrazine.

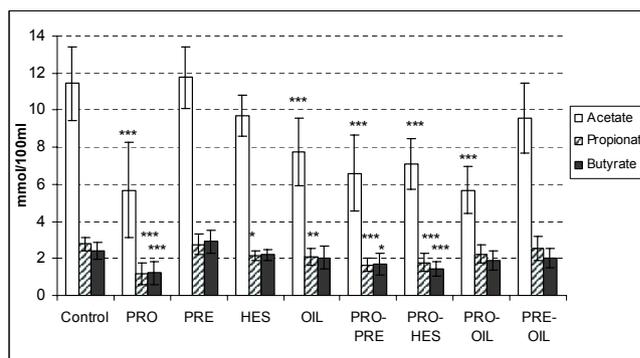


Fig.2. Main short-chain fatty acids in colon contents of rats.

Data presented as mean ± SD. Different from the control group, *p < 0.05; **p < 0.01; *** p < 0.001

Control group – high fat diet without any bioactive substances; PRO – high fat diet supplemented with probiotic *Lactobacillus plantarum*; PRE – high fat diet supplemented with inulin enriched with oligofructose; HES – high fat diet supplemented with plant extract of *Aesculus hippocastanum* L.; OIL – high fat diet comprised *Lini oleum virginale* (ω-3 polyunsaturated fatty acids); PRO-PRE – high fat diet supplemented with probiotic *L. plantarum* and inulin; PRO-HES – high fat diet supplemented with probiotic *L. plantarum* and plant extract of *Aesculus hippocastanum* L.; PRO-OIL – high fat diet supplemented with probiotic *L. plantarum* and *Lini oleum virginale*; PRE-OIL – high fat diet supplemented with inulin and *Lini oleum virginale*. All rats were administered with N,N dimethylhydrazine.

PRO, HES, OIL, PRO-HES and PRE-OIL was nonsignificantly increased (PRO group 6.51 ± 0.22; HES group 6.39 ± 0.15; OIL group 6.35 ± 0.22; PRO-HES group 6.34 ± 0.12; PRE-OIL group 6.33 ± 0.15).

Cytokines serum levels. In all treated groups were detected noticeably elevated serum TNFα levels in range 4.19 – 9.74 pg/ml (p<0.001) than in control group (1.16 ± 0.23 pg/ml) as illustrated Fig 3. The serum IL-6 levels were increased significantly in PRE group (142.24 ± 20,63 pg/ml; p<0.001) and nonsignificantly in PRO, HES and OIL groups in compare to the controls (98.70 ± 14,06 pg/ml). In the groups PRO-PRE, PRO-HES, PRO-OIL and PRE-OIL were the serum IL-6 levels (range 83.88 - 89.66 pg/ml) lower than in control group.

Effect of *Hypocastani extractum siccum* on the viability (%) of cancer cell lines.

Incubation of different cancer cell lines – Jurkat, CEM, HeLa, MCF-7 and CaCo 2 with HES at 0.125 mg/mL for 72h caused 38.8, 86.2, 79.6, 59.6 and 87.9 % reduction in cell survival. Moreover, HES in dose 0.062 mg/mL significantly decreased MCF-7 survival (70.5%, p<0.05), in comparison to controls.

Microbial analysis. The counts of *E.coli* and lactobacilli have been observed in the PRO, PRO-PRE, PRO-HES, PRO-OIL and PRE-OIL groups. In the control group was determined the count of *E.coli* 4.67 ± 1.03 log₁₀ CFU/g. The application of probiotic strain *Lactobacillus plantarum* in PRO, PRO-PRE and PRO-OIL groups decreased the count of *E.coli* (range 2,76- 3,34 log₁₀ CFU/g) with the exception of PRO-HES group

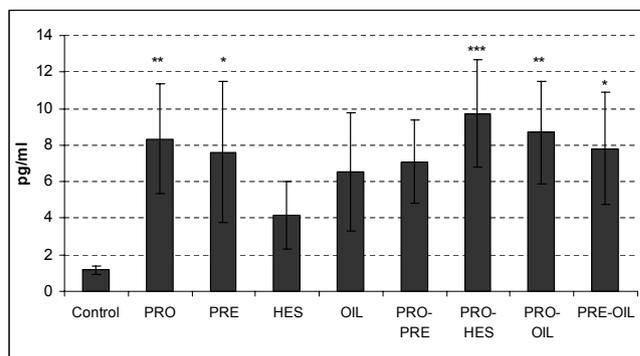


Fig.3. Serum tumor necrosis factor-alpha (TNFα) level in rats.

Data presented as mean ± SD. Different from the control group, *p < 0.05, **p < 0.01; *** p < 0.001

Control group – high fat diet without any bioactive substances; PRO – high fat diet supplemented with probiotic *Lactobacillus plantarum*; PRE – high fat diet supplemented with inulin enriched with oligofructose; HES – high fat diet supplemented with plant extract of *Aesculus hippocastanum* L.; OIL – high fat diet comprised *Lini oleum virginale* (ω-3 polyunsaturated fatty acids); PRO-PRE – high fat diet supplemented with probiotic *L. plantarum* and inulin; PRO-HES – high fat diet supplemented with probiotic *L. plantarum* and plant extract of *Aesculus hippocastanum* L.; PRO-OIL – high fat diet supplemented with probiotic *L. plantarum* and *Lini oleum virginale*; PRE-OIL – high fat diet supplemented with inulin and *Lini oleum virginale*. All rats were administered with N,N dimethylhydrazine

in which was the number of coliforms higher than in control group (4,84 ± 0,85 log₁₀ CFU/g). On the other side, the lowest

count of lactobacilli ($8.87 \pm 0.65 \log_{10}$ CFU/g) was determined in control group. In PRO group was observed higher count of lactobacilli (9.09 ± 0.62) in compare with the controls. The highest count of lactobacilli was found in combined PRO-OIL ($9.41 \pm 0.36 \log_{10}$ CFU/g, $p < 0.05$), PRO-PRE group ($9.33 \pm 0.32 \log_{10}$ CFU/g, $p < 0.05$) and PRE-OIL ($9.32 \pm 0.23 \log_{10}$ CFU/g, $p < 0.05$).

Discussion

Colorectal cancer represents a major public health problem accounting for over 1 million cases and about half a million deaths worldwide. Survival from colon cancer at 5 years has been found to vary demographically and estimated to be 65% in North America, 54% in Western Europe, 34% in Eastern Europe, and 30% in India [8]. The nutrition and physical activity patterns play remarkable role in the aetiology and prevention of chronic civilization diseases. Diet interventions and natural bioactive supplements have now been extensively studied to reduce the risks of colon cancer, as a cause of prevention instead of cure [9]. Within the frame of potentially protective food and natural bioactive compounds, as important dietary factors in colorectal cancer risk reduction, has been dedicated probiotics, prebiotics, plants and their extracts and polyunsaturated fatty acids.

From the viewpoint of the practical use of probiotics, it is of particular importance that probiotics have both local and general biomedical effects, an inhibitory effect against pathogens, an optimising effects on digestive processes, an immunostimulative effect, anti-tumor and cholesterol reducing activities [10, 11]. Probiotics are now clinically proven to have a number of health benefits including usefulness in irritable bowel syndrome [12], allergic conditions [13], dental health maintenance [14], immune functions [15] and liver functions [16], obesity management [17]. Probiotics inhibit carcinogen induced DNA damage in the rat colon [18] and that both probiotics and prebiotics have been shown to suppress preneoplastic lesions and tumors in the colons of rats treated with chemical carcinogens [19]. Considerable evidence from animal studies suggested that probiotic organisms can modulate the mucosal and systemic immune systems. This stimulation of host immunity is felt to relate to the ability of microorganisms to adhere to intestinal cells and interact with gut-associated lymphoid tissue. Cytokine expression in GALT, including TNF- α , IL-6 and IL-10, was modulated by dietary supplements. The inflammatory cytokines IL-1 and TNF- α exert cytotoxic and cytostatic effect on neoplastic cells in vitro models [20]. Application *Enterococcus faecium* CRL 183 in DMH treated rats enhanced the immune response by increasing TNF- α and other cytokines when compared with the DMH group [21]. It has also been demonstrated that *Bifidobacterium longum* and *B. animalis* promote the induction of inflammatory cytokines (IL-6, TNF- α) in mouse peritoneal cells [22]. Results at the present study show that the serum TNF- α and IL-6 levels were increased in probiotics

supplemented rats. In comparison to the control group, IL-6 levels in the treated groups with combination of probiotics with prebiotics (or oil, plant extract) was lower as recently revealed Kaminska et al. [23], which found that colon cancer patients had higher serum IL-6 levels than healthy controls and higher IL-6 levels were associated with increasing tumor stages and tumor size [24]. Elevated activity of bacterial enzymes is associated with an increased risk for various cancer. The activity of these enzymes with toxicological importance could be altered by the diet, ultimately results in potentially decreasing the risk of carcinogenesis. Supplementary ingestion of probiotic - *L. plantarum*, prebiotic - inulin, Hippocastani extractum siccum, Lini oleum virginale in DMH treated rats fed with HF diet 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 result revealed that the diet rich in fat as well as DMH application (control group) increased β -glucuronidase activity which could lead to a higher amount of toxic compounds in the colon.

Application of prebiotics can beneficially affect the colon microflora, improved bowel functions and metabolisms of the distal colon, including a reduced risk of colon cancer. The mechanisms by which they act are less clear, but it can be suggested that they may act through a combination of mechanisms involving an increase in short chain - fatty acids production, lower proliferative activity and a variation in the expression of some enzymes involved in the pathogenesis of colon cancer [25]. Inulin-type fructans with different degrees of polymerisation, extracted from chicory roots (*Cichorium intybus*) are prebiotic food ingredients, which in the gut lumen are fermented to lactic acid and SCFA. Research in experimental animal models revealed that inulin-type fructans and corresponding fermentation products have anticarcinogenic properties in rats [26] and in mice [4], therefore reduced the risks of colon cancer. Prebiotic - inulin in our experiment decreased the activity of bacterial enzymes and bile acids concentration. In human cells, inulin-derived fermentation products inhibited cell growth, modulated differentiation and reduced metastatic activities [27].

The combination of prebiotic and probiotic has synergistic effects because in addition to promoting growth of existing strains of beneficial bacteria in the colon, synbiotics also act to improve the survival, implantation and growth of newly added probiotic strains. The combination of *Bifidobacterium* and oligofructose synergistically suppressed colon carcinogenesis in rats compared to when both were given individually [28]. In our study was also observed that number of lactobacilli was the highest in combined probiotic - prebiotic and probiotic - oil treated groups of rats, while the count of Coliform organisms were significantly reduced in the same groups of rats.

Fatty acid composition of dietary fat, primarily the levels of omega-3 (e.g., flaxseed oil) and omega-6 (e.g., corn oil)

polyunsaturated fatty acids, has shown profound effect on colon tumor development in animal studies. Dwivedi et al. [28] in their study investigated the effects of dietary flaxseed oil and dietary corn oil on azoxymethane-induced colon tumor in rats. Their results indicated that dietary flaxseed oil is effective in preventing colon tumor development when compared with dietary corn oil containing omega-6 fatty acids in rats. Lini oleum virginale, flaxseed oil, is a drying oil obtained by cold expression from ripe flaxseeds of *Linum usitatissimum* L., contains substances that promote good health. One of these substances is alpha-linolenic acid (ALA), an essential fatty acid that appears to be beneficial for heart disease, inflammatory bowel disease, and other health conditions [29]. Flaxseed, in addition to ALA, contains a group of chemicals called lignans that may play a role in the prevention of cancer [30].

Natural products isolated from medicinal herbs have been the potential sources of novel anticancer drugs over the last few decades [31]. Extracts of horse chestnut (*Aesculus hippocastanum*) seed have been used in the treatment of chronic venous insufficiency, edema, and hemorrhoids [32]. β -Escin, a triterpene saponin, is one of the major active compounds in the extracts of horse chestnut seed. Recent studies suggest that β -escin may possess anti-inflammatory, anti-hyaluronidase and anti-histamine properties [33, 34]. Our results in presented experimental study - the changes in bacterial enzymes and bile acids and suppression cell proliferation in cancer cell lines testified opinion, that *Hyppocastani extractum siccum* may be the useful candidate agent for colon cancer chemoprevention and treatment.

Although epidemiological and experimental studies indicate an association of elevated faecal 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 [35, 36]. Elevated concentration of bile acids in the highest risk was significantly reduced by application of selected nutritional products.

In accordance with our results, probiotic microorganisms and selected bioactive compounds in different combinations decreased the concentration of bile acids in blood serum, activity of bacterial enzymes in colon contents, short-chain fatty acids, IL-6 levels and increased TNF- α level, in comparison with their individual application.

It is well established that diet has a major influence on the development of colon cancer. Several foods and certain nutritive factors have been associated with decreased risk of colon cancer. The results of this experiment indicate that probiotic microorganisms and bioactive compounds could exert a preventive effect on colon carcinogenesis induced by DMH.

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