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Influence of diet containing extract of black elder (*Sambucus nigra*) on colitis in rats

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Recent studies have indicated that oxidative stress plays an important role in the as yet unknown etiology of inflammatory bowel diseases. Based on the assumption that nutritional stimulation of the antioxidative defence of the organism could have beneficial effect on the course of inflammation, we studied the influence of one-month diet containing 4% of antioxidatively active extract of black elder (*Sambucus nigra*) on acute colitis in rats. Colitis was induced by intraluminal instillation of 4% acetic acid and after 48 hours the animals were killed and examined. The diet containing extract of black elder significantly decreased the disposition to colitis. The macroscopic damage score and the myeloperoxidase activity were one half lower in the experimental group than in the control diet group. Higher activities of lysosomal enzymes (acid phosphatase, cathepsin D) found in the group fed with black elder diet were considered to be an indication of higher integrity of the colonic mucosa. The significantly lower response to oxidative stress in the experimental group was documented also by a lower level of primary products of lipoperoxidation-conjungated dienes- in the liver and colon, accompanied by a significantly higher level of colonic reduced glutathione and by higher activities of glutathione-S-transferase in the erythrocytes. The results indicate that long-term diet with the antioxidatively active extract of black elder provided a protective effect on the rat colon exposed to acetic acid induced colitis.

Key words: rats, acetic acid, colitis, antocyanin.

Introduction

Despite intensive research, non-specific inflammatory bowel diseases (IBD) (ulcerative colitis and Crohn's disease) belong to diseases with yet unknown etiology and pathogenesis (Fiocchi, 1998). Recent data suggest that the inflamed intestine and/or colon may be subjected to considerable oxidative stress. The main sources of reactive oxygen species (ROS) production are phagocytic leukocytes present in large numbers in the inflamed mucosa, the xanthine oxidase pathway in colonocytes, and oxidation of arachidonic acid (Keshavarzian et al., 1990; Yamada & Grisham, 1991). Since the colonic mucosa contains a relatively small amount of antioxidant enzymes, (Yamada & Gr-

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ISHAM, 1991) the balance between prooxidant and antioxidant mechanisms may be easily disturbed, resulting in oxidative stress with subsequent tissue injury (LIH-BRODY et al., 1996; LOGUERCIO et al., 1996). The potential pathogenic role of ROS has recently been emphasised as being pivotal in the initiation of expression of several important genes (e.g. genes encoding proinflammatory cytokines) through regulating the activity of transcription factor NF-kB (BAUERLE & HENKEL, 1994). If ROS are important mediators of inflammatory damage then antioxidant treatment can be expected to have a protective effect. In this study we investigated whether a diet rich in antioxidatively active substances would be able to decrease the oxidative stress, and in the long run to moderate the course of colitis induced by intraluminal administration of acetic acid in rats. We used a diet containing 4% extract of black elder, known to be a rich source of antioxidatively active flavonoids (ABUJA et al., 1998).

Material and methods

General procedure

Male Wistar rats (Institute of Experimental Pharmacology, Slovak Academy of Sciences, Laboratory Animal Breeding Station Dobrá Voda) with initial body weight of 170–185 g ($n = 30$) were housed in wire-mesh cages and given standard laboratory rodent chow and tap water ad libidum. The rats were acclimatised for one week before experiment and then randomly assigned into two groups (15 animals in each group): the control group was fed with standard laboratory rodent chow (control diet) and the experimental group the same chow supplemented with 4% extract of black elder (*Sambucus nigra*) (experimental diet). The extract is marketed under the commercial name "Colouring concentrate from black elder pomace" and its preparation is included into the registered design application PÚV-32/98 (ŠILHÁR et al., 1998). The pomace was extracted by 70% ethanol and the extract was vacuum-concentrated to the 50–60% content of dry matter. The extract contains (%): saccharides 37, tanning matter 13, acids (expressed as citric acid) 12, antocyanins 5 (ŠILHÁR et al., 1998). The product is supposed to be used for colouring or supplemental colouring of drinks, fruit, confectionery and bakery products. The recommended concentration is 0.05–1.0%. After four-week feeding the above mentioned diets, colitis was induced by intracolonic instillation of acetic acid (NOSÁĽOVÁ & BAUER, 1996). The animals were weighed and anaesthetised by thiopental (50 mg/kg i.p.). After laparatomy 4% acetic acid (10 animals in each group) or saline (5 animals in each group) was administered in a volume of 2 mL. After 50 sec exposure the excess fluid was withdrawn and the abdomen was sutured. The rats were allowed to recover with food and water and the resulting injury was assessed after 48 hours.

Assessment of colonic damage

The rats were weighed, inspected for the presence of diarrhoea and sacrificed by decapitation in light diethylether anaesthesia. The colon was excised and opened longitudinally, rinsed with cold saline, and observed under a dissecting microscope. The colonic damage was scored on a 0–5 scale by NOSÁĽOVÁ & BAUER (1996). The scoring of damage was performed by an observer unaware of the treatment. Wet weight of colons were recorded.

Biochemical analyses

The content of conjugated dienes was estimated in plasma, erythrocytes, liver, and colon by the method of RECKNAGEL & GLENDE (1984).The following enzyme activities were determined in hepatic and colonic tissue: superoxide dismutase (SOD) (kit Randox Lab. Ltd., UK), catalase (CAT) (CAVAROCCHI et al., 1986), glutathione peroxidase (GSH-PX) (PAGLIA & VALENTINE, 1978) and glutathione-S-transferase (GST) (HABIG, 1974). The content of reduced glutathione (GSH) (BEUTLER et al., 1963) and protein content (LOWRY et al., 1951) were also estimated in these tissues. Samples of colonic tissue for the examination of myeloperoxidase (MPO) and lysosomal enzyme activities were taken from both the inflamed and noninflamed part of the colon. MPO activity was determined by the method of BRADLEY et al. (1982) modified by NOSÁĽOVÁ & BAUER (1996). Activities of lysosomal enzymes were measured in tissue extracts prepared by the method of NAVAROVÁ & NOSÁĽOVÁ (1994). Activity of acid phosphatase in the extracts was measured by the Bio-La ACP 60 kit and that of cathepsin D by a modified Anson's method (NAVAROVÁ & NOSÁĽOVÁ, 1994).

All tissue samples were taken immediately after excision of the organ and colonic damage scoring and kept along with plasma and isolated erythrocytes at −70 °C until the day of analysis. MPO activity was determined on the day of sacrifice. The results were statistically evaluated by Student's *t*-test (parametric data) or by the Mann-Whitney U test (nonparametric data).

Results

The final body weights of animals were not influenced by the presence of black elder extract in the diet. Intracolonic administration of acetic acid caused diffuse hyperaemia and bleeding with focal erosions and ulcerations. The colonic damage score was significantly lower by nearly 50% in the experimental diet group compared with the control group. The inflammatory process induced by acetic acid was accompanied by increase of wet colon weight which was significantly lower in the experimental group (37.9% vs 18.5%) (Tab. 1).

Table 1. Body and colon weights and colonic macroscopic damage score.

Parameter	Diet			
	Control		Extract of black elder	
	Intraluminal administration			
	Acetic acid	Saline	Acetic acid	Saline
N	10	5	10	5
Body weight (g)	313 ± 7	306 ± 7	305 ± 7	310 ± 9
Colon wet weight (mg/cm)	1.8 ± 0.1	1.3 ± 0.3^{D}	1.5 ± 0.2^{d}	1.2 ± 0.5^{D}
Colonic damage score	4.3 ± 0.7	–	2.4 ± 0.6^{a}	–

Values are means ± SEM for n animals in the group. ^{a,b,d}Statistically significant (experimental against control diet): $^{a}p < 0.05$ (Mann Whitney U test), $^{b}p < 0.02$, $^{d}p < 0.001$ (Student's *t*-test); ^{D}saline against acetic acid group within the same diet: $^{D}p < 0.0011$.

Table 2. Content of conjugated dienes and glutathione, and activity of antioxidative enzymes in rats.

Parameter		Diet			
		Control		Extract of black elder	
		Intraluminal administration			
		Acetic acid	Saline	Acetic acid	Saline
N		10	5	10	5
Conjugated dienes*					
Plasma	($d_{233}.mL^{-1}$)	0.24 ± 0.03	0.24 ± 0.05	0.29 ± 0.03	0.22 ± 0.04
Erythrocytes	($d_{233}.mL^{-1}$)	3.8 ± 0.6	4.1 ± 1.0	1.11 ± 0.16^{d}	1.3 ± 0.3^{a}
Liver	($d_{233}.g^{-1}$)	28.4 ± 4.3	19.7 ± 4.0	14.2 ± 0.8^{c}	14.5 ± 1.8
Colon	($d_{233}.g^{-1}$)	10.4 ± 0.8	8.5 ± 1.1	7.6 ± 1.0^{a}	7.8 ± 1.1
SOD**					
Erythrocytes	($U.mL^{-1}$)	182 ± 6	99 ± 6	172 ± 7	162 ± 5
Colon	($U.g^{-1}$)	72 ± 11	112 ± 10	81 ± 5	81 ± 5
CAT**					
Erythrocytes	($U.mL^{-1}$)	1186 ± 162	1207 ± 162	1419 ± 120	1323 ± 101
Colon	($u.g^{-1}.10^{2}$)	85 ± 5	88 ± 13	71 ± 6	68 ± 5
GSH-PX**					
Erythrocytes	($U.mL^{-1}$)	4.74 ± 0.23	5.03 ± 0.26	4.03 ± 0.15	4.23 ± 0.34
Colon	($U.g^{-1}.10^{2}$)	2.9 ± 0.7	3.6 ± 0.2	3.8 ± 0.4	4.2 ± 0.7
GST**					
Erythrocytes	($U.mL^{-1}$)	0.87 ± 0.04	1.11 ± 0.10	1.24 ± 0.07^{d}	1.04 ± 0.07
Colon	($U.mg^{-1}.10^{2}$)	4.5 ± 0.5	4.5 ± 0.3	3.5 ± 0.1	3.8 ± 0.4
GSH***					
Erythrocytes	($U.mL^{-1}$)	0.22 ± 0.01	0.19 ± 0.01	0.23 ± 0.01	0.23 ± 0.02
Colon	($U.g^{-1}$)	1.42 ± 0.09	1.73 ± 0.13	1.81 ± 0.11	2.13 ± 0.08^{d}

Values are means ± SEM for n animals in the group. *Values are expressed in optical density (d), measured at 233 nm, per mL of plasma, erythrocytes or g of tissue. **Values are expressed per mL of erythrocytes or mg of protein. ***Values are expressed per ml of erythrocytes or g of tissue. ^{a,b,c,d}Statistically significant (experimental against control diet): $^{a}p < 0.05$, $^{b}p < 0.02$, $^{c}p < 0.01$, $^{d}p < 0.001$ (Student's *t*-test).

In the inflamed segment of the colon MPO activity, considered to be the measure of neutrophil infiltration, was lower by nearly one half in the experimental group than in the control group. Activities of acid phosphatase and cathepsin D in the inflamed segment of the colon were significantly higher in the experimental group (by 90% and 40%). Activities of all the above mentioned enzymes were not influenced by the diet either in the noninflamed segment of colons or in colons of rats after intracolonic instillation of saline (sham operated) (Fig. 1).The diet containing black elder extract significantly decreased (by 30%–50%) the content of conjugated dienes in erythrocytes, liver and the inflamed colon. Except the significant increase of GST activity in erythrocytes, the black elder extract did not influence the activities of the other antioxidative enzymes examined in erythro-

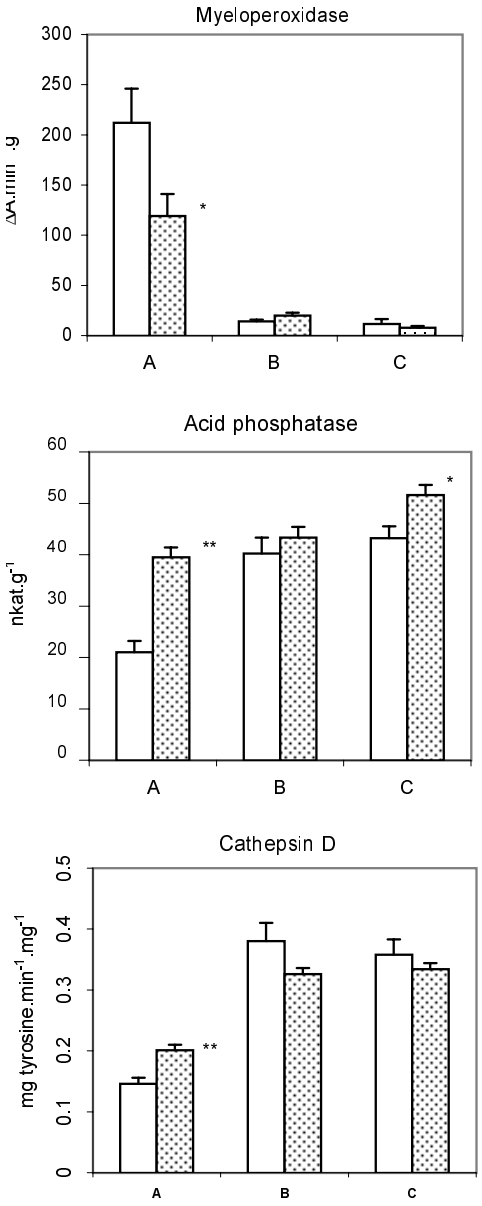

Fig. 1. Activity of myeloperoxidase, acid phosphatase and cathepsin D in rat colonic tissue. Empty columns present activities in colonic tissue of control group, dotted columns activities in colonic tissue of the experimental group fed with diet containing extract of black elder. Columns A and B present activities in the colonic tissue after administration of acetic acid: A – samples taken from the inflamed segment, B – samples taken from the noniflamed segment, columns C present activities in the colonic tissue after saline administration (sham operated rats). Values are expressed per g or per mg of tissue. Statistical significance against the control group (Student's t-test): $*p < 0.05$, $**$ $p < 0.001$.

cytes or in colonic tissue. Compared to controls the content of colonic GSH was higher in the group fed with the experimental diet, and the highest value was found in the colon of sham operated animals in the experimental group (Tab. 2).

Discussion

Dietary intervention is often a part of IBD therapy (Burke et al., 1997). Yet the role of specific nutrients in manipulating the antioxidant status has still to be defined. Our results showed that long-term feeding with a diet containing the antioxidatively active extract of black elder increased the resistance to the experimentally induced colitis. It is documented especially by the nearly one half lower macroscopic colonic damage score in animals fed with this diet. Accumulation of ROS from different sources in the inflamed colonic mucosa results in oxidative stress leading to lipoperoxidation and tissue injury. In our experiments, neutrophil infiltration into the colonic tissue, monitored by MPO activity, was significantly lower in the group fed with the black elder diet (experimental group) than in the control group. Activities of acid phosphatase and cathepsin D, lysosomal enzymes which are released into the extracellular space during inflammation (including colitis) and tissue injury, were significantly higher in the colonic tissue of the experimental group compared with controls. This fact indicates the higher integrity of the inflamed colonic tissue of the group fed with the diet containing black elder extract (Wallace et al., 1985; Mascolo et al., 1992; Lugering et al., 1998). The lower level of oxidative stress in the experimental group is documented also by the lower content of primary products of lipoxygenation – conjugated dienes – not only in the inflamed colon but also in erythrocytes and liver.

The findings suggest that a diet containing black elder extract may increase the level of antioxidative defence of the organism leading to higher resistance against induction of colitis. This idea is supported by the significantly higher level of the most important non-enzymatic intracellular antioxidant GSH in the colon of sham-operated animals, and higher activities of GST in erythrocytes in the group fed with black elder extract diet. GSH as a substrate of GSH-PX and GST participates in the most important step of detoxification of electrophilic decomposition products resulting from the attack of oxygen radicals on lipids and DNA (Ketterer, 1998).

The crucial antioxidative agents of the black

elder extract are evidently flavonoids. The orally administered phycocianin extract was found to reduce MPO activity and colonic damage in colitis induced by acetic acid (Gonzales et al., 1999). In the same rat model, the synthetic flavonoid DA-6034 decreased the macroscopic lesion score more effectively than did prednisolon or sulfasalazin, used as standard therapy in ulcerative colitis (Kim et al., 1999). Similarly, the orally administered flavonoid morin decreased colonic macroscopic damage, MPO activity and also the content of lipoperoxidation products in colitis induced by trinitrobenzenesulphonic acid in rats (Ocete et al., 1998). The beneficial effect of this flavonoid is, according to these authors, based on the inhibition of colonic leukotriene B4 synthesis and on antioxidant properties of morin, which partially prevents colonic glutathione depletion. A similar beneficial anti-inflammatory effect was observed using flavonoids of *Turnera olmifola* (Turneracae) in carrageenan induced colonic oedema (Antonio & Britto, 1998).

The latest studies concerning the anti-inflammatory effects of flavonoids in the treatment or prevention of inflammatory bowel diseases support the existing, though not generally accepted, shift from intravenous administration of nutrients to enteral feeding using elemental diets (Fiocchi, 1998). The antioxidatively active flavonoids of domestic sources can be a promising supplement of elemental diets.

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