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SYMPOSIUM

Reducing power of the natural polyphenols of *Sempervivum tectorum* in *vitro* and *in vivo*+

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ABSTRACT Unhealthy dietary habit *e.g.* lipid rich diet could result in inflammatory processes and necrosis in the gastrointestinal tract in which free radical reactions are involved. Higher plants have many free radical scavenger molecules and anti-inflammatory compounds in wonderful variations. *Sempervivum tectorum L.* is well known plant in folk medicine. In the case of complex plant extracts, such as *Sempervivum tectorum* ones, which contain several active compounds, it is impossible to discover the single mechanism of action, therefore practically the reducing activity have to be measured *in vitro* and in rat intestinal tract *in vivo.* The question was, whether the absorbed active compounds of *Sempervivum tectorum* extract were able to influence on the changed cellular redox states in mucosa of all part of intestinal tract in experimental bowel disease. This biochemical work presents the protective effect of natural polyphenols and flavonoids of *Sempervivum tectorum* extract in bowel disease - especially in jejunum and ileum - induced by high dietary triglyceride and cholesterol level in rats.

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KEY WORDS

Sempervivum tectorum
redox-homeostasis

It is generally agreed that the composition of diet plays a fundamental role in the induction of intestinal cancers. The redox homeostasis is very important for the equilibrium among cancer development, apoptosis, regeneration and necrosis. The moderate nutritional habits with natural antioxidants can help to restore the normal function of gastrointestinal tract, but the inadequate consumption of vitamins and polyphenol type antioxidants is contraindicated (Jadoul et al. 1993; Lugasi et al. 2000; Blázovics et al. 2002).

Cells and tissues have specific defence mechanism against pathological free radical processes. Certain activities or concentrations of antioxidants themselves (enzymes, vitamins or function groups) do not represent the antioxidant states of specimens (McCord 2000; Blázovics et al. 2002). Therefore a non-specific biochemical measurement, the reducing power was applied for the detection of antioxidant states of bowel parts to evaluate the redox states of mucosa homogenates in rats (Oyaizu 1986).

The judgement of biological and pharmacological effects of quercetin and kaempferol is extreme in literature, similarly to the polyphenolic compounds; their mutagenicity and carcinogenicity are supposed in biological relevant pH. In spite of that these two antioxidant flavonoids are ubiquitous in almost every foods of plant origin (Hertog et al. 1992; Bors et al. 1996; Hertog and Hollman 1998; Lugasi 2000).

Sempervivum tectorum extract contains approximately 20 different flavone and flavonol mono-and diglycosides (0.7 w/w%), mainly quercetin and kaempferol glycosides, polyphenolic compounds (4.2 w/w%), *e.g.* proanthocyanides, phenol carboxylic acids, ascobic acid, 11.2 w/w% polysaccharides and micronutrients, mainly Ca (76.52 mg/g), K (40.47 mg/g), Mg (17.85 mg/g). Characteristic monosaccharides are rhamnose, arabinose, xylose, mannose, galactose and uronic acids after strong hydrolysis. Alkaloids could not be detected in any examined samples (Blázovics et al. 1992 a,b,c, 1994; Abram and Donko 1999).

Data for toxicity after i.p. administration are: in male rats LD50 value is 2276 mg/bw kg and in female rats LD50 value is 2098 mg/bw kg, maximal tolerance i.p. 500 mg/bw kg and p.o. 5000 mg/bw kg in both sexes. These data indicate that the administration of *Sempervivum tectorum* extract represents a very low risk (Blázovics et al. 2002).

In previous studies we established that *Sempervivum tectorum* extract has not toxic effect at all on whole body in spite of its high quercetin and kaempferol contents in the applied concentration (2g/bw kg/day for 10 days) and we gave account of antioxidant, free radical scavenger (by EPR technique), membrane protecting, immune stimulating, serum lipid level lowering and HDL-cholesterol enhancing properties of *Sempervivum tectorum* extract *in vitro* and *in vivo* studies in concordance with literature.

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+In memory of Professor Béla Matkovics

Materials and Methods

The lyophilised extract of *Sempervivum tectorum* L. (Crassulaceae) was used for all examinations as a standardised preparation by 207657/1993 SOTE Patent. Serum bovine albumin was from Calbiochem AG (Lucerne). All other reagents were purchased from Reanal (Budapest).

Animal experiments

Forty Wistar rats (150-200 g) (obtained from Charles River Hungary Kft.) were divided into four groups (N=10-10) (control and fat rich diet fed and treated with extract in two adequate groups). Lipid rich diet (2% cholesterol, 0.5% cholic acid, 20 % sunflower oil added to the rat chow) was applied for the experiments. Extract was dissolved (2 g/bw kg) in the daily drinking water and added parallel with feedings for 10 days. At the end of treatment the animals were exsanguinated from abdominal vein in deep pentobarbital narcosis (55 mg/bw kg; Blázovics et al. 1992a).

Reducing power property

The reducing power of the samples was determined according to the method of Oyaizu (1986) based on the chemical reaction of Fe(III) => Fe(II). The absorbance of the reaction mixture was read at 700 nm. Increased absorbance indicated increased reducing power.

Preparation of bowel mucosa

After identification of bowel parts (duodenum, jejunum, ileum, coecum, colon, rectum), the bowel was cut and the content of actual bowel part was eliminated tenderly and washed three times with isotonic ice-cold NaCl solution. Mucosa was harvested by tender power with blunt knife using microscopic control.

Protein content

Protein content of mucosal homogenates was measured by Lowry et al (1951). Protein concentration of all bowel part mucosal homogenates was adjusted 10 mg/ml using bovine albumin as standard for the measurements.

Statistical analysis

Each value represents the mean ± SD of different measurements. In *in vitro* studies 5 parallel measurements were carried out, and in the animal experiments 2-2 parallel were in each measuring point in each animal. Significance was established at $p<0.05$ probability level.

Results

Reducing power was detected *in vitro* as a control for the *in vivo* experiments. Table 1. shows the reducing power of *Sempervivum tectorum* extract *in vitro* applied ascorbic acid as a standard. Reducing power was dependent on concentration.

Figure 1. shows the basic reducing power of different bowel mucosa. Significant difference can be seen in reducing capacity between small and large parts of intestinal tract. Injurious effect of fat rich diet changes the reducing power in all parts of intestinal tract (Figure 2). The significant difference is remained between small and large bowel, as well. Reducing power of duodenum, jejunum, ileum was significantly higher than in coecum, colon, and rectum.

The redox balance was not changed significantly, when the normolipidemic animals were treated with this extract. Results are not shown.

Antioxidant treatment caused a significant beneficial change in reducing power of ileum homogenate and non significant in coecum, colon and rectum as can be seen in Figure 3.

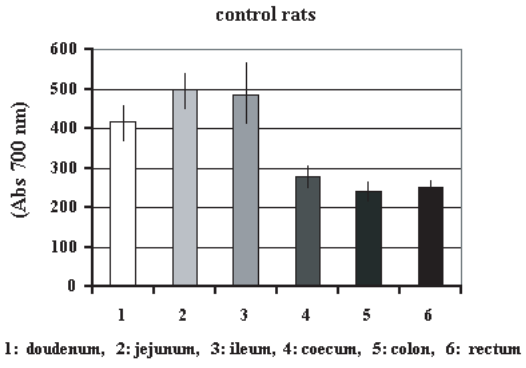

Figure 1. Reducing power in homogenates of small and large bowel mucosa in normolipidemic rats.

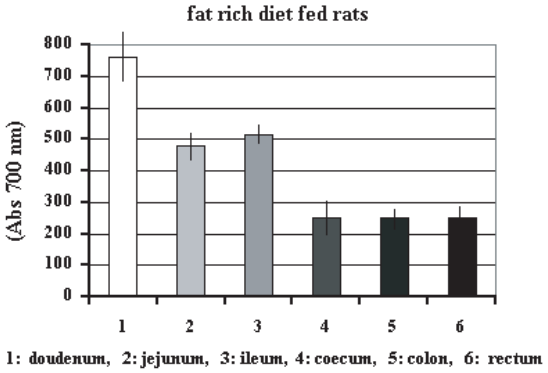

Figure 2. Reducing power in homogenates of small and large bowel mucosa in hyperlipidemic rats.

Table 1. Reducing power of ascorbic acid and *Sempervivum tectorum* extract *in vitro.*

Ascorbic acid Concentration (mg/ml)	Absorbance (700nm)	Extract Concentration (mg/ml)	Absorbance (700nm)
0.01	0.067 ± 0.067	0.25	0.211 ± 0.004
0.02	0.123 ± 0.005	0.50	0.321 ± 0.004
0.05	0.316 ± 0.003	1.25	0.659 ± 0.010
0.10	0.584 ± 0.009	2.50	0.668 ± 0.004

Discussion

Antioxidant, superoxide ($O^{\bullet}_2$) and hydroxyl radical ($^{\bullet}OH$) scavenger properties of *Sempervivum tectorum* extract were proved by EPR spectroscopic and chemiluminometric techniques (Blázovics et al. 1992c). Potential bioactive constituents were determined by chromatography (HPLC, TLC) and spectrometric (UV, UV-VIS) methods. It was established that activities of NAD(P)H reductases and content of cytochrome P450 were normalised in hyperlipidemic rat liver microsomes, if the animals were treated with this extract. Fatty acid composition in the liver was changed beneficially examined by HRGLC analysis. NADPH induced lipid peroxidation was also decreased in microsomes in *in vivo* and *in vitro* experiments. In the same time the components of *Sempervivum tectorum* had no significant influence on MFO system in normolipidemic animals and on cytochrome b5 concentration of microsome fractions of hyperlipidemic rats (Blázovics et al. 2000, 2002). Favourable changes of ion concentration were observed in the bile fluid of hyperlipidemic rats treated with extract (Szentmihályi et al. 1999, 2000).

Histological studies supported that enormous changes happened both in the liver and in jejunal mucosa of animals kept on fat rich diet. *Sempervivum tectorum* extract could restore the tissue structures (Blázovics et al. 1992, Fehér et al. 1992).

In the present experiments we justified the concentration dependent reducing ability of *Sempervivum tectorum* extract *in vitro* comparing with ascorbic acid and its beneficial effects on reducing property of bowel mucosa in animal experiments. The measurement of reducing power is suitable for study the complex effect of natural extracts as well as investigate the redox homeostasis of tissue homogenates.

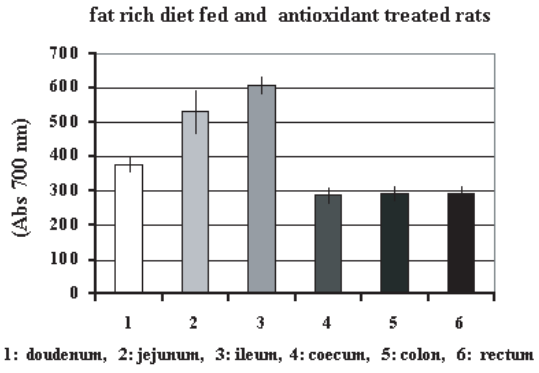

Figure 3. Reducing power in homogenates of small and large bowel mucosa in hyperlipidemic rats treated wit *Sempervivum tectorum* extract.

The reducing power was very high in duodenum of animals kept on fat rich diet, but the data were not changed significantly in jejunum, ileum, coecum, colon and rectum. The investigation of the reason of these changes needs further efforts.

Antioxidant compounds of *Sempervivum tectorum* extract caused significant elevation of reducing power of ileum mucosa in hyperlipidemic rats, and smaller reducing activity in parts of large bowel.

The relatively high essential metal ion content of extract (Blázovics et al. 2002) probably contribute to the favourable effect on bowel mucose. Especially zinc and magnesium may have a significant role. Zinc as an antioxidant element directly and magnesium by formation MgATP indirectly may participate in the operation of antioxidant defense system (Lakatos et al. 1997).

It can be known from the literature, that the fenolic acids, sourced from flavonoids can absorb in large bowel. Therefore, we concluded from the results that antioxidant compounds in basic form and after bacterial enzymatic transfor-mation in large bowel take part in redox homeostasis of intestinal tissues.

On the bases of our experimental data we proved that the antioxidant power of this drug extract was manifested in the bowel parts as well as in the liver.

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