Current Topics in Biophysics 2000, 24(2), 203–207

EFFECT OF NATURAL POLYPHENOLIC TYPE ANTIOXIDANTS (*SEMPERVIVUM TECTORUM* L. AND *RAPHANUS SATIVUS* L. *VAR NIGER* EXTRACTS) ON METAL ION CONCENTRATION IN RAT BILE FLUID

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***Sempervivum tectorum* and *Raphanus sativus* extracts were examined for antioxidant activity *in vivo*. Antioxidant activity and ion concentrations in bile fluid of six groups of male Wistar albino rats were investigated (control, control supplemented with extracts, hyperlipidemic, hyperlipidemic treated with the extracts). By the treatment with extracts, the relative light unit (RLU %) of the rat bile juice samples decreased compared to that in fatty liver (75.0 ± 13.4% for black radish root and 70.1 ± 9.9% for stonecrop extract) measured by chemiluminometry. Favourable changes in ion concentration were observed in the bile fluid of animals treated with the extracts. In both experiments the concentration of chromium, iron, phosphorus and sulfur in bile fluid changed significantly by the treatment with the extracts compared to that in hyperlipidemy. Other significant changes in the calcium and copper concentration have also been observed owing to the effect of *Sempervivum tectorum* or *Raphanus sativus* extract.**

INTRODUCTION

In folk medicine stonecrop (*Sempervivum tectorum*) and black radish root (*Raphanus sativus* L. *var niger*) extracts have been used in several fields, e.g. inflammation, against insufficient digestion, and for stimulation of bile function (Penso, 1982; Sátory, Petri, Kéry & Blazics, 1988; Varró, 1991; Terras, Goderis, Van Leuven, Vanderleyden, Cammue & Broekaert, 1992a). The extracts, which have antioxidant and free radical scavenging properties *in vitro*, are mixtures of organic and inorganic components. The phytochemical examination of *Raphanus sativus* and *Sempervivum tectorum* showed the presence of potentially antioxidant compounds: polyphenols (flavonoids), phenolcarboxylic acids, polysaccharides etc. (Terras, Schoofs, De Bolle, Van Leuven, Kees, Vanderleyden, Cammue & Broekaert, 1992b; Gawron-Gzella & Kowalewski, 1995a, b; Graewe, Bachhuber, Mock & Strack, 1992; Kowalewski, Gawron-Gzella & Jablonska, 1991). These components may effect several biochemical processes in the living systems. They may change e.g. the composition (amino acid, protein, fatty acid, macro-, meso-, micro element, etc.) and characteristics (e.g. antioxidant activity) of body fluids and cells favourably (Bendich, 1990).

The purpose of the study was to examine the changes in the antioxidant activity and ion concentration differences of bile fluid in normal rats, experimental hyperlipidemic rats and hyperlipidemic rats treated with antioxidant *Raphanus sativus* and *Sempervivum tectorum* extracts.

MATERIALS AND METHODS

Standardized squeezed juice from radish was prepared by PHARMA Company (Budapest) under the control of the Institute of Pharmacognosy, Semmelweis University. The preparation and standardization of the lyophilized extract of *Sempervivum tectorum* L was performed by the Institute of Pharmacognosy.

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Luminol, microperoxidase and hydrogen-peroxide were obtained from Sigma (USA), all other reagents were purchased from Reanal (Hungary). ICP standards were purchased from Merck.

Male Wistar albino rats (120–140 g) were used for the experiment. 6 groups of rats were examined with 10–10 animals: normolipidemic (I), hyperlipidemic (II), normolipidemic treated with *Sempervivum tectorum* extract (I/a) and with *Raphanus sativus* extract (I/b), hyperlipidemic treated with *Sempervivum tectorum* extract (II/a) and with *Raphanus sativus* extract (II/b). The animals in group I were fed with normal food (BIOFARM PROMT, BFP Kft, Hungary). The animals in group II were fed with fat rich diet (2% cholesterol, 20% sunflower oil and 0.5% cholic acid added to the control diet) for 9 days. The animals in group III were administered with extracts *ad libitum* (diluted ten times at a doze of 150 ml/kg in the case of black radish and 200 mg lyophilized extract/kg body weight dissolved in drinking water in the case of stonecrop). The rats were kept on the diets for 9 days. The animal group IV was the control supplemented with extract like group III. (Hyperlipidemia was checked by serum parameters of liver panel and fatty liver was proved by histologically as it was reported previously (Blázovics, Örsi, Kemény, Fehér, Barta & Fehér, 1997; Szentmihályi, Blázovics, Kocsis, Fehér, Lakatos & Vinkler, 2000)).

The rats were anaesthetized with urethane (1.2 g/kg) for deep narcosis at the 10th day. After laparotomy the biliary duct was cannulated with a plastic cannula. The bile was collected in a plastic tube for 2 hours. After operation the animals were killed by decapitation in general anesthesia.

Scavenging activity of the bile was measured in H_2O_2/˙OH-luminol system. Light emission was measured according to Blázovics, Fehér & Fehér (1992) using a Berthold Lumat LB-9501 luminometer. The composition of the reaction mixture was as follows: H_2O_2 (300 µl, 10^4 dilution), microperoxidase (300 µl, 3×10^{-7} M), luminol (50 µl, 7×10^{-7} M), sample and diluted with deionized water to 850 µl. The intensity of chemiluminescence light is given as the relative light unit (RLU) as percentage of that for the control group.

Inductively coupled plasma optical emission spectrometry (ICP-OES) was used for element measurements. Type of instrument: Atom Scan 25 (Thermo Jarrell Ash Co.). The following elements were determined in three parallel measurements: Ca, Cr, Cu, Fe, K, Mg, Mn, Na, P, S, Zn. Three times three sec. integration time, blank subtraction and background correction were applied during the measurements.

Sample preparation for the element measurement: the bile fluid samples (2.0 g) were digested with a mixture of HNO_3 (5 ml) and H_2O_2 (3 ml) in teflon vessels. After digestion the samples were diluted to 25 ml with deionised water.

Mean values and standard deviations (SD) were calculated from the results. For comparison of the means one-way analysis of variance (ANOVA) was used by GraphPAD software version 1.14 (1990).

RESULTS AND DISCUSSION

The chemiluminescence light intensity depends on the concentration of the microperoxidase and luminol, as well. The medicinal plant extracts investigated inhibited the enhanced chemiluminescence and the inhibition depended on their concentration *in vitro* as it was published earlier (Lugasi, Blázovics, Kéry & Dworschák, 1997; Lugasi, Dworschák, Blázovics & Kéry, 1998; Blázovics, Prónai, Fehér, Kéry & Petri, 1993).

In vivo investigations showed that the bile fluid of control and hyperlipidemic rats could scavenge the free radicals formed in H_2O_2/˙OH-luminol system. There was no significant difference in the antioxidant activity of control rats and control ones supplemented with extracts (control: 100 ± 12%, control + *Sempervivum tectorum* extract: 89.0 ± 4.3%, control + *Raphanus sativus* extract: 92.1 ± 2.0%), while the relative chemiluminescence light intensities of the lipid rich diet treated groups were significantly different at the level of $P < 0.05$. By the treatment with extracts, the relative light unit (RLU, %) of the rat bile juice samples decreased compared to that with control and fatty liver. The relative light unit (RLU, %) of the rat bile fluid decreased to 75.0 ± 13.4% compared to the control by the treatment with black radish root extract. In the group treated with *Sempervivum tectorum* extract the RLU % of the rat bile juice also decreased to 70.1 ± 9.9% (Fig. 1). That means a significant beneficial change in tissue antioxidant capacity.

Eleven elements in the bile fluid were measured by ICP-OES. There was no significant difference in the concentration of most element in bile fluid for control and control group treated with extracts (Table 1) while significant changes in concentration were observed in the bile fluid for hyperlipidemic and hyperlipidemic rats treated with extracts (Table 2). By the treatment with *Sempervivum*

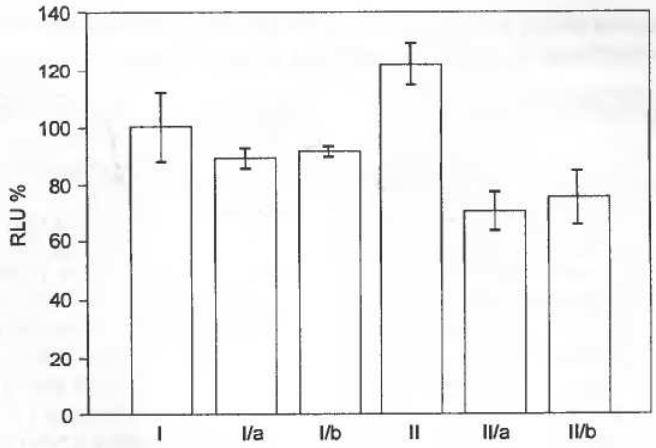

Fig. 1. H_2O_2/'OH scavenger activity of rat bile fluid in the percentage of control samples (control 100%, n = 10); I. control, I/a. control treated with *Sempervivum tectorum* extract, I/b. control treated with *Raphanus sativus* extract, II hyperlipidemy, II/a hyperlipidemy treated with *Sempervivum tectorum* extract, II/b hyperlipidemy treated with *Raphanus sativus* extract

Table 1. Element concentration in bile fluid for control and control rats treated with extracts ± standard deviation (μg/g, n = 10)

Elements	Control	Control + *Sempervivum tectorum* extract	Control + *Raphanus sativus* extract
Ca	83.03 ± 27.60	89.59 ± 12.06	87.08 ± 35.52
Cr	0.234 ± 0.094	0.471 ± 0.144	0.309 ± 0.022
Cu	0.793 ± 0.433	1.19 ± 0.49	0.212 ± 0.104
Fe	2.55 ± 0.74	4.06 ± 2.28	1.75 ± 0.58
K	181.4 ± 15.6	183.8 ± 31.3	198.2 ± 18.6
Mg	26.93 ± 5.91	20.42 ± 4.75	19.35 ± 1.73
Mn	0.391 ± 0.209	0.563 ± 0.152	0.412 ± 0.051
Na	3919 ± 185	3860 ± 407	3876 ± 170
P	168.2 ± 37.5	214.2 ± 61.0	162.0 ± 31.9
S	1132 ± 194	1365 ± 175	1470 ± 543
Zn	1.52 ± 1.04	2.67 ± 1.65	1.05 ± 0.77

tectorum extract, the concentration of Ca, Cr, Fe, P, S and Zn in bile fluid changed significantly ($P < 0.05$) compared to that in hyperlipidemy. The results compared to the values of control bile fluid, the concentration changes of elements mentioned above are also significant except for zinc. After administration of *Raphanus sativus* extract, the concentration of Cr, P, S and Zn in bile fluid changed significantly compared to that in hyperlipidemy. By comparing the results of three animal groups (control, hyperlipidemic, hyperlipidemic treated with black radish root extract), the concentration of Cr, Cu, Fe, P and S in the bile fluid was significant. The concentration of ions in most cases in the bile moved back to control values by the effect of extracts. According to the literature hypomagnesemia, hypokalemia and hypophosphatemia are common features of cirrhosis (Bogin, Avidar & Merom, 1986; Rocchi, Borella, Borghi, Paolillo, Pradelli, Farino & Casalgrandi, 1994). Decrease of magnesium level in serum, erythrocytes, lymphocytes, liver tissue, heart and skeleton muscles etc. is well known (Cohen, 1990). Magnesium depletion from the heart muscle and liver tissue is the fastest process. In our "short term" experiment the magnesium and potassium depletion could not be observed. Phosphorus depletion may be proved by elevated phosphorus concentration in bile fluid in hyperlipidemy and the tendency could not turn by the treatment with the extracts.

CONCLUSION

It has been proved that the extracts of *Sempervivum tectorum* and *Raphanus sativus* have free radical scavenging properties *in vivo*. Hyperlipi-

Table 2. Element concentration in bile fluid of rats in hyperlipidemy and hyperlipidemy treated with extracts, means ± standard deviation (μ/g, n = 10, STE: *Sempervivum tectorum* extract, RSE: *Raphanus sativus* extract)

Elements	Hyperlipidemy	Hyperlipidemy + STE (significance to hyperlipidemy)	Significance* (P < 0.05)	Hyperlipidemy + RSE (significance to hyperlipidemy)	Significance** (P < 0.05)
Ca	59.17 ± 31.69	79.02 ± 7.37 (+)	+	89.79 ± 37.17 (–)	–
Cr	< 0.02	0.299 ± 0.199 (+)	+	0.242 ± 0.186 (+)	+
Cu	0.290 ± 0.274	0.379 ± 0.191 (–)	–	0.302 ± 0.151 (–)	+
Fe	0.544 ± 0.371	3.46 ± 1.72 (+)	+	1.50 ± 1.19 (–)	+
K	198.3 ± 19.4	182.5 ± 13.0 (–)	–	182.0 ± 13.2 (–)	–
Mg	21.16 ± 2.69	25.48 ± 3.80 (–)	–	24.71 ± 6.12 (–)	–
Mn	0.173 ± 0.129	0.550 ± 0.354 (–)	–	0.396 ± 0.277 (–)	–
Na	4264 ± 660	3999 ± 456 (–)	–	3767 ± 263 (–)	–
P	263.6 ± 19.0	289.6 ± 15.75 (+)	+	230.9 ± 14.1 (+)	+
S	539.2 ± 59.1	817.6 ± 179.8 (+)	+	870.1 ± 241.1 (+)	+
Zn	0.209 ± 0.112	1.36 ± 0.96 (+)	–	1.11 ± 0.86 (+)	–

*Significance among the means of the control, hyperlipidemy group and hyperlipidemy group treated with *Sempervivum tectorum* extract

**Significance among the means of the control, hyperlipidemy group and hyperlipidemy group treated with *Raphanus sativus* extract

demic rats with fatty liver were treated with antioxidant and liver protective extracts. By the treatment, the free radical level of the rat bile juice decreased compared to the fatty liver. Favourable changes of ion concentration were observed in the bile fluid of animals treated with extracts. Both ion concentration and chemiluminescent values indicate the recovery of the normal functions of the liver thus may prove the liver protecting effect of extracts.

Acknowledgement

The study was supported by Ministry of Welfare ETT02-517 and ETT 250/2000.

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