**Volume 47(1-4):143-146, 2003**
**Acta Biologica Szegediensis**
http://www.sci.u-szeged.hu/ABS

**SYMPOSIUM**

# Effects of chicory on pancreas status of rats in experimental dislipidemia+

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**ABSTRACT** The well known medicinal plant, *Cichorium intybus* L. (Asteraceae) contains dietary oligofructose compounds which have beneficial effect on carbohydrate and lipid metabolism, other compounds e.g. polyphenol type derivatives could be responsible for the antioxidant properties reported in several studies before. Male Fischer rats were kept on normal and lipid rich diet supplemented with chicory extract in this study. Our aim was to investigate the influence of this supplementation on pancreas status of rats, especially in dislipidemia. Polyphenols, flavonoids and caffeic acid derivatives were measured in chicory extract by spectrophotometric and chromatographic methods. Antioxidant property of the plant extract was determined *in vitro* and the biological activity of antioxidant compounds of chicory was investigated *in vivo* by a luminometric technique.The effects of bioactive molecules of chicory extract influenced the lipid metabolism and the redox balance of pancreatic tissue of rats in experimental dislipidemia. **Acta Biol Szeged 47(1-4):143-146 (2003)**

**KEY WORDS**

*Cichorium intybus*
hyperlipidemy
pancreas
antioxidant status

*Cichorium intybus* L. (Asteraceae) as an important medicinal herb, has been used in folk medicine for liver disorders, gallstones and for inflammations of the urinary tract since the 17th century. It is well-known from the literature that the main active compounds of chicory are: inulin, fructooligosaccharides, caffeic acid derivatives, flavonoids and polyphe-nols.

Chicory fructooligosaccharides have been investigated in studies on the gastrointestinal system especially because of the inulin and dietary fiber content of this medicinal plant. Some oligosaccharides have functional effects similar to soluble dietary fiber such as enhancement of a healthy gastrointestinal tract, improvement of glucose control, and modulation of the metabolism of triglycerides (Roberfroid 2000; Roberfroid and Slavin 2000).

Dietary supplementation with oligofructose (100g/bwkg) a non-digestible oligomer of beta-D-fructose decreases triacylglycerols and VLDL in serum rats. By measuring the activity of key enzymes (*e.g.*: fatty acid syntase, phosphatidate phosphohydrolase) the authors found that long term feeding with oligofructose protected rats against liver triacylglycerols accumulation induced by fructose (Kok et al. 1996).

Oligofructose significantly alters liver lipid metabolism, resulting over time in a significant reduction in plasma triacylglycerols, phospholipids and cholesterol levels (Fiordaliso et al. 1995).

The water soluble antioxidant properties of *Cichorium intybus var. silvestre* were investigated and evaluated *in vitro* and *ex vivo* as protective activity against rat liver cell microsome lipid peroxidation (Gazzani et al. 2000).

The aim of our studies was to verify the effect of chicory decoct on lipid metabolism and antioxidant defend system in a short term animal experiment observing the changes in pancreas function through biochemical measurements. We also investigated the radical scavenger property of chicory extract *in vitro* with the help of chemiluminescence measurements.

Potential bioactive constituents ( polyphenols, flavonoids and caffeic acid derivatives) of chicory extract were measured by spectrophotometric and cromatographic techniques.

## Materials and Methods

In this short term experiment young male Fisher rats (weight: 200 ± 25 g) were kept on normal diet (20 animals) and lipid rich diet (20 animals). In the normolipidemic groups, rats were fed with normal chow (CRLT-N, Biopharm Prompt Kft., Hungary). In the hyperlipidemic groups, rats were kept on lipid rich diet (20% sunflower oil, 2.0% cholesterol, 0.5% cholic acid added to the normal chow) ad libitum according to their growth. Ten animals fed with normal diet and ten animals fed with lipid rich diet were treated by gastric tube with the solution of the lyophilised chicory decoction (2 g/ body weight kg) for 10 days.

The blood was collected by cannulation of posterior vena cava in deep Urethane anaesthesia (35 mg /body weight kg).

Accepted April 30, 2003
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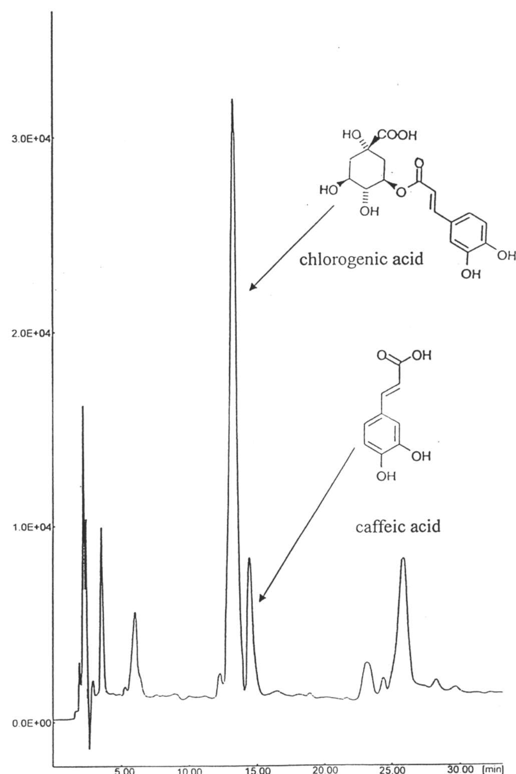
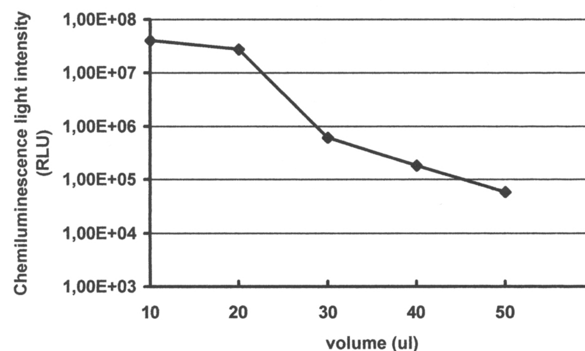



Figure 1. Qualitative evaluation of chicory extract by HPLC.




**Each measuring point represents the mean of five parallel data. The C.V. % was below 5% respectively.**

Figure 2. Changes of chemiluminescence light intensity in $H_2O_2$ /•OH microperoxidase system depending on the different volumes of Chicory decoct solution (1g %).

Sera for the biochemical measurements were separated by centrifugation at 2800 RPM for 10 minutes.

The total radical scavenger capacity (TSC) of pancreas tissue homogenates was measured by a chemiluminescence assay in $H_2O_2$/•OH-microperoxidase-luminol system adapted to Berthold Lumat 9501 instrument (Blázovics et al. 1999). During measurement, the program gave the integrated value of the light reaction. Light emission was initiated by the addition of 0.050 ml of 70 µmol $l^{-1}$ alkaline luminol solution ( pH 9.8 ), 0.30 ml $H_2O_2$ (1:10,000 dilution) and 0.30 ml of 1 mmol $l^{-1}$ micro-peroxidase solution as catalyst. The sensitivity of the instrument allows detection limit of <0.1 pg of material.

Enzyme activities (alpha-amylase (AMYL), lipase) and lipid parameters (cholesterol (CHOL), triacylglycerols (TG)) from the sera of animals were determined by Hitachi 717 automated chemical analyser using spectrophotometric enzymatic methods. For the measurement of alpha-amylase activity, ethylidien-$G_7$PNP was used as substrate in method of Lorentz et al. (Lorentz 1998). Lipase activity was measured by turbidimetry with colipase using the method of Lott, on DTN 400 spectrophotometer (Lott 1986). Cholesterol concentration was measured by enzymatic colorimetry (Richmond 1973) and concentration of triacylglycerols was determined by the enzymatic method of Nagele et al. (1984).

Pancreas tissue was homogenized in 0.9% NaCl solution, with a Potter-Elvehjem homogenizer, equalised by dilution to 5 mg% protein concentration. Protein content was measured by Lowry et al. (1951).

Wild growing *Cichorium intybus* L. (Asteraceae) were collected during flowering, identified in the Department of Pharmacognosy, Semmelweis University, where vouchers have been deposited. Diluted alcoholic (40 v %) extract (1:5) was prepared from the whole plant, which was then concentrated and lyophilised.

Plant extract was standardised for the potential bioactive constituents. Total polyphenol content was measured by the method of the Hungarian Pharmacopoeia (Ph.Hg. VII.), results were expressed as pyrogallol. Flavonoid content was determined by the modified method of the German Pharmacopoeia (DAB 10) by spectrophotometry following acidic hydrolysis. Result was expressed as hyperoside.

Table 1. Changes of the main lipid parameter concentrations in sera of rats in the different dietary groups.

| Dietary groups | CHOL (mmol/l) | TG (mmol/l) |
|---|---|---|
| Normal diet | 2,07*± 0,15 | 0,81 ± 0,06 |
| Normal diet supplemented with chicory extract | 2,11 ± 0,12 | 0,56 ± 0,05 |
| Lipid rich diet | 10,27* ± 1,13 | 1,23*± 0,20 |
| Lipid rich diet supplemented with chicory extract | 9,68 ± 0,48 | 0,64* ± 0,05 |

* significantly different (p<0.05)

Caffeic acid derivatives were measured by spectroscopic method (325 nm) after purification using caffeic acid for calibration. Results were expressed as caffeic acid.

Qualitative composition of the extract, used in the experiments was characterised by HPLC fingerprint. HPLC separation was performed with an ABL & JASCO system consisiting of PU-980 gradient pump and RHEODYNE 7725 (20 µl) injector. The instrument was equipped with a PU-980 UV-VIS detector in combination with an IBM-PC. A Hypersil ODS (5 µm) reverse phase C-18 column (250 x 4 mm) protected with a precolumn of the same material was used. Two solvent mixtures were employed for elution: Eluent A: AcCN, Eluent B: $H_2O$ : $CH_3COOH$ (40:1). Separation was achieved at ambient temperature with a flow rate 1.0 ml min$^{-1}$. Gradient elution was used. Data were collected at 325 nm. Peaks were identified with authentic standards by accordance to UV spectra and retention time.

The biochemical reagents were products of Roche Diagnostics, luminol and microperoxidase were purchased from Sigma Chemical (USA). All other reagents were obtained from Reanal (Hungary).

For the statistical analysis the STATISTICA 6.0 software package was used. The values were expressed as mean ± SD, at p< 0.05, the difference was considered significant.

## Results and Discussion

Numerous experimental studies published in the last decades investigated the possible involvement of reactive oxygen species in acute pancreatitis (Sanfey 1984; Nonaka 1989; Gough 1990; Tsai 1998). Most of them applied measurements for observing of the loss of endogenous antioxidants (ascorbic acid, glutathione) and the increase in markers of oxidative damage (malondialdehyde and 4-hydroxynoneal; Kruse et al. 2001).

Quantitative evaluation of chicory extract shows flavonoids as hyperoside 0.12 ± 0.02 g%, polyphenols as pyrogallol 12.98 ± 0.06 g%, caffeic acid derivatives as caffeic acid 16.7 ± 0.306 g%. Qualitative composition based on HPLC fingerprint showed chlorogenic acid, caffeic acid and the luteolin flavone as characteristic compounds (Fig. 1).These components may be responsible for the *in vitro* antioxidant property of the natural extract shown by Figure 2. The intensity of the emitted light in $H_2O_2/{}^{\bullet}OH$-luminol - microperoxidase system was diminished depending on the concentration of chicory decoct (Blázovics et al. 1999).

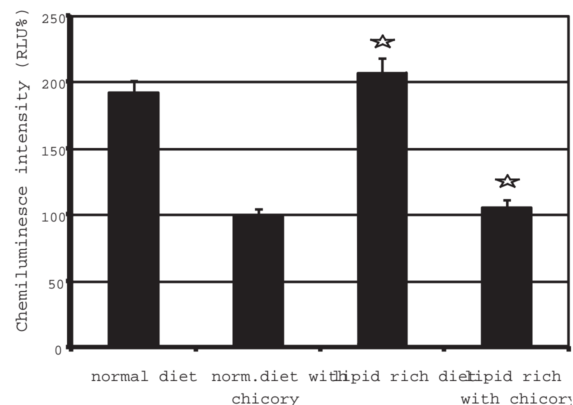



**Figure 3.** Changes in chemiluminescence intensity (RLU %) in pancreas tissue homogenate of rats in the different dietary groups. Signal * means significant difference (p<0,05).

The status of dislipidemia was verified by the increase of cholesterol and triacylglycerols concentrations in the sera of animals fed on lipid rich diet. In the dislipidemic group of animals, which received plant extract supplementation by their lipid rich diet, lower cholesterol and triacylglycerols concentrations were found (Table 1; Blázovics et al. 2000).

In the group of the animals fed on normal diet applying chicory extract supplementation, the activities of pancreas enzymes: alpha-amylase, and lipase in the sera showed slight increase, which were not significant. In the dislipidemic and plant extract treated animal group, lipase level was significantly lower than that of hyperlipidemic group. The alpha-amylase activities were not changed (Table 2).

These chemiluminescence measurement of the samples prepared from pancreas tissue homogenate of rats, as the intensity of the chemiluminescence light emission (RLU%) reduced significantly from 191.9% to 99.3% (p< 0.05) in the extract treated group by normal diet, and from 207.0% to 105.0% (p<0.05) in the dislipidemic animal group, showing the effect of decoct supplemented diet on pancreas status (Fig. 3).

On the basis of our results beneficial effect was verified on pancreas status in rats, using chicory extract supplementation either by normal or by lipid rich diet. We could reveal the change of the antioxidant status of pancreas tissue

**Table 2.** Changes of pancreas enzyme activities in sera of rats in the different dietary groups.

| Dietary groups | LIPASE (U/l) | AMYL (U/l) |
|---|---|---|
| Normal diet | 17,5 ± 3,12* | 6306,5 ± 289,7 |
| Normal diet supplemented with chicory extract | 12,71 ± 2,95 | 6675,8 ± 366,2 |
| Lipid rich diet | 29,33 ± 8,83* | 8603,2 ± 847,2 |
| Lipid rich diet supplemented with chicory extract | 15,6 ± 1,78* | 8660,2 ± 451,1 |

*significantly different (p< 0.05)

of rats to the effect of chicory extract supplementation, with the help of chemiluminescence method.

## Acknowledgment

The authors express their thanks to Ms. Sarolta Bárkovits and Ms. Edina Pintér for their excellent technical assistance. The research was supported by the Ministry of Welfare (ETT 250/2000) and 1/016 Széchenyi Project.

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