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The effect of *Smallanthus sonchifolius* leaf extracts on rat hepatic metabolism

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Abstract

Smallanthus sonchifolius (yacon), originating from South America, has become popular in Japan and in New Zealand for its tubers which contain β-1,2-oligofructans as the main saccharides. The plant is also successfully cultivated in Central Europe in the Czech Republic in particular. Its aerial part is used in Japan and in Brazil as a component in medicinal teas; while aqueous leaf extracts have been studied for their hypoglycemic activity in normal and diabetic rats. We have already demonstrated the high content of phenolic compounds in yacon leaf extracts and their *in vitro* antioxidant activity. In this paper, we present the effects of two organic fractions and two aqueous extracts from the leaves of *S. sonchifolius* on rat hepatocyte viability, on oxidative damage induced by *tert*-butyl hydroperoxide (*t*-BH) and allyl alcohol (AA), and on glucose metabolism and their insulin-like effect on the expression of cytochrome P450 (CYP) mRNA. All the extracts tested exhibited strong protective effect against oxidative damage to rat hepatocyte primary cultures in concentrations ranging from 1 to 1000 μg/ml, reduced hepatic glucose production via gluconeogenesis and glycogenolysis at 1000 μg/ml. Moreover, the effects of the organic fractions (200 and 250 μg/ml) and to a lesser extent, the tea infusion (500 μg/ml) on rat CYP2B and CYP2E mRNA expression, were comparable to those observed with insulin. The combination of radical scavenging, cytoprotective and anti-hyperglycemic activity predetermine *S. sonchifolius* leaves for use in prevention and treatment of chronic diseases involving oxidative stress, particularly diabetes.

Abbreviations: AA, allyl alcohol; CYP, cytochrome P450; DEC, decoction from *S. sonchifolius* leaves; DMSO, dimethyl sulfoxide; HPTLC, high-performance thin-layer chromatography; INF, infusion from *S. sonchifolius* leaves; LDH, lactate dehydrogenase; MTT, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide; OF1 and OF2, organic fractions 1 and 2 from *S. sonchifolius* leaves; SDS, sodium dodecyl (lauryl) sulphate; SSC, standard saline citrate; TBA, thiobarbituric acid; TBARS, thiobarbituric acid reacting substances; *t*-BH, *tert*-butyl hydroperoxide

Introduction

Yacon (*Smallanthus sonchifolius,* Asteraceae) is an interesting plant originating from the Andes and related to the Jerusalem artichoke which has been used for centuries by the original Peruvian population as a traditional folk medicament to treat hyperglycemia, kidney pro-

blems and for skin rejuvenation (Grau and Rea, 1997; Valentová et al., 2001; Valentová and Ulrichová, 2003). In Japan, yacon tubers have become popular as a dietary supplement for people suffering from diabetes mellitus (Tsukihashi, 1999). This plant can also be agriculturally cultivated in the European climate.

The only studies reported to date on *S. sonchifolius* leaves include isolation of four kaurenoids (Kakuta et al., 1992) and four sesquiterpene lactones (Inoue et al., 1995) from the leaves and an investigation of the hypoglycemic activity of an aqueous extract in normal and streptozocin-induced diabetic rats (Aybar et al., 2001). Phenolic compounds have been isolated from the tubers of yacon – mainly chlorogenic acid (Yan et al., 1999) and other caffeic acid derivatives (Takenaka et al., 2003; Takenaka and Ono, 2003) – and from the leaves of related *Smallanthus fruticosus* (centaureidin, Beutler et al., 1993). We have already reported on the presence of caffeic, chlorogenic and ferulic acids detected using HPTLC in ethyl acetate extract from the leaves of yacon (Valentová et al., 2003). The presence of the phenolic acids was then confirmed by HPLC coupled with electrochemical detection (HPLC-ECD) (Jirovsky et al., 2003). More recently, we presented a detailed analysis of phenolic compounds from *S. sonchifolius* leaves extracts (Simonovska et al., 2003).

We have also provided evidence for the antioxidant activity of two extracts in relation to the content of phenolics. Moreover, we showed that these extracts exhibited cytoprotective effect against *tert*-butyl hydroperoxide induced oxidative damage to rat hepatocytes (Valentová et al., 2003).

In this paper, we present the effects of four different extracts of yacon leaves on rat hepatocyte viability, on oxidative damage induced by *tert*-butyl hydroperoxide (*t*-BH) and allyl alcohol (AA) and on glucose metabolism in these cells.

Given the insulin-like effects of these extracts we investigated their effects on hepatic functions known to be regulated by insulin. Contrary to rat cultured hepatocytes, the rat hepatoma cell line does not require specific insulin supplementation of the culture medium and is particularly convenient for the study of insulin regulation. It has been reported that insulin directly down-regulated the expression of CYP2E and CYP2B mRNA in these cells (de Waziers et al., 1995). We showed in this work that three of the tested extracts effectively produced the same effect as insulin on CYP2B and CYP2E mRNA expression.

Experimental

Plant material

Yacon plants, originally purchased from Ecuador, were grown at the Potato Research Institute in Havlíčků Brod. Voucher specimens are deposited in our collection at the Institute of Medical Chemistry and Biochemistry, Olomouc, Czech Republic. The leaves were collected in October 2000 at harvest time of the tubers and dried at ambient temperature.

Extraction procedure

Dried yacon leaves of (20 g) were extracted as follows:

1. *Organic fraction 1 (OF1)*. Using Soxhlet extractor with MeOH, chlorophyll removal with petroleum ether, the aqueous layer was then acidified (0.01 mol/L H_3PO_4) and extracted by ethyl acetate. Extract yield after evaporation of the solvent was 0.26 g.

2. *Organic fraction 2 (OF2)*. The dried drug was extracted by cool percolation with methanol/water (3:7); the extract was then acidified (0.01 mol/L H_3PO_4) and extracted by ethyl

acetate. Extract yield after evaporation of the solvent was 0.25 g.

3. Decoction (DEC). The material was kept in boiling water under reflux for 20 min and then left to cool at room temperature. Extract yield after freeze-drying was 3.30 g.

4. Tea infusion extract (INF). Boiling water (1000 ml) was poured onto the leaves and then allowed to extract for 20 min while cooling. Extract yield after freeze-drying was 6.06 g.

All extracts were dried until constant weight was achieved. The extraction procedure was repeated 3 times for each sample and the reproducibility of the procedure was controlled by the measurement of total phenolic content in the samples.

Animals

Male Wistar rats weighing 200–250 g were conditioned in standard boxes for 15 days before the experiments. They were fed a standard laboratory diet, provided with water *ad libitum* and kept on a 12:12 h light–dark cycle. For the gluconeogenesis study, rats were food deprived for 24 h before hepatocyte isolation.

Reagents

Tert-butyl hydroperoxide (*t*-BH, 70% in water), 2-thiobarbituric acid (TBA, 98%), trypan blue, dimethyl sulfoxide (DMSO) for cell cultures, metformin (1,1-dimethylbiguanide), Williams' medium E, MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide), fetal calf serum and additives were purchased from Sigma-Aldrich Ltd, Czech Republic. Collagenase was from Sevapharma, Czech Republic. Other chemicals and solvents were of analytical grade from Pliva-Lachema, Czech Republic.

Phenolic content analysis

Total phenolics in all extracts were determined using Folin-Ciocalteau reagent (Singleton and Rossi, 1965). Twenty-five µl of the tested fraction in distilled water was mixed with 500 µl of Folin-Ciocalteau reagent (previously diluted 10-fold with distilled water) and maintained at room temperature for 5 min; 500 µl of sodium bicarbonate (75 g/L) was added to the mixture. After 90 min at 30°C, absorbance was measured at 725 nm. Results were expressed as gallic acid equivalents.

Rat hepatocyte primary cultures

Rat hepatocytes were isolated by two-step collagenase perfusion of rat liver (Moldeus et al., 1978). The cell viability was determined by measuring trypan blue exclusion. Yields of $2–4 \times 10^8$ cells/liver with a viability greater than 80% were routinely obtained. The hepatocytes were then dispersed in sterile conditions in Williams' medium E supplemented by penicillin (10 IU/ml), streptomycin (0.1 mg/ml), dexamethasone (1 µmol/L), insulin (0.1 µmol/L) and glutamine (2 mmol/L) and then cultivated in collagen-coated 12-well dishes in a humidified atmosphere of 5% CO_2 and 37°C. For culture stabilization, 10% fetal calf serum was added to the cultivation medium for the first 4 h. The cultivation medium was replaced by the serum-free one and the tested samples (final concentrations 1–1000 µg/ml) were added to the incubation medium in DMSO (max 0.5%) or in the cell culture medium (aqueous extracts).

For cytotoxicity studies, the hepatocyte monolayers were incubated with the tested samples for 4, 24 and 48 h and viability of the cells was assessed by the MTT test (Sieuwerts et al., 1995).

To study cytoprotective effects against *t*-BH- or AA-induced damage, the primary cultures were intoxicated by *t*-BH for 1.5 h (final

concentration 0.5 mmol/L) or AA for 4 h (final concentration 0.2 mmol/L) after pre-incubation with the tested samples (0.5 h). Positive and negative controls were prepared also. The quality of the culture was controlled by the following parameters: cell viability (MTT test), level of released lactate dehydrogenase LDH (Bergmeyer and Bernt, 1974b) and of lipoperoxidation products (thiobarbituric acid reacting substances, TBARS, Buedge and Aust, 1978) in the medium.

Rat hepatocytes suspensions – study of glucose metabolism

Rat hepatocytes were isolated as described above. Hepatocytes (2×10^6 cells/ml) were incubated at 37°C in 2 ml of Krebs–Henseleit bicarbonate buffer (pH 7.4) for 1 h in a gyratory shaking water bath. Each experiment was performed in triplicate. The tested samples were dissolved in DMSO or in Krebs–Henseleit bicarbonate buffer. Metformin (1,1-dimethylbiguanide), an antihyperglycemic agent capable of reducing hepatic gluconeogenesis (Fulgencio et al., 2001) was used as positive control.

The rates of gluconeogenesis were determined in hepatocytes from 24 h-food-deprived rats after a 1-h incubation period in presence or absence (endogenous) of lactate/pyruvate (10/1 mmol/L) or dihydroxyacetone (10 mmol/L) or alanine (10 mmol/L) as gluconeogenetic precursors and of tested samples or metformin as control. The rates of glycogenolysis were determined in hepatocytes isolated from *ad libitum* fed rats after a 1-h incubation period in presence or absence of tested samples or metformin. Quality of the suspension was monitored by trypan blue exclusion. The incubation was ended by adding 0.2 ml $HClO_4$ (40% v/v).

Glucose level in supernatants after centrifugation was measured by the glucose oxidase enzymatic method as described previously (Bergmeyer and Bernt, 1974a). Data were expressed as % of control.

Fao cells – study of CYP mRNA expression

Fao cells are differentiated derivatives of the clonal cell line H4IIEC3, which was derived from the Reuber H35 rat hepatoma (Deschatrette and Weiss, 1974). They were grown in monolayer culture as previously described (Pavé-Preux et al., 1990). Cells were exposed to insulin (0.1 μmol/L), the usual supplement to primary hepatocyte culture medium or to yacon extracts OF1 (50 and 200 μg/ml), DEC (100 to 500 μg/ml), INF (250 and 500 mg) and OF2 (250 and 400 mg/ml) for 6 h during their exponential phase of growth. Untreated cultures were used as controls except for OF1 and OF2 treatments for which control cells were treated by DMSO (0.3%).

Total RNA from Fao cells was extracted using the Rneasy Mini kit (Qiagen, Courtaboeuf, France). Subsequently, 10 to 20 μg of total RNA were subjected to electrophoresis in denaturing formaldehyde/1.2% agarose gels and transferred to nylon membranes. These membranes were prehybridized and then hybridized with several ^{32}P-labeled cDNA probes: CYP2E, CYP2B1 and 18S rRNA. CYP2E1 and CYP2B1 probes were generously provided by Dr FJ Gonzalez (National Cancer Institute, Bethesda, MD). After hybridization, the filters were washed at 65°C for 30 min successively with 2× standard saline citrate (SSC) (300 mmol/L sodium chloride, 30 mmol/L sodium citrate, pH 7.0)/0.1% SDS, then 0.5× SSC/0.1% SDS and sometimes 0.1× SSC/0.1% SDS then processed for autoradiography. The relative intensities of the hybridization signals were determined by scanning with a phosphorimager Storm 840 (Molecular Dynamics).

Statistics

Data were analyzed with one-way ANOVA using the StatView Statistical Package. Differences were considered statistically significant when $*p$ was <0.05 and $**p<0.01$ compared with the control.

Results

Phenolic content analysis

The phenolic content in the extracts were $24.6\pm0.6\%$ for OF1, $22.1\pm3.4\%$ for OF2, $10.7\pm0.4\%$ for DEC, and $11.8\pm0.4\%$ for INF. The content in the two organic fractions were significantly different from both the aqueous extracts, while OF1 was not significantly different from OF2 and DEC did not differ from INF.

Cytotoxicity

The basal cytotoxicity was determined in the model of rat hepatocytes primary cultures by measuring the ability of the cells to reduce 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) into violet-coloured formazane (MTT test, Sieuwerts et al., 1995) after 4, 24 and 48 h of incubation. In the concentrations tested (1, 10, 100, and 1000 μg/ml), no significant toxicity was noted after 4 and 24 h, and after 48 h, only organic fractions OF1 and OF2 exhibited significant cytotoxicity in the highest concentration (viability 26.5% and 57.3%, respectively). For all cytoprotectivity studies, incubation periods shorter then 24 h were utilized.

Cytoprotectivity

For the protectivity measurement, the stabilised rat hepatocyte primary cultures were preincubated with the tested extracts for 30 min. The oxidative damage of the cultures was induced by *tert*-butyl hydroperoxide (*t*-BH, 0.5 mmol/L and 1.5 h, Figure 1) and allyl alcohol (AA, 0.2 mmol/L and 4 h, Figure 2).

In a non-treated typical cell culture, the MTT test gave $A_{540\ nm} = 1.009\pm0.030$, the activity of LDH was 4.70 ± 0.50 μkat/L, and TBARS level in culture medium was 0.35 ± 0.05 μmol/L. After intoxication with *t*-BH, these values changed to 0.193 ± 0.010 (MTT), 21.65 ± 1.74 μkat/L (LDH), and 4.44 ± 0.19 μmol/L (TBARS). If the cell culture was preincubated with 1000 μg/ml of OF2 before *t*-BH application, $A_{540\ nm} = 0.928\pm0.033$, LDH activity = 6.27 ± 1.67 μkat/L, and TBARS level was 1.97 ± 0.82 μmol/L. In the case of AA, the toxic effect was less pronounced. The MTT test gave $A_{540\,nm} = 0.234\pm0.047$, LDH activity = 11.97 ± 0.25 μkat/L, and the TBARS level was 1.03 ± 0.03 μmol/L. OF2 at 1000 μg/ml restored the control values to 1.095 ± 0.033 (MTT), 5.66 ± 0.59 μkat/L (LDH), and 0.38 ± 0.21 μmol/L (TBARS).

For comparison of the activity of different extracts at various concentrations, we expressed cytoprotective effects as percentage of controls (Figures 2 and 3). All the extracts tested showed dose dependent and in most cases significant protective activity in this model. The highest activity was noted with the fraction OF2 in the highest concentration tested (1000 μg/ml), reaching nearly 100% protectivity. With aqueous extracts INF and DEC, the cytoprotective effect was lower, about 50% in the highest concentration tested.

Glucose metabolism

In freshly isolated hepatocytes from 24 h-food-deprived rats (gluconeogenesis, Figure 3a), glucose production depended on the gluconeogenetic precursor provided to the cells: 1.64 ± 0.03 (without precursor), 16.06 ± 0.78 (lactate/pyruvate), 85.75 ± 5.20 (dihydroxyace-

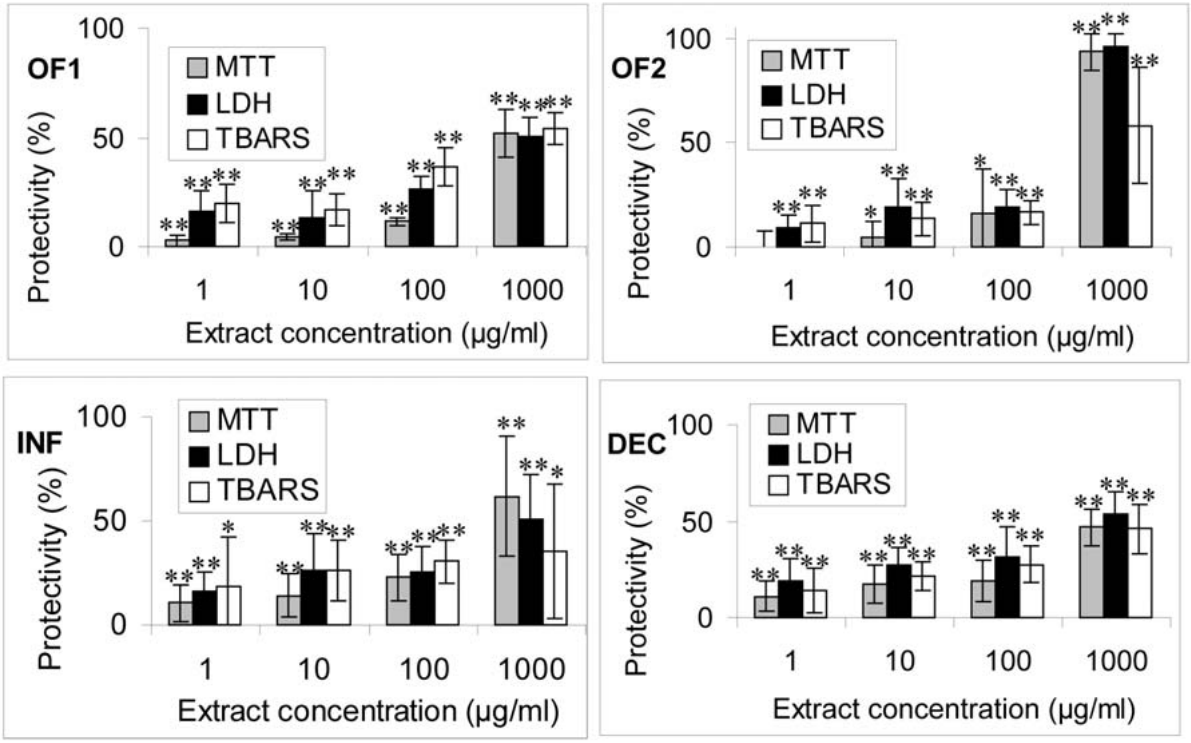

Figure 1. Protective effect of extracts on *tert*-buty lhydroperoxide-induced damage of rat hepatocyte primary cultures. After 30 min preincubation with tested extracts, the cell monolayers were treated with 0.5 mmol/L *tert*-butyl hydroperoxide for 1.5 h. Results are expressed as mean $\pm$ SD, n = 9; maximum protective effect is considered to be 100%. Significant differences from control (*t*-BH-intoxicated) cells (*$p<0.05$, **$p<0.01$) are indicated.

tone), and 17.92 ± 1.22 (alanine) nmol/h/10^6 cells. In hepatocytes from *ad libitum*-fed rats (glycogenolysis, Figure 3b), glucose production was typically 73.58 ± 3.59 nmol/h/10^6 cells. All the extracts tested reduced glucose production in both models as compared with the control cells incubated in the absence of the extracts (45–90% of controls).

CYP mRNA expression

Insulin and also extracts from *S. sonchifolius* leaves reduced CYP mRNA expression maximally to 35% of the controls. The most pronounced effect on CYP2B and CYP2E mRNA was observed in organic fractions OF1 (200 µg/ml) and OF2 (250 µg/ml), whilst the aqueous infusion demonstrated weaker effect. In water decoction, the effect on mRNA expression was not significant even at the highest concentration tested (500 µg/ml). The effects of the two organic fractions were with the same order of magnitude ($p<0.01$) than insulin (0.1 µmol/L, Figure 4).

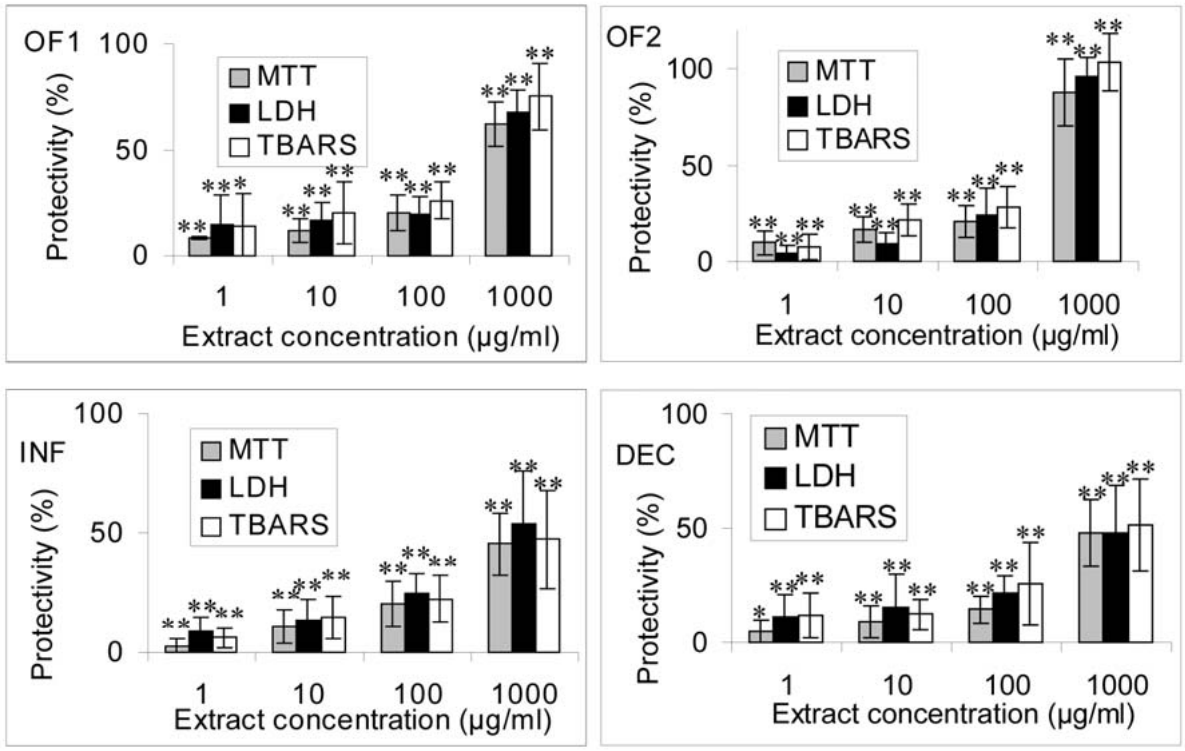

Figure 2. Protective effect of extracts on allyl alcohol-induced damage of rat hepatocyte primary cultures. After 30 min preincubation with tested extracts, the cell monolayers were treated with 0.2 mmol/L allyl alcohol for 4 h. Results are expressed as mean ± SD, $n = 9$; maximum protective effect is considered to be 100%. Significant differences from control (AA-intoxicated) cells (*$p < 0.05$, **$p < 0.01$) are indicated.

Discussion

The protective effect of the extracts observed in rat hepatocyte primary cultures during our experiments was probably due to the high content of phenolic compounds (phenolics). Antioxidant and protective effect on *t*-BH-induced oxidative damage of U937 human monocytic cells (Nardini et al., 2001) and of rat mitochondria and hepatocytes (Psotová et al., 2003) of phenolics was described previously. Moreover, this effect is in accordance with our previous *in vitro* study of antioxidant effect (DPPH and superoxide radical scavenging, inhibition of lipoperoxidation) and with our preliminary results on protective effect of two extracts (identical to the organic fractions OF1 and OF2) on *t*-BH-induced oxidative damage of rat hepatocyte primary cultures (Valentová et al., 2003). The protection is most important for the organic fraction OF2, which exhibited almost 100% protectivity at the highest concentration used (1000 µg/ml). Concerning the aqueous extracts, the weaker effect compared to OF2 is in agreement with its lower content of phenolic compounds. The effect observed corresponds with total phenolic content in the extracts (22.1 ± 3.4% for OF2 versus 10.7 ± 0.4% for DEC or 11.8 ± 0.4 % for INF).

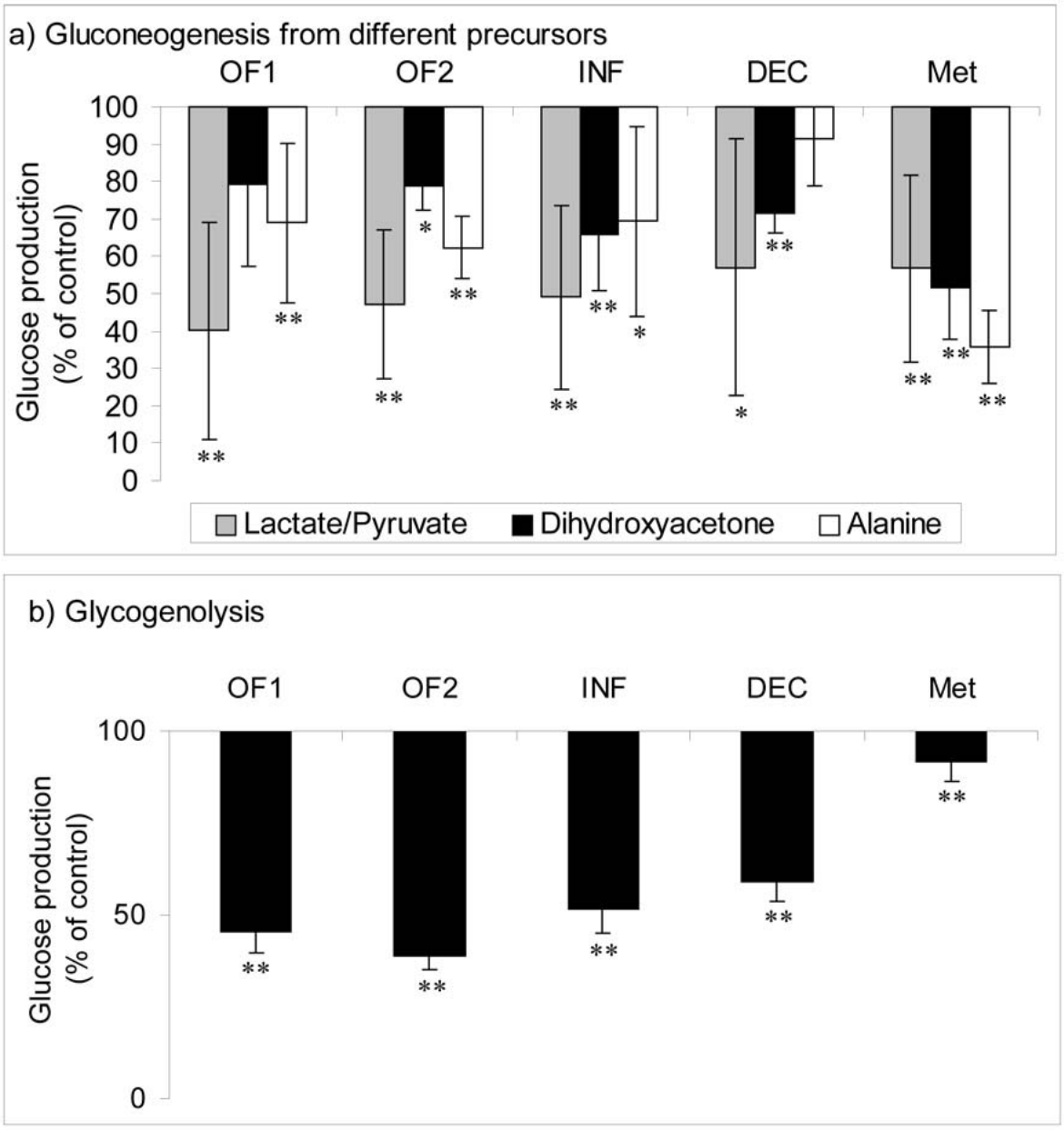

Figure 3. Effect of extracts (1 mg/ml) and metformin (met, 5 mmol/L) on glucose metabolism in suspensions of rat hepatocytes. After 60 min incubation in the absence/presence of tested extracts and gluconeogenetic precursors, the production of glucose was measured in the incubation medium. Results are expressed as mean ± SD, $n = 9$; glucose production in control cells was considered to be 100%. Significant differences from control cells (*$p < 0.05$, **$p < 0.01$) are indicated.

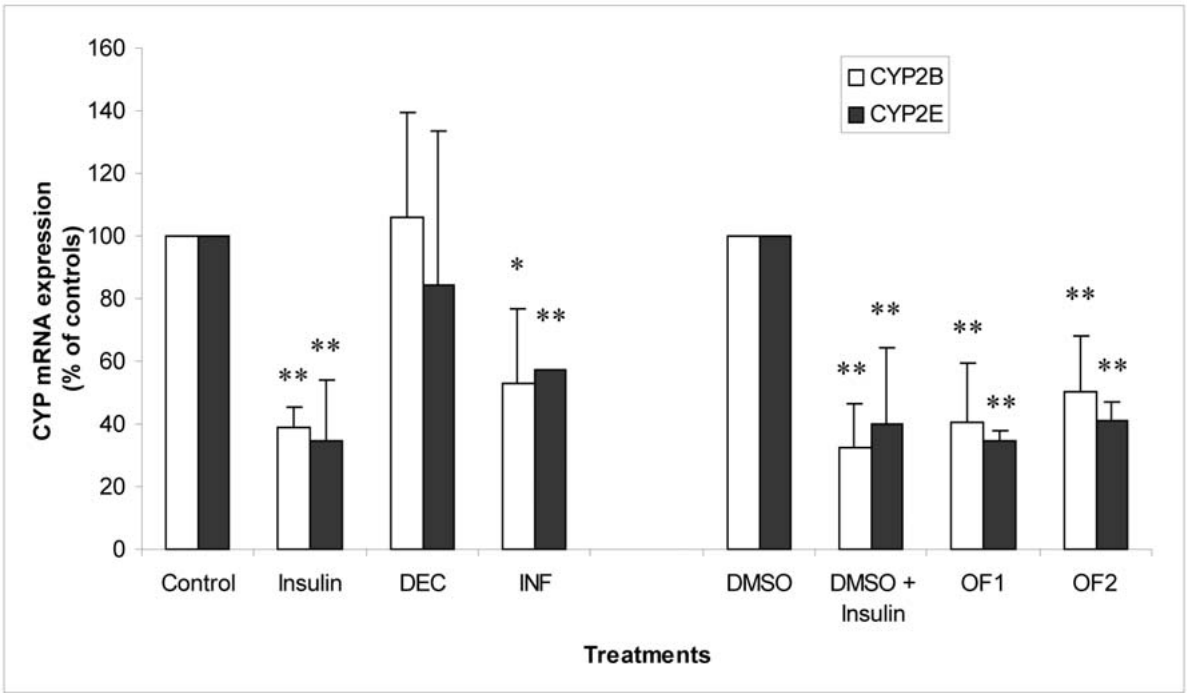

Figure 4. Effects of insulin, *Smallanthus sonchifolius* extracts on CYP2B and CYP2E mRNA expression in rat hepatoma H4IIEC3 cell line. The results for cells treated with the extracts DEC, INF (500 μg/ml), OF1 (200 μg/ml), and OF2 (250 μg/ml) or insulin (0.1 μmol/L) are expressed as mean ± SD; the mRNA expression in control cells was considered to be 100%. Concentrations of the extracts for which the effect was the most similar to insulin were chosen for the figure, OF1 and OF2 were dissolved in DMSO. Significant differences from control cells (*$p < 0.05$, **$p < 0.01$) are indicated.

All extracts tested were able to reduce hepatic glucose production. In the case of glycogenolysis, the effect is even more pronounced than for metformin, a frequently used antihyperglycemic drug. This effect is in accordance with previous findings, that metformin inhibits glucose production only by inhibition of gluconeogenesis and its effect on glycogenolysis is weak (Hansen and McCormack, 2002). Our results suggest that the antidiabetic effect of *Smallanthus sonchifolius* aqueous extracts proved *in vivo* (Aybar et al., 2001) is probably due not only to an increase in plasma insulin concentration, but also to inhibition of hepatic gluconeogenesis and glycogenolysis.

The rats with streptozotocin-induced diabetes used previously for yacon aqueous extracts testing *in vivo* (Aybar et al., 2001) are more sensitive than control animals to several procarcinogens presumably due, at least in part, to increased levels of hepatic CYP2B and CYP2E expression and STZ-induced diabetes may modulate the metabolic activation of some toxic chemical compounds (Favreau and Schenkman, 1988; Ioannides et al., 1988; Yamazoe et al., 1989). These changes can be reversed by daily insulin treatment (Favreau and Schenkman 1988). Fao cells possess numerous insulin receptors by which insulin regulates the expression of several genes (Messina 1990, Aggerbeck et al., 1993) and express CYP2B and CYP2E (de Waziers et al., 1992). In this *in vitro* model, insulin has been shown to directly down-regulate the expression of

these genes by shortening the half-life of their mRNAs (de Waziers et al., 1995).

All the *S. sonchifolius* extracts tested, except for water decoction (DEC), were able to down-regulate the expression of CYP2B and CYP2E mRNA. The effects of the organic fractions OF1 and OF2 (200 and 250 μg/ml, $p < 0.01$) and to a lesser extent, the tea infusion extract INF (500 μg/ml, $p < 0.02$) on rat CYP2B and CYP2E mRNA expression were not significantly different from those observed with insulin. These effects observed in the Fao cell line confirm their insulin-like properties previously observed in their ability to reduce glucose production. Further experiments to evaluate the effect of extracts on glucose metabolism intermediates and enzymes level are in progress.

The phytochemical investigation of yacon leaves (Jirovsky et al., 2003; Lachman et al., 2003; Simonovska et al., 2003; Valentová et al., 2003) revealed chlorogenic, dicaffeoylquinic and caffeic acids as main phenolic compounds. Chlorogenic and caffeic acids, known predominantly for their antioxidant and free radical scavenging properties (Kono et al., 1998; Nardini et al., 1995, 1997; Chen et al., 1997), have recently been identified also as active components in regulation of glucose metabolism. Thus, caffeic acid lowered plasma glucose in streptozocin-induced diabetic rats (Hsu et al., 2000), chlorogenic acid and its derivatives are competitive inhibitors of glucose-6-phosphatase (Arion et al., 1997, 1998; Hemmerle et al., 1997) and subsequently of glucose production in isolated perfused rat liver (Hemmerle et al., 1997). *In vivo*, chlorogenic acid was found to improve glucose tolerance and insulin resistance, decrease some plasma and liver lipids and improve mineral pool distribution in obese, hyperlipidemic and insulin resistant (*fa/fa*) Zucker rats (type 2 diabetes animal model). It did not stimulate insulin release, thus it is not possible for chlorogenic acid to cause sustained hypoglycemia (de Sotillo et al., 2002).

Botanical products can improve glucose metabolism in persons with diabetes not only by direct hypoglycemic effects, but also by improving lipid metabolism, antioxidant status and capillary function (Broadhurst et al., 2000). Increased oxidative stress is a widely accepted participant in the development and progression of diabetes mellitus and its complications; diabetes is usually accompanied by increased production of free radicals or impaired antioxidant defences (Maritim et al., 2003). In rats with streptozocin-induced diabetes, renal antioxidant enzymes superoxide dismutase and catalase mRNA levels are increased (Sechi et al., 1997) and non-enzymatically glycated proteins have been found to produce superoxide radical (Sakurai and Tsuchiya, 1988).

Extracts of many common culinary herbs and spices demonstrated insulin-like or insulin potentiating action in adipocytes *in vitro* and over 200 pure phytochemicals (among them compounds with aromatic hydroxyl groups, polysaccharides and peptidoglycans) are known to be hypoglycemic (Broadhurst et al., 2000); the hypoglycemic activity of black tea has been ascribed to both polyphenolics and polysaccharides (Gomes et al., 1995). Many of the phytochemicals were found to be hypoglycemic due to metabolic or hepatic toxicity (Broadhurst et al., 2000). In as much as we used only non-toxic concentrations; toxicity is probably not involved in the mechanism of hypoglycemic and insulin-like activity of *S. sonchifolius* extracts.

In view of the complexity of the processes regulating glucose metabolism as well as the complexity of plant extracts, it is difficult to evaluate correctly the effects observed at the *in vitro* level. The activity of *S. sonchifolius* leaf extracts reported in this work may be attributed not only to phenolic compounds, but also to other phytochemicals that they contain, and the effect on glucose metabolism might, but does not need, to be mediated by the insulin-

like effect or by improvement in antioxidant status. In all cases, the combination of radical scavenging, cytoprotective and anti-hyperglycemic activity make *S. sonchifolius* leaves a good candidate for use in prevention and treatment of chronic diseases involving oxidative stress, particularly diabetes mellitus.

Acknowledgments

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