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# Induction of Glucokinase mRNA by Dietary Phenolic Compounds in Rat Liver Cells in Vitro

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Diabetes and its complications, including oxidative stress, are major reasons for medical intervention and one of the most frequent causes of death in developed countries. Several lines of data suggest that the use of certain dietary polyphenolic compounds may alter glucose metabolism, thus decreasing the risk for type 2 diabetes. In this paper, we present the effect of phenolic acids (caffeic, chlorogenic, rosmarinic, and ferulic) and extracts from *Smallanthus sonchifolius* and *Prunella vulgaris* on glucose production in rat hepatocytes and on glucokinase, glucose-6-phosphatase, and phosphoenol–pyruvate carboxykinase mRNA expression in rat hepatoma Fao cells. The phenolics at 500 $\mu$M and after 1 h incubation lowered glucose production via both gluconeogenesis (10 mM alanine or dihydroxyacetone as precursors) and glycogenolysis compared with metformin. Most of the phenolics increased the level of glucokinase mRNA after 24 h in the same way as insulin ($10^{-7}$ M).

**KEYWORDS: Glucose metabolism; hepatocytes; Fao cells; phenolics; plant extracts**

## INTRODUCTION

Diabetes and its complications, including oxidative stress, are major reasons for medical intervention and one of the most frequent causes of death in developed countries. Considering the heterogeneity of this disease, current therapies are often limited. For this reason, the exploration of novel compounds with improved antidiabetic action is of paramount importance.

Phenolic compounds, widely distributed in food plants, act as primary antioxidants and thus may be beneficial for improving and/or preventing a number of chronic diseases (*1*). Moreover, in addition to their antioxidant effects, several lines of data suggest that certain dietary polyphenolic compounds may alter glucose metabolism (*1, 2*). For instance, recent studies have linked heavy regular use of coffee to decreased risk for type 2 diabetes (*3–5*). Caffeine itself seems unlikely to mediate this benefit since the apparent effect has been observed in those who use decaffeinated coffee. For this reason, chlorogenic acid present in coffee or its hydrolytic product, caffeic acid, may be responsible. Moreover, caffeic acid is known to have antidiabetic effects in streptozotocin-induced diabetic rats (*6*).

The liver plays an important role in regulating plasma glucose levels and is therefore, apart from the pancreas, a target organ for the prevention and treatment of glucose metabolism disorders. We have previously shown that phenolic extracts of yacon (*Smallanthus sonchifolius* [Poepp. & Endl.] H. Robinson, Asteraceae) leaves were able to reduce glucose production in suspensions of rat hepatocytes. Moreover, these extracts displayed insulin-like effects on the expression of cytochrome P450 (CYP) 2B1 and 2E1 mRNA in rat hepatoma Fao cells (*7*). As the phytochemical investigation of yacon leaves (*8–11*) revealed chlorogenic and caffeic acids, as main phenolic compounds, we have hypothesized that these phenolic acids were responsible for the hypoglycemic activity of the leaf extracts. No effect on glucose metabolism was up to date described for other bioactive constituents of *S. sonchifolius* leaves (*7*). With the aim of confirming this hypothesis, we present here the effects of several purified phenolic acids (caffeic (CAF), chlorogenic (CHLOR), rosmarinic (ROS) and ferulic (FER, **Figure 1**)) and mixed phenolic compounds contained in plant extracts (*S. sonchifolius* leaves (SSL) and tubers (SST), *Prunella vulgaris* aerial parts (PVA)) on glucose production in rat hepatocytes. Furthermore,

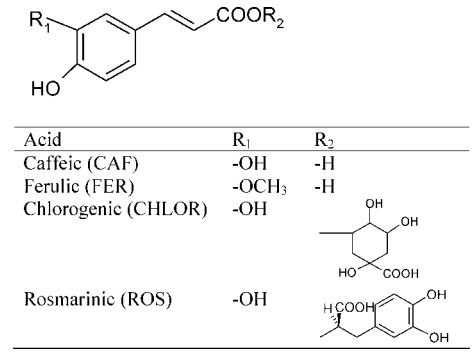


**Figure 1.** Structures of phenolic acids studied.

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in rat hepatoma cell lines, the effects of these different compounds were measured on the mRNA expression of genes encoding enzymes involved in glucose metabolism: phosphoenol–pyruvate carboxykinase (E.C. 4.1.1.32, PEPCK), glucose-6-phosphatase (E.C. 3.1.3.9, G6Pase), and glucokinase (E.C. 2.7.1.2, GK).

## MATERIALS AND METHODS

**Chemicals.** Alanine, 8-bromo-cAMP, chlorogenic acid, dexamethasone, dihydroxyacetone, dimethyl sulfoxide (DMSO), ethidium bromide, gallic acid, metformin (1,1-dimethylbiguanide), and trypan blue were purchased from Sigma-Aldrich Ltd., Czech Republic, collagenase from *Clostridium histolyticum* (0.263 U/mg) was purchased from Serva, Czech Republic, human biosynthetic insulin (humulin) was from Lilly, France, and ferulic acid was from Merck, Germany; caffeic and rosmarinic acids were isolated from PVA by a procedure described elsewhere (*12*). Other chemicals and solvents were of analytical grade from Pliva-Lachema, Czech Republic.

**Plant Material.** *S. sonchifolius* plants (originally purchased from Ecuador) were grown at the Potato Research Institute in Havlíčkův Brod, Czech Republic. The leaves and tubers were collected in October 2000 and identified as *Smallanthus sonchifolius* [Poepp. & Endl.] H. Robinson by the Department of Botany, Palacký University, Olomouc, Czech Republic (*13*). The leaves were dried separately from the tubers at room temperature; the tubers were first peeled, cut into 8 × 8 mm cubes, predried for 12 min at 115 °C, and then dried (31 min at 107 °C, 96 min at 100 °C, and 100 min at 75 °C) according to the standardized commercial procedure as for carrots at SEVEROFRUKT Trávčice Co., Czech Republic.

*P. vulgaris* aerial parts were collected in bloom in July 2001 in Markvartice, Czech Republic, and identified as *Prunella vulgaris* L. by Planta Naturalis, Markvartice, Czech Republic (*12*). The plant was dried in a drying cabinet with forced ventilation at 40 °C for 4 days.

Voucher specimens were deposited at the Department of Medical Chemistry and Biochemistry, Palacký University Olomouc.

**Extraction Procedures.** *1. S. sonchifolius Leaves (SSL).* Dried yacon leaves (200 g) were extracted with MeOH (1 L) at 60 °C for 24 h. The extract was evaporated under reduced pressure at 60 °C, suspended in 1% aqueous sulfuric acid (1 L), and successively extracted with petroleum ether (3 L). The petroleum ether layer was not further processed. The aqueous layer was then extracted successively with ethyl acetate (3 L). The ethyl acetate layer was evaporated to dryness in vacuo (*12*) to give 2.6 g of a solid residue SSL with total phenolic content 24.6 ± 0.6% of gallic acid equivalents (GAE, determined by Folin-Ciocalteu reagent as described hereafter).

*2. S. sonchifolius Tubers (SST).* Dried yacon tubers (200 g) were extracted with MeOH at 60 °C for 24 h. The extract was evaporated under reduced pressure at 60 °C, suspended in 1% aqueous sulphuric acid (1 L), and successively extracted with ethyl acetate (3 L). The ethyl acetate layer was evaporated to dryness in vacuo to give 4.4 g of a solid residue SST (4.54 ± 0.58% GAE).

*3. P. vulgaris Aerial Parts (PVA).* The dried and powdered herb (200 g, 2.2% w/w of rosmarinic acid) was extracted as *S. sonchifolius* leaves to give 11.2 g of solid residue of PVA, which was characterized by its rosmarinic and caffeic acid content (25.7% and 0.32% w/w, respectively; determined by HPLC (*12*) and 101.71 ± 8.08% GAE).

**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 (KrmiMo Mohelsky, Czech Republic), provided with water ad libitum and kept on a 12/12 h light–dark cycle. Use of animals for hepatocyte isolation was approved by the Ethics Committee, Ministry of Education, Czech Republic.

**Phenolic Content Analysis.** Total phenolics in all extracts were determined using the Folin-Ciocalteau reagent (*14*). 5 μL of the tested fraction in distilled water was mixed with 100 μL of the Folin-Ciocalteau reagent (previously diluted 10-fold with distilled water) and maintained at room temperature for 5 min; 100 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.

**Table 1.** Oligonucleotide Primer Sequences Used in RT-PCR Analysis

| | oligonucleotide | sequences |
|---|---|---|
| glucose-6-phosphatase | forward primer | 5′-CTTGTGGTTGGGATACTGG-3′ |
| | reverse primer | 5′-GAGGCTGGCATTGTAGATG-3′ |
| glucokinase | forward primer | 5′-GAGCAGAAGGGAACAACATC-3′ |
| | reverse primer | 5′-TGGCGGTCTTCATAGTAGC-3′ |
| calmodulin | forward primer | 5′-TGATGACAGCGAAGTGAAG-3′ |
| | reverse primer | 5′-AGGTTGAAGGACAATGACAG-3′ |
| PEPCK | forward primer | 5′-TGTTGGCTGGCTCTCACTG-3′ |
| | reverse primer | 5′-ACTTTTGGGGATGGGCAC-3′ |

**Rat Hepatocyte Suspensions: Study of Glucose Production.** Rat hepatocytes were isolated by two-step collagenase perfusion of rat liver (*15*). The cell viability was determined by measuring trypan blue exclusion. The production of glucose in 60 min was determined in hepatocytes isolated from ad libitum fed rats (glycogenolysis) and in hepatocytes isolated from 24 h food-deprived rats (gluconeogenesis) in the presence of alanine (10 mM) or dihydroxyacetone (10 mM) as glucose precursors. Hepatocytes ($2 \times 10^6$ cells/mL) were incubated 1 h in 2 mL of Krebs-Henseleit bicarbonate buffer in the presence or absence of tested samples (500 μM for phenolic acids and 0.3 mg/mL for plant extracts) (*7*). Metformin (1,1-dimethylbiguanide) (5 mM) (*16*) was used as positive control. The incubation was terminated by centrifugation (500 g, 5 min). The glucose level in supernatants after centrifugation was measured by the glucose oxidase enzymatic method (*17*). Data were expressed as percent of control (DMSO treated or untreated for MET) and presented as mean ± SD from three independent hepatocyte isolations; incubations were performed in triplicate.

**Fao Cells: Study of mRNA Expression.** Fao cells are differentiated derivatives of the clonal cell line H4IIEC3, which was derived from the Reuber H35 rat hepatoma (*18*). They were grown in monolayer culture as described previously (*19*) and seeded on six-well plates at a density of $0.3 \times 10^6$ cells by well. One day later, the medium was changed and replaced with a medium without glucose but supplemented with 8-bromo-cAMP (0.5 mM) and dexamethasone (0.1 μM). The following day, cells were incubated with tested samples for 24 h. Phenolic acids and insulin, dissolved in DMSO, and metformin were added to culture medium to give a final concentration of 100 μM caffeic acid (CAF), 100 μM chlorogenic acid (CHLOR), 100 μM ferulic acid (FER), 30 μg/mL *P. vulgaris* (PVA, corresponding to 21 μM rosmarinic acid), 25 μM rosmarinic acid (ROS), 10 μg/mL *S. sonchifolius* leaves (SSL), 10 μg/mL *S. sonchifolius* tubers (SST), 0.1 μM insulin (INS), and 100 μM metformin (MET). DMSO concentration in culture medium was 0.1% (v/v). According to the treatment used, untreated (for INS and MET) or DMSO-treated cells were used as controls.

Total RNA from Fao cells was extracted using the Rneasy Mini kit (Qiagen, Courtaboeuf, France) and RNase-free DNase set (Qiagen) according to the supplier's recommendations. The amount of RNA was estimated by the absorbance at 260 nm. The quality of the RNA samples was determined by the absorbance ratio at 260/280 nm and by electrophoresis through denaturing agarose gels and staining with ethidium bromide. The 18S and 28S rRNA subunits were visualized under ultraviolet light.

Reverse transcription (RT) was performed on 2 μg of RNA samples using the cDNA High-Capacity Archive kit (Applied Biosystems, Courtaboeuf, France) in a final volume of 25 μL according to the manufacturer's instructions. Specific oligonucleotide primers were designed using oligoexplorer software (Oligosoftware), and to avoid amplification of contaminating genomic DNA, one of the two primers spanned an exon–exon junction. PEPCK primers, provided by Dr. C. Benelli (INSERM U747), were designed using the same criteria. For each primer pair, we performed no-template control (NTC) and no-reverse-transcriptase control (RT negative) assays, which produced negligible signals (usually >40 in Ct value), suggesting that primer–dimer formation and genomic DNA contamination effects were negligible. Gel electrophoresis was performed to verify the size of the specific amplicon and the absence of additional PCR fragment. Oligonucleotide primer sequences are presented in **Table 1**. Quantitative changes in mRNA expression were assessed with a real-time quantita-

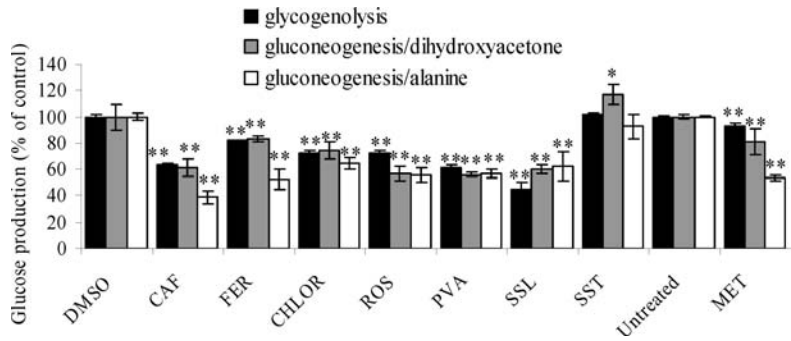


**Figure 2.** Effect of phenolic compounds on glucose metabolism in suspensions of rat hepatocytes. After 60 min incubation in the absence/presence of tested samples and gluconeogenetic precursors, the production of glucose was measured in the incubation medium. Results are expressed as mean ± SD from three independent hepatocyte isolations; incubations were performed in triplicate, and glucose production in control cells was considered to be 100%. CAF = caffeic acid, FER = ferulic acid, CHLOR = chlorogenic acid, ROS = rosmarinic acid (all 500 $\mu$M), PVA = *Prunella vulgaris* aerial parts, SSL = *Smallanthus sonchifolius* leaves, SST = *Smallanthus sonchifolius* tubers (organic fractions, all 0.3 mg/mL), and MET = metformin 5 mM. * $p < 0.05$, ** $p < 0.01$ compared to DMSO-treated or untreated (for MET) controls.

tive RT-PCR using SYBR-Green detection consisting of SYBR Green PCR Master Mix (ABGene, Courtaboeuf, France), 300 nM of each primer and 4 ng of cDNA in a final volume of 10 $\mu$L. PCR cycles proceeded as follows: denaturation 15 min at 95 °C, followed by 40 cycles at 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s. We also quantified transcripts of the calmodulin gene as the endogenous control, and each sample was normalized on the basis of its calmodulin content. Relative quantification of the mRNA expression levels of target genes was calculated using the $2^{-\Delta\Delta Ct}$ method, where $\Delta\Delta Ct = (Ct_{\text{gene studied}} - Ct_{\text{calmodulin}})_{\text{treated}} - (Ct_{\text{gene studied}} - Ct_{\text{calmodulin}})_{\text{control}}$. The parameter Ct (threshold cycle) is defined as the fractional cycle number at which the fluorescence, generated by SYBR green dye–amplicon complex formation, passes a fixed threshold above baseline. The increase in fluorescent signal associated with exponential growth of PCR products was detected by the laser detector of the ABI Prism 7900 Sequence Detection System (Perkin-Elmer Applied Biosystems, Foster City, CA), using PE Biosystems analysis software according to the manufacturer's manuals.

**Statistical Analysis.** Statistical significance was tested by the distribution-free Wilcoxon paired *t* test between untreated cells or DMSO-treated cells and phenolic acids, insulin, or metformin-treated cells. The minimal level of significance accepted was $<0.05$.

## RESULTS

The first goal of this work was to look at the acute effect of several phenolics and metformin on glucose production in 1 h incubated hepatocytes. In freshly isolated hepatocytes from 24 h food-deprived rats (gluconeogenesis), glucose production depended on the gluconeogenetic precursor provided to the cells: 1.64 ± 0.03 (without precursor), 85.75 ± 5.20 (dihydroxyacetone), and 17.92 ± 1.22 (alanine) nmol/h/$10^6$ cells. In hepatocytes from ad libitum fed rats (glycogenolysis), glucose production was typically 73.58 ± 3.59 nmol/h/$10^6$ cells. All the phenolics tested, except SST, lowered glucose production ($p < 0.01$) from glycogen (glycogenolysis) and also from dihydroxyacetone and alanine (gluconeogenesis). The most pronounced effect was observed with 500 $\mu$M caffeic acid and alanine as precursors (**Figure 2**). None of the samples tested influenced hepatocyte viability in the suspensions as controlled by trypan blue retention (data not shown).

Given the effects observed on glucose production, we have undertaken to research potential effects of these phenolic acids and plant extracts on mRNA levels of key enzymes involved in glucose metabolism: PEPCK, G6Pase, and GK. Fao cells possess an active gluconeogenic pathway, and when they grow in glucose-free medium, they release glucose for over 8 h. Addition of 8-bromo-cAMP and dexamethasone results in an increase in glucose release which can be significantly suppressed by insulin (*20*, *21*). Preliminary experiments were then performed to choose concentrations of phenolic acids and plant extracts without any detrimental effect on growth and viability (using trypan blue exclusion) of Fao cells.

None of the phenolic acids influenced PEPCK mRNA expression which was down-regulated by insulin. G6Pase mRNA was decreased by insulin, increased by PVA, and remained unaffected by other treatments. Most of the phenolic acids tested (CAF, CHLOR, ROS, SSL) and insulin increased the level of glucokinase mRNA (**Figure 3**). Metformin had no effect on mRNA levels of any of the genes studied.

## DISCUSSION

Before considering the effect of phenolic compounds in vitro, we must emphasize that the concentrations used in incubated hepatocytes are much higher than levels founds in peripheral blood plasma after eating phenolic rich food (ca. 1 $\mu$M). The experimental model used required high concentration of tested compounds and extracts because of single short time period of exposure but proved previously (*16*) to be relevant for this kind of studies more than cultured hepatocytes, which quite rapidly lose their differentiated functions. In the present research, the phenolic acids (CAF, FER, CHLOR, ROS) and also organic fractions from aerial parts of *S. sonchifolius* (SSL) and *P. vulgaris* (PVA) reduced glucose production in freshly isolated hepatocytes regardless of the nutritional status of the rat (fed ad libitum or 24 h food deprived). These results confirm our earlier hypothesis that phenolic acids are mainly responsible for the antihyperglycemic activity of extracts and fractions of yacon leaves (*7*) and they verify previous results showing that metformin reduced the rates of glucose production from alanine and dihydroxyacetone (*16*). These results suggest that not only yacon leaves but also other plants rich in phenolic compounds, such as *P. vulgaris*, could be used in the prevention and auxiliary treatment of diabetes and related disorders. In case of some substances (CAF, FER, MET), their inhibitory effect on gluconeogenesis was higher when glucose was formed from alanine compared to dihydroxyacetone. This finding suggests involvement of some metabolic processes in the gluconeogenetic pathway between alanine– pyruvate and dihydroxyacetone phosphate (**Figure 4**).

In order to elucidate the mechanism of phenolic action on glucose metabolism, the effects of these substances on key regulatory enzyme mRNA expression from glucose metabolism (**Figure 4**) were examined. Rat hepatocytes in suspension required high concentrations of tested compounds because of

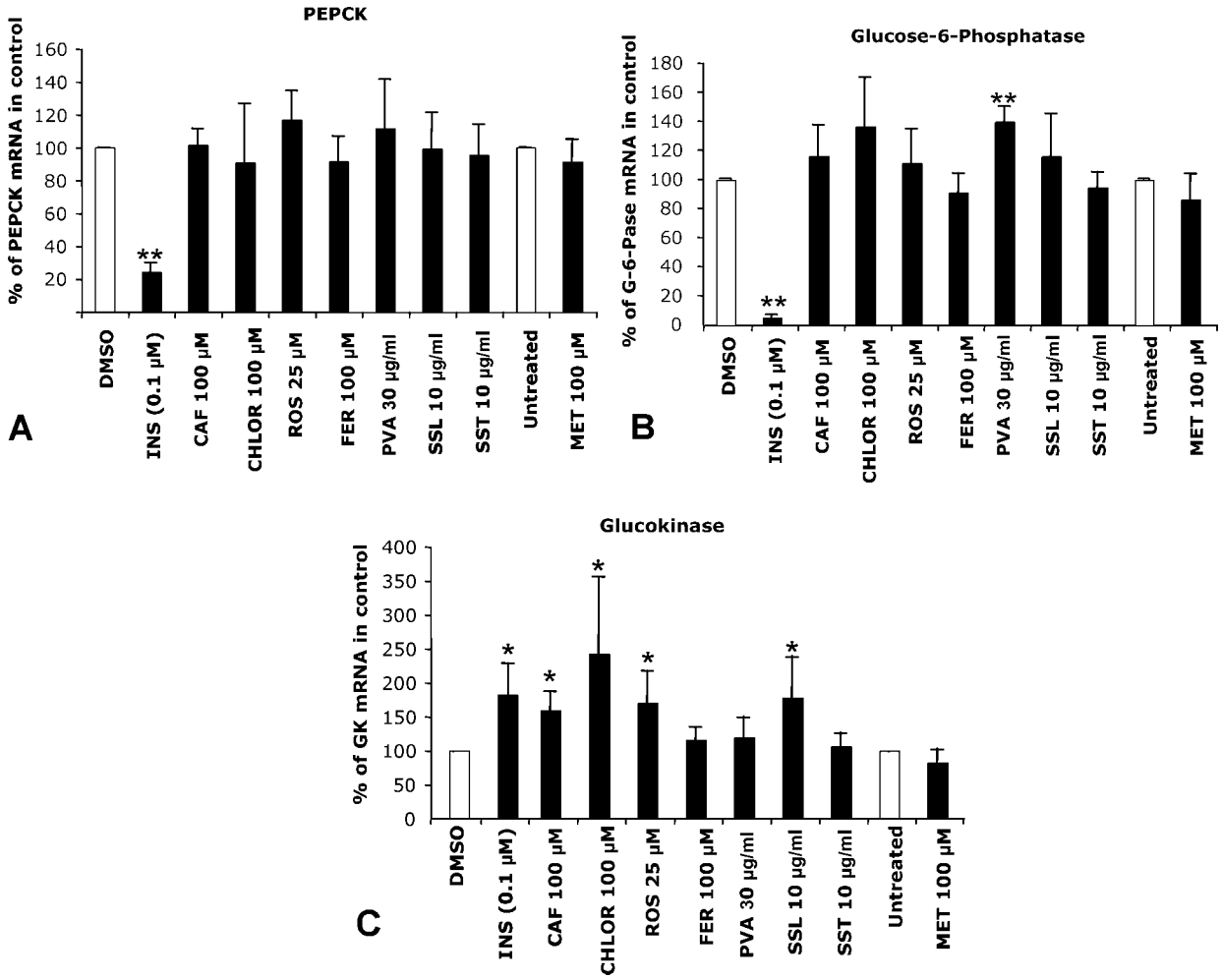


**Figure 3.** Effect of phenolic compounds on selected mRNA production in Fao cells. The cells were treated with the tested compounds for 24 h. Relative quantification of the mRNA expression levels of target genes was calculated using the $2^{-\Delta\Delta Ct}$ method (see experimental section) using the calmodulin gene as the endogenous control. Results are expressed as mean ± SD from four or five independent experiments performed in triplicate. CAF = caffeic acid, FER = ferulic acid, CHLOR = chlorogenic acid, ROS = rosmarinic acid, PVA = *Prunella vulgaris* aerial parts, SSL = *Smallanthus sonchifolius* leaves, SST = *Smallanthus sonchifolius* tubers (organic fractions), MET = metformin, PEPCK = phosphoenolpyruvate carboxykinase. * $p < 0.05$, ** $p < 0.01$.

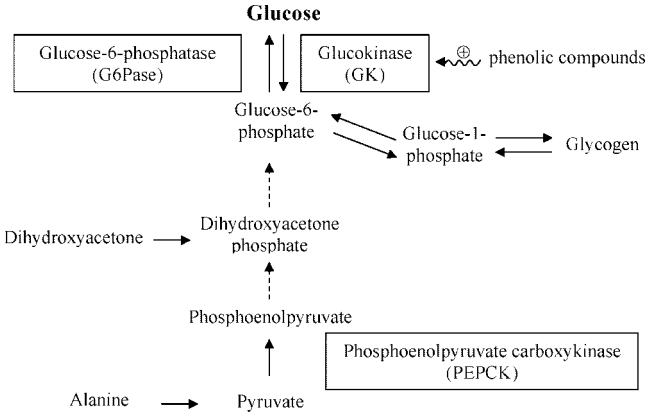


**Figure 4.** Main pathways of glucose production in hepatocytes. Glucokinase is the target of caffeic, chlorogenic, and rosmarinic acids and organic fraction from *Smallanthus sonchifolius* leaf extract (→ direct transformation, --→ transformation through intermediate(s), ⇝ influence).

quite short period of exposure (1 h). Longer exposure is needed to induce an effect in gene expression. The concentrations used in freshly isolated hepatocytes induced severe toxicities in Fao cells, and before the second part of the experimental work we performed to select, for each product, doses with no effect on the growth and viability of these cells over 24 h: 10–100 $\mu$M (CHLOR, ROS, FER, CAF, MET), 5–10 $\mu$g/mL (SST, SSL), and 10–30 $\mu$g/mL of PVA (corresponding 7–35 $\mu$M ROS).

As anticipated from the literature (*20*–*23*), insulin decreased PEPCK, and G6Pase mRNA and increased GK mRNA expression. Insulin stimulation of GK expression at the transcriptional level was described previously (*23*, *24*), and recently So-Youn et al. (*25*) demonstrated that insulin increased the binding of SREBP-1c on two response elements SREa and SREb in rat liver GK promoter, resulting in increase in the gene expression. It is known that metformin decreases hepatic glucose production mainly by inhibition of hepatic G6Pase activity (*26*). Fulgencio et al. (*16*), in addition to this effect, showed a decrease in G6Pase and PEPCK mRNA and an increase in GK, but these effects were only significant at 500 $\mu$M, whereas at 100 $\mu$M, the dose used in this study, metformin did not affect the gene expression as confirmed by our results in **Figure 3**. This concentration was much higher than those observed in human therapy (10–50 nM) (*27*) but was chosen to be of the same order of magnitude as the doses used for the phenolic acids. At the dose used in this study, metformin may act mainly by decreasing G6Pase activity without significant effect on the gene expression involved in glucose metabolism.

Most of the phenolics tested (CAF, CHLOR, ROS, SSL) and insulin increased the level of glucokinase mRNA (**Figure 3**). As glucokinase is responsible for glucose phosphorylation and further utilization of glucose-6-phosphate, GK induction logically results in lower glucose release by hepatocytes (*28*) (**Figure 4**). It was recently shown that a GK activator displayed an antihyperglycemic effect (*29*). Mutations in GK gene were found responsible for mild hyperglycemia without ketoacidosis in young children (*30*). Restoration of hepatic glucokinase expression in mice with liver-specific phosphoinositide-dependent kinase-1 deficiency induced insulin-like effects in the liver and almost completely normalized the fasting hyperinsulinemia and postprandial hyperglycemia in these animals (*31*).

Chlorogenic acid is a major component of coffee, and it has been shown that intake of chlorogenic acid lowers plasma glucose concentrations in rats (*32*), mice (*33*), and humans (*34*). Since most of the chlorogenic acid absorbed in the small intestine is absorbed intact, it is metabolized extensively in the liver (*35*). Chlorogenic acid was found to be a reversible linear competitive inhibitor of G6Pase in rat liver microsomes (*36*). In addition to this effect on enzyme activity, our results demonstrated that chlorogenic acid significantly enhanced GK mRNA expression. Both effects may contribute to the reduced glucose production found (**Figure 4**).

This is the first report on the possible effect of *P. vulgaris* on glucose metabolism. The literature on this plant describes its radical scavenging, cytoprotective (*12*, *37*, *38*), anti-HIV (*39*, *40*), antiallergic, anti-inflammatory (*41*), and immune modulatory effects (*42*, *43*). The only report related to disorders of glucose metabolism deals with reduction in oxidative stress induced by a high-sucrose diet in hereditary hypertriglyceridemic rats (*44*). It was involved here for comparison with previously studied *S. sonchifolius* as a plant having high content of phenolic compounds. Rosmarinic and caffeic acids were extracted from solid residue PVA by organic solvent and represented about 26% and 0.3% w/w of total extract, respectively. Both compounds significantly increased GK mRNA expression (**Figure 3**). Jung et al. (*45*) also observed, in C57BL/KsJ-db/db mice, that hepatic mRNA levels of GK and hepatic GK activity were significantly higher in the caffeic acid-supplemented group than the control group. However, whether ROS and CAF significantly increased GK mRNA concentrations, PVA did not affect this gene expression. Differences observed between these compounds could be due to other unknown components of the complex PVA extract that could differently affect glucose metabolism.

Organic fractions of *S. sonchifolius* tubers did not affect glucose production or mRNA levels of the studied genes. Fractions extracted from leaves of this plant reduced glucose production and this could be explained by increase in GK mRNA expression.

In conclusion, the present study demonstrated that CAF, FER, CHLOR, ROS, PVA, and SSL significantly decreased glucose production in rat hepatocytes. This decrease could be due, at least in part, to the induction of GK mRNA expression for CAF, CHLOR, ROS, and SSL as reported for insulin. This finding is reported here for the first time. More experiments in hepatocytes and in vivo are needed to elucidate the role of food derived phenolic compounds in glucose metabolism.

## ABBREVIATIONS USED

CAF, caffeic acid; CHLOR, chlorogenic acid; CYP, cytochrome P450; DMSO, dimethyl sulfoxide; FER, ferulic acid; G6Pase, glucose-6-phosphatase; GAE, gallic acid equivalents; GK, glucokinase; INS, insulin; MET, metformin; PEPCK, phosphoenol–pyruvate carboxykinase; PVA, phenolic fraction from Prunella vulgaris aerial parts; ROS, rosmarinic acid; SSL, phenolic fraction from Smallanthus sonchifolius leaves; SST; phenolic fraction from *S. sonchifolius* tubers.

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