Polyphenolic compounds from *Artemisia dracunculus* L. inhibit PEPCK gene expression and gluconeogenesis in an H4IIE hepatoma cell line

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**METHODS**

**TYPE 2 DIABETES** has reached epidemic proportions not only in the US, but worldwide (15a). The major factors contributing to the hyperglycemia of diabetes are multifactorial but are secondary to the failure of the insulin resistance, characterized by decreased whole body insulin-mediated glucose utilization and elevated hepatic glucose output. With regard to enhanced glucose production by the liver, phosphoenolpyruvate carboxykinase (PEPCK) is the key enzyme catalyzing the first step in hepatic gluconeogenesis (5). Glucagon and stress hormones, such as glucocorticoids, upregulate PEPCK gene expression in hepatocytes via a cyclic AMP (cAMP)-dependent pathway (5). Alternatively, insulin strongly represses PEPCK transcription through the activation of the phosphoinositide-3-kinase (PI3K) pathway (4).

Normally, the increase in blood glucose levels after food intake stimulates the secretion of insulin from the pancreas. This increase in blood insulin concentration then leads to the downregulation of PEPCK gene expression and, subsequently, the cessation of gluconeogenesis by the liver (4). Insulin-resistant hepatocytes, however, are unable to effectively convey the insulin signal, leading to a decrease in PEPCK mRNA transcription. Thus, the de novo glucose synthesis persists despite a high blood glucose concentration (12). The compounds that are able to repress PEPCK expression and overcome insulin resistance could constitute a new class of glucose-lowering agents.

Plants have traditionally been a rich source of medicinal compounds for many indications, including diabetes. In fact, metformin is one of the most prescribed glucose-lowering medicines currently used and is derived from a chemical isolated from a plant (19). Recently, a number of polyphenolic substances from different plant sources were shown to decrease PEPCK expression in vitro (11), but in vivo data on the effects of these compounds have not been reported.

There are a number of reports (15) about use of plants from the genus *Artemisia* as a traditional treatment for diabetes. *Artemisia dracunculus* L. (*A. dracunculus*), or Russian tarragon, is a perennial herb with a long history of medicinal and culinary use. Recently, the ethanolic extract of *A. dracunculus* was shown (13) to significantly decrease blood glucose levels in both genetic and chemically induced murine models of diabetes. In addition, the extract significantly decreased PEPCK mRNA expression in the streptozotocin (STZ)-induced diabetic rats, suggesting a potential mode of action (13).

The extract also inhibited aldose reductase, an enzyme involved in many diabetic complications (9). A phenoxychromone and dihydronaldehyde were identified as the specific polyphenolics responsible for most of the glucose-lowering activity of the *Artemisia dracunculus* extract.

**Chemicals and biochemicals.** 8-(4-Chlorophenylthio)-cAMP (8-CPT-cAMP), 5-aminooimidazole-4-carboxamide-1-β-d-ribofuranoside, PI3K inhibitor LY-294002, rapamycin, sodium lactate, sodium pyruvate, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were purchased from Sigma Chemicals (St. Louis, MO). The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Human insulin (Humulin R) was purchased from Eli Lilly (Indianapolis, IN). All other chemicals, including cell culture media, were obtained from Invitrogen (Carlsbad, CA). PD-184352 was purchased from Toronto Research Chemicals (Toronto, ON, Canada) and compound C from EMD Biosciences (San Diego, CA). Reagents used in RT-PCR, including enzymes, were supplied by Stratagene (La Jolla, CA). Phospho-Akt (Ser473) and Akt2 rabbit MAb were purchased from Cell Signaling Technology (Danvers, MA). The H4IIE cell line (ATCC CRL-1548) was provided by American Type Culture Collection (Manassas, VA).

Plant material. The seeds of *Artemisia dracunculus* were purchased from Sheffield’s Seed (Locke, NY). The plants were grown in hydroponics, and the shoots were harvested and stored at −20°C.

**Extraction.** Two kilograms of the shoot material was heated to 80°C with 10 liters of 80% ethanol for 2 h. The extraction was continued for an additional 10 h at 20°C. The extract was filtered through a cheese cloth and evaporated to 1 liter using a rotary evaporator. The extract was then diluted with 1 liter of water and partitioned three times with 2 liters of ethyl acetate. The ethyl acetate fraction was then dried to a slurry using a rotary evaporator, followed by freeze-drying for 48 h.

**Purification and isolation of bioactive compounds.** Purification, isolation, and identification of bioactive compounds were done as in Longendra et al. (9). One gram of the dried extract was dissolved in 5 ml of 60% ethanol and 0.5 ml of acetonitrile and purified using a preparatory HPLC. For the initial purification, a Waters 19 × 300 mm symmetry prep C8 reverse-phase column with a particle size of 7 µm was used. The mobile phases consisted of two components: solvent A [0.5% American Chemical Society (ACS) grade acetic acid in double-distilled deionized water, pH 3–3.5] and solvent B (100% acetonitrile). For the initial separation, a gradient run of 5–95% solvent B over 35 min was used at a flow rate of 8 ml/min. Ten fractions at 5-min intervals were collected and tested for PEPCK gene expression. The fractions and subfractions that showed higher inhibition of gene expression were further purified using different conditions. Fractions 7 and 7-8 were purified by altering the gradient conditions described above, with flow rates ranging from 1 to 8 ml/min. Fraction 7-1 was purified using an isocratic condition, with a mobile phase consisting of three components: solvent A (0.5% ACS grade acetic acid in double-distilled deionized water, pH 3–3.5), solvent B (100% acetonitrile), and solvent C (100% methanol) at a ratio of 5:3:2. Purification of the bioactive subfractions 7-1 and 7-8 gave 6-demethoxycapillarisin and 2’,4’-dihydroxy-4-methoxydihydrochalcone, respectively. The ultraviolet profiles were monitored at wavelengths of 210 and 290 nm.

**Identification of compounds.** The bioactive compounds were identified using liquid chromatography-mass spectrometry (LC-MS) and 1H-, 13C-, and 2D-NMR spectroscopic data. LC-MS system used for analysis includes the Waters (Milford, MA) LC-MS integrity system consisting of a solvent delivery system with a W616 pump and W600S controller, W717 plus autosampler, W996 photodiode array (PDA) detector, and Waters TMD Thermabeam electron impact (EI) single quadrupole mass detector with fixed ionization energy of 70 eV. Data were collected and analyzed with the Waters Millennium version 3.2 software and linked with the sixth edition of the Wiley Registry of mass spectral data, containing 229,119 EI spectra of 200,500 compounds. After the W996 PDA detector the eluent flow was split into two equal flow paths with an adjustable flow splitter (model no. 600-PO10-06; Analytical Scientific Instruments, El Sobrante, CA). One of them was to the Thermabeam EI mass detector and the other to a Varian 1200L (Varian, Palo Alto, CA) triple quadrupole mass detector with electrospray ionization interface operated in either positive or negative ionization mode. The electrospray voltage was −4.5 kV, heated capillary temperature was 240°C, and sheath gas was air for the negative mode, and electrospray voltage was 5 kV and sheath gas was nitrogen for the positive ionization mode; the mass detector was used in scanning mode from 110 to 1,400 atomic mass units. Data from the Varian 1200L mass detector were collected and compiled using Varian’s MS Workstation, version 6.41, SP2. The 1H- and 13C-NMR spectra and 2D-NMR experiments were recorded using a Bruker Avance AV-300 NMR spectrometer at 300 (1H) and 75 MHz (13C). The 2D experiments 1H-1H COSY (Correlation Spectroscopy), heteronuclear multiple-bond correlation, and edited heteronuclear single quantum coherence were acquired using standard Bruker software. All compounds were measured in CD3OD.

**Cell culture and treatment.** The H4IIE hepatoma cells (CRL-1600; American Type Culture Collection) were plated in 24-well tissue culture plates (Greiner Bio One) and were grown to near confluence in Dulbecco’s modified Eagle’s medium containing 2.5% (vol/vol) newborn calf serum and 2.5% (vol/vol) fetal calf serum. Cells were treated for 8 h with 500 nM dexamethasone and 0.1 mM 8-CTP-cAMP (Dex-cAMP) to induce PEPCK gene expression together with different concentrations of test compounds, plant extract, or 10 nM insulin. The fractions were tested at 50 µg/ml medium, and the

![Fig. 1. Bioactivity guided fractionation of the *Artemisia dracunculus* extract.](http://ajpendo.physiology.org/)

The phosphoenolpyruvate carboxykinase (PEPCK) mRNA was upregulated in all samples except for negative control by incubation H4IIE rat hepatoma cells with 500 nM dexamethasone and 0.1 mM cAMP (Dex-cAMP). PEPCK mRNA was normalized to β-actin mRNA. The effect of different fractions and subfractions of *Artemisia dracunculus* extract or 10 nM insulin on PEPCK gene expression is represented as a ratio of PEPCK mRNA level relative to the response to Dex-cAMP activation alone (fold ratio is equal to 1.0). Lower fold ratio values represent greater inhibitory effect. The data represent the average of 4 experiments ± SE. **A:** the effect of 10 different fractions (Fr1–10) of *Artemisia dracunculus* extract on PEPCK mRNA levels in rat hepatoma cells at 25 µg/ml. Each of the 10 fractions was collected as sequential 10-min elutions from the preparatory HPLC column. **B:** the effect of subfractions of fraction 7 (Fr7-1 to 7-9) on PEPCK mRNA levels in rat hepatoma cells at 25 µg/ml.
compounds were tested at doses of 2.5, 5, 10, and 25 μg/ml. Three wells were allocated for each treatment as well as for a negative control (untreated cells). For inhibitory assays, cells were pretreated with 20 μM LY-294002 for 30 min, washed with phosphate-buffered saline, and incubated for an additional 7 h with Dex-cAMP together with 20 μM LY-294002 for 30 min, washed with phosphate-buffered saline, and incubated for an additional 7 h with Dex-cAMP together with different concentrations of test compounds, plant extract, or 10 nM insulin.

Cell viability assay and dose range determination. Cell viability was measured by the MTT assay (10). The MTT (Sigma) tetrazolium dye assay was performed to measure cell survival in culture after incubation with treatments. MTT (100 μg/ml) was added to the medium in each well, and plates were incubated in the cell growth chamber for 5 h. Medium was then removed, and dimethyl sulfoxide (150 μl) was added to each well to solubilize the purple formazan crystals created by mitochondrial dehydrogenase reduction of MTT. After 5 min of additional incubation, absorbance was read at 550 nm on a microplate reader spectrophotometer ( Molecular Devices, Sunnyvale, CA). The concentrations of test reagents that showed significant cell viability compared with that of the control (dimethyl sulfoxide, 0.1%) were further selected for in vitro gene expression assays. All treatments were performed in duplicate.

Total RNA extraction, purification, and cDNA synthesis. Total RNA was extracted from H4IIE rat hepatoma cells using Trizol reagent (Invitrogen), following the manufacturer’s instructions. RNA was quantified spectrophotometrically by absorbance measurements at 260 and 280 nm using the NanoDrop system (NanoDrop Technologies). Quality of RNA was assessed by separation in gel electrophoresis. RNA was then treated with DnaseI (Invitrogen), following the manufacturer’s guidelines, to remove any traces of DNA contamination. The cDNAs were synthesized with 2.5 μg of RNA for each sample, using Stratascript reverse transcriptase (Stratagene), following the manufacturer’s protocol.

Quantitative PCR and data analysis. The synthesized cDNAs were diluted fourfold. Five microliters of each of these diluted samples was used for PCR reactions of 25 μl final volume. The other components of the PCR reactions were 0.5 μl of 6 μM gene-specific primers (synthesized by IDT, Coralville, IA) and 12.5 μl of Brilliant SYBR Green PCR master mix (2×; Stratagene) containing green jump-start Taq ready mix. ROX (Stratagene) was used as a reference dye. The primers were selected using the Primer Express version 2.0 software (Applied Biosystems, Foster City, CA) as follows: β-actin: forward primer 5′-GGGAAATCGTGCGTGACATT-3′, reverse primer 5′-GCGGCAGTGCCCATGC-3′; PEPCK: forward primer 5′-GCA-GAGCATAGGCGCAAGGT-3′, reverse primer 5′-TTGCCGAAATGTGTAAGAAA-3′.
β-Actin primers were selected from the RefSeq sequence with the accession no. NM_031144. Both primers reside on exon 4 of the rat β-actin gene (Rat Genomic Sequence Consortium, assembly version accession no. NM_198780). The intron-spanning forward primer was selected to cover the exon 9–10 boundary. The reverse primer was selected from exon 10. The oligos were synthesized by IDT. These primers generated a 76-bp product from /H9252-actin gene (Rat Genomic Sequence Consortium, assembly version accession no. NM_031144). Both primers reside on exon 4 of the rat gene. 

Quantitative PCR (qPCR) amplifications were performed on an MX3000p system (Stratagene) using one cycle at 50°C for 2 min and one cycle of 95°C for 10 min, followed by 40 cycles of 15 s at 95°C and 1 min at 60°C. The dissociation curve was completed with one cycle of 1 min at 95°C, 30 s at 55°C, and 30 s at 95°C. Non-RT control and no-template control were included in each experiment as quality control steps.

PEPCK mRNA expressions were analyzed using the ΔΔCT method and normalized with respect to the expression of the β-actin housekeeping gene. The ΔΔCT values obtained from these analyses directly reflect the relative mRNA quantities for a specific gene in response to a treatment as relative to a calibrator. The Dex-cAMP treatment (positive control) served as the calibrator sample in this study. The PEPCK gene expression of the calibrator sample was assigned to a value of 1.0. A value of <1.0 indicates transcriptional downregulation (inhibition of gene expression) relative to the calibrator. Amplification of specific transcripts was further confirmed by obtaining melting curve profiles. All samples were run in duplicate.

AMPKα1 and -α2 activity assay. AMP-activated protein kinase (AMPK) activity was assayed as previously described (6). Briefly, AMPK was immunoprecipitated from 200 μg of H4IIE cell lysate using α-AMPKα1 (Upstate Biotecnoology) or α-AMPKα2 (Santa Cruz Biotechnology, Santa Cruz, CA) antibodies in 500 μl of buffer A (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 50 mM NaF, 5 mM sodium pyrophosphate, 1 mM EDTA, 1 mM EGTA, 1 mM dithiothreitol, 0.1 mM benzamidine, 1 mM phenylmethylsulfonyl fluoride, 5 μg/ml aproatin) at 4°C for 2 h. Immunocomplexes were washed with buffer A three times, buffer B containing 0.5 M NaCl and 62.5 mM NaF, and then reaction buffer (50 mM HEPES, pH 7.4, 1 mM dithiothreitol) three times. AMPK activity of immunocomplexes was determined by phosphorylation of SAMS peptide in reaction buffer containing 0.25 mM SAMS, 5 mM MgCl2, and 10 μCi of [γ-32P]ATP for 10 min at 30°C with or without 200 μM AMP stimulation. The reaction was terminated by spotting reaction mixtures onto P81 filter paper and rinsed in 1% (vol/vol) phosphoric acid with gentle stirring to remove free ATP. Phosphorylated substrate was measured by scintillation counting.

Western blot analysis. Cells were cultured as described above, and whole cell extracts were prepared in ice-cold lysis buffer (62.5 mM Tris-HCl (pH 6.8), 2% wt/vol SDS, 10% glycerol, 50 mM DTT, 0.01% wt/vol bromophenol blue) and centrifuged at 12 000 g for 20 min at 4°C. Equal amounts of protein (50 μg) from the supernatants were separated on SDS 10% polyacrylamide gels and blotted onto nitrocellulose membrane. Western blot analysis was performed with monoclonal phospho-Akt (Ser473) antibodies according to the manufacturer’s instructions (Cell Signaling Technology, Danvers, MA). After being washed, the blots were incubated with an anti-rabbit peroxidase-labeled secondary antibody and visualized using ECL Western Blotting Detection Reagent (GE Healthcare, Piscataway, NJ). After being stripped, the blots were probed with Akt2 (5B5) antibodies.

AMPK activity was assayed as previously described (6). Briefly, AMPK was immunoprecipitated from 200 μg of H4IIE cell lysate using α-AMPKα1 (Upstate Biotecnoology) or α-AMPKα2 (Santa Cruz Biotechnology, Santa Cruz, CA) antibodies in 500 μl of buffer A (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 50 mM NaF, 5 mM sodium pyrophosphate, 1 mM EDTA, 1 mM EGTA, 1 mM dithiothreitol, 0.1 mM benzamidine, 1 mM phenylmethylsulfonyl fluoride, 5 μg/ml aproatin) at 4°C for 2 h. Immunocomplexes were washed with buffer A three times, buffer B containing 0.5 M NaCl and 62.5 mM NaF, and then reaction buffer (50 mM HEPES, pH 7.4, 1 mM dithiothreitol) three times. AMPK activity of immunocomplexes was determined by phosphorylation of SAMS peptide in reaction buffer containing 0.25 mM SAMS, 5 mM MgCl2, and 10 μCi of [γ-32P]ATP for 10 min at 30°C with or without 200 μM AMP stimulation. The reaction was terminated by spotting reaction mixtures onto P81 filter paper and rinsed in 1% (vol/vol) phosphoric acid with gentle stirring to remove free ATP. Phosphorylated substrate was measured by scintillation counting.

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Fig. 4. Phosphoinositide 3-kinase (PI3K) inhibitor LY-294002 reverses effect of the active ingredients from the Artemisia dracunculus. L. dracunculus extract on Dex-cAMP-activated PEPCK gene expression (A and B) and on Akt2 Ser473 phosphorylation (C). The PEPCK mRNA was upregulated in all samples except for negative control by incubation H4IIE rat hepatoma cells with Dex-cAMP. PEPCK mRNA was normalized to β-actin mRNA. The effect of the active compounds applied in different doses or 10 nM insulin on PEPCK gene expression is represented as a ratio of PEPCK mRNA level relative to the active compounds applied in different doses or 10 nM insulin on PEPCK gene expression. The data represent the average of 4 experiments ± SE. *P < 0.05 (ANOVA comparison with the samples treated only by Dex-cAMP). A: effect of PI3K inhibitor LY-294002 on the PEPCK mRNA downregulation caused by 70 μM 6-demethoxycapillarisin. B: effect of PI3K inhibitor LY-294002 on the PEPCK mRNA downregulation caused by 70 μM 2′,4′-dihydroxy-5′-methoxy-dihydrochalcone. C: effect of 20 μM LY-294002 on the Akt2 phosphorylation caused by 70 μM 6-demethoxycapillarisin was evaluated as described in METHODS.
**RESULTS**

The ethylacetate extract of *A. dracunculus* and its fractions were tested for inhibitory activity of Dex-cAMP-induced PEPCK gene expression in an H4IE rat hepatoma cell line (Fig. 1). The membrane-permeable cAMP analog 8-CTP-cAMP and the synthetic glucocorticoid dexamethasone were used to upregulate the PEPCK gene. A decrease in relative PEPCK mRNA level in cells treated with the test compounds or with insulin indicated an inhibitory effect and potential antidiabetic activity from the treatment. Untreated cells were used to measure the basal level of PEPCK expression. The β-actin gene expression was chosen as an internal standard. The data represent the average of 4 experiments ± SE. One-way ANOVA was used to determine the significance of treatments. Tukey’s multiple means comparison test was performed to determine the significance of the difference between the control and treatments. Treatments were considered significantly different if \( P < 0.05 \).

At 100 μg/ml, the total extract repressed PEPCK expression by 60% (data not shown). Ten basic fractions, separated on the basis of time by HPLC fractionation of the total extract of *A. dracunculus*, were tested for PEPCK inhibitory activity. Fraction 7 (Fig. 1A) exhibited the highest inhibitory activity over PEPCK expression (PEPCK mRNA decreased >2-fold). Therefore, it was fractionated further into eight subfractions, and their effect on PEPCK transcription was evaluated (Fig. 1B). Additional purification of active subfractions 7-1 and 7-8 yielded two bioactive compounds, identified by LC-MS and NMR as 6-demethoxyxciparilisin and 2’,4’-dihydroxy-4-methoxydihydrochalcone, respectively (Fig. 2C). The dose response of the two isolated bioactive compounds on the PEPCK mRNA expression from fraction 7 was then evaluated over nontoxic concentration ranges of 8.7-70 μM for 6-demethoxyxciparilisin and 36.8-92 μM for 2’,4’-dihydroxy-4-methoxydihydrochalcone (Fig. 2, A and B). 6-Demethoxyxciparilisin possessed stronger inhibitory activity on the PEPCK gene expression than 2’,4’-dihydroxy-4-methoxydihydrochalcone, with IC\textsubscript{50} equal to 43 and 61 μM, respectively. In addition, 6-demethoxyxciparilisin in combination with 10 nM insulin decreased the PEPCK gene expression twice as much as insulin alone (Fig. 3), whereas the effects of 2’,4’-dihydroxy-4-methoxydihydrochalcone and insulin on the PEPCK gene expression were not additive.

The inhibitory effect of insulin over PEPCK transcription is mediated by the activation of PI3K and can be reversed by specific PI3K inhibitors such as LY-294002. The activity of this specific inhibitor can be used to determine whether the insulin-like effects of other bioactive compounds are also mediated through the PI3K pathway. Indeed, preincubation of
the culture cells with 20 μM LY-294002 also reversed the downregulation of PEPCK gene expression by 70 μM 6-demethoxycapillarisin (Fig. 4A), but only slightly by 75 μM 2',4'-dihydroxy-4-methoxydihydrochalcone (Fig. 4B). Like insulin, 6-demethoxycapillarisin-induced Akt2 phosphorylation within the carboxy terminus at Ser473 is significantly decreased by the PI3K inhibitor LY-294002 (Fig. 4C). Other inhibitors were also incorporated into the assays to further characterize the mechanisms of action of the isolated compounds. Neither rapamycin, an inhibitor of p70 S6 kinase (p70S6k), or PD-184352, an inhibitor of mitogen-activated protein kinase (MAPK), exerted any significant effect on the PEPCK mRNA downregulation caused by the active compounds from A. dracunculus extract (Fig. 5). On the other hand, compound C, an inhibitor of AMPK (21), completely reversed the effect of 2',4'-dihydroxy-4-methoxydihydrochalcone on Dex-cAMP-stimulated PEPCK gene expression in H4IIE cells (Fig. 6C). This compound was able to increase the activity of the AMPK-stimulated AMPKα2 catalytic subunit by 150%, whereas both

![Graph](image_url)
to the downregulation of PEPCK gene expression. Two polyphenolic substances, 6-demethoxy-capillarisin and 2’,4’-dihydroxy-4-methoxy-dihydrochalcone, were isolated from active fraction 7 of the extract and shown to be the primary factors responsible for the downregulation of PEPCK gene expression. With the IC50 values equal to 43 and 61 µM, respectively, these compounds are more potent inhibitors of PEPCK mRNA transcription than metformin or thiazolidinediones, which have active doses ranging from 100 to 5,000 µM in hepatoma cells (2, 20). Although these identified compounds are primarily responsible for the PEPCK inhibitory activity of the total extract, partial inhibitory activity of other fractions and subfractions suggests that other inhibitors may also be present in the extract.

Polyphenols of plant origin, such as (−)-epigallocatechin gallate, were recently shown to mimic the effect of insulin on PEPCK gene expression by increasing PI3K, MAPK, and p70S6k activities (17). The PI3K pathway is a primary component of the insulin-signaling cascade in hepatocytes (1). The effect of insulin or (−)-epigallocatechin gallate on the expression of its target genes, including PEPCK, can be reversed by the administration of specific inhibitors of PI3K, such as LY-294002. LY-294002 is a potent and selective cell-permeable inhibitor of PI3K (16) shown to abolish the insulin inhibition of Dex-cAMP-induced PEPCK gene transcription in hepatoma cells (1). The inhibitory effect of 6-demethoxy-capillarisin on PEPCK mRNA was significantly lower in the presence of this specific PI3K inhibitor, suggesting that it may suppress PEPCK gene expression by affecting the upstream components of the insulin-signaling pathway, such as PI3K. The effect of 2’,4’-dihydroxy-4-methoxy-dihydrochalcone, to the contrary, was not significantly influenced by the LY-294002 inhibitor and, therefore, is not likely to involve the PI3K insulin-like pathway. 6-Demethoxy-capillarisin, like insulin, increases phosphorylation of Akt2 protein kinase at Ser473. LY-294002 significantly inhibits Akt2 phosphorylation induced by 6-demethoxy-capillarisin or insulin (Fig. 4C). The effect of Artemisia polyphenols on PEPCK gene expression was not affected by the inhibition of the the p70S6k pathway with rapamycin (Fig. 5B). Likewise, although activated Ras can downregulate cAMP-induced PEPCK gene expression in an insulin-independent manner through MAPK pathway (1), pretreatment with the MAPK inhibitor PD-184352 did not affect the PEPCK downregulation caused by Artemisia active compounds (Fig. 5A).

The biguanidine drugs, such as metformin, and thiazolidinediones such as troglitazone were shown to exert a PI3K-dependent downregulation of basal PEPCK gene expression in the liver of insulin-resistant patients (2, 20) through an AMPK-dependent mechanism. This pathway leads to the insulin-independent suppression of hepatic gluconeogenesis by phosphorylation and cytoplasmic sequestration of the mammalian target of rapamycin complex 2 transcriptional coactivator (8). Both active compounds from the A. dracunculus extract are capable of increasing basal activity of ubiquitously expressed AMPKα1 catalytic subunit, but only dihydroxy-4-methoxy-dihydrochalcone significantly increases AMP-stimulated activity of the liver-specific AMPKα2 catalytic subunit. Moreover, downregulation of PEPCK mRNA expression by the dihydrochalcone is completely reversed by AMPK inhibitor compound C (Fig. 6).

In conclusion, 6-demethoxy-capillarisin and 2’,4’-dihydroxy-4-methoxy-dihydrochalcone from an A. dracunculus extract were identified as active compounds responsible for decreasing PEPCK overexpression in the liver cells of diabetic rodents. These compounds may act through insulin-like and insulin-independent pathways to achieve the suppression of both PEPCK gene expression and gluconeogenesis in the hepatocytes. Preliminary clinical (14) testing also suggested that the extract attenuated hyperinsulinemia in mildly diabetic patients. Thus, the A. dracunculus extract, or the compounds contained therein, may be useful for the prevention and treatment of diabetes and related disorders.
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GRANTS

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