

Caffeic Acid as Active Principle from the Fruit of *Xanthium strumarium* to Lower Plasma Glucose in Diabetic Rats

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Abstract: The antihyperglycemic effect of caffeic acid, one of the phenolic compounds contained in the fruit of *Xanthium strumarium*, was investigated. After an intravenous injection of caffeic acid into diabetic rats of both streptozotocin-induced and insulin-resistant models, a dose-dependent decrease of plasma glucose was observed. However, a similar effect was not produced in normal rats. An insulin-independent action of caffeic acid can thus be considered. Otherwise, this compound reduced the elevation of plasma glucose level in insulin-resistant rats receiving a glucose challenge test. Also, glucose uptake into the isolated adipocytes was raised by caffeic acid in a concentration-dependent manner. Increase of glucose utilization by caffeic acid seems to be responsible for the lowering of plasma glucose.

Key words: *Xanthium strumarium*, Compositae, caffeic acid, antihyperglycemic effect, insulin-independent action, glucose challenge test, glucose uptake.

Introduction

The fruit of *Xanthium strumarium* (Compositae), named as Chang'ErZi or Chang'ErChao in Chinese (1), is known to contain carboxyatractyloside, xanthanol, isoxanthanol and hydroquinone (2). A previous report indicated the hypoglycemic effect in animals receiving the extract of this herb (3). In this paper, we report the isolation and identification of caffeic acid as one of the active principles for lowering plasma glucose.

Materials and Methods

General experimental procedures

M.p.s were determined on a Yanagimoto micromelting point apparatus and are uncorrected. Optical rotations were measured with a Jasco DIP-140 digital polarimeter. IR spectra were obtained with a Jasco A-100 spectrometer. The ¹H- and ¹³C-NMR spectra were taken with a Bruker AM-300WB spectrometer operating at 300 MHz and 75 MHz, respectively, using TMS as an internal standard. FAB-MS was performed in a Jeol JMS-HX 110 and X-ray crystallography was determined by

means of a CAD4 under Kappa Axis single crystal XRD. TLC was carried out on a precoated Kieselgel 60 F254 plates (0.2 mm thick, Merck) with the solvent system C₆H₆-HCOOEt-HCOOH (1:7:1 and 3:6:1); TLC detection was made by UV illumination and by spraying with 10% H₂SO₄ followed by heating (for thiazine) and FeCl₃ reagent (for phenolics).

Plant material

The fruits of *Xanthium strumarium* (Compositae), *Xanthii fructus*, were purchased from a folk medicinal drug store in Taipei and authenticated by Mr. M. T. Kao, Department of Botany, National Taiwan University. The voucher specimens (TMCP85-10) have been deposited in the herbarium of the School of Pharmacy, Taipei Medical College, Taipei City, Taiwan.

Extraction and isolation

Xanthii fructus (6 kg) were chopped into small pieces and extracted three times with 70% aqueous acetone at room temperature. The extract, after removal of the acetone by evaporation in vacuo, was fractionated with column chromatography on Diaion HP-20, eluted with H₂O-MeOH with increasing MeOH content to afford 3 fractions. Through the assay of hypoglycemic activity, fr. 2 was found to be the effective one. The fr. 2 was further chromatographed on a Sephadex LH-20 column (4 × 80 cm) with 60% aqueous MeOH (3 L), and with acetone to afford caffeic acid (260 mg) (4). NMR analysis indicated the *trans*-form of caffeic acid. The purity of this compound was not less than 99.8%.

Animals

Male Wistar rats, weighing 230–250 g, were obtained from the animal center of National Cheng Kung University Medical College. They were maintained in a temperature-controlled room (25 ± 1 °C) and kept on a 12:12 light-dark cycle (light on at 06:00 h). Food (Purina Rat Chow) and water were available ad libitum. Rats were treated in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Detection of plasma glucose

Plasma glucose was measured in duplicate with the enzymatic glucose method by an autoanalyzer (Quik-Lab, Ames, Miles Inc., USA) using a blood sample from the femoral vein of rats

under anesthesia with pentobarbital (30 mg/kg, *i.p.*). Rats were made diabetic by a bolus injection of streptozotocin (60 mg/kg) dissolved in citrate buffer (pH 4.5) according to our previous method (5). In addition to polyuria, rats with the plasma glucose levels higher than 400 mg/dL were considered diabetic for the experiments. Also, insulin-resistance was induced in rats aged 8 weeks by the *i.p.* injections of long-acting human insulin (Monotard® HM) at 0.5 IU/kg three times daily as described previously (6). Rats with the plasma glucose level higher than 120 mg/dL in addition to the symptoms of hyperphagia and/or increase of body weight were considered as diabetic. This model is considered similar to patients with non-insulin-dependent diabetes mellitus (NIDDM) (7).

Assay of antihyperglycemic activity

Antihyperglycemic activity was determined in fasted rats that received an intravenous (*i.v.*) injection of aqueous solution containing caffeic acid at desired doses. Results were calculated as percentage decrease of initial value according to the formula: $\{[(Gi - Gt) / Gi] \times 100\%$ where G_i was the initial value and G_t indicated the value after treatment. Plasma glucose was measured every 30 min except when stated otherwise in the results. Values of plasma glucose were then compared with the control group that was injected with the same volume of vehicle. After the investigation, animals were left for more than 3 days. Then, the diabetic rats with a plasma glucose value that had returned to the initial value within ± 10 mg/dL were identified as recovery from the effect and they were used for other studies. As a positive control, metformin was used in the present study.

Glucose challenge test

The glucose challenge test was performed in NIDDM rats, as described previously (8). In pentobarbital-anesthetized rats, glucose (60 mg/kg) was injected into the femoral vein using a 50% (W/V) solution. Blood samples (0.25 mL) were taken 10 min before, and every 10 min after glucose injection for 90 min. Blood loss was replaced by injection of equivalent volumes of isotonic saline solution. Changes of plasma glucose in rats treated with caffeic acid were compared with those from the vehicle-treated controls.

Determination of glucose uptake

The epididymal fat pads were removed from the Wistar rats under anesthesia with pentobarbital (30 mg/kg, *i.p.*). The adipose tissue was minced and digested with collagenase (Type I, Sigma) in Krebs's Ringer buffer (KRB) containing 1% (W/V) bovine serum albumin as described in our previous report (9). After digestion for 30 min at 37 °C under shaking (120 cycles/min), fatty cells were obtained from the supernatant by centrifugation (1,200 rpm, 5 min). They were filtered and washed three times in KRB. The packed cells were adjusted to a suitable dilution (around 105 to 106 cells/mL) for experiments. Uptake of glucose was measured as described previously (15) using 2-deoxy-D-[¹⁴C]glucose (2-DG) (323 mCi/mmol, NEN) as indicator. Fat-cell suspensions were incubated in plastic vials (final volume 1 mL) in which drugs were added. Uptake was initiated by the addition of 2-DG (0.002 mM) and stopped by addition of 0.1 mM phloretin (0.75 mL, Sigma). Each vial was mixed with 0.5 mL silicone oil ($D = 0.99$) to obtain adipocytes in the super-

natant after centrifugation (6,000 rpm, 3 min). The adipocytes were placed in scintillation vials and 3 mL scintillation fluid was added and the radioactivity was counted. Nonspecific uptake, extracellular 2-DG present in the obtained fraction, was assayed with cells treated under similar conditions except that 50 mM cytochalasin B (Sigma) was incubated. Specific uptake of glucose was calculated after subtraction of the nonspecific uptake from total uptake, samples were not treated with cytochalasin B in parallel. The effect on spontaneous uptake of glucose was obtained from adipocytes that have been co-incubated with the caffeic acid at the indicated concentration for 30 min followed by an incubation with 2-DG for 5 min to obtain the specific uptake. The specific uptake from samples incubated with vehicle (KRB) at the same volume was taken as 100% control. All the data of specific uptake were expressed as percentage of that from a control run in parallel.

Statistical analysis

All values, expressed as mean $\pm$ s.e., were obtained from the number (N) of samples. Student's *t*-test for paired comparisons between prior to and after administration, or unpaired observation for between vehicle-treated control and tested data were carried out for statistical evaluation of the differences; *p* values of 0.05 or less were considered significant.

Results and Discussion

By using Diaion HP-20 and Sephadex LH-20 gel column chromatography, caffeic acid was isolated from the aqueous acetone extract of *Xanthium Fructus*. Based on the NMR spectral data of a previous report (4), the obtained compound was identified as *trans*-caffeic acid.

Intravenous injection of caffeic acid (0.5–3 mg/kg) into both streptozotocin-induced diabetic rats (STZ-rats) and rats with insulin-resistance (NIDDM-rats), produced a marked plasma glucose lowering effect in a dose-dependent manner (Fig. 1). Antihyperglycemic activity by caffeic acid at 3 mg/kg in STZ-rats was $23.8 \pm 8.8\%$ which was similar ($P > 0.05$) to that in NIDDM-rats ($33.4 \pm 5.7\%$). As the positive control, oral administration of metformin at 100 mg/kg produced $18.4 \pm 3.1\%$ antihyperglycemic activity in STZ-rats. Similar treatment with metformin at 10 mg/kg produced $28.2 \pm 4.5\%$ antihyperglycemic activity in NIDDM-rats. The effects of caffeic acid reached a plateau within 30 min and were maintained for 40 min or more. The duration of effect for caffeic acid was progressively lengthened as the dosage was increased but the effects were completely reversed within 90 min (data not shown). No irreversible decrease in plasma glucose level was observed. However, the plasma glucose level in normal rats, Wistar rats, was not modified by caffeic acid at the same doses (Fig. 1). Lowering of plasma glucose may be induced by the release of insulin, an endogenous peptide in regulation of blood sugar (10). Activity of insulin was considered negligible in this STZ-induced diabetic rat (11) and rats with insulin-resistance (7). An insulin-related mechanism in the obtained antihyperglycemic effect seems unlikely. Caffeic acid is introduced as an antioxidant (12) that has been used to prevent human low-density lipoprotein oxidation (13). Actually, involvement of oxidative stress in diabetic disorders has been documented (14) and renal antioxidant enzyme mRNA levels were increased in the diabetic rat (15). However, antihyperglycemic effect of caffeic acid has not been mentioned before.

Then, a glucose challenge test was performed in rats with insulin-resistance according to the previous method (8). An intravenous injection of glucose produced hyperglycemia rapidly within 10 min in insulin-resistant rats that received only vehicle and the plasma glucose level returned to the initial value at 60 min after glucose injection. Pretreatment with caffeic acid (1 mg/kg, i.v.) attenuated this elevation of plasma glucose (Fig. 2). Inhibition of this glucose elevation in plasma can be due to an increase of glucose utilization in animal (8). An increase of glucose uptake into isolated adipocytes by caffeic acid was also obtained in a concentration-dependent manner (Fig. 3). Caffeic acid at 10 μ M made an increase of glucose uptake into adipocytes (143 $\pm$

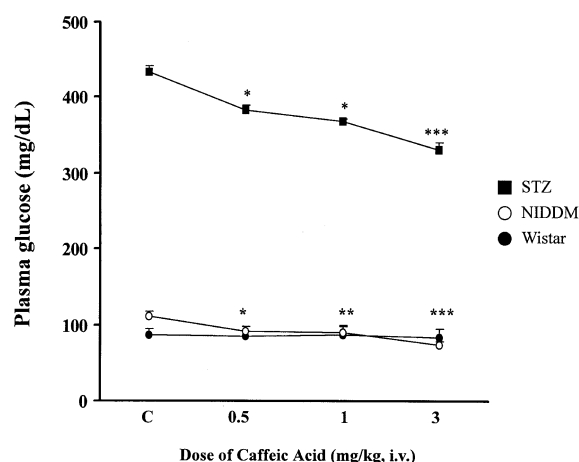


Fig. 1 Effect of caffeic acid on the plasma glucose level in normal rats (Wistar), streptozotocin-induced diabetic rats (STZ) and rats with insulin-resistance (NIDDM). Caffeic acid was injected into rats intravenously at the indicated dose to compare with vehicle-treated control (C). Each point indicates the mean of value from 6–8 rats with error (s.e.) bar. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ vs. control (C), respectively. As a positive control, oral administration of metformin at 100 mg/kg decreased the plasma glucose by 76.5 ± 4.3 mg/dL in streptozotocin-induced diabetic rats (STZ) and similar treatment at 10 mg/kg lowered 42.5 ± 4.9 mg/dL in rats with insulin-resistance (NIDDM).

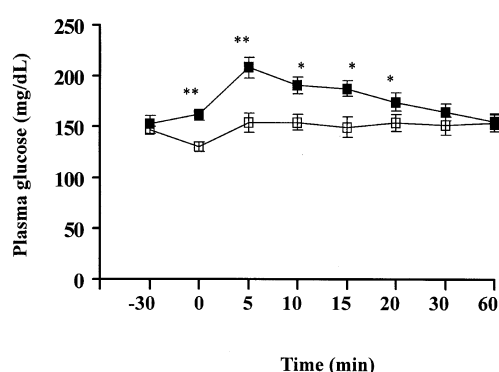


Fig. 2 Effect of caffeic acid on the plasma glucose level in insulin-resistant (NIDDM) rats receiving the glucose challenge test. Caffeic acid (1 mg/kg) injected into the femoral vein (open square) was compared with the control group that received a similar injection of saline at the same volume (solid square). Then the glucose challenge test was performed by an intravenous injection of glucose (60 mg/kg) into two groups of NIDDM rats at 30 min later and the plasma glucose in samples obtained immediately was indicated as 0 min. Values of mean with error (s.e.) bar were obtained from 6 rats in each group. * $P < 0.05$ and ** $P < 0.01$ vs. data from control group, respectively.

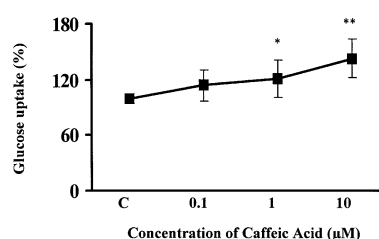


Fig. 3 Effect of caffeic acid on the glucose uptake into isolated adipocytes. Each point indicating the percentage of control represents the mean from 6 samples with s.e. bar. * $P < 0.05$ and ** $P < 0.01$ vs. control (C), respectively.

21.4%) in a way similar ($P > 0.05$) to that induced by 0.1 IU/ml of insulin ($159.1 \pm 34.4\%$). In the *in vitro* assay, involvement of endogenous insulin is negligible. Increase of glucose utilization by caffeic acid through an insulin-independent action can thus be considered.

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