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The anti-diabetic effects of ethanol extract from two variants of *Artemisia princeps* Pampanini in C57BL/KsJ-*db*/*db* mice

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Abstract

The anti-diabetic effects of two variants of *Artemisia princeps* Pampanini, sajabalssuk (SB) and sajuarissuk (SS), were investigated in type 2 diabetic animal using their ethanol extracts. Male C57BL/KsJ-*db*/*db* (*db*/*db*) mice were divided into control, SB ethanol extract (SBE), SS ethanol extract (SSE), or rosiglitazone (RG) groups and their age-matched littermates (*db*/+) were used. Supplementation of the SBE (0.171 g/100 g diet), SSE (0.154 g/100 g diet), and RG (0.005 g/100 g diet) improved glucose and insulin tolerance and significantly lowered blood glycosylated hemoglobin levels, as compared to the control group. Plasma insulin, C-peptide and glucagon levels in *db*/*db* mice were higher in the *db*/+ mice, however these values were significantly lowered by SBE, SSE or RG-supplement. Hepatic GK activity was significantly lower in the *db*/*db* mice than in the *db*/+ mice, while hepatic G6Pase activity was vice versa. Supplementation of SBE, SSE and RG reversed these hepatic glucose-regulating enzyme activities. In addition, SBE and SSE markedly increased the hepatic glycogen content and muscle ratio as compared to the control group, but they did not alter the food intake, body weight and plasma leptin level. The RG group, however, showed a significant increase in the food intake, body weight and plasma leptin. These results suggest that SBE and SSE exert an anti-diabetic effect in type 2 diabetic mice.

Keywords: *Artemisia princeps* Pampanini; Glucose tolerance; Insulin tolerance; Glucose regulating enzymes; C57BL/KsJ-*db*/*db* mice

1. Introduction

Diabetes is a major public health problem which affects about 5% of the population (Taylor, 1999). In particular, type 2 diabetes is the most common, accounting for 90% of patients. It is caused by a resistance to the action of insulin combined with a deficiency in insulin secretion (DeFronzo, 1997). At present, oral therapy for type 2 diabetes relies upon insulin secretagogues such as glibenclamide (Codina et al., 1978), and insulin sensitizers such as thiazolidinedione (Kobayashi et al., 1992). Recently, there has been a growing interest in alternative therapies and in the therapeutic use of natural products for diabetes, especially those derived from plants (Kato et al., 1994; Kakuda et al., 1996). This is because plant sources are usually considered to be less toxic with fewer side-effects than synthetic ones.

Abbreviations: BW, body weight; *db*/*db* mice, C57BL/KsJ-*db*/*db* mice; GK, glucokinase; G6Pase, glucose-6-phosphatase; PEPCK, phosphoenolpyruvate carboxykinase; RG, rosiglitazone; SBE, sajabalssuk ethanol extract; SSE, sajuarissuk ethanol extract.

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Among the various plants, about 500 plants belong to the genus *Artemisia* (McArthur, 1979). Most *Artemisia* herbs are perennials that, growing in the northern hemisphere and are used for various purposes, such as medicine, food, spices, and ornaments. The medicinal effects of *Artemisia* herbs are extremely diverse and include the following: Cell protection from peptic ulcers, liver protection, antimalarial, anti-tumor, and anti-diabetic effects (Gilani and Janbaz, 1994; White, 1994; Kim et al., 1997; Tahraoui et al., 2007). Several *Artemisia* herbs, for instance *Artemisia herbe-alba* (AI-Waili, 1986), *Artemisia santonicum* (Korkmaz and Gurdal, 2002), *Artemisia pallens* (Subramoniam et al., 1996), have been reported to be beneficial to experimental animal or people with diabetes. Among *Artemisia* herbs, *Artemisia princeps* has been widely used in Korean traditional medicine in treating of colic, vomiting and diarrhea, and irregular bleeding form the uterus (Zhao et al., 1994). Sajabalssuk (SB) and sajuarissuk (SS), two variants of *A. princeps* Pampanini, particularly are cultivated commercially in Ganghwa County in Korea. They contain a high-content of flavonoids such as eupatilin and jaceosidin compared to the *Artemisia* herbs from other places such as China (Ryu et al., 2005). Certain dietary polyphenolic compounds show growing interest in altering glucose metabolism in addition to their lipid-lowering and anti-oxidant effects (Jung et al., 2004; Scalbert et al., 2005; Okutan et al., 2005; Jung et al., 2006). The present study evaluated the anti-diabetic effects of SB and SS ethanol extracts on C57BL/KsJ-*db*/*db* (*db*/*db*) mice that display many of the characteristics of type 2 diabetic patients including hyperplasia, hyperglycemia, hyperinsulinemia and obesity (Hummel et al., 1966). Effects of SB and SS ethanol extracts were compared with those of rosiglitazone, an insulin sensitizer for the treatment of type 2 diabetes.

2. Materials and methods

2.1. The preparation of A. princeps Pampanini and an analysis of the general composition

Two variants of the *A. princeps* Pampanini, Sajabalssuk (SB) and Sajuarissuk (SS), were provided from Ganghwa Agricultural R&D Center, Incheon, Korea, which was harvested at Ganghwa County in 2003 and stored for 2 years in the air. The extraction with ethanol was performed as follows: 2 kg of the finely powdered materials from the dried aerial parts of SB and SS were soaked in ethanol (1:10 w/v) for 8 h, respectively. Each ethanol extract was filtered through Whatman No. 1 filter paper, evaporated under vacuum at 40 °C. The ethanol extract yields after vacuum evaporation of SB and SS were 68.3 g and 61.6 g per 2 kg of powdered material, respectively. The total polyphenol contents in the dried SB and SS, determined using the Folins–Ciocalteus method (Aline et al., 2005), were 9.5 mg/g and 7.4 mg/g, respectively. The total flavonoid contents in two variants were 4.3 mg/g for SB and 3.6 mg/g for SS, respectively, when determined using the spectroscopic method (Lee et al., 2001).

For the analysis of quantity of eupatilin and jaceosidin, the two major flavonoids in these plants, HPLC analysis was carried out. HPLC was performed on a Shimadzu LC-20 (Shimadzu, Japan) system using an Atlantis dC18 column (3.0 × 250 mm, 5 μm, Waters, USA). The mobile phase using isocratic elution was a solvent mixture of 70% MeOH containing 0.1% TFA. The flow rate was 1.5 mL/min with an injection volume of 20 μl and UV detection was observed at 340 nm. The eupatilin was eluted at retention time of 5.11 min, and the jaceosidin was at 6.97 min. According to these measurements, SB contains 204.6 mg eupatilin and 104.6 mg jaceosidin per 100 g and SS contains 146.1 mg eupatilin and 89.6 mg jaceosidin per 100 g, respectively.

2.2. Animals and diets

Five week old, male C57BL/KsJ-*db*/*db* (*db*/*db*) mice and their non-diabetic litter mates (*db*/+) were purchased from Jackson Laboratory (Bar Harbor, ME) and maintained under standard light (12 h light/dark), temperature (22 ± 2 °C) and humidity (40 ± 10%) condition. The forty *db*/*db* mice and ten *db*/+ mice were fed a pelletized commercial chow diet for a period of two weeks after arrival, then the *db*/*db* mice were randomly divided into 4 groups ($n = 10$). Thereafter, the *db*/+ group and control group of the *db*/*db* mice were fed a standard semisynthetic diet (AIN-76) (American Institute of Nutrition, 1977; American Institute of Nutrition, 1980), while the other three groups of *db*/*db* mice were fed a standard semisynthetic diet with rosiglitazone (RG; Avandia; GlaxoSmithKline, UK; 0.005 g/100 g diet,), SBE (0.170 mg/100 g diet) or SSE (0.154 g/100 g diet) for five weeks (Table 1). The SBE and SSE were given as a supplement and each amount was based on 5% dried powder of SB or SS per 100 g diet. The animals were given food and distilled water *ad libitum*. Food consumption and weight gain were measured daily and weekly, respectively. At the end of the experimental period, the mice were anesthetized with ketamine after withholding food for 12 h, and blood samples were taken from the inferior vena cava to determine the plasma biomarkers. Also, the liver and muscles were removed after collecting blood, rinsed with a physiological saline solution, and immediately stored at −70 °C. The mice were treated in accordance with Kyungpook National University guidelines for the care and use of laboratory animals.

2.3. Blood glucose, glycosylated hemoglobin, intraperitoneal glucose tolerance test (IPGTT), and intraperitoneal insulin tolerance test (IPITT)

Blood glucose concentration was monitored in the venous blood taken from the tail vein using a glucometer (Arkary, Japan) every week after a 6 h fast. The blood glycosylated hemoglobin concentration was measured with an analyzer (Micromat™ I Hemoglobin A_{1c} Test, Bio-Rad, USA).

Glucose tolerance and insulin tolerance tests were performed after fasting overnight. Mice were injected intraperitoneally with either glucose (0.5 g/kg body weight) or insulin (2 units/kg body weight). Blood glucose levels were determined from the tail vein at 0, 30, 60, and 120 min after the glucose injection and at 0, 30 and 60 min after the insulin injection.

2.4. Plasma biomarker analyses

Blood was collected in a heparin-coated tube and centrifuged at 1000*g* for 15 min at 4 °C. Plasma insulin (DSL-1600 Insulin RIA kit, Diagnostic Systems Laboratories, USA), C-peptide (C-peptide RIA kit, Diagnostic Systems Laboratories, USA), leptin (Mouse leptin RIA kit, Linco Research, USA) and glucagon (Glucagon RIA kit, Packard, USA) levels were measured based on a radio-immunometric assays.

2.5. Hepatic glycogen content and glucose regulating enzyme activities

The glycogen concentration was determined, as previously described by Seifter et al. (1950) with modifications. Briefly, liver tissue was homogenized in five volumes of an ice-cold 30% (wt./vol.) KOH solution and dissolved in a boiling water-bath (100 °C) for 30 min. The glycogen was precipitated with ethanol, pelleted, washed, and resolubilized in distilled water. The glycogen concentration was then determined by treatment with an anthrone reagent (2 g anthrone/1 L of 95% (vol./vol.) H_2SO_4) and the absorbance was measured at 620 nm.