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Dieckol isolated from brown seaweed *Ecklonia cava* attenuates type II diabetes in *db/db* mouse model

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ABSTRACT

In the present study, the attenuation of type II diabetes by dieckol, a phlorotannin derivative isolated from brown seaweed, *Ecklonia cava* was investigated in C57BL/KsJ-*db/db*, a type II diabetes mouse model. Dieckol was administered intraperitoneal injection at doses of 10 and 20 mg/kg body weight diabetes mice for 14 days. The blood glucose level, serum insulin level and body weight were significantly reduced in the dieckol administered group, compared to that of the saline administered group. Furthermore, reduced thiobarbituric acid reactive substraces (TBARS), as well as increased activities of antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-px) in liver tissues were observed in the dieckol administered group. In addition, increased levels of the phosphorylation of AMPK and Akt were observed in the muscle tissues of the dieckol administered group in a Western blotting analysis. According to the findings of this study, it could be suggested that, dieckol can be developed as a therapeutic agent for type II diabetes.

1. Introduction

Diabetes mellitus (DM) is a common endocrine system disease that causes metabolic disorders that leads to a multiple organ damage syndrome. Clinical admiral diabetes are divided into two types, with more than 90% of patients having type II diabetes and are one of the major public health challenges of the 21st century (Kim et al., 2011; Palanuvej et al., 2009; Adams et al., 2011). It is characterized by an increase in the blood glucose level due to insulin resistance (Prabhakar and Doble, 2008; Devalaraja et al., 2011; Leo et al., 2011). Insulin resistance is characterized by the failure of tissues to respond to insulin, resulting in reduced glucose intake in the peripheral tissues and increased hepatic glucose output (Ha et al., 2010; Lebovitz and Banerji, 2004). Due to the multiplication of diabetics worldwide, a great consideration is given to the disease by health care management whole over the world (Umar et al., 2010). The AMP-activated protein kinase (AMPK) system is one of the targets for the anti-diabetic drugs (Viollet et al., 2007). Thus, the relationship between AMPK activation and beneficial metabolic effects provide the fundamental reason for the development of new therapeutic strategies in metabolic disorders.

Since the natural products have great value as a source of therapeutic agents, there is a greater interest in the scientific community to evaluate secondary metabolites isolated from plant materials in experimental studies (Hamza et al., 2010). In addition, this trend obviously leads to the development of new drugs. In general, seaweed-derived compounds have been exhibited a variety of biological activities including antioxidant, anti-cancer, anti-inflammation and anti-hypertension (Ahn et al., 2007; Athukorala and Jeon, 2005; Heo and Jeon, 2008; Kotake-Nara et al., 2005; Lee et al., 2010a,b; Mayer and Hamann, 2004; Nagayama et al., 2002). Hence, seaweeds have always been considered to be a possible alternative and rich source of bioactive metabolites.

Ecklonia cava, brown seaweed (*Laminariaceae*) has long been utilized as a traditional food and traditional folk herb in Korea (Kim et al., 2006; Shim et al., 2009). Recent researches provide the evidence that *E. cava* has exhibited various biological activities both *in vitro* and *in vivo* (Kang et al., 2012; Kang et al., 2010; Lee et al., 2010a,b). Phlorotannins are found only in brown algae and they are a subgroup of tannins, produced entirely by polymerization of phloroglucinol (Kang et al., 2007). Moreover, brown seaweeds have been recognized as a potential source of phlorotannins and it is expected that a high content of phlorotannins is present in *Ecklonia*

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species (Wijesekara et al., 2010). In contrast, the total phlorotannin content of *E. cava* is higher than the other brown algae (Heo et al., 2003). These naturally occurring phlorotannins are known to have various biological activities and found to be one of the most significant groups of active compounds in *E. cava* (Wijesinghe and Jeon, 2012). Previously, several evidences have demonstrated that supplementation of *E. cava* extracted showed anti-diabetes effect by hepatic glucose and lipid metabolism through the improvement of insulin sensitivity in type II diabetes in a *db/db* mouse model (Lee et al., 2012).

Taken together, the objective of the present study is to evaluate the anti-diabetic effect of dieckol, a phlorotannin isolated from brown seaweed *E. cava,* using *in vivo* model for the first time.

2. Materials and methods

2.1. Materials

C57BL/KsJ-*db/db* mice (6 weeks of age; purchased from Joong Ang Lab Animal Co, Seoul, Korea) were used. The thiobarbituric acid-reactive substances (TBARS), superoxide dismutase (SOD), catalase (CAT), reduced glutathione peroxidase (GSH-px) in the liver were determined using a commercially available kit from Sigma Chemical co (St. Louis, MO, USA). All the chemicals and reagents used were of analytical grade and obtained from commercial sources. Antibodies against phosphor-AMP-activated protein kinase (Thr172), Protein kinase B (Akt) and β-actin were obtained from Cell signaling Technology (Bedford, Massachusetts, USA).

2.2. Isolation of dieckol from E. cava

Dried *E. cava* powder (500 g) was extracted with 80% aqueous ethanol and filtered. The filtrate was evaporated at 40 °C to obtain the ethanol extract, which was suspended in water, then extracted with EtOAc. The EtOAc extract (47.85 g) was mixed with Celite. The mixed Celite was dried and packed into a glass column, and eluted in the order of hexane, methylene chloride, diethyl ether, and methanol. The diethyl ether fraction (27.39 g) was subjected to Sephadex LH-20 column chromatography using stepwise gradient chloroform/methanol (2/1→0/1) solvents system, and then finally purified by reversed-phase HPLC (Waters Spherisorb® DOS-2 RP-18, 4.6 × 250 mm, 5 μm, waters Co) to give dieckol. The purified compound, dieckol was confirmed by comparing their LC/MS, ^{1}H NMR data to the investigated.

Dieckol: LC/MS data (M^+, *m/z*: 742.0 Calcd for $C_{36}H_{22}O_{18}$). ^{1}H NMR (400 MHz, DMSO-*d6*) δ9.71(1H, s, OH-9), 9.61 (1H, s, OH-900), 9.51 (1H, s, OH-400), 9.46 (1H, s, OH-4), 9.36 (2H, s, OH-300, 500), 9.28 (1H, s, OH-200), 9.23 (1H, s, OH-2), 9.22 (1H, s, OH-700), 9.15 (2H, s, OH-30, 50) 6.17 (1H, s, H-300), 6.14 (1H, s, H-3), 6.02 (1H, d, J = 2.7 Hz, H-8), 5.98 (1H, d, J = 2.7 Hz, H-800), 5.95 (1H, s, H-2000, 6000), 5.82 (1H, d, J = 2.7 Hz, H-6), 5.81 (1H, d, J = 2.7 Hz, H-600), 5.80 (1H, t, J = 2.0 Hz, H-40), 5.78 (2H, d, J = 2.0 Hz, H-20, 60).

The purity of dieckol (Fig. 1) was >95%, based on the peak area of all components molecular weight in LC/MS analysis. Dieckol was dissolved in DMSO and used for experiments adjusting the final concentration of DMSO in culture medium to <0.01%.

2.3. Animals

Male C57BL/KsJ-*db/db* mice, weighing 50–55 g, were purchased from Jung Ang Lab Animal Inc (Seoul, Korea). Animals were acclimated to temperature (22 °C) and humidity (55%) controlled rooms with a 12 h light/dark cycle for 1 week prior to use in the experiment. Male C57BL/KsJ-*db/db* mice of nine-week-old were randomly divided into 3 groups, saline treatment (saline, 200 μl), dieckol treatment (10 mg/kg, mouse) and dieckol treatment (20 mg/kg mice). Each group consisted of 7 mice. During the experimental period, the body weights and blood glucose levels were investigated daily. After 14 days, the mice were anesthetized and blood samples were collected to determine biochemical parameters. The remaining livers and muscles were frozen in liquid nitrogen and stored at −70 °C for biochemical assays.

2.4. Biochemical assays

Blood glucose levels were determined by detecting serum glucose content. Glucose contents were measured in tail blood using a glucometer (Accu-Chek Instant; Boehringer Mannheim, Seoul, Korea). The livers and muscles were minced into small pieces, and rinsed twice with ice-cold homogenization buffer [250 mM sucrose, 50 mM Tris–HCl, pH 7.4, 1 mM ethylenediaminetetraacetic acid (EDTA)] at 4 °C. The livers and muscle were homogenized in five volumes of ice-cold homogenization buffer with a motor-driven Teflon pestle. The homogenates were centrifuged at 1000*g* for 10 min. The pellets were discarded, and the supernatants were centrifuged at 12,000*g* for 30 min. The final supernatant protein contents were determined using the bicinchoninic acid protein assay reagents.

Lipid peroxidation productions were estimated by measuring the concentration of thiobarbituric acid-reactive substances (TBARS) in fluorescence at 530 nm. Activities of superoxide dismutase (SOD) and catalase (CAT) were measured according to the previously described method (Kakkar et al., 1984; Sinha, 1972). The liver homogenates were diluted 1:20 (g ml^{-1}) in 5% metaphosphoric acid. The homogenates were then centrifuged at 2500*g* and 4 °C, for 10 min. Reduced glutathione peroxidase (GSH-px) is expressed as micro-molar per milligram of protein (μM mg protein^{-1}).

2.5. Plasma insulin

Blood samples from the inferior vena cava were collected into heparin-coated tubes. After centrifugation at 1000*g* for 15 min at 4 °C, the plasma was carefully removed from the sample. The levels of plasma insulin were determined using radioimmunoassay with enzyme-linked immunosorbent assay ELISA kit (Linco Research Inc., Billerica, MA, USA).

2.6. Protein extraction and Western blot analysis

The muscles in mice lysates were prepared by using lysis buffer (50 mmol/l Tris–HCl (pH 7.4), 150 mmol/l NaCl, 1% Triton X-100, 0.1% SDS and 1 mmol/l EDTA). The protein concentrations of the cell lysates were determined by using a BCATM protein assay kit (Thermo Scientific, Rockford, USA). The protein (30 μg/well) preparations were loaded into each lane of sodium dodecyl sulfate–polyacrylamide gels and electrophoresed under denaturing conditions. Subsequently, the proteins were electro-transferred onto nitrocellulose transfer membranes (Schleicher and Schuell, Keene, NH, USA). After blocking with 5% bovine serum albumin (BSA) for 1 h, the blots were incubated with AMPK subunit and phosphorylated (Ser 79) and Akt (1:1000 dilution, all from Cell Signaling Technology, Beverly, MA, USA) protein antibody for 60 min followed by incubation with horseradish peroxidase-conjugated anti-mouse or anti-rabbit IgG (1:2000, Santa Cruz Biotechnology, Inc) as secondary antibody for 45 min. Visualization was achieved by using ECL reagents (Amersham Life Science, Buckinghamshire, UK). Signal intensities were determined by densitometric analysis using Odyssey IR imaging system software (Scion Image software, version beta 4.0.3; Frederick, MD, USA).

2.7. Statistical analysis

All the data were expressed as mean ± standard deviation (SD) of three determinations. Statistical comparison was performed via a one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT). P-values of less than 0.005 ($P < 0.005$) and 0.05 ($P < 0.05$) was considered as significant.

3. Results

3.1. The effect of dieckol on body weight and blood glucose level

Throughout the experiment, the body weights of all the experimental rats were monitored daily. Fig. 2A shows the observations of body weight of experimental rats during the experiment. As shown in the Fig. 2A the body weights of all the tested groups (control and sample treated) were reduced up to 2 days. Then, the body weights of the control group were continuously increased, and at the end of the experimental period, there was a little weight gain, compared to the initial weight. At the same time, considerably lower body weights were observed in the dieckol treated groups during the tested period. In addition, the effect of dieckol on blood glucose level was investigated in diabetic mice. Significantly lower blood glucose levels were observed in the dieckol treated groups, compared to that of the control group (Fig. 2B). However, the effects of dieckol on both body weights and blood glucose levels were not dose-dependent.

3.2. The effect of dieckol on blood insulin level

The effect of dieckol on blood insulin level was shown in Fig. 3. As the Fig. 3 indicates, significantly lower insulin levels were observed in the dieckol treated groups in a dose-dependent manner.