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Effects of brown alga, *Ecklonia cava* on glucose and lipid metabolism in C57BL/KsJ-*db*/*db* mice, a model of type 2 diabetes mellitus

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ABSTRACT

Recently, there has been a growing interest in alternative therapies of marine algae for diabetes. Therefore, the anti-diabetic effects of brown alga, *Ecklonia cava* was investigated in type 2 diabetic animal. Male C57BL/KsJ-*db*/*db* (*db*/*db*) mice were divided into control, dieckol rich extract of *E. cava* (AG-dieckol), or rosiglitazone (RG) groups. The blood glucose, blood glycosylated hemoglobin levels, and plasma insulin levels were significantly lower in the AG-dieckol and RG groups than in the control *db*/*db* mice group, while glucose tolerance was significantly improved in the AG-dieckol group. AG-dieckol markedly lowered plasma and hepatic lipids concentration compared to the control *db*/*db* mice group. The antioxidant enzyme activities were significantly higher in the AG-dieckol group than in the control *db*/*db* mice group, yet its TBARS level was markedly lower compared to the RG group. With regard to hepatic glucose regulating enzyme activities, glucokinase activity was enhanced in the AG-dieckol group mice, while glucose-6-phosphatase and phosphoenolpyruvate carboxykinase activities in the AG-dieckol group mice were significantly lowered than those in the control *db*/*db* mice group. These results suggest that AG-dieckol exert an anti-diabetic effect in type 2 diabetic mice by improving the glucose and lipid metabolism and antioxidant enzymes.

1. Introduction

Diabetes mellitus is the most serious and chronic disease that is developing with an increasing obesity and aging in the general population over the world. Diabetes mellitus is a complex disorder that is characterized by hyperglycemia. It is largely classified into insulin-dependent diabetes mellitus (type 1 diabetes) and non-insulin-dependent diabetes mellitus (type 2 diabetes). In particular, type 2 diabetes is an increasing worldwide health problem and is the most common type of diabetes (Zimmet et al., 2001). Hyperglycemia plays an important role in the development type 2 diabetes and complications associated with the diseases such as micro-vascular and macro-vascular diseases (Baron, 1998). Therefore, the effective control of blood glucose level is the key to prevent or reverse diabetic complications and improve the quality of the life in diabetic patients (DeFronzo, 1999).

Currently available drugs for type 2 diabetes have a number of limitations, such as adverse effects and high rates of secondary failure. Therefore, recently, there has been a growing interest in alternative therapies and in the therapeutic use of natural products for diabetes, especially those derived from herbs (Chang et al., 2006; Jung et al., 2007). This is because plant sources are usually considered to be less toxic with fewer side effects than synthetic ones.

Marine algae are known to provide an abundance of bioactive compounds with great pharmaceutical foods and biomedical potential. In particular, the brown algae have a variety of biological compounds such as pigments, fucoidans, phycocolloids, and polyphenolic compounds. *Ecklonia cava*, a kind of brown alga is popular in Korea and Japan as a food ingredient and marine herb. It contains biologically active compounds such as polyphenols called

Abbreviations: IPGTT, intraperitoneal glucose tolerance test; GK, glucokinase; G6Pase, glucose-6-phosphatase; PEPCK, phosphoenolpyruvate carboxykinase; SOD, superoxide dismutase; CAT, catalase.

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phlorotannins including phloroglucinol, eckol, dieckol, and triphlorethol-A (Kang et al., 2005; Heo et al., 2009). Among these phlorotannins, dieckol is one of the major and active compounds. Its attributes include antioxidant activity, antiallergic activity, inhibition of human immunodeficiency virus-1 reverse transcriptase, and inhibition of the expression of matrix metalloproteinase-1 (MMP-1) (Ahn et al., 2007, 2004; Joe et al., 2006; Le et al., 2008).

Previously, we demonstrated that dieckol isolated from *E. cava* is a potential therapeutic agent that will reduce the damage caused by hyperglycemia-induced oxidative stress associated with diabetes (Lee et al., 2010a). In addition, we investigated the effect of dieckol on postprandial hyperglycemia in *in vivo* test and evidenced prominent effect of dieckol in both streptozotocin-induced diabetic mice and normal mice (Lee et al., 2010b). Therefore, the purpose of this study was to evaluate the anti-diabetic effects of dieckol rich extract of *E. cava* on C57BL/KsJ-*db*/*db* (*db*/*db*), type 2 diabetic mice, and the efficacy was compared with an oral anti-diabetic agent, rosiglitazone.

2. Materials and methods

2.1. Chemicals

D-Glucose-6-phosphate solution, glucose dehydrogenase, inosine 5′-diphosphate sodium salt (IDP), glucose-6-phosphate dehydrogenase, phospho(enol)pyruvic acid, monosodium salt hydrate, and ATP were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All others chemicals and reagents used were of analytical grade and obtained from commercial sources.

2.2. Preparation of E. cava AG-dieckol and analysis of the general composition

The brown alga *E. cava* was collected from the coast of Jeju Island, South Korea. A voucher specimen has been deposited in the author's laboratory and taxonomic identification of *E. cava* was performed by Prof. Ki-Wan Lee at Jeju National University, Republic of Korea. The samples were washed three times with tap water to remove the salt, sand, and epiphytes attached to the surface. Then, the samples were rinsed carefully with fresh water and freeze-dried. The preparation of dieckol-enriched extract was conducted by Aqua Green Tech Co. (Jeju, Korea) processing. One kilogram of the ground dried *E. cava* powder was homogenized with 10 l of distilled water and mixed 10 ml of Celluclast (Novo Nordisk, Bagsvaerd, Denmark). The reaction with this enzyme was conducted at 50 °C for 24 h. As soon as the enzymatic reaction is completed, the digest was boiled for 10 min at 100 °C to inactivate the enzyme. The product was clarified by centrifugation (3000*g* for 20 min) to remove any unhydrolyzed residue. Subsequently, the enzymatic digest was separated by ultrafiltration membranes with different molecular weight cut-offs according to their molecular sizes. The smaller molecular weight fraction through ultrafiltration membranes was mixed with 70% ethanol and placed in shaking incubator for 24 h at room temperature. The ethanol extract was centrifuged at 3500*g* for 20 min at 4 °C and filtered with Whatman No. 1 (Whatman Ltd., Maidstone, England) filter paper to remove the residue, there after evaporated under vacuum at 40 °C. The ethanol extract (AG-dieckol) yield after freeze-drying was 15 g/100 g of powdered smaller molecular weight fraction. The total polyphenol content in the dried Ag-dieckol, determined using the Folins–Ciocalteus method (Aline et al., 2005), was 459.9 mg/g.

For the analysis of quantity of dieckol, the major polyphenol in the AG-dieckol, HPLC analysis was carried out. HPLC was performed on a Acme 9000 (YoungLin Instrument, Gyeonggi, Korea) system using an RP-C18 column (250 × 4.6 mm, 5 μM, Waters, Milford, MA, USA). The mobile phases used in the gradient elution consisted of primary eluant (A) consisting of 10% methanol and 0.04% trifluoroacetic acid (TFA), and a secondary eluant (B) consisting of 100% methanol and 0.04% trifluoroacetic acid (TFA). 10% solvent A changed, in the linear gradient, to 100% of B 40 min after injection. The flow rate was 1 ml/min with injection volume of 20 μl and UV detection was observed at 230 nm. The dieckol was eluted at retention time of 25 min. According to measurements of quantity of dieckol, AG-dieckol contains 100 mg dieckol/g. AG-dieckol from *E. cava* showed noticeable amount of dieckol. The HPLC chromatogram are shown in Fig. 1.

2.3. Animals and diets

Male C57BL/KsJ-*db*/*db* (*db*/*db*) mice (5 weeks of age; purchased from Joong Ang Lab Animal Co., Korea) were used and maintained under a stand light (12 h light/dark), temperature (22 ± 1 °C), and humidity (50 ± 5%) condition. The *db*/*db* mice were fed with a pelletized commercial chow diet for 2 weeks after arrival, then the *db*/*db* mice were randomly divided into three groups (n = 7). Thereafter, control group of *db*/*db* mice were fed with a standard semisynthetic diet (AIN-93G), while the other two groups of *db*/*db* mice were fed with a standard semisynthetic diet containing rosiglitazone (RG; Avandia; GlaxoSmithKline, UK; 0.005 g/100 g diet) or AG-dieckol (0.5 g/100 g diet) for 6 weeks (Table 1). The mice had free access to food and 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 ethyl ether after withholding food for 12 h, and blood samples were taken from the inferior vena cava to determine the plasma biomarkers. Also, the liver was removed after collecting the blood, rinsed with physiological saline solution, and immediately stored at −70 °C. The experimental protocol was approved by the Laboratory Animal Administration Committee of Pusan National University and performed according to the University Guidelines for Animal Experimentation.

2.4. Fasting blood glucose, glycosylated hemoglobin (HbA_{1c}), and intraperitoneal glucose tolerance test (IPGTT)

Every week after 12 h fasting, the blood glucose concentration was monitored in the venous blood from the tail vein using a glucometer (Roche Diagnostics Gmbh, Mannheim, Germany). The blood glycosylated hemoglobin (HbA_{1c}) concentration was measured after hemolysis anticoagulated whole blood specimen and blood glycosylated hemoglobin (HbA_{1c}) concentration was determined with an analyzer (Nyco Card HbA_{1c} test, Axis-shield, Norway). An intraperitoneal glucose tolerance test (IPGTT) was performed during the last week of the experimental period. Following an overnight fast, the mice were injected intraperitoneally with glucose (0.5 g/kg body weight), and the blood glucose levels were determined in tail blood samples 0, 30, 60, and 120 min after the glucose administration. Areas under the curve (AUC) were calculated using the trapezoidal rule.

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. Homeostatic index of insulin resistance and quantitative insulin sensitivity check index

Homeostatic index of insulin resistance (HOMA-IR) and quantitative insulin sensitivity check index (QUICKI) were determined as surrogates of insulin sensitivity (Haffner et al., 1997; Katz et al., 2000). The HOMA-IR was calculated by using the homeostasis model assessment as follows (Eq. (1)):

$$\text{HOMA-IR} = \text{fasting glucose(mmol/l)} \times \text{fasting insulin(IU/ml)}/22.51 \quad (1)$$

QUICKI was derived by using the inverse of the sum of the logarithms of fasting insulin and fasting glucose as follows (Eq. (2)):

$$\text{QUICKI} = 1/\log(\text{fasting glucose mg/dl}) + \log(\text{fasting insulin IU/ml}) \quad (2)$$

2.7. Plasma and hepatic lipid concentrations

The plasma total cholesterol (TC), HDL-cholesterol (HDL-C) and triglyceride concentrations were determined using an enzymatic method (Asan Pharmaceutical corp., Korea), while the plasma free fatty acid (FFA) concentration was determined using an enzymatic colorimetric method (Wako, Japan). The hepatic lipids were extracted using the procedure developed by Folch et al. (1957) and the hepatic cholesterol and triglyceride concentrations analyzed with the same enzymatic kit as used in the plasma analysis.

2.8. Hepatic tissue processing

The hepatic enzyme source was prepared according to the method developed by Hulcher and Oleson (1973) with a slight modification. A 20% (w/v) homogenate was prepared in a buffer containing 0.1 M of triethanolamine, 0.02 M of EDTA, and 2 mM of dithiothreitol, pH 7.0. The homogenates were centrifuged at 600*g* for 10 min to discard any cell debris, and then the supernatant was centrifuged at 10,000*g* followed by 12,000*g* at 4 °C for 20 min to remove the mitochondria pellet. Finally, the supernatant was ultracentrifuged twice at 100,000*g* for 60 min at 4 °C to obtain the cytosolic supernatant. The mitochondrial and microsomal pellets were then redissolved in 1 ml of a homogenization buffer and the protein content determined by the Bradford method (1976) using bovine serum albumin as the standard.

2.9. Lipid peroxidation and antioxidant enzymes

The hepatic lipid peroxidation was determined by the method of Ohkawa et al. (1979) based on TBA reactivity. The degree of lipid peroxide formation was assessed by MDA, which is accepted as an index of lipid peroxide. Superoxide dismutase (SOD) was determined by the method of Marklund and Marklund (1974). Catalase (CAT) was determined by monitoring the decomposition of hydrogen peroxide as described by Aebi (1974).