Sargassum coreanum extract alleviates hyperglycemia and improves insulin resistance in db/db diabetic mice

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BACKGROUND/OBJECTIVES: The goal of this study was to examine the effect of *Sargassum coreanum* extract (SCE) on blood glucose concentration and insulin resistance in C57BL-KsJ-db/db mice.
MATERIALS/METHODS: For 6 weeks, male C57BL/KsJ-db/db mice were administrated SCE (0.5%, w/w), and rosiglitazone (0.005%, w/w).
RESULTS: A supplement of the SCE for 6 weeks induced a significant reduction in blood glucose and glycosylated hemoglobin concentrations, and it improved hyperinsulinemia compared to the diabetic control db/db mice. The glucokinase activity in the hepatic glucose metabolism increased in the SCE-supplemented db/db mice, while phosphoenolpyruvate carboxykinase and glucose-6-phosphatase activities in the SCE-supplemented db/db mice were significantly lower than those in the diabetic control db/db mice. The homeostatic index of insulin resistance was lower in the SCE-supplemented db/db mice than in the diabetic control db/db mice.
CONCLUSIONS: These results suggest that a supplement of the SCE lowers the blood glucose concentration by altering the hepatic glucose metabolic enzyme activities and improves insulin resistance.

INTRODUCTION

Diabetes mellitus is a chronic disease representing one of the world's most serious health concerns. Primarily, diabetes mellitus has been classified into type 1 diabetes and type 2 diabetes. The incidence of type 2 diabetes is increasing around the globe [1]. Type 2 diabetes is mostly a defect that is characterized by high blood glucose due to insulin resistance and a reduced sensitivity to insulin in muscle, adipose, and liver cells [2,3]. Currently available drugs for type 2 diabetes include insulin secretagogues, such as sulfonylurea, and insulin sensitizers, such as thiazolinedione [4]. However, pharmacological agents for type 2 diabetes exhibit a number of limitations, such as side effects and high rates of secondary failure [5]. Thus, person with diabetes and healthcare professionals are interested in alternative therapies and natural products with the therapeutic potential to treat diabetes, particularly those derived from marine algae or plants because these sources are regarded to be less toxic with fewer side effects compared to their synthetic counterparts.

Marine algae are known to generate an abundance of bioactive compounds with great potential in the pharmaceutical, food, and biomedical industries. In particular, brown algae have many different bioactive compounds, including phycocolloids, pigments, and polyphenolic compounds (e.g., phlorotannins) [6]. The brown alga *Sargassum coreanum* is produced on Jeju Island in Korea [7]. It has many biological benefits, including its antioxidant effects in free-radical mediated oxidative systems [8] and its inhibitory effect on human immunodeficiency virus [9].

However, the effect of *S. coreanum* extract on type 2 diabetes has not yet been investigated, especially with respect to alleviating blood glucose concentration, improving insulin resistance, and its effect on the activities of the enzymes involved in hepatic glucose metabolism. Therefore, the present study was conducted to investigate whether *S. coreanum* extract alleviates hyperglycemia and improves insulin resistance in type 2 diabetes mellitus mice. The efficacy was compared with an oral anti-diabetic agent, rosiglitazone (an insulin sensitizer), for type 2 diabetes.

MATERIALS AND METHODS

Preparation of Sargassum coreanum extract

S. coreanum were collected from the coast of Jeju Island, Korea. The samples were initially washed 3 times with tap water to remove salt, epiphytes, and sand attached to the surface and then carefully rinsed with fresh water. Thereafter, the samples were lyophilized and homogenized with a grinder. The dried *S. coreanum* powder was extracted 3 times with 80% methanol and then filtered. Subsequently, the methanol extract was filtered through Whatman No.1 filter paper and evaporated under a vacuum at 40℃, and 30 g of extract per 200 g of powdered *S. coreanum* was obtained. After freeze-drying, the *S. coreanum* extract was powdered and used in the experiment.

Animals and diets

Male C57BL/KsJ-db/db mice were purchased from Japan SLC (Hamamatsu, Japan). The 5-week-old db/db mice were fed a pelletized commercial chow diet for 2 weeks after arrival. The mice were then randomly divided into 3 groups (n = 8). For 6 weeks, the db/db mice in the control of diabetes mellitus group were fed a standard semi-synthetic diet (AIN-93G), while those in the other 2 groups were fed a standard AIN-93G diet with either rosiglitazone (0.005%, w/w) or *S. coreanum* extract (SCE; 0.5%, w/w) (Table 1). Rosiglitazone and SCE doses were based on references and dosage for human type 2 diabetes patients. Type 2 diabetic patients (BW: 60 kg) take 2 tablet rosiglitazone a day (1 tablet: 2 mg). In mouse dosage terms (BW: 30 g), the daily food intake is 0.002 mg. And then, we were multiply that by human safety index 100. The mice were fed a standard AIN-93G diet with either rosiglitazone (0.005%, w/w). Also, we multiply drug dosage by safety index 100 to decide SCE dose. The mice were fed a standard AIN-93G diet with either SCE (0.5%, w/w). The mice were housed individually in stainless steel cages with a randomized complete block design at a temperature of 23 ± 1℃ and humidity of 53 ± 2% in a light-controlled room with a 12 h light-dark cycle. The mice had access to food and water *ad libitum*. Food and water intakes were measured

Table 1. Composition of the experimental diets

	DMC	SCE	Rosiglitazone
Casein	20	20	20
Cornstarch	39.7486	39.7486	39.7486
Dyetrose	13.2	13.2	13.2
Sucrose	10	9.5	9.995
Cellulose	5	5	5
Soybean Oil	7	7	7
t-Butylhydroquinone	0.0014	0.0014	0.0014
Salt Mix	3.5	3.5	3.5
Vitamin Mix	1	1	1
L-Cystine	0.3	0.3	0.3
Choline Bitartrate	0.25	0.25	0.25
Sargassum coreanum extract		0.5	
Rosiglitazone			0.005
Total (%)	100	100	100

DMC: C57BL/KsJ-db/db mice supplemented with AIN-93G diet; rosiglitazone: C57BL/KsJ-db/db mice supplemented with rosiglitazone (0.005 g/100 g diet); SCE: C57BL/KsJ-db/db mice supplemented with SCE (0.5 g/100 g diet).

periodically manually. After a defined time elapses (24 h), the food and water were reweighed and the amount consumed was calculated by difference. At the end of the experimental period, the mice were anesthetized with ether after withholding food for 12 h, and blood samples were taken from the inferior vena cava to determine the level of plasma biomarkers. Furthermore, the liver was removed after collecting the blood and was rinsed with a physiological saline solution. All procedures were approved by the animal ethics committee of pusan national university (PNU-2012-0078).

Blood glucose and glycosylated hemoglobin concentrations

Glucose concentration in the venous blood drawn from the tail vein was determined using a glucometer (Roche Diagnostics GmbH, Mannheim, Germany) once a week for 6 weeks after a 12 h fast. The anticoagulated whole blood specimen was hemolyzed and the blood glycosylated hemoglobin concentration was measured. Glycosylated hemoglobin levels were determined by immunoturbidimetry.

Plasma insulin concentration

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

Homeostatic index of insulin resistance and quantitative insulin sensitivity check index

The homeostatic index of insulin resistance (HOMA-IR) [10] and quantitative insulin sensitivity check index (QUICKI) [11] were determined as surrogates of insulin sensitivity. The HOMA-IR was calculated by using the homeostasis model assessment as follows:

Eq. (1): HOMA-IR
= [fasting glucose (mmol/L) × fasting insulin (IU/mL)]/22.51

QUICKI was derived by using the inverse of the sum of the logarithms of fasting insulin and fasting glucose as follows:

Eq. (2): QUICKI
= 1/[log (fasting glucose (mg/dL)) + log (fasting insulin (U/mL))]

Intraperitoneal glucose tolerance test

An intraperitoneal glucose tolerance test (IPGTT) was performed during the last week of the experimental period. Twelve-hour-fasted mice received an intraperitoneal injection of glucose (0.5 mg of glucose/g of body weight), and blood samples in the diabetes mellitus control (DMC), SCE, and rosiglitazone db/db mice groups were obtained for glucose measurement at 0, 30, 60, and 120 min. The glucose concentration in the venous blood drawn from the tail vein was determined using a glucometer (Roche Diagnostics GmbH, Mannheim, Germany) every week after a 12 h fast.

Hepatic tissue processing

The livers were perfused via the portal vein with cold 0.25 M sucrose and then excised, blotted, weighed, minced, and homogenized in 9 volumes of 0.25 M sucrose. Each homogenate

was subjected to cell fractionation. The cytosolic, mitochondrial, and microsomal fractions were isolated by a sucrose linear density-gradient centrifugation method [12] and stored at -80°C. All of the isolation procedures were performed at 2-4°C. The cytosolic, mitochondrial, and microsomal fractions (hepatic subcellular fractions) were used for the enzyme assays.

Hepatic glucose metabolic enzyme activities

Glucokinase activity was determined by a continuous spectrophotometric assay, as described by Davidson and Arion [13] with a slight modification, wherein the formation of glucose-6-phosphatase at 37°C was coupled to its oxidation by glucose-6-phosphate dehydrogenase and NAD+. The glucose-6-phosphatase activity was determined according to the method of Alegre *et al.* [14] with a slight modification; the activity was determined using a spectrophotometric assay containing 100 mmol/L sodium Hepes (pH 6.5), 26.5 mmol/L glucose-6-phosphate, and 1.8 mmol/L EDTA (adjusted to pH 6.5), 2 mmol/L NADP+, 0.6 IU/mL mutarotase, and 6 IU/mL glucose dehydrogenase. The phosphoenolpyruvate carboxykinase activity in the direction of oxaloacetate synthesis was estimated using the spectrophotometric assay developed by Bentle and Lardy [15] with a slight modification; a 1-mL final volume of the purified enzyme was pipetted into a reaction mixture (pH 7.0) containing 50 mmol/L sodium Hepes, 1 mmol/L IDP, 1 mmol/L $MnCl_2$, 1 mmol/L dithiothreitol, 0.25 mmol/L NADH, 2 mmol/L phosphoenolpyruvate, 50 mmol/L $NaHCO_3$, and 7.2 units of malic dehydrogenase. The enzyme activity was then measured at 25°C based on a decrease in the absorbance at 340 nm.

Hepatic glycogen assay

The glycogen concentration was determined as previously described by Seifter *et al.* with some modifications [16]. Briefly, the liver tissue was homogenized in 5 volumes of a 30% (w/v) ice-cold KOH solution and dissolved in a boiling water bath (100°C) for 30 min. The glycogen was precipitated with ethanol and then pelleted, washed, and resolubilized in distilled water. This solution was treated with an anthrone reagent [2 g anthrone/1 L of 95% (v/v) H_2SO_4, and its absorbance was measured at 620 nm.

Plasma lipid concentration

The plasma total cholesterol (TC), HDL-cholesterol (HDL-C) and triglyceride concentrations were determined using an enzymatic method (Asian Pharmaceutical Corp., Korea), while the plasma free fatty acid (FFA) concentration was determined using an enzymatic colorimetric method (Wako, Japan).

Hepatic function tests

Asparate aminotransferase and alanine aminotransferase activities were determined using a kit (Asian Pharm Co., Korea) by the modified Reitman-Frankel method.

Statistical analysis of data

The data were represented as the mean ± SD. Statistical analyses were performed using the SAS software. The values were evaluated by one-way analysis of variance (ANOVA) followed by post-hoc Duncan's multiple range tests.

RESULTS

Body weight and food and water intake

At the start of the study, the db/db mice did not differ significantly in their body weight among the control of diabetes mellitus, rosiglitazone-supplemented, and SCE-supplemented db/db mice groups. During the 6-week period, body weight increased gradually. The body weights of the control of diabetes mellitus, rosiglitazone-supplemented, and SCE-supplemented db/db mice were significantly different at the end of the study. The rosiglitazone-supplemented db/db mice had higher body weight compared to the control of diabetes mellitus and SCE-supplemented db/db mice (Table 2). The daily food intake of these db/db mice did not differ significantly. The rosiglitazone- and SCE-supplemented db/db mice had a significantly lower daily intake of water than the control of diabetes mellitus db/db mice.

Blood glucose and glycated hemoglobin concentrations

The effects of the SCE and rosiglitazone supplement on blood glucose concentrations are shown in Fig. 1. There were no significant differences in blood glucose concentrations among the groups at the beginning of the experiment. Throughout the experimental period, the blood glucose concentrations of the control of diabetes mellitus db/db mice increased steeply,

Table 2. The effects of SCE supplement on food intake and body weight in C57BL/KsJ-db/db mice

	DMC	SCE	Rosiglitazone
Food intake (g/day)	6.09 ± 0.33[NS]	5.09 ± 0.69	4.61 ± 1.36
Water intake (mL/day)	20.98 ± 1.47[a]	13.89 ± 2.49[b]	6.16 ± 1.95[c]
Body weights (g)			
Initial	34.34 ± 1.45[NS]	33.51 ± 1.01	34.82 ± 1.87
Final	42.07 ± 4.48[NS]	43.90 ± 1.95	49.92 ± 3.11

Values are mean ± SD (n = 8). Superscripts in the same row not sharing a common superscript are significantly different between groups (*P* < 0.05). DMC: C57BL/KsJ-db/db mice supplemented with AIN-93G diet; rosiglitazone: C57BL/KsJ-db/db mice supplemented with rosiglitazone (0.005 g/100 g diet); SCE: C57BL/KsJ-db/db mice supplemented with SCE (0.5 g/100 g diet).
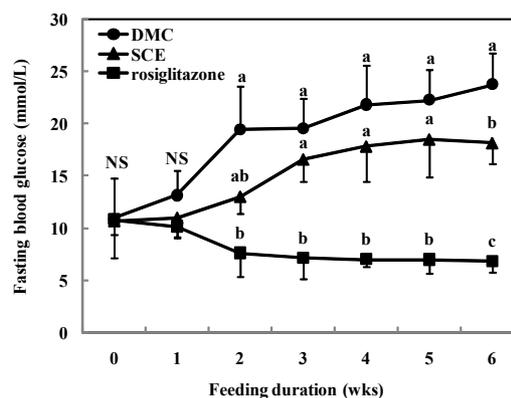
Fig. 1. Changes in the blood glucose concentrations of C57BL/KsJ-db/db mice supplemented with SCE for 6 weeks. DMC: C57BL/KsJ-db/db mice supplemented with AIN-93G diet; rosiglitazone: C57BL/KsJ-db/db mice supplemented with rosiglitazone (0.005 g/100 g diet); SCE: C57BL/KsJ-db/db mice supplemented with SCE (0.5 g/100 g diet). Values are mean ± SD (n = 8); Superscripts with different alphabets in same time point differ significantly at *P* < 0.05 as analyzed via ANOVA with Duncan's multiple range test.

Table 3. The effects of SCE supplement on glycated hemoglobin, plasma insulin, fasting blood glucose, and insulin resistance in C57BL/KsJ-db/db mice

	DMC	SCE	Rosiglitazone
HbA1c (mmol/mol Hb)	112.24 ± 6.30[a]	74.08 ± 9.18[b]	59.02 ± 4.83[b]
Plasma insulin (pmol/L)	239.65 ± 34.51[a]	168.70 ± 16.97[b]	158.57 ± 29.46[b]
Fasting blood glucose (mmol/L)	23.73 ± 3.03[a]	18.16 ± 2.05[b]	6.81 ± 1.04[c]
HOMA-IR	25.50 ± 1.32[a]	13.54 ± 2.29[b]	7.05 ± 0.01[b]
QUICKI	0.25 ± 0.01[b]	0.27 ± 0.01[a]	0.29 ± 0.01[a]

Values are mean ± SD (n = 8). Superscripts in the same row not sharing a common superscript are significantly different between groups ($P < 0.05$). DMC: C57BL/KsJ-db/db mice supplemented with AIN-93G diet; rosiglitazone: C57BL/KsJ-db/db mice supplemented with rosiglitazone (0.005 g/100 g diet); SCE: C57BL/KsJ-db/db mice supplemented with SCE (0.5 g/100 g diet). HOMA-IR: homeostatic index of insulin resistance; QUICKI: quantitative insulin sensitivity check index.

but those of SCE-supplemented db/db mice increased gradually and to a lesser extent than the control of diabetes mellitus db/db mice. The blood glycated hemoglobin concentration was significantly lower in the SCE-supplemented db/db mice than in the control of diabetes mellitus db/db mice. The blood glycated hemoglobin concentration was effectively reduced in the SCE-supplemented db/db mice, even if the level in these db/db mice was higher than that of the rosiglitazone-supplemented db/db mice (Table 3).

Homeostatic index of insulin resistance, quantitative insulin sensitivity check index, and intraperitoneal glucose tolerance test
Table 3 shows that the plasma insulin concentrations of the SCE- and rosiglitazone-supplemented db/db mice were significantly lower than that of the control of diabetes mellitus db/db
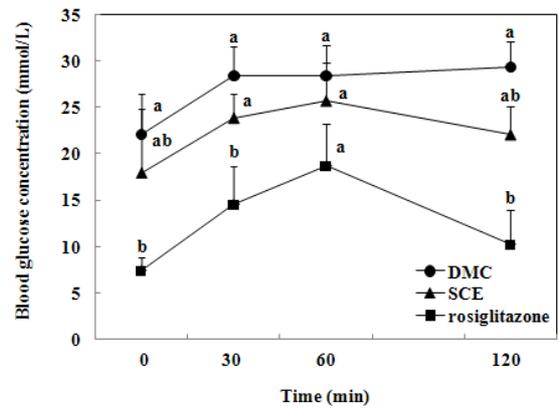
Fig. 2. The effect of SCE supplement on glucose tolerance test in C57BL/KsJ-db/db mice. After a 12 h fast, male mice were intraperitoneally injected with glucose (0.5 g/kg body weight). The blood glucose concentration was measured at the indicated times and presented as actual value injection. DMC: C57BL/KsJ-db/db mice supplemented with AIN-93G diet; rosiglitazone: C57BL/KsJ-db/db mice supplemented with rosiglitazone (0.005 g/100 g diet); SCE: C57BL/KsJ-db/db mice supplemented with SCE (0.5 g/100 g diet). Values are mean ± SD (n = 8); Superscripts with different alphabets in same time point differ significantly at $P < 0.05$ as analyzed via ANOVA with Duncan's multiple range test.

mice. Furthermore, the homeostatic index of insulin resistance and quantitative insulin sensitivity check index were significantly different among the control of diabetes mellitus, rosiglitazone-supplemented, and SCE-supplemented db/db mice. The homeostatic index of insulin resistance was significantly lower in the SCE- and rosiglitazone-supplemented db/db mice than in the control of diabetes mellitus db/db mice. In addition, the quantitative insulin sensitivity check index was significantly
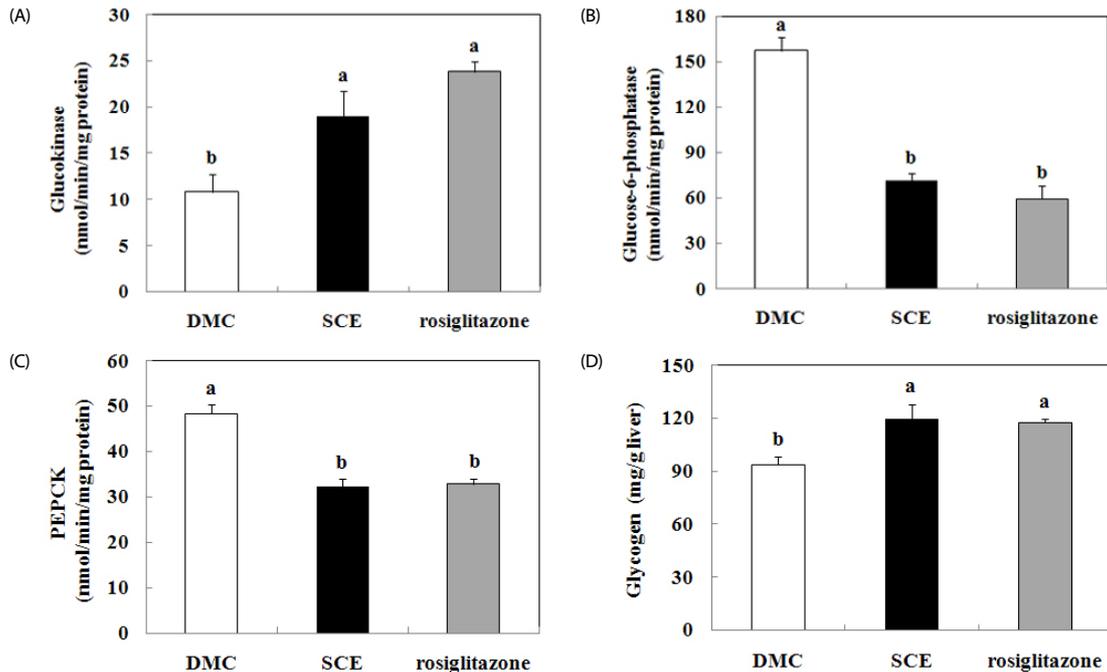
Fig. 3. The effects of SCE supplementation on the hepatic glucokinase (A), glucose-6-phosphatase (B), and phosphoenolpyruvate carboxykinase activities (C), and glycogen content (D) in C57BL/KsJ-db/db mice. DMC: C57BL/KsJ-db/db mice supplemented with AIN-93G diet; rosiglitazone: C57BL/KsJ-db/db mice supplemented with rosiglitazone (0.005 g/100 g diet); SCE: C57BL/KsJ-db/db mice supplemented with SCE (0.5 g/100 g diet). Values are mean ± SD (n = 8); Superscripts with different alphabets differ significantly at $P < 0.05$ as analyzed via Duncan's multiple range test.

Table 4. The effects of SCE supplement on plasma lipid concentration in C57BL/KsJ-db/db mice

	DMC	SCE	Rosiglitazone
Total cholesterol (mg/dL)	327.15 ± 69.61[a]	198.30 ± 53.79[ab]	184.05 ± 21.87[b]
Triglyceride (mg/dL)	289.47 ± 17.92[a]	216.87 ± 21.35[b]	199.03 ± 21.80[b]
FFA (mmol/L)	1.02 ± 0.13[a]	0.42 ± 0.12[b]	0.49 ± 0.15[b]
HDL-C (mg/dL)	49.13 ± 9.86[b]	104.12 ± 7.63[a]	78.01 ± 16.30[ab]
LDL-C (mg/dL)	220.12 ± 56.17[a]	74.06 ± 68.56[b]	70.02 ± 25.22[b]
AI (mg/dL)	5.66 ± 0.31[a]	1.14 ± 0.13[b]	1.41 ± 0.22[b]

Values are mean ± SD (n = 8). Superscripts in the same row not sharing a common superscript are significantly different between groups (*P* < 0.05). DMC: C57BL/KsJ-db/db mice supplemented with AIN-93G diet; rosiglitazone: C57BL/KsJ-db/db mice supplemented with rosiglitazone (0.005 g/100 g diet); SCE: C57BL/KsJ-db/db mice supplemented with SCE (0.5 g/100 g diet). AI: Atherogenic index.

Table 5. The effects of SCE supplement on hepatic function tests in C578BL/KsJ-db/db mice

	DMC	SCE	Rosiglitazone
ALT (karmen/ml)	14.06 ± 0.55[NS]	13.09 ± 0.51	13.14 ± 0.17
AST (karmen/ml)	12.24 ± 0.67[NS]	11.90 ± 0.61	12.48 ± 0.21

Values are mean ± SD (n = 8). DMC: C57BL/KsJ-db/db mice supplemented with AIN-93G diet; rosiglitazone: C57BL/KsJ-db/db mice supplemented with rosiglitazone (0.005 g/100 g diet); SCE: C57BL/KsJ-db/db mice supplemented with SCE (0.5 g/100 g diet). ALT: alanine aminotransferase; AST: aspartate aminotransferase; NS: not significant.

higher in the SCE- and rosiglitazone-supplemented db/db mice than in the control of diabetes mellitus db/db mice.

Glucose tolerance was monitored by an intraperitoneal glucose tolerance test at 6 weeks after supplementation with SCE or rosiglitazone (Fig. 2). There was a significant difference in the progression of nonlinear patterns among the control of diabetes mellitus, rosiglitazone-supplemented, and SCE-supplemented db/db mice. In terms of the change point analysis, it was confirmed that the SCE- and rosiglitazone-supplemented db/db mice had a change point at 60 min. Specifically, the blood glucose concentrations in the rosiglitazone-supplemented db/db mice peaked at 60 min and almost recovered to the basal value at 120 min. Similarly, the blood glucose concentrations in SCE-supplemented db/db mice peaked at 60 min and presented with a declined value at 120 min. However, the blood glucose concentrations in the control of diabetes mellitus db/db mice gradually increased and remained at a constant, high level with minimal changes occurring over the course of 30-120 min. In other words, when the mice were injected with glucose, the rates of increase in the blood glucose concentration were similar among the groups during the first 60 min. Subsequently, the blood glucose concentration became significantly higher in the control of diabetes mellitus db/db mice compared to the SCE- and rosiglitazone-supplemented db/db mice.

Hepatic glucose regulating enzyme activities and glycogen levels

Hepatic glucokinase activity was significantly higher in the SCE- and rosiglitazone-supplemented db/db mice than in the control of diabetes mellitus db/db mice. In contrast, glucose-6-phosphatase activity was remarkably lower in the SCE- and rosiglitazone-supplemented db/db mice than in the control of diabetes mellitus db/db mice. Phosphoenolpyruvate carboxykinase

activity was also significantly lower in the SCE- and rosiglitazone-supplemented db/db mice than in the control of diabetes mellitus db/db mice. The hepatic glycogen levels in the SCE- and rosiglitazone-supplemented db/db mice were significantly higher than that in the control of diabetes mellitus db/db mice (Fig. 3).

Plasma lipid

The plasma triglyceride, free fatty acid, total cholesterol, LDL-cholesterol, and atherogenic index levels of the SCE- and rosiglitazone-supplemented db/db mice were significantly lower than those of the control of diabetes mellitus db/db mice, while the plasma HDL-cholesterol was significantly higher in the SCE- and rosiglitazone-supplemented db/db mice than in the control of diabetes mellitus db/db mice (Table 4).

Hepatic function

The levels of ALT and AST were not significantly different among the control of diabetes mellitus, rosiglitazone-supplemented, and SCE-supplemented db/db mice groups (Table 5). The ALT and AST levels of each were within the normal range.

DISCUSSION

Type 2 diabetes mellitus and its related complications have arisen as serious health problems in modern societies. In this study, we demonstrated the anti-diabetic effect of SCE supplementation in C57BL/KsJ-db/db mice. These db/db mice have characteristics that are similar to human type 2 diabetes, including hyperglycemia, obesity, and insulin resistance [16-18]. The body weights increased in db/db mice after 6 weeks. The rosiglitazone-supplemented db/db mice experienced significantly greater body weight gain compared to the SCE-supplemented and control of diabetes mellitus db/db mice. These results may be due to increased adipose tissue mass, which has been shown for rodents and humans receiving rosiglitazone treatment [5]. Food intake did not have a significant effect on the groups, but water intake was significantly lower in the SCE- and rosiglitazone-supplemented db/db mice than in the control of diabetes mellitus db/db mice. The typical symptoms of type 2 diabetes are polyphagia, polyuria, and polydipsia. Supplements of SCE and rosiglitazone improved the symptoms of type 2 diabetes compared to the control of diabetes mellitus db/db mice; particularly polydipsia.

Rosiglitazone is an anti-diabetic drug in the thiazolinedione class, and was used as the positive control for SCE in the present study. Rosiglitazone works as an insulin sensitizer by binding to the Peroxisome proliferator-activated receptor receptors in fat cells and making the cells more responsive to insulin [19]. That is, it increases insulin sensitivity and improves glycemic control in type 2 diabetes. However, this compound induces adipogenesis in cell culture systems [20] and raises weight gain in rodents and humans [5]. In addition, it has several side effects, such as headaches, hypoglycemia, edema, hypertension, and liver toxicity [21].

Hyperglycemia is a crucial factor in the onset of type 2 diabetes and the complications associated with the disease [22]. Thus, the effective control of hyperglycemia is key for preven-

ting diabetic complications and improving the quality of life in patients with type 2 diabetes [23]. Hyperglycemia is an independent risk factor for vascular complication disease, and a therapeutic medication may be required in order to reach hyperglycemic control targets in patients who have type 2 diabetes [24]. Fasting blood glucose concentration in the control of diabetes mellitus db/db mice increased steeply, but the concentration in the SCE-supplemented db/db mice increased gradually up to the second week of the experimental diet. Also, rosiglitazone supplementation slowly decreased the fasting blood glucose concentration over a 6-week period. It is well known that rosiglitazone supplement improves glycemic control in db/db mice [20]. The SCE supplement in the db/db mice significantly alleviated rising blood glucose levels compared to the control of diabetes mellitus db/db mice over the entire experimental period. In addition, the SCE supplement in the db/db mice significantly lowered the glycated hemoglobin concentrations compared to the control of diabetes mellitus db/db mice. Glycated hemoglobin is primarily measured to identify average blood glucose concentrations over prolonged periods of time. This serves as a marker for average blood glucose concentrations over the previous months and is useful for monitoring glycemic control in patients with type 2 diabetes [25]. The findings in this study suggest that the SCE supplement improved blood glucose concentration in type 2 diabetes mellitus mice.

In general, db/db mice exhibit an initial phase of hyperinsulinemia and progressively develop insulinopenia with age, a feature that is commonly observed in the late stages of type 2 diabetes [26]. The results of this study showed that the plasma insulin level in the control of diabetes mellitus db/db mice was higher than those in the SCE- and rosiglitazone-supplemented db/db mice, indicating that hyperinsulinemia was still expressed in the control of diabetes mellitus db/db mice. It has been reported that rosiglitazone improves the actions of insulin, thereby ameliorating glucose tolerance and lowering hyperinsulinemia in animals and humans with type 2 diabetes [27].

The homeostatic index of insulin resistance and the quantitative insulin sensitivity check index are simple indexes of insulin resistance. These were calculated using insulin and fasting glucose levels. The homeostatic index of insulin resistance is used as a biomarker of insulin resistance; values should increase with increasing insulin resistance. It is a useful index of insulin resistance [28]. Even though the homeostatic index of insulin resistance has several limitations in terms of accuracy and reliability [12], it essentially expresses insulin resistance [29]. The quantitative insulin sensitivity check index is an index of insulin sensitivity; values should decrease with increasing insulin resistance. In this study, the blood glucose level, plasma insulin level, and homeostatic index of insulin resistance were significantly lower in the SCE-supplemented db/db mice than in the control of diabetes mellitus db/db mice.

The intraperitoneal glucose tolerance test was performed after supplementing SCE in db/db mice for 6 weeks. Twelve-hour-fasted mice received an intraperitoneal injection of glucose, and blood samples in the control of diabetes mellitus, rosiglitazone-supplemented, and SCE-supplemented db/db mice were obtained for glucose measurement at 0, 30, 60, and 120 min.

The rosiglitazone-supplemented db/db mice results indicated a near recovery of blood glucose levels at 120 min after glucose loading when expressed as actual values. SCE also improved the glucose-handling ability of db/db mice by lowering blood glucose level at 120 min, whereas glucose tolerance in the control of diabetes mellitus db/db mice became impaired. Thus, the SCE- and rosiglitazone-supplemented db/db mice ameliorated their glucose tolerance when the data were presented as actual blood glucose levels in the intraperitoneal glucose tolerance test. Subjects with impaired glucose tolerance generally have a heightened risk of macrovascular disease [30-32]. The diagnosis of impaired glucose tolerance has important prognostic implications for cardiovascular disease risk factors with respect to detecting hypertension, dyslipidemia, and central obesity [33].

Abnormal hepatic glucose metabolism is a major symptom of type 2 diabetes, and it contributes to postprandial hyperglycemia [34]. Elevated hepatic glucose production is critical for the occurrence of fasting and postprandial hyperglycemia [35]. While the level of plasma insulin in the db/db mice was high, hepatic glucose production was elevated, indicating a relative insensitivity of the liver to insulin [36]. Hepatic glucokinase plays a major role in controlling blood glucose homeostasis and its activity is low in type 2 diabetes [37]. Glucose-6-phosphatase is a key enzyme controlling hepatic gluconeogenesis and glucose output in the liver, and is normally suppressed by the actions of insulin [38]. Due to their strategic positions in hepatic glucose metabolism, both glucokinase and glucose-6-phophatase are the target enzymes regulating hepatic glucose production [39]. In this study, hepatic glucokinase activity was significantly higher in the SCE-supplemented db/db mice than in the control of diabetes mellitus db/db mice. Furthermore, hepatic glucose-6-phosphatase activity was significantly lower in the SCE-supplemented db/db mice than in the control of diabetes mellitus db/db mice, thereby decreasing hepatic gluconeogenesis. The SCE supplement in db/db mice also lowered the activity of hepatic phosphoenolpyruvate carboxykinase compared with the control of diabetes mellitus db/db mice. Thus, the hypoglycemic effects of the SCE supplement may be partly controlled through enhanced glucokinase activity and suppressed glucose-6-phosphatase and phosphoenolpyruvate carboxykinase activity in the liver of db/db mice.

Both lipolysis and circulating free fatty acids increase in an insulin resistance condition [19,40]. Also, elevated plasma free fatty acid levels account for up to 50% of insulin resistance in obese patients with type 2 diabetes [41]. In the present study, the SCE supplement significantly lowered the plasma free fatty acid, triglyceride, total cholesterol, and LDL-cholesterol levels compared to the control of diabetes mellitus db/db mice. A few studies have reported that free fatty acid and LDL-cholesterol levels were significantly reduced by supplementation with natural products containing polyphenol in type 2 diabetes mellitus animals [42-44]. In this study, the results also indicate that SCE supplementation enhances the ameliorating effect of insulin resistance by decreasing plasma lipid levels in db/db mice.

In conclusion, we investigated the anti-diabetic effect of SCE on blood glucose concentration and insulin resistance in male C57BL/KsJ-db/db mice. Fasting blood glucose level, glycated

hemoglobin concentration, and insulin resistance were ameliorated in the SCE-supplemented db/db mice compared to those in the control of diabetes mellitus db/db mice. Furthermore, SCE supplementation elevated the activity of glucokinase, while the activities of glucose-6-phosphatase and phosphoenolpyruvate carboxykinase were significantly reduced in the SCE-supplemented db/db mice compared to the control of diabetes mellitus db/db mice. The plasma lipid level was also improved in the SCE-supplemented db/db mice compared to the control of diabetes mellitus db/db mice. These results suggest that SCE diet supplementation lowers the blood glucose concentration and improves insulin resistance. Thus, it seems likely that SCE, the extract from *Sargassum coreanum,* is a potential anti-diabetic resource that will be helpful for the alleviating the symptoms of type 2 diabetes.

REFERENCES

1. Zimmet P, Alberti KG, Shaw J. Global and societal implications of the diabetes epidemic. Nature 2001;414:782-7.

2. Kumar V, Abbas AK, Fausto N, Robbins SL, Cotran RS. The skin. In: Robbins and Cotran Pathologic Basis of Disease. 7th ed. Philadelphia (PA): Elsevier Saunders; 2005. p.1194-5.

3. DeBruyne LK, Whitney EN, Pinna K. Nutrition and Diet Therapy. 7th ed. Belmont (CA): Thomson Wadsworth; 2008.

4. Kobayashi M, Iwanishi M, Egawa K, Shigeta Y. Pioglitazone increases insulin sensitivity by activating insulin receptor kinase. Diabetes 1992;41:476-83.

5. Min KH, Kim HJ, Jeon YJ, Han JS. Ishige okamurae ameliorates hyperglycemia and insulin resistance in C57BL/KsJ-db/db mice. Diabetes Res Clin Pract 2011;93:70-6.

6. Halliwell B, Gutteridge JM. Antioxidant defences. In: Free Radicals in Biology and Medicine. 3rd ed. Oxford: Oxford University Press; 1999. p.105-59.

7. Lee IK, Kang JW. Check list of the marine Algae in Korea. Korean J Phycol 1986;1:311-25.

8. Zou Y, Qian ZJ, Li Y, Kim MM, Lee SH, Kim SK. Antioxidant effects of phlorotannins isolated from Ishige okamurae in free radical mediated oxidative systems. J Agric Food Chem 2008;56:7001-9.

9. Ahn MJ, Yoon KD, Kim CY, Kim JH, Shin CG, Kim J. Inhibitory activity on HIV-1 reverse transcriptase and integrase of a carmalol derivative from a brown Alga, Ishige okamurae. Phytother Res 2006;20:711-3.

10. Haffner SM, Miettinen H, Stern MP. The homeostasis model in the San Antonio Heart Study. Diabetes Care 1997;20:1087-92.

11. Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, Quon MJ. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. J Clin Endocrinol Metab 2000;85:2402-10.

12. Kwak CS, Kwak JS. Cell fractionation method of the rat liver. Keimyung Med J 1986;5:45-53.

13. Davidson AL, Arion WJ. Factors underlying significant underestimations of glucokinase activity in crude liver extracts: physiological implications of higher cellular activity. Arch Biochem Biophys 1987;253:156-67.

14. Alegre M, Ciudad CJ, Fillat C, Guinovart JJ. Determination of glucose-6-phosphatase activity using the glucose dehydrogenase-coupled reaction. Anal Biochem 1988;173:185-9.

15. Bentle LA, Lardy HA. Interaction of anions and divalent metal ions with phosphoenolpyruvate carboxykinase. J Biol Chem 1976;251:2916-21.

16. Seifter S, Dayton S, Novic B, Muntwyler E. The estimation of glycogen with the anthrone reagent. Arch Biochem 1950;25:191-200.

17. Kim DJ, Jeong YJ, Kwon JH, Moon KD, Kim HJ, Jeon SM, Lee MK, Park YB, Choi MS. Beneficial effect of chungkukjang on regulating blood glucose and pancreatic beta-cell functions in C75BL/KsJ-db/db mice. J Med Food 2008;11:215-23.

18. Simon SF, Taylor CG. Dietary zinc supplementation attenuates hyperglycemia in db/db mice. Exp Biol Med (Maywood) 2001;226:43-51.

19. Fujiwara T, Yoshioka S, Yoshioka T, Ushiyama I, Horikoshi H. Characterization of new oral antidiabetic agent CS-045. Studies in KK and ob/ob mice and Zucker fatty rats. Diabetes 1988;37:1549-58.

20. Cantello BC, Cawthorne MA, Haigh D, Hindley RM, Smith SA, Thurlby PL. The synthesis of BRL 49653 - a novel and potent antihyperglycemic agent. Bioorg Med Chem Lett 1994;4:1181-4.

21. Kim KR, Lee JH, Kim SJ, Rhee SD, Jung WH, Yang SD, Kim SS, Ahn JH, Cheon HG. KR-62980: a novel peroxisome proliferator-activated receptor gamma agonist with weak adipogenic effects. Biochem Pharmacol 2006;72:446-54.

22. Baron AD. Postprandial hyperglycaemia and alpha-glucosidase inhibitors. Diabetes Res Clin Pract 1998;40 Suppl:S51-5.

23. DeFronzo RA, Gunnarsson R, Björkman O, Olsson M, Wahren J. Effects of insulin on peripheral and splanchnic glucose metabolism in noninsulin-dependent (type II) diabetes mellitus. J Clin Invest 1985;76:149-55.

24. Van Gaal LF, De Leeuw IH. Rationale and options for combination therapy in the treatment of type 2 diabetes. Diabetologia 2003;46 Suppl 1:M44-50.

25. Tahara Y, Shima K. Kinetics of HbA1c, glycated albumin, and fructosamine and analysis of their weight functions against preceding plasma glucose level. Diabetes Care 1995;18:440-7.

26. Fujiwara T, Wada M, Fukuda K, Fukami M, Yoshioka S, Yoshioka T, Horikoshi H. Characterization of CS-045, a new oral antidiabetic agent, II. Effects on glycemic control and pancreatic islet structure at a late stage of the diabetic syndrome in C57BL/KsJ-db/db mice. Metabolism 1991;40:1213-8.

27. Patel J, Miller E, Hu J, Granett J. BRL 49653 (a thiazolidinedione) improves glycemic control in NIDDM patients. Diabetes 1997;46:150A.

28. Uno T, Ohsawa I, Tokudome M, Sato Y. Effects of Goshajinkigan on insulin resistance in patients with type 2 diabetes. Diabetes Res Clin Pract 2005;69:129-35.

29. Bonora E, Targher G, Alberiche M, Bonadonna RC, Saggiani F, Zenere MB, Monauni T, Muggeo M. Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: studies in subjects with various degrees of glucose tolerance and insulin sensitivity. Diabetes Care 2000;23:57-63.

30. Shaw JE, Hodge AM, De Courten M, Chitson P, Zimmet PZ. Isolated post-challenge hyperglycaemia confirmed as a risk factor for mortality. Diabetologia 1999;42:1050-4.

31. Tominaga M, Eguchi H, Manaka H, Igarashi K, Kato T, Sekikawa A. Impaired glucose tolerance is a risk factor for cardiovascular disease, but not impaired fasting glucose. The Funagata Diabetes Study. Diabetes Care 1999;22:920-4.

32. Perry RC, Baron AD. Impaired glucose tolerance. Why is it not a disease? Diabetes Care 1999;22:883-5.

33. Zimmet PZ, Alberti KG. The changing face of macrovascular disease in non-insulin-dependent diabetes mellitus: an epidemic in progress. Lancet 1997;350 Suppl 1:SI1-4.

34. Basu A, Basu R, Shah P, Vella A, Johnson CM, Jensen M, Nair KS, Schwenk WF, Rizza RA. Type 2 diabetes impairs splanchnic uptake of glucose but does not alter intestinal glucose absorption during enteral glucose feeding: additional evidence for a defect in hepatic glucokinase activity. Diabetes 2001;50:1351-62.

35. DeFronzo RA, Bonadonna RC, Ferrannini E. Pathogenesis of NIDDM. A balanced overview. Diabetes Care 1992;15:318-68.

36. Coleman DL, Hummel KP. Studies with the mutation, diabetes, in the mouse. Diabetologia 1967;3:238-48.

37. Postic C, Shiota M, Niswender KD, Jetton TL, Chen Y, Moates JM, Shelton KD, Lindner J, Cherrington AD, Magnuson MA. Dual roles for glucokinase in glucose homeostasis as determined by liver and pancreatic beta cell-specific gene knock-outs using Cre recombinase. J Biol Chem 1999;274:305-15.

38. Nordlie RC, Bode AM, Foster JD. Recent advances in hepatic glucose 6-phosphatase regulation and function. Proc Soc Exp Biol Med 1993;203:274-85.

39. Mithieux G. New knowledge regarding glucose-6 phosphatase gene and protein and their roles in the regulation of glucose metabolism. Eur J Endocrinol 1997;136:137-45.

40. Robinson C, Tamborlane WV, Maggs DG, Enoksson S, Sherwin RS, Silver D, Shulman GI, Caprio S. Effect of insulin on glycerol production in obese adolescents. Am J Physiol 1998;274:E737-43.

41. Boden G. Interaction between free fatty acids and glucose metabolism. Curr Opin Clin Nutr Metab Care 2002;5:545-9.

42. Park SA, Choi MS, Cho SY, Seo JS, Jung UJ, Kim MJ, Sung MK, Park YB, Lee MK. Genistein and daidzein modulate hepatic glucose and lipid regulating enzyme activities in C57BL/KsJ-db/db mice. Life Sci 2006;79:1207-13.

43. Park SA, Choi MS, Kim MJ, Jung UJ, Kim HJ, Park KK, Noh HJ, Park HM, Park YB, Lee JS, Lee MK. Hypoglycemic and hypolipidemic action of Du-zhong (Eucommia ulmoides Oliver) leaves water extract in C57BL/KsJ-db/db mice. J Ethnopharmacol 2006;107:412-7.

44. Jung UJ, Baek NI, Chung HG, Bang MH, Jeong TS, Lee KT, Kang YJ, Lee MK, Kim HJ, Yeo J, Choi MS. Effects of the ethanol extract of the roots of Brassica rapa on glucose and lipid metabolism in C57BL/KsJ-db/db mice. Clin Nutr 2008;27:158-67.