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Hypolipidemic effect of *Salicornia herbacea* in animal model of type 2 diabetes mellitus*

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Abstract

To control blood glucose level as close to normal is a major goal of treatment of diabetes mellitus. Hyperglycemia and hyperlipidemia are the major risk factors for cardiovascular complications, the major cause of immature death among the patients with type 2 diabetes. The purpose of this study is to determine the hypoglycemic and hypolipidemic effects of *Salicornia herbacea* in animal model of type 2 diabetes and to investigate the possible mechanisms for the beneficial effects of *S. herbacea. S. herbacea* was extracted with 70% ethanol and desalted with 100% ethanol. Three week-old db/db mice (C57BL/KsJ, n=16) were fed AIN-93G semipurified diet or diet containing 1% desalted ethanol extract of *S. herbacea* for 6 weeks after 1 week of adaptation. Fasting plasma glucose, triglyceride, and total cholesterol were measured by enzymatic methods and blood glycated hemoglobin (HbA_{1C}) by the chromatographic method. Body weight and food intake of *S. herbacea* group were not significantly different from those of the control group. Fasting plasma glucose and blood glycated hemoglobin levels tended to be lowered by *S. herbacea* treatment. Consumption of *S. herbacea* extract significantly decreased plasma triglyceride and cholesterol levels ($p<0.05$). The inhibition of *S. herbacea* extract against yeast α-glucosidase was 31.9% of that of acarbose at the concentration of 0.5 mg/mL *in vitro*. The inhibitory activity of ethanol extract of *S. herbacea* against porcine pancreatic lipase was 59.0% of that of orlistat at the concentration of 0.25 mg/mL *in vitro*. Thus, these results suggest that *S. herbacea* could be effective in controlling hyperlipidemia by inhibition of pancreatic lipase in animal model of type 2 diabetes.

Key Words: Diabetes mellitus, *Salicornia herbacea*, cholesterol, triglyceride, db/db mouse

Introduction

Diabetes mellitus is characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. Cardiovascular disease (CVD) is a major complication and the leading cause of premature death among patients with diabetes (Centers for Disease Control and Prevention, 1999). The primary risk factor for CVD among patients with type 1 diabetes is hyperglycemia, although other risk factors, such as hypertension and dyslipidemia, may occur secondary to uncontrolled hyperglycemia (Bate & Jerums, 2003). In contrast, the pathophysiology of cardiovascular complications of type 2 diabetes involves elements of hyperglycemia, dyslipidemia, hypertension, and obesity. It was also reported that tight control of hyperglycemia and aggressive therapeutic treatment of diabetic dyslipidemia are associated with the reduced risk of CVD in diabetic patients (American Diabetes Association, 2003). Although there have been enormous improvements in medication, strategy to cure diabetes mellitus completely has not been established so far. Therefore, numerous studies have been carried out to evaluate natural products, including plant materials, as alternative treatments for diabetes to be used in addition to conventional treatments (Bailey & Day, 1989).

In line of remedy from natural substances, it was reported that *Salicornia herbacea* L. could exert hypoglycemic and hypolipidemic effects. *S. herbacea* is an annual herb growing in the salt mashes and on the muddy seashores. Ethanol extract of *S. herbacea* was found to prevent the onset of the hyperlipidemia induced by high fat diet in mice (Park *et al*., 2006). *S. herbacea* powder supplementation decreased blood glucose and triglyceride levels in streptozotocin (STZ)-induced diabetic rats (Bang *et al*., 2002; Kim, 2007a). Consumption of water extract of enzyme-treated *S. herbacea* showed hypocholesterolemic effect in rat fed

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high cholesterol diet (Kim *et al*., 2006). *S. herbacea* was also reported to have strong antioxidative effect *in vitro* (Lee *et al*., 2004; Oh *et al*., 2007) and *in vivo* (Bang *et al*., 2002; Jang *et al*., 2007; Kim, 2007b). Since oxidative stress generated by free radical species plays a major role in the progression of diabetes (Kaneto *et al*., 2005), *S. herbacea* could be beneficial for the prevention and treatment of diabetes via its antioxidative effects. However, the beneficial effects of *S. herbacea* on carbohydrate and lipid metabolism in type 2 diabetes were not studied. Therefore, the present study was aimed to investigate the effect of *S. herbacea* on hyperglycemia and hyperlipidemia in animal model of type 2 diabetes mellitus.

Materials and Methods

Reagents

Assay kits for glucose, cholesterol, and triglyceride were purchased from Asan Co (Seoul, South Korea) and a glycated hemoglobin (HbA_{1C}) assay kit from BioSystems (Barcelona, Spain). Cornstarch was acquired from Daesang Co. (Seoul, Korea). Casein, L-cystine, mineral mixture, and vitamin mixture were purchased from ICN Pharmaceuticals Inc. (Costa Mesa, CA, USA) and *tert*-butyl hydroquinone from Fluka Co. (Milwaukee, WI, USA). Sucrose and soybean oil were obtained from Cheiljedang Co. (Seoul, Korea). Acarbose and orlistat were purchased from Bayer Korea (Seoul, Korea) and Roche Korea Co. (Seoul, Korea), respectively. Yeast α-glucosidase, porcine pancreatic lipase, *p*-nitropheny-α-D-glucopyranoside, 4-methylumbelliferyl oleate (4-MU oleate), and all other reagent grade chemicals were purchased from Sigma Chemical Co (St. Louis, MO, USA).

Preparation of the desalted ethanol extract

S. herbacea was obtained from a local market in Busan, Korea. *S. herbacea* was freeze-dried, powdered and extracted with ten volumes of 70% ethanol for 12 h three times at room temperature. The solvent was removed by rotary evaporation at 50℃. The dry extract was redissolved in 200 volumes of 100% ethanol and filtered using filter paper (No. 2, Whatman Inc., Florham Park, NJ, USA). The solvent was removed by rotary evaporation at 50℃. The yield of the desalted ethanol extract of *S. herbacea* was 4.2%. The dry extract was used for *in vivo* study and redissolved in dimethylsulfoxide (DMSO) to be used as a test material for the *in vitro* study.

Animals and experimental design

Three-week-old male C57BL/KsJ db/db mice (n=16) were purchased from the Korea Research Institute of Bioscience and Biotechnology (Daejeon, South Korea). After 1 week of adaptation during which the animals had free access to commercial chow, they were randomly divided into a control and a *S. herbacea* group. The mice in the control group were offered a standard AIN-93G diet, which contained 39.8% cornstarch, 20% casein, 13.2% dextrinized cornstarch, 10% sucrose, 7% soybean oil, 5% Alphacel, 3.5% mineral mixture, 1% vitamin mixture, 0.3% l-cystine, 0.25% choline bitartrate, and 0.0014% *tert*-butyl hydroquinone, whereas the *S. herbacea* group was offered the same diet supplemented with 1% (wt/wt, final concentration) of ethanol extract of *S. herbacea ad libitum* for 6 weeks. The mice were housed individually in plastic cages and located in a room where temperature (23-27℃), humidity (50-60%), and lighting cycle (0600-1800 hr light and 1800-0600 hr dark) were controlled. Body weight and food intake were measured three times a week. At the end of the experimental period, the mice were sacrificed by heart puncture after an overnight fast. Blood HbA_{1C} levels were measured by the chromatographic method using a commercial assay kit. Blood samples were centrifuged at 3000 × g for 15 min; plasma was removed and frozen at -70℃ for further analysis. Plasma glucose, triglyceride, and total cholesterol were measured by enzymatic methods using commercial assay kits. The experiments were performed according to the guidelines of animal experimentation approved by the Animal Resource Center at Inje University, Korea.

Enzyme inhibition assay

Yeast α-glucosidase inhibitory activity was measured by the chromogenic method developed by Watanabe *et al*. (1997) using a microplate reader (model 550, Bio-Rad, Hercules, CA, USA). Yeast α-glucosidase (0.7 U) dissolved in 100 mM phosphate buffer (pH 7.0) containing 2 g/L bovine serum albumin and 0.2 g/L NaN_3 and 5 mM *p*-nitrophenyl-α-D-glucopyranoside in the same buffer (pH 7.0) were used as an enzyme and a substrate solution, respectively. The final concentration of the *S. herbacea* extract and acarbose, a positive control, was 0.5 mg/mL. The measurement was performed in triplicate.

The inhibitory activity against pancreatic lipase was measured by using 4-MU oleate as a substrate (Bitou *et al.,* 1999). Porcine lipase (25 μL) was added to the reaction mixture which consisted of 50 μL of 0.1 mM 4-MU oleate, 20 μL of a McIlvane buffer (0.1 M citrate-Na_2 HPO_4, pH 7.4) and 5 μL of a sample solution. After the final volume was adjusted to 0.1 mL, the reaction mixture was incubated at 37℃ for 10 min. The amount of 4-MU released by the lipase was measured using fluorescence multi-detection reader (Bio-Tek Instruments, Inc., Winooski, VT, USA) at an excitation wavelength of 320 nm and an emission wavelength of 450 nm. The final concentration of the *S. herbacea* extract and orlistat, a positive control, was 0.25 mg/mL. The measurement was performed in triplicate.

Statistical analysis

All values were expressed as mean ± standard deviation (SD). All statistical analyses were performed using SAS (version

Table 1. Body weight, food intake, and feed efficiency ratio of the animals

	Control	*S. herbacea* group
Initial body weight (g)	25.4 ± 2.5	24.4 ± 3.0^{ns}
Final body weight (g)	39.3 ± 1.7	40.0 ± 2.6^{ns}
Body weight gain (g/d)	0.33 ± 0.04	0.37 ± 0.07^{ns}
Food intake (g/d)	4.8 ± 0.4	5.0 ± 0.3^{ns}
Feed efficiency ratio (%)*	7.0 ± 1.0	7.5 ± 1.1^{ns}

Values represent mean ± SD (n=8)
* Feed efficiency ratio (%)=(body weight gain (g)/food intake (g))×100
^{ns}Not significant

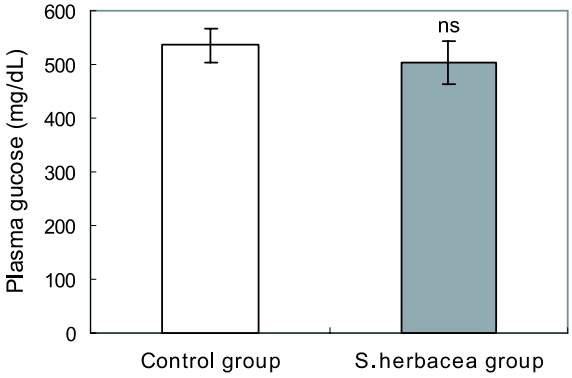

Fig. 1. Effect of *S. herbacea* extract on the levels of fasting blood glucose of db/db mice. Values are mean ± SD. ^{ns}Not significant.

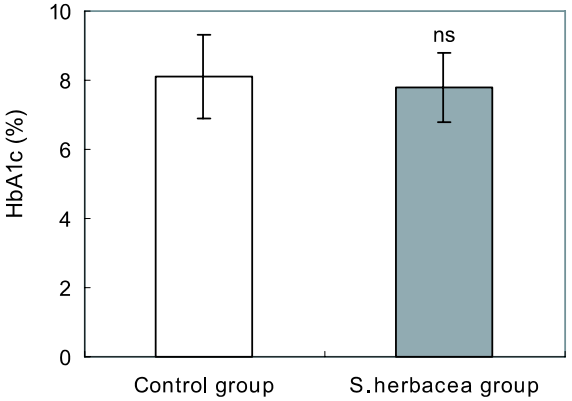

Fig. 2. Effect of *S. herbacea* extract on the levels of glycated hemoglobin (HbA_{1c}) of db/db mice. Values are mean ± SD. ^{ns}Not significant.

8.02). Differences between the control and *S. herbacea* groups were assessed by Student's *t*-test and significance was defined at $p<0.05$.

Results

Body weight and food intake of the mice are shown in Table 1. Chronic consumption of *S. herbacea* extract at the level of 1% of the diet did not significantly influence body weight, food intake and feed efficiency ratio in db/db mice. The effect of *S. herbacea* extract on glycemic control is shown in Fig. 1 and 2. The fasting plasma glucose level of the *S. herbacea* group

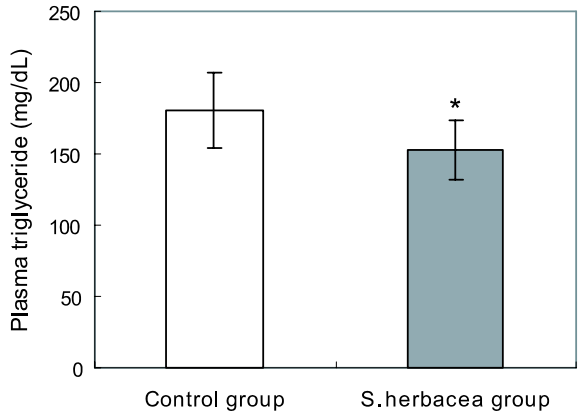

Fig. 3. Effect of *S. herbacea* extract on the levels of plasma triglyceride of db/db mice. Values are mean ± SD. *p<0.05.

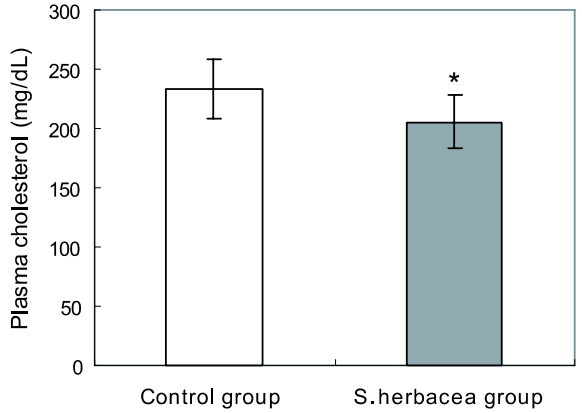

Fig. 4. Effect of *S. herbacea* extract on the levels of plasma cholesterol of db/db mice. Values are mean ± SD. *p<0.05.

Table 2. Inhibitory activities of the ethanol extract of *S. herbacea* on yeast α-glucosidase and porcine pancreatic lipase

	α-Glucosidase inhibitory activities (%)	Pancreatic lipase inhibitory activities (%)
S. herbacea extract	10.2 ± 0.5	56.2 ± 0.4
Acarbose	31.8 ± 0.4	-
Orlistat	-	95.4 ± 0.3

Values represent mean ± SD

(509.7 ± 40.9 mg/dL) tended to be lowered compared with the control group (535.6 ± 35.4 mg/dL, Fig. 1). Blood glycated hemoglobin level of *S. herbacea* group (7.8 ± 1.0%) was not significantly different from the control group (8.1 ± 1.2%, Fig. 2). The effect of *S. herbacea* extract on lipid profile is shown in Fig. 3 and 4. Consumption of *S. herbacea* extract significantly decreased plasma triglyceride (152.7 ± 21.0 mg/dL, Fig. 3) and total cholesterol levels (205.7 ± 22.1 mg/dL, Fig. 4) compared with the control group (180.7 ± 26.7 mg/dL and 233.5 ± 25.2 mg/dL, respectively, $p<0.05$).

The inhibitory activities of the ethanol extract of *S. herbacea* against yeast α-glucosidase and porcine pancreatic lipase are shown in Table 2. The ethanol extract of *S. herbacea* inhibited

yeast α-glucosidase activity by 10.2% at the concentration of 0.5 mg/mL *in vitro*. Acarbose, an α-glucosidase inhibitor, which is used for an oral hypoglycemic agent inhibited the enzyme activity by 31.8%. Inhibitory activities of *S. herbacea* extract against porcine pancreatic lipase were 56.2% at the concentration of 0.25 mg/mL. Orlistat, a pancreatic lipase inhibitor, showed inhibition of 95.4% at the same concentration.

Discussion

S. herbacea has been shown to exert hypoglycemic and hypolipidemic effects in rodents fed a high fat diet or in animal models of type 1 diabetes (Bang *et al*., 2002; Kim *et al*., 2006; Kim, 2007a). However, its hypoglycemic and hypolipidemic effects in animal model of type 2 diabetes have not been reported. Achieving near-normal glycemic control and lowering plasma lipid levels lead to a decrease in the risk of cardiovascular complications of diabetes which is the leading cause of premature death of patients with type 2 diabetes (UKPDS, 1998). In this study we determined antidiabetic effect of *S. herbacea* in db/db mice which exhibit type 2 diabetes and obesity. Consumption of ethanol extract of *S. herbacea* led to a significant decrease in plasma triglyceride and cholesterol. *S. herbacea e*xtract showed strong inhibitory activity against porcine pancreatic lipase *in vitro*. Inhibitory activity of *S. herbacea e*xtract against pancreatic lipase was 59.0% of that of orlistat at the concentration of 0.25 mg/mL *in vitro*. Inhibitor of pancreatic lipase could interfere with digestion of dietary triglycerides and retard absorption of fatty acids (Ballinger & Peikin, 2002). Chronic consumption of orlistat, a potent inhibitor of pancreatic lipase, reduced body weight, plasma triglyceride and cholesterol in obese patients (Lucas *et al*., 2003). In this study, *S. herbacea* with pancreatic lipase inhibitory activity showed triglyceride and cholesterol-lowering effect but did not influence body weight significantly in db/db mice. *Dioscorea nipponica* Makino (Kwon *et al*., 2003) and onion skin extract (Kim, 2007c) with pancreatic lipase inhibitory activity also decreased blood triglyceride and cholesterol in rats fed high fat diet. Orlistat has been reported to show side effects such as diarrhea (Ballinger & Peikin, 2002). Thus natural product with lipase inhibitory activity without side effects such as *S. herbacea* could be useful hypolipidemic agents which could reduce risks for diabetic complications. Plasma glucose and blood glycated hemoglobin levels of *S. herbacea* group tended to be decreased compared with the control group, but the differences between the two groups were not significant. It was reported that *S. herbacea* powder supplementation at the level of 5% of the diet showed hypoglycemic effect in STZ-induced diabetic rats (Bang *et al*., 2002). Kim reported supplementation of *S. herbacea* powder at the level of 20% of the diet significantly decreased blood glucose in STZ-induced diabetic rats and suggested that dietary fiber contained in *S. herbacea* could be responsible for the hypoglycemic effect (2007a). However, consumption of ethanol extract of *S. herbacea* did not affect glycemic control in db/db mice in this study. *S. herbacea e*xtract showed mild inhibitory activity against yeast α-glucosidase *in vitro*. Inhibitory activity of *S. herbacea e*xtract against α-glucosidase was 31.9% of that of acarbose at the concentration of 0.5 mg/mL *in vitro*. It appears that the hypoglycemic effect of *S. herbacea* could be stronger than that of *S. herbacea* extract if dietary fiber is the active component exerting hypoglycemic effect.

In conclusion, ethanol extract of *S. herbacea* reduced plasma triglyceride and cholesterol in animal models of type 2 diabetes and the hypolipidemic effect could be partly due to inhibitory activity against pancreatic lipase.

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