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Salicornia herbacea Prevents High Fat Diet-Induced Hyperglycemia and Hyperlipidemia in ICR Mice

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Salicornia herbacea L. (Chenopodiaceae) has been used as a seasoned vegetable by living in coastal areas. *S. herbacea* (SH) has been demonstrated to stimulate cytokine production, nitric oxide release, and to show anti-oxidative effect. In a series of investigations to develop potential anti-diabetic and/or anti-hyperlipidemic agents from Korean indigenous plants, 50% ethanol extract of *Salicornia herbacea* was found to prevent the onset of the hyperglycemia and hyperlipidemia induced by high fat diet in ICR mice. At 6 week old, the ICR mice were randomly divided into five groups; two control and three treatment groups. The control mice were to receive either a regular diet (RD) or high-fat diet (HFD), and the treatment groups were fed a high fat diet with either 350 mg/kg, 700 mg/kg of SH (SH350 and SH700) or 250 mg/kg of metformin (MT250) for a 10-week period. SH not only reduced body weight but also corrected associated hyperglycemia and hyperlipidemia in a dose dependent manner. SH exerted beneficial effects on the plasma glucose and lipid homeostasis possibly ascribed to its specific effects on lipogenesis related genes (SREBP1a, FAS, GAPT), and PEPCK, glucose 6-phosphatase gene expressions in liver. Ethanol extract of *S. herbacea* has potential as a preventive agent for type 2 diabetes (and possibly hyperlipidemia) and deserves future clinical trial.

Key words: *Salicornia herbacea*, High fat diet, Type 2 diabetes, Lipogenesis related genes, PEPCK, Glucose 6-phosphatase

INTRODUCTION

Type 2 diabetes is characterized by high concentrations of glucose in the blood, which is caused by decreased secretion of insulin from the pancreas and decreased insulin action (Cavaghan *et al*, 2000). This condition is prevalent worldwide and is associated with morbidity and mortality secondary to complications such as myocardial infarction, stroke and end-stage renal disease. The importance of tight control of blood glucose in either preventing or delaying the progression of complications is recognized (The Diabetes Control and Complication Trial Research group, 1993; UK Prospective Diabetes Study Group, 1998). Currently available pharmacological agents for type 2 diabetes have a number of limitations, such as adverse effects and high rates of secondary failure (Inzucchi, 2002). Due to these factors, diabetic patients and healthcare professionals are increasingly considering complimentary and alternative approaches, including the use of medicinal herbs with antihyperglycemic activities (Attele *et al.*, 2002).

In a series of investigations to develop potential anti-diabetic and/or anti-hyperlipidemic agents from Korean indigenous plants, 50% ethanol extract of *Salicornia herbacea* was found to prevent the onset of the hyperglycemia and hyperlipidemia induced by high fat diet in ICR mice. *Salicornia herbacea* L. (*Chenopodiaceae*), an annual succulent shrub growing in salt marshes and on muddy along the western and southern seashores of the Korea peninsula (Kim and Song, 1983; Lee, 1997), has been used as a folk medicine as well as a seasoned vegetable by people living in coastal areas. This plant has previously been shown to stimulate cytokine production, nitric oxide release and expression of surface molecular (Im *et al.*, 2003), and also recently showed an anti-oxidative effect (Han and Kim, 2003). The present study was carried out to examine the anti-hyperglycemic and anti-hyperlipidemic activities as well as mechanisms of *S. herbacea.*

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MATERIALS AND METHODS

Plant material

Salicornia herbacea L. (SH, Chenopodiaceae) was obtained from Korea Herbal Science Institute (Seoul, Korea), and identified and authenticated with the assistance of Prof. Chang S. Yook at the Department of Oriental Pharmaceutical Science, Kyung Hee University. A voucher specimen of SH was deposited at the Medicinal Plants Herbarium of the School of Pharmacy, Kyung Hee University, with registration number 241. The air-dried powdered material of *S. herbacea* (1 kg) was extracted with 50% ethanol under reflux. After removal of the solvent in vacuo, the residue (110 g) was freeze-dried and was stored in a deep freezer until use.

Animals and treatment

Five week-old male ICR mice were purchased from the Orient (Seoul, Korea) and were acclimatized for 1 week before being randomly assigned into the experimental groups. The animals were housed under standard conditions (24±1°C, 55±5% humidity and a 12 h light/dark cycle) and maintained with free access to water and a regular diet. At 6 week-old, the ICR mice were randomly divided into five groups; two control and three treatment groups. The control mice continued to receive either a regular diet (RD) or high-fat diet (HFD), and the treatment groups were fed a high-fat diet with either 350 mg/kg or 700 mg/kg of SH (SH350 and SH700) for a 10-weeks period. As a positive control, metformin was administrated with the high-fat diet at a dose of 250 mg/kg (MT250). Regular and high fat diets were purchased from Dyets Inc (Bethlehem, U.S.A.), and the nutrition contents of high-fat diet were similar to those of the regular diet except low carbohydrate and addition of beef tallow (Table I). Body weights were measured weekly, and at every other week blood was collected for blood glucose analysis. At the end of the study, blood was also collected for determination of plasma insulin and lipid levels. At the time killed, sera, periepididymal fat and liver tissues were collected. Tissues were either fixed in buffered formalin and processed for histology or snap frozen in liquid nitrogen and stored at -80°C until RNA and TG concentrations were determined. The experimental protocols were conducted in accordance with internationally-accepted principles for laboratory animal use and care as found in the US guidelines.

Blood sampling and plasma assay

Blood was withdrawn from the orbital venous plexus every other week, using a heparinized capillary tube without anesthesia. The blood samples were placed on ice, centrifuged, and plasma stored at -80°C until assay. The plasma glucose concentration was determined using the glucose oxidase method (Trinder, 1969). The plasma insulin concentration was measured according to the protocol described by the manufacturer of the mouse insulin ELISA kit (Shibayagi Co., Japan). Plasma triglyceride and total cholesterol were determined using commercially available kits (Asan Phramaceutical Co., Korea), and plasma nonesterified fatty acid (NEFA) concentrations were assayed using a NEFAZYMES-S kit (Eiken, Japan).

Table I. Composition of the diets

	Regular diet (g/kg diet)	High fat diet (g/kg diet)
Ingredient		
Casein	200	200
DL-Methionine	3	3
Corn Starch	150	150
Sucrose	500	150
Cellulose	50	-
Corn oil	50	-
Beef tallow	-	400
Mineral mixture[1]	35	35
Vitamin mixture[1]	10	10
Choline bitartrate	2	2
Energy, kJ/g	0.91	1.30
Protein, % kcal/kg	13.3	13.3
Carbohydrate, % kcal/kg	47.4	19.8
Fat, % kcal/kg	8.0	65.7
Fiber, % kcal/kg	8.0	-
Other	23.3	1.3

[1] AIN 76A Rodent Purified Diet

Determination of hepatic TG

For determination of triglycerides in liver, 50~70 mg of liver from each mouse was homogenized in 4 mL of chloroform-methanol (2:1). 0.8 mL of 50 mM NaCl was added to each homogenate, and the solution was incubated at 4°C for 12 h. The sample was centrifuged for 5 min at 1,300 g. The organic layer was removed and dried using Speed Vac (Savant, U.S.A.). The resulting pellet was dissolved in phosphate buffer saline containing 1% Triton X-100 and then assayed for triglyceride (Cohen *et al.*, 2001).

RNA extraction and RT-PCR

The total RNA from liver tissue was prepared using a guanidine thiocyanate-water saturated phenol/chroloform extraction method and subsequent precipitation with acidic sodium acetate (Chomczynski and Sacchi, 1987). The total RNA in the aqueous phase was precipitated with ice-cold isopropyl alcohol, and isolated RNA was determined by spectrophotometric analysis at 260 nm and 280 nm. 1 μg of total RNA was reverse transcribed into cDNA using the Moloney murine leukemia virus transcriptase and random hexamers as primers. The list of specific primers were : sterol regulatory element binding protein 1

(SREBP1a) sense GCG CTA CCG GTC TTC TAT CA, anti-sense TGC TGC CAA AAG ACA AGG G, accession number AF374266; fatty acid synthase (FAS) sense GAT CCT GGA ACG AGA ACA C, anti-sense AGA CTG TGG AAC ACG GTG GT, accession number X13135; glycerol-3-phosphate acyltransferase (GPAT) sense GGT AGT GGA TAC TCT GTC GTC CA, anti-sense CAT CAG CAA CAT CAT TCG GT, accession number NM_008149; steroyl-CoA desaturase (SCD)-1 sense CGA CGG TTG GTT GTT GAT CTG T, anti-sense ATA GCA CTG TTG GCC CTG GA, accession number NM_009127; glyceraldehyde 3-phosphate dehydrogenase (GAPDH) sense CAA CTT TGG CAT TGT GGA AGG, anti-sense ATG GAA ATT GTG AGG GAG ATG C accession number BC083149. The primers were added at final concentration of 0.5 μM to a 25 μL reaction mixture containing 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$, 0.5 mM each dNTP, 5 μL of cDNA, and 2.5 units of Taq DNA polymerase. The PCR conditions were denaturation at 94°C for 1 min, annealing at 57.5°C for 1 min, and extension at 72°C for 1 min. The RT-PCR products were electrophorosed in 1% agarose gels under 100 V and was stained with 0.5 μg/mL ethidium bromide. GAPDH was amplified as a control gene. The density of the PCR product was measured using a GS-700 imaging densitometer. The level of mRNA was expressed as the ratio of signal intensity for each gene relative to that of GAPDH.

Northern blot analyses of PEPCK and glucose 6-phosphatase

The cRNA probes for PEPCK, glucose 6-phosphatase and cyclophilin were synthesized in vitro from linearized expression vectors which contained SP6 or T7 viral promoter. 1 μg of linearized plasmid was mixed with RNA labelling mixture containing ATP, CTP, GTP, Dig-labeled-UTP, transcription buffer, and SP6 or T7 RNA polymerase. After incubation at 37°C for 2 h, the mixture was co-incubated with DNase I (RNase free) at 37°C for 15 min, precipitated in ethanol containing lithium chloride at -70°C for 30 min, and washed with 70% chilled ethanol. Extracted RNA samples were dissolved in 30~50 μL water and 500 X diluted RNA solution was measured to spectrophotometric analysis at 260 and 280 nm. 10 μg of the total RNA and equal volume of RNA loading buffer, containing 50% glycerol and 1 mM EDTA, were denatured in 65°C for 30 min and subsequently cooled on ice. The denaturated RNA samples were electrophorosed in 1% agarose-formaldehyde gels under 60-80 V and transferred to nylon hybond-N hybridization membrane and cross-linked with UV. After the membranes were pre-hybridized at 68°C for 1 h in prehybridization buffer (5 X SSC, 50% formamide, 0.02% SDS, 0.1% sodium N-lauroyl sarcosine, and 2% blocking reagent), the membranes were incubated overnight at 68°C in prehybridization buffer with the digoxigenin (DIG)-labeled probe, using the hybridization incubator. The membrane was washed twice for 30 min in 2 X washing solution (2 X SSC and 0.1% SDS) at room temperature and washed for 1 h in 0.1 X washing solution. After equilibrating the membrane in Buffer I (100 mM maleic acid, 150 mM NaCl, and pH 7.5) for 5 min, the membrane was gently shaked in Buffer II (1% blocking reagent in Buffer I) for 60 min. The membrane was hybridized with the diluted anti-DIG-alkaline phosphatase (1:10,000, 75 mU/mL) in Buffer II for 30 min. After washing the membrane twice for 20 min per wash in 0.3% Tween 20 (in Buffer I), the membrane was equilibrated in Buffer III (100 mM Tris-HCl, 100 mM NaCl, and 50 mM $MgCl_2$, pH 9.5) for 2 min. CSPD solution was spread over the surface of the membrane. After incubation of the membrane at 37°C for 15-30 min, the membrane was exposed to Hyperfilm-ECL for detection of the chemiluminescent signal. For rehybridization, the membrane was washed for 20 min at room temperature in sterilized water, and shaked for overnight at 65°C in 50 mM Tris-HCl (pH 8.0), 50% dimethylformamide and 1% SDS to remove the hybridized probe. After remove the former probe, the membrane was rehybridized to the DIG-labeled rat cyclophilin cRNA probe to normalize.

Periepididymal fat histology

Wedges of periepididymal fat from representative mice in each group were fixed overnight in buffered formalin, embedded in paraffin, cut into thin section (6 ìm) and mounted on glass slides. Hematoxylin and eosin staining was done to demonstrate general fat morphology.

Statistical analysis

Data are expressed as mean ± SEM, and ANOVA was conducted by using the program STATVIEW (Abacus Concepts, Berkeley, CA). When a significant F ratio was obtained ($P < 0.05$) a post hoc analysis was conducted between groups by using a multiple comparison procedure with a Bonferroni/Dunn correction of means (ANOVA) or a Dunnett's post hoc comparison. P values < 0.05 were considered significant.

RESULTS

Body weight and weight gain

Body weight was determined once a week. The body weight of the mice in the RD group gradually increased during the 10-week period. In contrast, the body weight of animals on the HFD showed a rapid increase. Weight gains in RD and HFD control groups during the 10-week period were 7.7±2.1 g (28% increase over the initial body weight) and 13.4±2.0 g (48% increase over the initial body

weight), respectively (Table II). Subjects fed the HFD and SH showed a gradual increase in body weight, but the increase was significantly less than that measured for the HFD control in spite of continued and prolonged access to the high fat diet. SH700 and MT250 groups prevented the weight gains by 34% and 39%, respectively, relative to the weight gain evident in the HFD control group.

Plasma glucose, insulin and insulin resistance index

Fasting plasma glucose levels were determined every other week, and plasma insulin levels were measured at the end of the treatment. A modest but significant hyperglycemia was developed in the HFD group compared to the RD group for 10-week trial (Table III). SH administered mice, however, showed a mild decrease in plasma glucose levels relative to the vehicle control mice in a dose dependent manner. Plasma insulin levels at 10^{th} week were increased by 1.9 fold in the HFD group compared to the RD group. As a result of increased plasma glucose and insulin levels, homeostatic model assessment values for insulin resistance (HOMA-IR), calculated by insulin (μU/mL) x glucose (mM)/22.5 (Matthews *et al.,* 1985), of the HFD control group was 3.1 times higher than that of the RD group. In spite of feeding HFD, the insulin resistance indices of SH350 and SH700 groups were reduced by 27% and 25%, respectively, when compared to the HFD control group. Although insulin resistance indices of SH treated groups were reduced, they were not statistically significant compared to that of HFD control group, resulting from the relatively high insulin levels in SH treated groups.

Table II. Effect of *S. herbacea* ethanol extract on body weight and weight gain

Group	Body weight (g)		Weight gain (g)
	Initial	Final	
RD	27.2 ± 0.7	34.9 ± 2.2	7.7 ± 2.1
HFD	28.1 ± 1.1	41.5 ± 2.7††	13.4 ± 2.0†††
HFD+SH350	28.1 ± 1.8	41.4 ± 3.1	13.4 ± 1.7
HFD+SH700	28.5 ± 1.5	37.4 ± 1.9*	8.9 ± 1.8**
HFD+MT250	28.1 ± 1.6	36.3 ± 1.9*	8.2 ± 1.2***

Values represent the mean ± SD (n=8). $^{\dagger\dagger}P < 0.01$, $^{\dagger\dagger\dagger}P < 0.001$ vs. RD; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. HFD

Table III. Effect of *S. herbacea* ethanol extract on plasma glucose, plasma insulin, and homeostasis model assessment values for insulin resistance (HOMA-IR)

Group	Plasma glucose (mmol/l)	Insulin (iU/ml)	HOMA-IR
RD	3.4 ± 0.2	40.9 ± 5.6	6.2 ± 0.6
HFD	5.7 ± 0.2†††	76.2 ± 8.4††	19.3 ± 2.2†††
HFD+SH350	5.5 ± 1.2	57.7 ± 13.5	14.1 ± 4.0
HFD+SH700	4.6 ± 0.4*	75.0 ± 25.2	14.5 ± 5.0
HFD+MT250	3.5 ± 0.4***	23.6 ± 2.3***	4.0 ± 0.6***

Values represent the mean ± SE (n=8). Homeostasis Model Assessment was used to calculate an index of insulin resistance as insulin (iU/ml) x glucose (mM)/22.5. $^{\dagger\dagger}P < 0.01$, $^{\dagger\dagger\dagger}P < 0.001$ vs. RD; $^{*}P < 0.05$, $^{***}P < 0.001$ vs. HFD

Plasma lipid levels

The effects of SH on plasma lipid levels were examined at the end of the treatment (Table IV). The plasma lipid levels in HFD fed mice were significantly increased compared to the levels in RD fed mice. In the HFD control group, plasma non-esterified fatty acid (NEFA) was increased by 1.2-fold (1558.8 to 1790.1 mg/dl), triglyceride (TG) increased by 1.4-fold (112.2 to 157.4 mg/dl), total cholesterol (TC) increased by 1.5-fold (151.0 to 222.8 mg/dl) and LDL cholesterol (LDL-C) increased by 2.3-fold (43.8 to 101.9 mg/d) compared to those in the RD group. SH350 and SH700 groups showed a marked reduction of the NEFA, TG, TC, and LDL-C levels in a dose dependent fashion (29%, 33%, 27%, and 69% inhibition in the SH700 group). Metformin also remarkably improved high fat induced hyperlipidemia, and lipid levels in MT250 group were comparable to those in SH700 group except NEFA.

Hepatic triglycerides

The increase in the intracellular deposition of TG in muscles, liver and pancreas in subjects prone to diabetes is well documented and demonstrated to attenuate glucose metabolism by interfering with insulin signaling and insulin secretion. To determine an ectopic accumulation in liver, the hepatic TG levels were compared between groups (Fig. 1). The hepatic triglyceride concentration of the HFD group was dramatically increased by 3.0 fold relative to the RD group (113.8 ± 10.5 vs. 37.8 ± 4.3 mg/g wet tissue). SH350 and SH700 groups, however, showed 72% and 83% decrease in hepatic triglycerides compared to the

Table IV. Effect of *S. herbacea* ethanol extract on plasma lipid levels

Group	NEFA (mg/dl)	Triglyceride (mg/dl)	Total Cholesterol (mg/dl)	LDL Cholesterol (mg/dl)
RD	1558.8± 83.7	112.2±8.2	151.0± 9.6	43.8± 7.7
HFD	1790.1± 57.9†	157.4±1.5†††	222.8± 6.4†††	101.9±17.0††
HFD+SH350	1274.8±146.3**	105.7±3.5**	189.8±12.1*	50.0± 6.4*
HFD+SH700	1006.3± 73.3***	132.4±9.2*	163.5±10.5***	32.0±16.1**
HFD+MT250	1669.4± 74.6	136.3±5.3**	173.5± 6.1***	38.0± 6.1**

Values represent the mean ± SE (n=8). LDL-cholesterol (mg/dl) = Total cholesterol – HDL cholesterol – triglyceride/5. $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$, $^{\dagger\dagger\dagger}P < 0.001$ vs. RD; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. HFD.

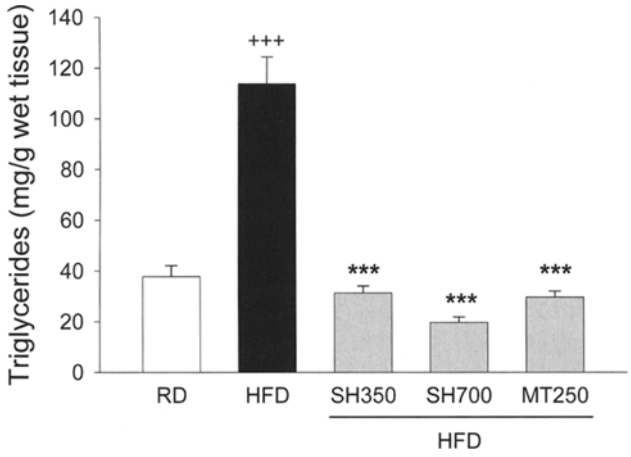

Fig. 1. Effect of SH and metformin on hepatic triglycerides. Values represent the mean ± SE (n=5). $^{\dagger\dagger\dagger}P < 0.001$ vs. RD; $^{***}P < 0.001$ vs. HFD

HFD control mice in a dose dependent manner. The hepatic triglyceride concentration of metformin treated group was reduced to the level similar to the SH350 group.

Lipogenic genes in liver

To elucidate the molecular mechanism by which SH induces physiological changes, we probed for differential changes in gene expressions involved in hepatic lipogenesis by RT-PCR. In the HFD fed group, mRNA expressions of SREBP1a and its target enzymes such as FAS, GPAT and SCD-1 were all significantly enhanced compared to those in RD fed group (Fig. 2). SH700 decreased the mRNA expression of SREBP1a by 64% ($p < 0.01$). SH subsequently suppressed FAS and GPAT mRNA levels (SCD-1 mRNA expression tended to reduce although not to the same degree as those seen in other hepatic lipogenic enzymes). On the other hand, SH had no significant effect on the level of genes involved in fatty acid oxidation, i.e., carnitine palmitoyltransferase (CPT-1a) and acylCoA oxidase (ACOx), and on mRNA level of the fatty acid transporter CD36 (data not shown).

PEPCK and G6Pase expressions in hepatocyte

The quantitative analysis of phosphoenolpyruvate carboxykinase (PEPCK) and glucose 6-phosphatase (G6Pase),

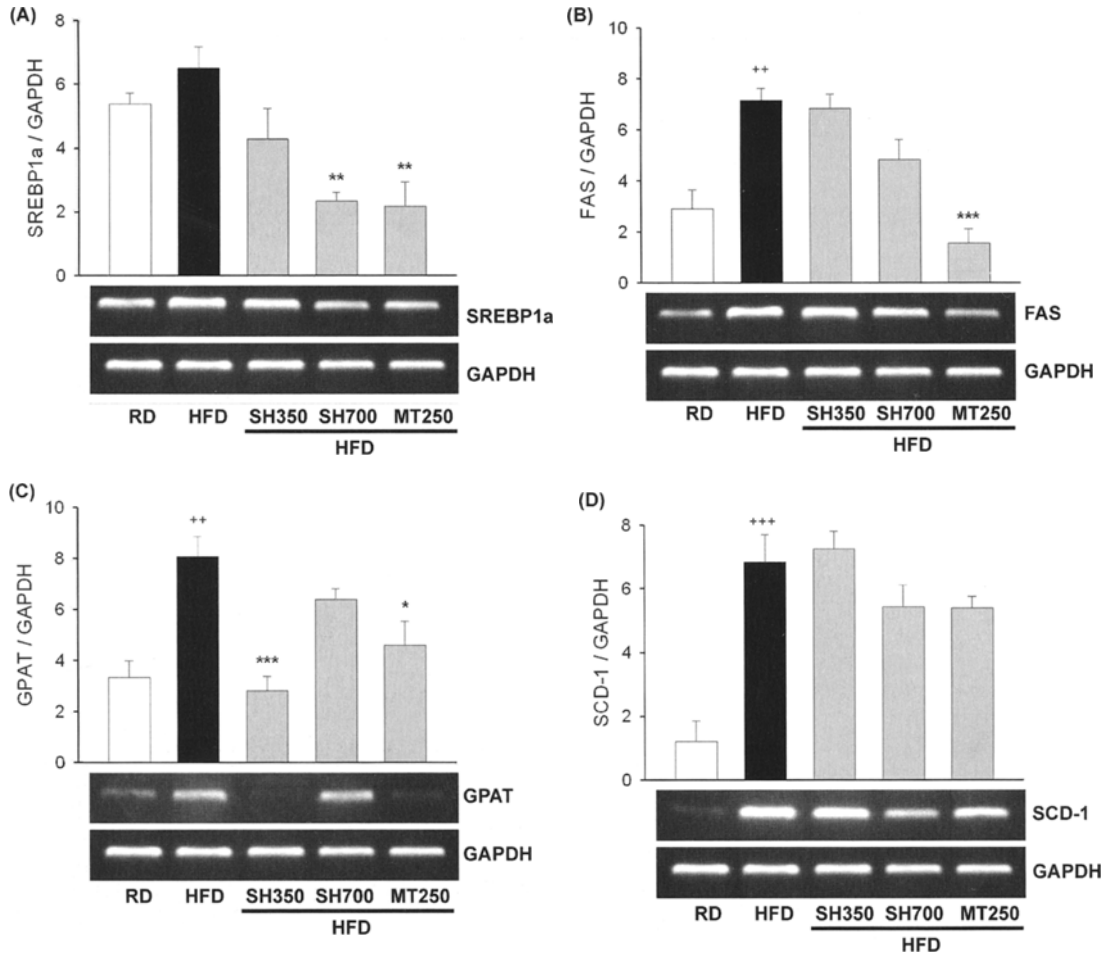

Fig. 2. Effect of SH and metformin on either SREBP1a A), FAS (B), GPAT (C) or SCD-1 (D) mRNA expression in HFD fed mice liver. $^{\dagger\dagger}P < 0.01$ vs. RD; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. HFD.

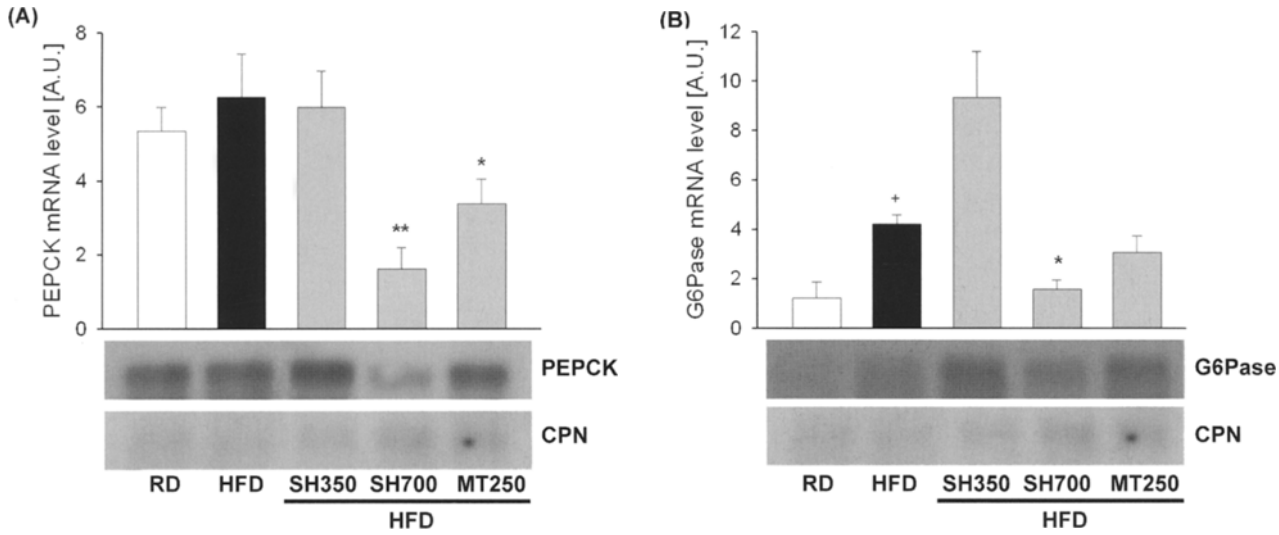

Fig. 3. Effect of SH and metformin on PEPCK (A) and G6Pase (B) mRNA expressions in hepatocytes. Values represent the mean ± S (n=4). †P < 0.05 vs. RD; * P <0.05, ** P <0.01 vs. HFD

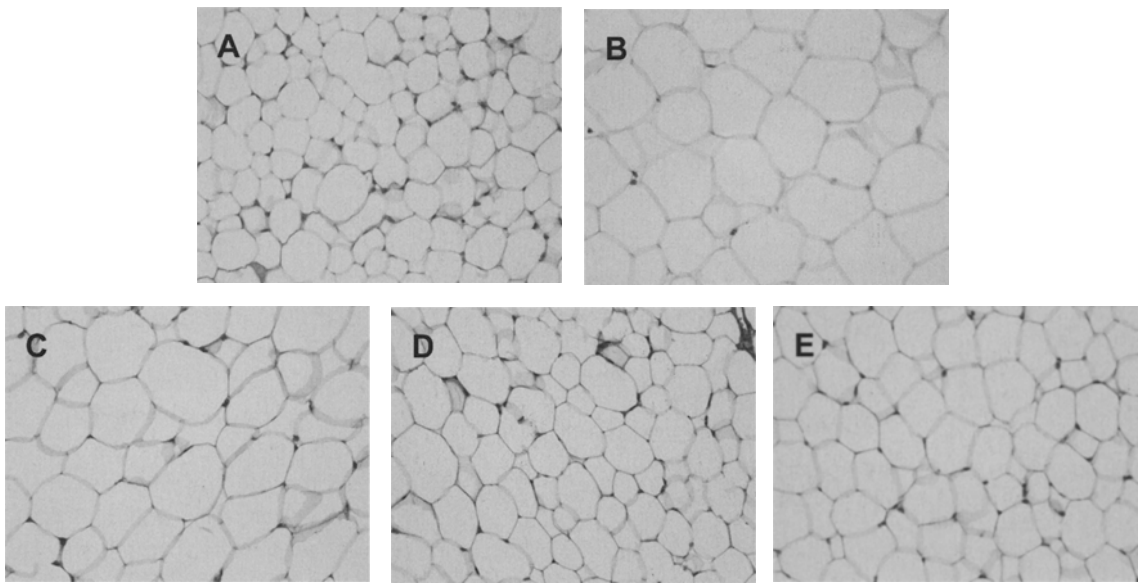

Fig. 4. Morphology of periepididymal fat pad. Hematoxylin and eosin-stained paraffin sections of liver from RD (A), HFD (B), EA350 (C), EA700 (D), and MT250 (E). Magnification x400

which are rate-limiting enzymes primarily regulated by insulin in hepatic gluconeogenic pathway (Mithieux, 1997; Davies *et al.,* 2001), was performed using Northern blotting. mRNA level of G6Pase in the HFD control group was increased by 3.8-fold compared to that of the RD control group, but there was no significant difference between the RD and HFD group for PEPCK mRNA level (Fig. 3). On the other hand, the mRNA expressions of PEPCK and G6Pase in SH700 group were significantly decreased by 75% and 62% compared to the HFD control mice, respectively. PEPCK mRNA level in MT250 group was also reduced by 46% compared to the HFD control mice.

Fat mass and morphology of periepididymal fat pad

The body weight of HFD fed mice was increased by 19% compared to the RD fed mice (Table II). Notably, there was 3.1 fold increase in the mass of periepididymal fat pad of the HFD group, compared to the RD group (Table V). Histological analysis of periepididymal fat pad further confirmed this result and indicated that the increases of area and diameter of periepididymal fat pad in the HFD (3.8-fold and 2.0-fold) result mainly from accumulation of lipids (Fig. 4). On the other hand, the area and diameter of periepididymal fat pad were decreased by 65% and 45% in the SH700 group, respectively,

Table V. Effect of *S. herbacea* ethanol extract on area and diameter of periepidydimal fat pad

Group	Mass (g)	Area (μm^2)	Diameter (μm)
RD	0.67 ± 0.09	1181.3 ± 73.9	42.7 ± 1.4
HFD	2.08 ± 0.13†††	4521.8 ± 80.0†††	83.7 ± 1.3†††
HFD+SH350	1.70 ± 0.12**	3169.8 ± 374.0**	62.5 ± 2.4***
HFD+SH700	1.04 ± 0.13***	1591.9 ± 130.8***	46.2 ± 1.1***
HFD+MT250	1.16 ± 0.21***	2328.6 ± 91.5***	52.9 ± 1.3***

Values represent the mean ± SD (n=8). $^{\dagger\dagger\dagger}P < 0.001$ vs. RD; $^{**}P < 0.01$, $^{***}P < 0.001$ vs. HFD

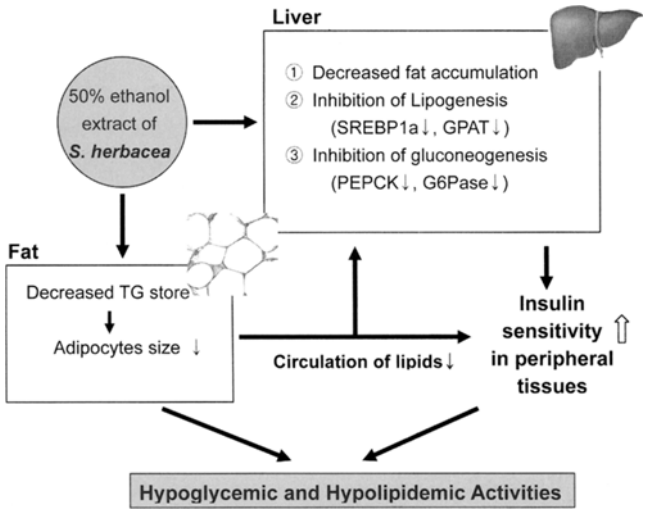

Fig. 5. Plausible hypoglycemic and hypolipidemic mechanisms of SH ethanol extract

compared to those in HFD control group.

DISCUSSION

It is energy intake that matters in relation to the development of overweight, and energy intake is often high when high fat diet (HFD) are consumed in large amounts (Astrup, 2001). The obesity in rodent mice is developed by feeding HFD, and the obese mice have the characters of hyperglycemia, insulin resistance and hepatic steatosis (Surwit *et al.*, 1988; Thupari *et al.*, 2004; Yun *et al.*, 2004). HFD-induced hyperglycemia in ICR mice could rely upon the development of obesity that follows protracted access to the HFD. Although many kinds of animal model, like a *Lep*^{ob}, *Lepr*^{db} and ZDF, are used to develop the new drugs for T2DM, most of all show serious obesity and hyperglycemia, and are suitable to investigation for treating established diabetes (Sone *et al.*, 2001). On the other hand, HFD-induced animal models show mild obesity and hyperglycemia, and are appropriate to develop the preventive agent for T2DM.

As a result of feeding HFD to ICR mice for 10-week, HFD control mice could develop a prediabetic state associated with overweight, mild hyperglycemia, hyperinsulinemia, insulin resistance and dyslipidemia. However, SH treated mice inhibited the subsequent development of obesity and hyperglycemia in spite of continued access to the HFD. SH significantly improved the homeostasis of plasma glucose and lipid in HFD-induced hyperglycemic and hyperlipidemic mice. Patients with T2DM exhibit a marked reduction in insulin-mediated glucose disposal. Therefore, most drug treatments focus on stimulation of insulin secretion and enhancement of insulin sensitivity (Inzucchi, 2002). SH was able to increase insulin sensitivity by decreasing blood glucose and insulin levels at a fasting state. Enhanced insulin sensitivity increased the ability of insulin to inhibit the hepatic gluconeogenic pathway (suppressed mRNA expressions of PEPCK and G6Pase as shown in Fig. 3, is closely associated with reduction of hepatic glucose production), and was helpful to maintain glucose homeostasis and clear the fasting hyperglycemia.

A high fat intake and increased circulation of FFA might lead to insulin resistance, and ultimately to diabetes mellitus in genetically prone subjects through the mechanism of lipotoxicity (Lebovitz, 2004; Manco *et al.*, 2004). On the way to prevent or treat type 2 diabetes, one of the target organs should be fat depots. Reducing visceral fat on the one hand, or increasing peripheral fat on the other hand, will prevent deposition of fat or direct fat out of muscle, liver and pancreas, improving insulin sensitivity and preventing beta cell dysfunction and apoptosis. As our results were shown in Table IV and Fig. 1, plasma lipid levels and hepatic TG concentration were significantly reduced by SH administration, and these effects ameliorated insulin resistance. To further explore the mechanisms of SH, we examined the mRNA expressions of hepatic genes engaged in lipogenesis and lipolysis. Through suppressing of genes involved in hepatic lipogenesis, such as SREBP1a, FAS and GPAT, SH treated mice improved insulin resistance by mitigation of lipotoxicity in liver (Fig. 2). Lin *et al.* demonstrated that metformin also reduced hepatic FAS expression in ob mice (Lin *et al.*, 2000). Markedly reduced expression of GPAT, which catalyzes the first step in triacylglyceride synthesis, was able to decrease accumulation of fat in non-adipose tissue such as the liver. Increased TG store and lipolysis in adipocytes were caused by hyperinsulinemia (Manco *et al.*, 2004). Because the repressed plasma insulin levels via improvement of insulin resistance by SH treatment decreased TG store, and the area and diameter of adipocytes were thus consequently reduced.

Overweight and obesity are the result of excessive adipogenesis. Therapeutically and historically, antidiabetic drugs have focused on hyperglycemia, while largely ignoring the overweight or obesity problem. Even worse,

most of the antidiabtic agents promote weight gain, i.e., adipogenesis, while reducing blood glucose (Moller, 2001). In comparisons, reducing hyperglycemia, hyperlipidemia, and hyperinsulinemia without increasing adiposity or with a reduction of body weight would constitute a much preferred treatment alternative. As shown in Table II, IV and Fig. 4, SH was able to prevent the weight gain and adipocyte hypertrophy as well as to ameliorate the associated hyperglycemia. SH exerted beneficial effects on glucose and lipid homeostasis in diabetes that are not secondary to its ability to decrease food intake but its specific effects on hepatic lipogenesis related genes and PEPCK, G6Pase gene expressions, as proposed in Fig. 5. Recently, Lee *et al.* demonstrated that isorhamnetin-3-*O*-β-D-glucoside, isolated from ethylacetate fraction of *Salicornia herbacea*, inhibits rat lens aldose reductase and sorbitol accumulation in streptozotocin-induced diabetic rats (Lee *et al.,* 2005). Although this report may be the first demonstrating for SH (isorhamnetin-3-*O*-β-D-glucoside) as a potential drug for the treatment and/or prevention of diabetes and its complications, diabetic animal model they used is the streptozotocin-induced chemical model, which is not appropriate for type 2 diabetes, and their study was largely focused on diabetic complications. Therefore, our study is the first to demonstrate that 50% ethanol fraction of SH showed two noble activities (inhibiting weight gain and hyperglycemia) and makes SH ideally suited as a prototype material to develop noble pharmaceuticals treating symptoms such as hyperglycemia, hypertriglycemia without concomitant weight gain. A drug with such a combination of properties should be a much better option for up to 90% of Type 2 diabetic patients who have diabetes-associated weight problems.

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REFERENCES

Astrup, A., Healthy lifestyles in Europe: prevention of obesity and type II diabetes by diet and physical activity. *Public Health Nutr.,* 4, 499-515 (2001).

Attele, A. S., Wu, J. A., and Yuan, C. S., Multiple pharmacological effects of ginseng. *Biochem. Pharmacol.,* 58, 1685-1693 (1999).

Cavaghan, M. K., Ehrmann, D. A., and Polonsky, K. S., Interactions between insulin resistance and insulin secretion in the development of glucose intolerance. *J. Clin. Invest.,* 106, 329-333 (2000).

Chomczynski, P. and Sacchi, N., Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.,* 162, 156-159 (1987).

Cohen, P., Zhao, C., Cai, X., Montez, J. M., Rohani, S. C., Feinstein, P., Mombaerts, P., and Friedman, J. M., Selective deletion of leptin receptor in neurons leads to obesity. *J. Clin. Invest.,* 108, 1113-1121 (2001).

Davies, G. F., Khandelwal, R. L., Wu, L., Juurlink, B. H., and Roesler, W. J., Inhibition of phosphoenolpyruvate carboxykinase (PEPCK) gene expression by troglitazone: a peroxisome proliferator-activated receptor-gamma (PPARgamma)-independent, antioxidant-related mechanism. *Biochem. Pharmacol.,* 62, 1071-1079 (2001).

Han, S. K. and Kim, S. M., Antioxidative effect of Salicornia herbacea L. grown in closed sea beach. *J. Korean Food Sci. Nutr.,* 32, 207-210 (2003).

Im, S. A., Kim, G. W., and Lee, C. K., Immunomodulatory activity of *Salicornia herbaceae* L. components. *Nat. Prod. Sci.,* 9, 273-277 (2003).

Inzucchi, S. E., Oral antihyperglycemic therapy for type 2 diabetes: scientific review. *J. Am. Med. Assoc.,* 287, 360-372 (2002).

Kim, C. S. and Song, T. G., Ecological studies on the halophyte communities at western and southern coasts in Korea.. *Kor. J. Eco.,* 6, 167-176 (1983).

Lee, Y. N., Flora of Korea. Kyohak Publishing Co., Seoul, pp.144 (1997).

Lee, Y. S., Lee, S., Lee, H. S., Kim, B.-K., and Ohuchi, K., Inhibitory effects of 3-*O*-β-D-glucoside from Salicornia herbecea on rat lens aldose reductase and sorbitol accumulation in streptozotocin-induced diabetic rat tissues. *Biol. Pharm. Bull.,* 28(5), 916-918 (2005).

Lin, H. Z., Yang, S. Q., Kujhada, F., Ronnet, G., and Diehl, A. M., Metformin reverses nonalcoholic fatty liver disease in obese leptin-deficient mice. *Nature Medicine* 6, 998-1003 (2000).

Manco, M., Calvani, M., and Mingrone, G., Effects of dietary fatty acids on insulin sensitivity and secretion. *Diabetes Obes. Metab.,* 6, 402-413 (2004).

Matthews, D. R., Hosker, J. P., Rudenky, A. S., Naylor, B. A., Treacher, D. F., and Turner, R. C., Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentration in man. *Diabetologia* 28, 412-419 (1985).

Mithieux, G., New knowledge regarding glucose-6-phosphatase gene and protein and their roles in the regulation of glucose metabolism. *Eur. J. Endocrinol.*, 136, 137-145 (1997).

Moller, D. E., New drug targets for type 2 diabetes and the metabolic syndrome. *Nature (Lond.)*, 414, 821-827 (2001).

Sone, H., Suzuki, H., Takahashi, A., and Yamada, N., Disease model: hyperinsulinemia and insulin resistance. Part A-targeted disruption of insulin signaling or glucose transport. *Trends Mol. Med.,* 7, 320-322 (2001).

Surwit, R. S., Kuhn, C. M., Cochrane, C., McCubbin, J. A., and Feinglos, M. N., Diet-induced type II diabetes in C57BL/6J mice. *Diabetes,* 37, 1163-1167 (1988).

The Diabetes Control and Complication Trial Research Group. The effect of intensive treatment of diabetes in the development and progression of long term complications in insulin-dependent diabetes mellitus. *New Engl. J. Med.,* 329, 977-986 (1993).

Thuresson, E. R., Inhibition of glycerol-3-phosphate acyltransferase as a potential treatment for insulin resistance and type 2 diabetes. *Curr. Opin. Invest. Drugs* 5, 411-418 (2004).

Trinder, P., Determination of blood glucose using an oxidase-peroxidase system with a non-carcinogenic chromogen. *J. Clin. Pathol.,* 22, 158-161 (1969).

Thupari, J. N., Kim, E. K., Moran, T. H., Ronnett, G. V., and Kuhajda, F. P., Chronic C75 treatment of diet-induced obese mice increases fat oxidation and reduces food intake to reduce adipose mass. *Am. J. Physiol-Endoc M.,* 287, 97-104 (2004).

UK prospective Diabetes Study Group (UKPDS), Intensive blood glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPSD 33). *Lancet,* 352, 837-853 (1998).

Yun, S. N., Moon, S. J., Ko, S. K., Im, B. O., and Chung, S. H., Wild ginseng prevents the onset of high-fat diet induced hyperglycemia and obesity in ICR mice. *Arch. Pharm. Res.,* 27, 790-796 (2004).

Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L., and Friedman, J. M., Positional cloning of the mouse obese gene and its human homologue. *Nature*, 372, 425-432 (1994).