Anti-Obesity Properties of a Sasa quelpaertensis Extract in High-Fat Diet-Induced Obese Mice

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This study explores the anti-obesity properties of a Sasa quelpaertensis leaf extract (SQE) in high-fat diet (HFD)-induced obese C57BL/6 mice and mature 3T3-L1 adipocytes. SQE administration with HFD for 70 d significantly decreased the body weight gain, adipose tissue weight, and serum total cholesterol and triglyceride levels in comparison with the HFD group. SQE administration also reduced the serum levels of glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, lactate dehydrogenase, and the accumulation of lipid droplets in the liver, suggesting a protective effect against HFD-induced hepatic steatosis. SQE administration restored the HFD-induced decreases with phosphorylation of AMP-activated protein kinase (AMPK) and acetyl-CoA carboxylase (ACC) in epididymal adipose tissue. SQE also induced AMPK phosphorylation in mature 3T3-L1 adipocytes. These results suggest that SQE exerted an anti-obesity effect on HFD-induced obese mice by activating AMPK in adipose tissue and reducing lipid droplet accumulation in the liver.

Key words: Sasa quelpaertensis; high-fat diet; AMP-activated protein kinase; anti-obesity; 3T3-L1 adipocytes

Obesity, as a major risk factor for many chronic diseases, including hypertension, hyperlipidemia, cardiovascular disease, and type 2 diabetes,3) is a major obstacle in efforts to improve human health and the quality of life. Obesity is characterized by excessive fat deposition associated with morphological and functional changes in adipocytes.4) Studies of adipose tissue biology have led to an improved understanding of the mechanisms that link metabolic disorders with altered adipocyte functions.5) Lipid accumulation is caused by both adipose tissue hypertrophy and adipose tissue hyperplasia.4) Although the molecular basis for these associations remains unclear, experimental evidence suggests that some metabolic disorders might be treatable or preventable by inhibiting adipogenesis and modulating the adipocyte function.6)

High-fat feeding has commonly been used to induce visceral obesity in rodent animal models,7,8) because the pathogenesis of obesity is similar to that found in humans.7) 3T3-L1 preadipocytes are frequently used to study the function of adipocytes in vitro due to their ability to differentiate into mature adipocytes.9) The drugs currently available for treating obesity have undesirable side effects, and the high demand for a safe but therapeutically potent anti-obesity drug has therefore increased interest in the search for anti-obesity phytonutrients that effectively reduce the visceral fat mass.9,10)

The genus Sasa (Poaceae) comprises perennial plants commonly known as bamboo grasses, and various Sasa species are widely distributed in Asian countries, including Korea, Japan, China, and Russia.11) Sasa leaves have been used in traditional medicine due to their anti-inflammatory, antipyretic, and diuretic properties.12) They have also been used in a clinical setting to treat hypertension, cardiovascular disease, and cancer.13) Many recent studies on Sasa species leaves have described such beneficial effects on health as anti-inflammation, anti-cancer, and improved insulin resistance.14–16)

Sasa quelpaertensis Nakai is a bamboo grass native to Korea that grows only on Mt. Halla on Jeju Island in South Korea. Young leaves of S. quelpaertensis are used in a popular bamboo tea, but their beneficial health effects on lipid metabolism have not been fully evaluated. We evaluated in this study the anti-obesity potential of a S. quelpaertensis leaf extract (SQE) in mice fed with a high-fat diet (HFD) and in murine 3T3-L1 adipocytes.

Materials and Methods

Reagents. Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), and penicillin-streptomycin were obtained from Gibco (Grand Island, NY, USA). Phosphate-buffered saline (PBS, pH 7.4), 3-isobutyl-1-methylxanthine (IBMX), dexamethasone, insulin, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). The lactate dehydrogenase (LDH) cytotoxicity detection kit was...
performed at 30°C. The major polyphenols contained in SQE were analyzed by LC-MS/MS. Leaves of S. quelpaertensis were collected from Mt. Halla on Jeju Island in South Korea. One kilogram of dried leaves was mixed with water (13L) and incubated for 30 min. The mixture was incubated at 90°C for 4 h on a platform shaker. The extract (SQE) was filtered and concentrated on a rotary evaporator under reduced pressure, and then freeze-dried to a powder. The yield of SQE from dried leaves was approximately 8.5%. SQE was stored at −20°C until needed. The phytochemicals contained in SQE were analyzed by high-performance liquid chromatography (HPLC), using a 2695 Alliance system (Waters Corp., Milford, MA, USA) equipped with a system controller, auto-injector, column oven, and Waters 2998 photodiode array detector. A qualitative analysis of SQE was performed at 30°C with a Sunfire RP 18 column (250 × 4.6 mm ID; 5 μm), using a mobile phase consisting of acetonitrile (A) and water containing 10 μM acetic acid (B). The gradient elution program was as follows: a 52-min gradient was started using 15% A, linearly increased to 100% A over 40 min, held for 5 min, then finally returned to the initial conditions for 5 min with a flow rate at 0.8 mL/min, the eluate being monitored at 200–600 nm.

Animals. The animal study protocol was approved by the Institutional Animal Care and Use Committee of Jeju National University. After their purchase, 4-week-old male C57BL/6 mice (Nara Biotech Co., Seoul, Korea) were adapted for 1 week to specific temperature conditions: a 52-min gradient was started using 15% A, linearly increased to 100% A over 40 min, held for 5 min, then finally returned to the initial conditions for 5 min with a flow rate at 0.8 mL/min, the eluate being monitored at 200–600 nm.

Measurement of body weight, food intake, epididymal adipose tissue weight, and perirenal adipose tissue weight. The mice were sacrificed by an ether inhalation method. The epididymal adipose tissue and perirenal adipose tissue were weighed after being anesthetized with diethyl ether after an overnight fast. The epididymal adipose tissue weight, and perirenal adipose tissue weight.

Cell culture and differentiation. 3T3-L1 adipocytes obtained from the American Type Culture Collection (Rockville, MD, USA) were cultured in DMEM containing 100 unit/mL of penicillin, 100 μg/mL of streptomycin (PS) and 10% bovine calf serum (Gibco) at 37°C in a 5% CO2 atmosphere. To induce differentiation, 2-d post-confluent adipocytes (designated day 0) were cultured for 2 d in an MDI differentiation medium (DMEM containing PS, 10% FBS, 0.5 mM IBMX, 1 μM dexamethasone, and 5 μg/mL of insulin). The cells were then cultured for another 2 d in DMEM containing PS, 10% FBS, and 5 μg/mL of insulin, before being maintained in a post-differentiation medium (DMEM containing PS and 10% FBS) which was replaced every 2 d.

The effect of SQE on the cell viability and cytotoxicity was determined by MTT and LDH assays. Mature 3T3-L1 adipocytes were cultured for 24 h in DMEM containing PS, 10% FBS, and SQE. MTT (400 μg/mL) was added to each culture well at 0.1% CMC. The liquid on the plate was removed, and dimethyl sulfoxide was added to dissolve the MTT-formazan complex. The optical density was then measured at 540 nm. The effect of SQE on the cell viability was evaluated by comparing the relative absorbance with that of a control culture. The cytotoxic effect of SQE was measured by using an LDH cytotoxicity detection kit. The LDH activities in the medium and cell lysate were measured to evaluate cytotoxicity according to the kit manufacturer’s protocol (LDH released into the medium/maximal LDH release × 100).

RNA preparation and quantitative real-time reverse-transcription polymerase chain reaction (real-time RT-PCR) analyses. Total RNA was extracted from the adipose tissue and 3T3-L1 adipocytes by using the TRIzol reagent according to the manufacturer’s instructions, and then treated with DNase (Wako Pure Chemical Industries, Osaka, Japan). cDNA was synthesized from 1 μg of total RNA in a 20-μL reaction by using a Maxima RT PreMix kit (Intron Biotechnology, Seongnam, Kyunggi, Korea). The following primers were used for the real-time RT-PCR analyses: adiponectin, 5'-GAC CTG GCC ACT TTC TCC TC-3' and 5'-GTC ATC TTC GGC ATG ACT GG-3'; carnitine palmitoyltransferase-1a (CPT-1a), 5'-GTC ATC TTC GGC ATG ACT GG-3'; 3T3-L1 CPT-1a; 5'-GTC ATC TTC GGC ATG ACT GG-3'; 3T3-L1 CPT-1a; 5'-GTC ATC TTC GGC ATG ACT GG-3'; and 5'-ACC CAA GGA AGG CGT GA-3'. Samples were prepared by using iQ SYBR Green Supermix (Bio-Rad Laboratories) according to the manufacturer’s instructions. The expression of adiponectin, CPT-1a, and β-actin mRNAs was measured by quantitative real-time RT-PCR with the Chromo4 real-time PCR system (Bio-Rad Laboratories). The formation of a single product was verified by a melting curve analysis. The expression levels of adiponectin and CPT-1a were normalized to that of β-actin, all data being analyzed by using Opticon Monitor ver. 3.1 software (Bio-Rad Laboratories).

Statistical analyses. Each value is expressed as the mean ± standard deviation (SD) or standard error (SE). A one-way analysis of variance

produced and encapsulated using a Micropack (BPC Biosed, Rome, Italy). Treatment with the Kuadro automatic blood analyzer (Asanpharm, Seoul, Korea) and a Kuadro automatic blood analyzer (Beverly, MA, USA). All other reagents were purchased from Sigma Chemical Co. unless otherwise noted.
ANOVA was used for multiple comparisons. The treatment effects were analyzed by a paired t-test or Tukey’s test with SPSS ver. 12.0 software (SPSS, Chicago, IL, USA). Differences are considered statistically significant at \( p < 0.05 \).

Results

HPLC analysis of SQE

Four compounds (\( p \)-coumaric acid, tricin, chlorogenic acid, and iso-orientin), which were already known to be present in Sasa species leaves, were analyzed by HPLC. Among these compounds, two were detected in SQE as shown in Fig. 1 (350 nm). The retention times were 11.103 min (\( p \)-coumaric acid) and 18.396 min (tricin). The respective contents of \( p \)-coumaric acid and tricin in SQE were 23.706 mg/g (SD/C6 1:776) and 0.028 mg/g (SD ± 0.017). \( p \)-Coumaric acid was therefore confirmed as the major compound in SQE (Fig. 1).

SQE improved the high-fat diet-induced obesity

After 70 d feeding with HFD, the mean body weight gain in the HFD group was more than 176.1% higher than that in the ND group, indicating that the HFD induced obesity (Fig. 2 and Table 1). SQE administration (150 mg/kg/d) significantly decreased the body weight gain in the HFD+SQE group (31.0% lower) relative to that in the non-SQE-treated HFD group. The weights of the epididymal and perirenal adipose tissue were also significantly higher in the HFD group (134.9% and 146.0%, respectively) than in the ND group. However, the epididymal and perirenal adipose tissue weights were significantly lower in the HFD+SQE group (29.7% and 21.1%, respectively) than in the HFD group. A histological analysis of the epididymal adipose tissue confirmed that the adipocyte size was markedly higher in the HFD group than in the ND group after 70 d, whereas the adipocyte size was markedly less in the HFD+SQE group than in the HFD group (Fig. 3). The food intake did not significantly differ among the HFD groups. However, the serum levels of T-CHO and TG were significantly lower in the HFD+SQE group (11.55 and 39.7%, respectively) than in the HFD group (Table 2).

SQE reduced damage to the liver in the high-fat diet-induced obese mice

We next examined the effect of SQE on the levels of serum GPT, GOT, and LDH in the HFD mice. SQE administration significantly reduced the levels of these markers of cell damage. The levels of serum GPT and GOT were significantly lower in the HFD+SQE group (52.1% and 23.4%, respectively) than in the HFD group (Table 2), and the serum LDH level was also significantly lower in the HFD+SQE group (42.3%) than in the HFD group (Table 2). Figure 3 presents photomicrographs of liver-tissue samples stained with hematoxylin and eosin. The hematoxylin and eosin analysis of the liver revealed the accumulation of lipid droplets in the HFD group when compared with the ND group; however, no accumulation of lipid droplets was apparent in the liver of the HFD+SQE group.

SQE restored AMPK the phosphorylation and adiponectin expression in epididymal adipose tissue

We next tested the protein expression responsible for fatty acid \( \beta \)-oxidation in the epididymal adipose tissue. Figure 4A and B show that the protein expression by the phosphorylated forms of AMPK and its immediate substrate (the phosphorylated form of ACC) was significantly higher in the HFD+SQE group than in the HFD group after 70 d. On the other hand, the gene expression of adiponectin was significantly lower in the HFD group than in the ND group (Fig. 4C), but was restored in the HFD+SQE group.

SQE activated the AMPK pathway in mature 3T3-L1 adipocytes

The adipocytes generated from 3T3-L1 preadipocytes exhibit most of the key features of adipocytes in vivo. We therefore investigated the effect of SQE on the AMPK signaling pathway in mature 3T3-L1 adipocytes. First of all, the effects of SQE on the cell viability and cytotoxicity of mature 3T3-L1 adipocytes was evaluated by MTT and LDH assays. An SQE concentration of 1,000 mg/mL did not affect the viability (97.33 ± 6.08% vs. the control) or cytotoxicity (−1.15 ± 2.85% vs. the control) of the mature 3T3-L1 adipocytes (Fig. 5A). We treated mature 3T3-L1 adipocytes with
Fig. 2. Effect of SQE on the Body Weight Change in Mice Fed with ND, HFD, and HFD+SQE (150 mg/kg/d).

Body weight was measured at 5-d intervals for 70 d. Results are shown as the mean ± standard error (n = 10). Values with different letters in each assay were significantly different from each other between the ND, HFD, and HFD+SQE groups by Tukey’s test (p < 0.05).

Table 1. Effects of SQE Supplementation on Body Weight Gain, Food Intake, Epididymal Adipose Tissue Weight, and Perirenal Adipose Tissue Weight in High-Fat Diet-Induced Obese Experimental Group after 70 d

<table>
<thead>
<tr>
<th>Group</th>
<th>ND</th>
<th>HFD</th>
<th>HFD+SQE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (g)</td>
<td>22.61 ± 0.53</td>
<td>22.60 ± 0.42</td>
<td>22.55 ± 0.36</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>28.26 ± 0.99a</td>
<td>38.20 ± 1.12b</td>
<td>33.31 ± 1.12b</td>
</tr>
<tr>
<td>Body weight gain (g)</td>
<td>5.65 ± 0.60a</td>
<td>15.60 ± 0.78c</td>
<td>10.76 ± 0.59b</td>
</tr>
<tr>
<td>Intake of SQE (mg/kg of body weight/d)</td>
<td>—</td>
<td>—</td>
<td>150</td>
</tr>
<tr>
<td>Food intake (g/cage/5 d)</td>
<td>26.57 ± 0.40a</td>
<td>21.19 ± 0.28b</td>
<td>21.04 ± 0.28b</td>
</tr>
<tr>
<td>Epididymal adipose tissue (g)</td>
<td>0.86 ± 0.05a</td>
<td>2.02 ± 0.12b</td>
<td>1.42 ± 0.06b</td>
</tr>
<tr>
<td>Perirenal adipose tissue (g)</td>
<td>0.50 ± 0.06a</td>
<td>1.23 ± 0.07b</td>
<td>0.97 ± 0.06b</td>
</tr>
<tr>
<td>Epididymal adipose tissue (mg/g of body weight)</td>
<td>29.46 ± 1.47a</td>
<td>55.38 ± 1.73c</td>
<td>41.97 ± 1.72b</td>
</tr>
<tr>
<td>Perirenal adipose tissue (mg/g of body weight)</td>
<td>16.88 ± 1.54a</td>
<td>33.83 ± 1.50c</td>
<td>28.51 ± 1.34b</td>
</tr>
</tbody>
</table>

Value expressed as mean ± standard error (n = 10). Values with different letters in each assay are significantly different from each other between the ND, HFD, and HFD+SQE groups by Tukey’s test (p < 0.05).

Fig. 3. Effect of SQE on the Accumulation of Lipid Droplets in Epididymal Adipose Tissue and the Liver of Mice Fed with ND, HFD, and HFD+SQE (150 mg/kg/d).

Hematoxylin and eosin-stained photomicrographs of the epididymal adipose and liver tissue sections are shown at 50×, 100×, or 200× magnification.

Table 2. Effects of SQE Supplementation on Serum Profile in High-Fat Diet-Induced Obese Experimental Group after 70 d

<table>
<thead>
<tr>
<th>Group</th>
<th>ND</th>
<th>HFD</th>
<th>HFD+SQE</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-CHO (mg/dL)</td>
<td>119.71 ± 4.09a</td>
<td>179.14 ± 3.90a</td>
<td>158.57 ± 2.38b</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>92.29 ± 4.86a</td>
<td>138.43 ± 9.15a</td>
<td>83.43 ± 3.03a</td>
</tr>
<tr>
<td>GPT (IU/L)</td>
<td>8.57 ± 0.85a</td>
<td>17.29 ± 3.03a</td>
<td>8.29 ± 0.65a</td>
</tr>
<tr>
<td>GOT (IU/L)</td>
<td>42.29 ± 1.62a</td>
<td>57.43 ± 3.14a</td>
<td>44.00 ± 1.21a</td>
</tr>
<tr>
<td>LDH (IU/L)</td>
<td>440.86 ± 80.48a</td>
<td>981.86 ± 117.44a</td>
<td>566.57 ± 26.43a</td>
</tr>
</tbody>
</table>

Value expressed as mean ± standard error (n = 10). Values with different letters in each assay are significantly different from each other between the ND, HFD, and HFD+SQE groups by Tukey’s test (p < 0.05).
various concentrations of SQE to characterize the effects of SQE on the phosphorylation of AMPK and ACC in vitro. Consistent with the in vivo data, SQE markedly induced the phosphorylation of AMPK and ACC in a dose-dependent manner (Fig. 5B and C). SQE also increased the mRNA level of CPT-1a which is involved in fatty acid oxidation (Fig. 5D).

Discussion

Adipose tissue is a dynamic organ that plays an important role in the energy balance and changes in mass according to the metabolic requirements of an organism. We examined the effects of SQE on HFD-
induced fat accumulation in the adipose tissue of C57BL/6 mice. The body weight gain, adipose tissue weight, and T-CHO and TG serum levels were significantly lower in the SQE-treated mice than in the HFD group, with no difference in the amount of food ingested. Moreover, a histological analysis revealed a greater number of large cells in the epididymal adipose tissue of the HFD group, a typical sign of obese adipose tissue, while the epididymal adipose tissue of the HFD+SQE group exhibited a small number of large cells. Adiponectin is a protein hormone that modulates a number of metabolic processes, including glucose regulation and fatty acid catabolism. Obesity, diabetes, and atherosclerosis have been associated with a reduced adiponectin level. We therefore examined the effects of SQE on the level of adiponectin mRNA in adipose tissue of the HFD-induced mice. SQE recovered the expression of adiponectin mRNA in the present study which had been lowered in the C57BL/6 mice fed with HFD. These results indicate that SQE might have had an anti-obesity effect in vivo, without affecting the amount of food ingested.

We also analyzed the effects of SQE on the development of fatty liver which is strongly associated with obesity. The histological analysis of the liver of the HFD group exhibited the accumulation of numerous lipid droplets, a typical sign of fatty liver. However, the liver of the HFD+SQE group exhibited a much smaller degree of lipid accumulation. Moreover, the serum GPT, GOT, and LDH levels are clinically and toxicologically important indicators, and rise as a result of tissue damage caused by toxicants or disease conditions. The activities of the liver function markers in the HFD group, including serum GPT, GOT, and LDH, were significantly higher than those in the ND group and were improved by SQE supplementation. These results indicate that the administration of SQE could dramatically suppress the development of HFD-induced fatty liver.

AMPK is known to play a major role in glucose and lipid metabolism and to control such metabolic disorders as diabetes, obesity, and cancer. AMPK has emerged as a therapeutic target for metabolic disorders. We examined the effects on AMPK signaling in epididymal adipose tissue and mature 3T3-L1 adipocytes to detect other specific molecular targets through which SQE might exert inhibitory effects on obesity. AMPK is known to be a metabolic master switch that is activated by LKB1 under intracellular stress conditions, including glucose deficiency, hypoxia, and reactive oxygen species activity. AMPK activation is associated with metabolic organs, including the liver, skeletal muscle, pancreas, and adipose tissue; thus, AMPK has been targeted in the development of drugs to treat metabolic diseases. SQE recovered the expression of the phosphorylated forms of AMPK in the present study which had been reduced in the C57BL/6 mice fed with HFD. Furthermore, treatment with SQE induced AMPK phosphorylation in a dose-dependent manner, and the activation of this kinase led to the phosphorylation of its substrate, ACC, in mature 3T3-L1 adipocytes. These results suggest that SQE influenced metabolic processes related to the AMPK signaling pathway. AMPK activation increases fatty acid oxidation by reducing malonyl-CoA through the inhibition of ACC, and this process up-regulates CPT-1a expression. CPT-1a also regulates long-chain fatty acid transport across the mitochondrial membrane. SQE enhanced the expression of CPT-1a mRNA in a dose-dependent manner in mature 3T3-L1 adipocytes. This result suggests that SQE promoted fatty acid β-oxidation by activating AMPK in the HFD-induced obese mice and 3T3-L1 adipocytes, although the mechanism by which SQE activated this remains unclear.

Several recent studies have described the beneficial health effects of *Sasa* species leaves which have been used as an alternative medicine. Alkaline extracts containing polysaccharides, lignin, chlorophyllin, and flavonoids have exerted strong anti-tumor activity and protective effects on spontaneous mammary tumorigenesis. However, in this present study, the bioactive compounds responsible for the anti-obesity activity in *Sasa* leaves were not identified. SQE is a mixture of such phytonutrients as polysaccharides, amino acids, and polyphenols, including p-coumaric acid (23.706 ± 1.776 mg/g) and tricin (0.028 ± 0.017 mg/g). These polyphenols have such biological activities as anti-cancer and anti-oxidative effects. Further research is therefore necessary to clarify the relationship between the anti-obesity effects and the active compounds in SQE.

In summary, we have shown that administering SQE to mice with HFD-induced obesity reduced the body weight gain, adipose tissue weight, cell size in adipose tissue, and the accumulation of lipid droplets in the liver. SQE reduced the serum T-CHO and TG levels, thereby regulating lipid metabolism. SQE also increased the phosphorylation of AMPK and ACC in epididymal adipose tissue and mature 3T3-L1 adipocytes. Taken together, these results suggest that SQE may protect against HFD-induced obesity by activating the AMPK pathway in adipose tissue and inhibiting the lipid droplet accumulation in liver tissue.

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