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Acanthopanax senticosus Reverses Fatty Liver Disease and Hyperglycemia in ob/ob Mice

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Non-alcoholic fatty liver disease (NAFLD) is common in obesity. However, weight reduction alone does not prevent the progression of NAFLD to end-stage disease associated with the development of cirrhosis and liver disease. In a previous experiment, 50% ethanol extract of *Acanthopanax senticosus* stem bark (ASSB) was found to reduce body weight and insulin resistance in high fat diet-induced hyperglycemic and hyperlipidemic ICR mice. To evaluate the anti-steatosis action of ASSB, insulin-resistant ob/ob mice with fatty livers were treated with ASSB ethanol extract for an 8 week-period. ASSB ethanol extract reversed the hepatomegaly, as evident in reduction of % liver weight/body weight ratio. ASSB ethanol extract also specifically lowered circulating glucose and lipids, and enhanced insulin action in the liver. These changes culminated in inhibition of triglyceride synthesis in non-adipose tissues including liver and skeletal muscle. Gene expression studies confirmed reductions in glucose 6-phosphatase and lipogenic enzymes in the liver. These results demonstrate that ASSB ethanol extract is an effective treatment for insulin resistance and hepatic steatosis in ob/ob mice by decreasing hepatic lipid synthesis.

Key words: *Acanthopanax senticosus* stem bark, Non-alcoholic fatty liver disease, Insulin resistance, Lipogenic enzymes, Glucose 6-phosphatase

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the most common cause of abnormal results in liver-function tests. This clinical situation is really a continuum of diseases that includes simple (benign) fatty liver, nonalcoholic steatohepatitis (NASH), cirrhosis, and hepatocellular carcinoma (Tolman *et al.*, 2004). NAFLD is defined as liver steatosis in patients who do not consume enough alcohol to cause liver injury. Although some drugs or genetic abnormalities can cause NAFLD, the majority of cases are associated with obesity, insulin resistance, and type 2 diabetes. Given the epidemics of obesity and diabetes, there is concern that liver disease will emerge as a major cause of morbidity and mortality (Angulo, 2002).

Current therapy for NAFLD is aimed at correcting the risk factors, but no treatment has been scientifically proven to ameliorate NAFLD lesions or to avert its progression. However, some studies have suggested that antidiabetic agents, e.g., metformin and insulin- sensitizing thiazolidinediones may improve NAFLD (Lin *et al.*, 2000; Neuschwander-Tetri *et al.*, 2003). Recently, some clinicians noted improved liver function in a few patients with fatty liver disease who were treated with metformin. However, liver disease is often considered to be a contraindication for these drugs because lactic acidosis is a frequent side effect of metformin and hepatotoxicity is one of the complications of thiazolidinediones (Cusi *et al.*, 1996).

Acanthopanax senticosus, also called Siberian ginseng, has long been used in empirical traditional medicine for many ailments. This medicinal plant was first discovered in the search for an inexpensive substitute for Korean ginseng. But it was shown that phytochemically the main components of *Acanthopanax senticosus* (lignans) markedly differ from those of the Korean ginseng (saponins). In Japan, in order to preserve natural resources from the point of exhaustion, the stem bark of *Acanthopanax senticosus* has been also used as Siberian ginseng in place of the root bark. It was observed that water extract

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from the stem bark prolonged the exercise time to exhaustion in chronic swimming stressed rats (Nishioka *et al.*, 1990).

We have previously demonstrated that ethanol extract of *Acanthopanax senticosus* stem bark (ASSB) remarkably decreases body weight, glucose, and lipids in high fat diet-induced obese and hyperlipidemic ICR mice (unpublished data). Thus, we hypothesized that ethanol extracts of ASSB might reverse NAFLD by correcting the underlying factors, i.e. obesity, hyperglycemia, and hyperlipidemia. The aim of this study was to test the efficacy of ethanol extract of ASSB in ob/ob mice, a well-known model of obesity, insulin resistance, diabetes, and hepatic steatosis (Lin *et al.*, 2000; Cohen *et al.*, 2002).

MATERIALS AND METHODS

Plant material

Acanthopanax senticosus stem bark (ASSB) was collected in September 2002 from Jangsu-gun in Jeonbuk province, 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 ASSB was deposited at the Medicinal Plants Herbarium of the School of Pharmacy, Kyung Hee University, with registration number 221. Siberian ginseng, or ASSB (1 kg), was left to dry in the shade at room temperature, and extracted with 50% ethanol under reflux. After removal of the solvent in vacuo, the residue was freeze-dried (yield was 92.3 g) and stored in airtight jars until use.

Animals and treatment

Male, C57BL/6J ob/ob mice were purchased at 8 weeks old from the Korea Research Institute of Bioscience and Biotechnology (Deajeon, Korea) and were acclimatized for 1 week before being randomly assigned into 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 standard rodent chow (Lab diets, U.S.A.). At 9 weeks old, the C57BL/6J ob/ob mice were randomly divided into four groups: a diabetic control group and three treatment groups. The lean (C57BL/6J, lean control, LC) and obese (obese control, OC) mice continued to receive a standard rodent chow, and the treatment groups were fed with either 400 or 800 mg/kg of ASSB ethanol extract (AS400 and AS800) for an 8-week period. As a positive control, metformin was administered at a dose of 300 mg/kg (MT300). Body weights were measured weekly, and blood was collected for blood glucose analysis every other week. At the end of the study, blood was also collected for determination of plasma insulin and lipid levels. After these blood samples were taken, the mice were killed. Liver, skeletal muscle, and pancreas tissues were immediately removed, instantly soaked in liquid nitrogen, and stored at -70°C for morphological examination and mRNA analyses. The experiments were conducted in accordance with internationally accepted experimental protocols for laboratory animal use and care as found in the U.S. 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 stored as plasma 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).

Oral glucose tolerance test

Oral glucose tolerance test (OGTT) was performed at the end of the treatment. Prior to testing, animals were fasted for 10 h, and glucose (1.5 g/kg) was then orally administered. Blood glucose levels were determined from the orbital venous plexus at 0 (before glucose challenge), 30, 60, and 120 minutes after glucose challenge.

Determination of triglycerides in non-adipose tissues

For determination of triglycerides in non-adipose tissues, 50-70 mg of liver, skeletal muscle, or pancreas was obtained from each mouse and homogenized in 4 mL of chloroform-methanol (2:1). An aliquot of 0.8 mL of 50 mM NaCl was added to each homogenate, and the solution was incubated at 4°C for 12 h. Samples were centrifuged for 5 minutes at 1,300 g, and organic layers were removed and dried using Speed Vac (Savant, U.S.A.). The resulting pellet fractions were dissolved in phosphate buffer saline containing 1% Triton X-100 and then assayed for triglyceride as described by Cohen *et al* (2001).

RNA extraction and RT-PCR

Total RNA from liver tissue was prepared using a guanidine thiocyanate-water saturated phenol/chloroform extraction method and subsequently precipitated with acidic sodium acetate (Chomczynski and Sacchi, 1987). Total RNA in the aqueous phase was precipitated with ice-cold isopropyl alcohol, and the isolated RNA was

determined by spectrophotometric analysis at 260 nm and 280 nm. 1 μg of total RNA was reverse transcribed into cDNA using Moloney murine leukemia virus transcriptase and random hexamers as primers. The list of specific primers were as follows: sterol regulatory element binding protein 1a (SREBP1a) sense GCG CTA CCG GTC TTC TAT CA with anti-sense TGC TGC CAA AAG ACA AGG G, fatty acid synthase (FAS) sense GAT CCT GGA ACG AGA ACA C with anti-sense AGA CTG TGG AAC ACG GTG GT, glycerol-3-phosphate acyltransferase (GPAT) sense GGT AGT GGA TAC TCT GTC GTC CA with anti-sense CAT CAG CAA CAT CAT TCG GT, steroyl-CoA desaturase (SCD)-1 sense CGA GGG TTG GTT GTT GAT CTG T with anti-sense ATA GCA CTG TTG GCC CTG GA, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) sense CAA CTT TGG CAT TGT GGA AGG with anti-sense ATG GAA ATT GTG AGG GAG ATG C. The primers were added at a 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 of each dNTP, 5 μL of cDNA, and 2.5 units of Taq DNA polymerase. The PCR conditions were denaturation at 94°C for 1 minute, annealing at 57.5°C for 1 minute, and extension at 72°C for 1 minute. The RT-PCR products were electrophoresed in 1% agarose gels at 100 V, and then stained with 0.5 μg/mL ethidium bromide. PCR product densities were measured using a GS-700 imaging densitometer. All mRNA levels were expressed as relative signal intensities for each gene versus GAPDH mRNA.

Northern blot analyses of phosphoenolpyruvate carboxykinase and glucose 6-phosphatase

The cRNA probes for phosphoenolpyruvate carboxykinase (PEPCK), glucose 6-phosphatase (G6Pase), and cyclophilin were synthesized *in vitro* from linearized expression vectors that contained SP6 or T7 viral promoter. Linearized plasmid in the amount of 1 μg 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 minutes, precipitated in ethanol containing lithium chloride at -70°C for 30 minutes, 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 at 260 and 280 nm. Of the total RNA, 10 μg and an equal volume of RNA loading buffer containing 50% glycerol and 1 mM EDTA were denatured at 65°C for 30 minutes and then cooled on ice. Denatured RNA samples were subject to electrophoresis in 1% agarose-formaldehyde gels under 60-80 V, transferred to nylon hybond-N hybridization membranes, and cross-linked with UV. Membranes were pre-hybridized at 68°C for 1 h in prehybridization buffer (5 × SSC, 50% formamide, 0.02% SDS, 0.1% sodium N-lauroyl sarcosine, and 2% blocking reagent). The membranes were then incubated overnight at 68°C in prehybridization buffer with the digoxigenin (DIG)-labeled probe, using the hybridization incubator. Membrane was washed twice for 30 minutes in 2 × washing solution (2 × SSC and 0.1% SDS) at room temperature and washed for 1 h in 0.1 × washing solution. After equilibrating in Buffer I (100 mM maleic acid, 150 mM NaCl, and pH 7.5) for 5 minutes, membrane was gently shaken in Buffer II (1% blocking reagent in Buffer I) for 60 minutes and hybridized with diluted anti-DIG-alkaline phosphatase (1:10,000, 75 mU/mL) in Buffer II for 30 minutes. After washing twice for 20 minutes per wash in 0.3% Tween 20 (in Buffer I), membrane was equilibrated in Buffer III (100 mM Tris-HCl, 100 mM NaCl, and 50 mM $MgCl_2$, pH 9.5) for 2 minutes. CSPD solution was then spread over the surface of the membrane. After incubation at 37°C for 15-30 minutes, membrane was exposed to Hyperfilm-ECL for detection of chemiluminescent signal. For rehybridization, membrane was washed for 20 minutes at room temperature in sterilized water, and shaken overnight at 65°C in 50 mM Tris-HCl (pH 8.0), 50% dimethylformamide, and 1% SDS to remove the hybridized probe. After removing the probe, membrane was rehybridized to the DIG-labeled rat cyclophilin cRNA probe to normalize.

Hepatic histology

After fasting overnight, the mice were deeply anesthetized with urethane (0.9 mL/100 g body weight of 20% solution) and then perfused transcardially with 10% buffered formalin. The mice were sacrificed by decapitation and their livers were removed to be embedded in paraffin. Paraffin sections (6 μm) were prepared using a microtome (RM2125RT, Leica, Germany) and mounted on slides. Tissue sections were deparaffinized and then stained with hematoxylin and eosin

Statistical analysis

Data is expressed as mean ± SEM, and ANOVA was conducted by STATVIEW (Abacus Concepts, Berkeley, CA). When a significant F ratio was obtained ($P < 0.05$) a post hoc analysis was conducted to compare the 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

Effects of ASSB on body weight and fatty liver

Body weight was measured once a week. The body weight of the mice in the lean control (LC) group gradually increased during the 8-week period. On the other hand,

the body weight of obese control (OC) showed a rapid increase. Weight gains in LC and OC groups during the 8-week period are shown in Table I. Subjects fed the ethanol extract of ASSB showed a gradual increase in body weight, but the increase was significantly less than that of the obese control in spite of hyperphagia. Treatment with AS400 and AS800 prevented the kind of weight gains evident in the obese control group. AS400 reduced weight gain by 54% and AS800 treatment resulted in 32% lower weight gains relative to the weight gain observed in the obese control mice. Compared with their lean littermates, the obese mice had hepatomegaly. The mean liver weight/body weight ratio in obese mice was 7.1%, higher than the 4.2% seen in lean mice of the same age and gender. Both ASSB ethanol extract and metformin reduce hepatomegaly in obese mice, although the AS400 group showed the greatest effect (63% reduction, Fig. 1A). Indeed, after 8 weeks of AS400 treatment, the liver/body weight ratio became normal. Hepatic histology (Fig. 1B) is presented as diffuse micro- and macro-vesicular steatosis in obese mice. However, steatosis had virtually disappeared from the livers of mice treated with ASSB ethanol extract (C and D of Fig. 1B). Thus, ASSB ethanol extract reversed fatty liver disease in obese, ob/ob mice.

Table I. Effect of ASSB ethanol extract on body weight and weight gain

Group	Body weight (g)		Weight gain (g)
	Initial	Final	
LC	21.0 ± 0.6	25.2 ± 0.3	4.2 ± 0.7
OC	38.5 ± 2.2	52.7 ± 2.7†††	14.2 ± 2.1†††
AS400	38.9 ± 2.4	45.4 ± 4.7**	6.5 ± 2.9***
AS800	38.4 ± 2.7	48.0 ± 2.3**	9.6 ± 1.1**
MT300	39.1 ± 2.7	52.2 ± 2.9	13.0 ± 0.5

Values represent the mean ± SD (n=6). †††P < 0.001 vs. LC; **P<0.01, ***P < 0.001 vs. OC.

Effects of ASSB on plasma glucose, insulin and insulin resistance index

As reported previously by Lin *et al.* (2000), ob/ob mice had significantly elevated glucose and insulin levels (Table II). As a result of increased plasma glucose and insulin levels, homeostatic model assessment values for the insulin resistance (HOMA-IR) of the obese control group was 14.7 times higher than that of the LC group as calculated by insulin (μU/mL) x glucose (mM)/22.5 (Matthews *et al.*, 1985). ASSB ethanol extract-treated mice, however, showed marked decreases in plasma glucose and insulin levels relative to the obese control mice. In spite of

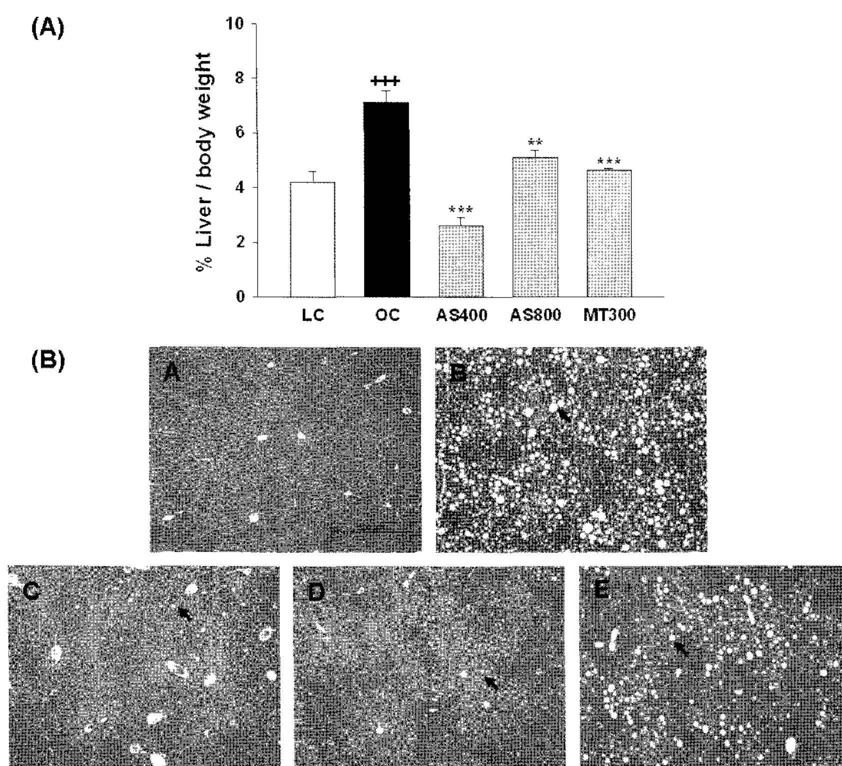

Fig. 1. Effect of the ethanol extract of ASSB on fatty liver disease in ob/ob mice. (A) Liver weight/body weight ratio in lean control (LC), obese mice (OC) and in the three treatment groups (n=6). †††P < 0.001 vs. LC; **P < 0.01, ***P < 0.001 vs. OC. (B) Hepatic histology in one representative mouse from each group. Final magnification was × 100.

hyperphagia, the insulin resistance index of the AS400 group was reduced by 58% and that of the AS800 group was 28% lower than the insulin resistance index of the obese control group. Amelioration of insulin resistance in the AS400 group was significant and even better than that in the MT300 group.

Effect of ASSB on oral glucose tolerance test (OGTT)

To examine whether the insulin resistance of ASSB ethanol extract-treated mice was improved (Table II), an oral glucose tolerance test (OGTT) was carried out at the end of the experiment (Fig. 2). Glucose challenge dramatically increased the blood glucose levels in the OC group over those in the LC group. However, the ASSB ethanol extract-treated groups showed significantly reduced blood glucose levels, especially at 30 and 60 minutes after glucose intake (Fig. 2A). When area under the curve (AUC) was compared between groups, the groups treated with Siberian ginseng extract showed reductions of 34% with AS400 and 28% with AS800 when compared to the OC group (Fig. 2C). The improved glucose tolerance of the ASSB ethanol extract-treated group was similar to that of MT300 group (32% reduction compared to the OC group). Insulin response during OGTT was also considerably greater in the OC group than in the LC group,

Table II. Effect of ASSB ethanol extract on plasma glucose, plasma insulin, and homeostasis model assessment values for insulin resistance (HOMA-IR)

Group	Plasma glucose (mmol/L)	Plasma insulin (μU/mL)	HOMA-IR
LC	6.6 ± 0.4	21.2 ± 7.6	6.4 ± 2.2
OC	10.0 ± 0.4†††	212.5 ± 7.1†††	93.8 ± 3.0†††
AS400	5.9 ± 0.8***	153.6 ± 23.8*	39.7 ± 7.9***
AS800	7.2 ± 0.2***	212.4 ± 13.4	68.0 ± 4.7***
MT300	7.3 ± 0.5***	181.1 ± 8.5*	60.3 ± 3.6***

Values represents means ± SE (n=6). Homeostasis Model Assessment was used to calculate an index of insulin resistance as glucose (mM/L) × insulin (μU/mL) / 22.5. †††P < 0.001 vs. LC; *P < 0.05, ***P < 0.001 vs. OC.

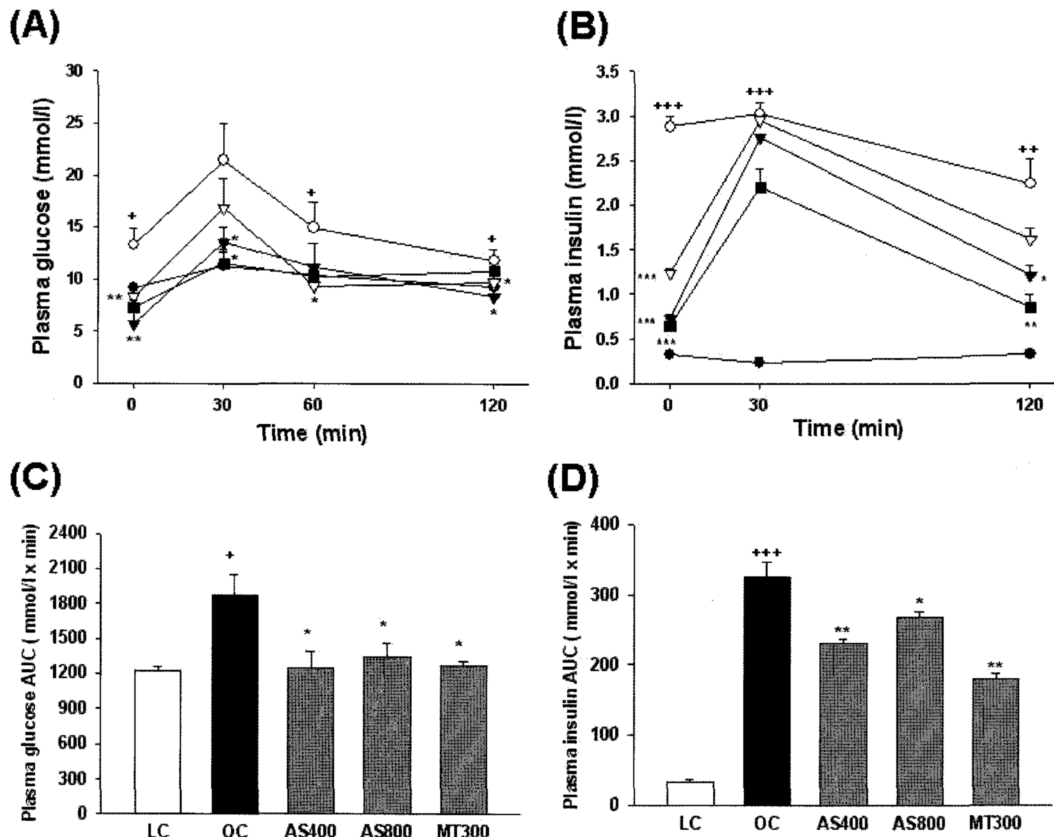

Fig. 2. Plasma glucose (A) and insulin (B) responses to an oral glucose challenge (1.5 g/kg) after 10 h of food deprivation after 8 weeks of treatment. Area under the curve (AUC) of plasma glucose (C) and insulin (D) during oral glucose tolerance test. Values represent the means ± SE of 6 mice in each group. LC (●), lean control; OC (○), obese control; AS400 (▼), mice treated with 400 mg/kg of ASSB ethanol extract; AS800 (▽), mice treated with 800 mg/kg of ASSB ethanol extract; MT300 (■), mice treated with 300 mg/kg of metformin. †P < 0.05, ††P < 0.01, †††P < 0.001 vs. LC; *P < 0.05, **P < 0.01, ***P < 0.001 vs. OC.

Table III. Effect of ASSB ethanol extract on plasma lipid levels

Group	NEFA (mg/dl)	Triglyceride (mg/dl)	Total Cholesterol (mg/dl)	LDL Cholesterol (mg/dl)
LC	1193.8 ± 50.3	79.0 ± 4.1	85.5 ± 4.7	11.2 ± 3.2
OC	1254.3 ± 45.8	100.8 ± 3.6††	196.3 ± 10.1†††	81.4 ± 4.5†††
AS400	1100.3 ± 48.5*	71.0 ± 2.5***	171.5 ± 4.6*	68.3 ± 4.9*
AS800	1061.8 ± 68.9*	82.5 ± 7.0*	177.8 ± 20.6	71.8 ± 14.2
MT300	1201.3 ± 96.9	61.3 ± 2.8***	136.0 ± 3.0**	27.0 ± 5.3***

Values represent the mean ± SE (n=6). LDL-cholesterol (mg/dl) = Total cholesterol – HDL cholesterol – triglyceride/5. ††P < 0.01, †††P < 0.001 vs. LC; *P < 0.05, **P < 0.01, ***P < 0.001 vs. OC.

2B). Plasma insulin AUC levels were also lowered from levels seen in the OC group, by 29% in the AS400 and by 18% in the AS800 groups (Fig. 2D).

Effects of ASSB on plasma lipid levels

The effect of ASSB ethanol extract on plasma lipid levels was examined at the end of the treatment (Table III). The levels of plasma lipid, with the exception of NEFA, in OC mice were significantly higher than in LC mice. In the obese control group, plasma triglyceride (TG) was increased 1.3-fold (79.0 to 100.8 mg/dl), total cholesterol (TC) increased 2.3 -fold (85.5 to 196.3 mg/dl), and LDL cholesterol (LDL-C) increased 7.3-fold (11.2 to 81.4 mg/d). On the other hand, groups treated with ASSB ethanol extract showed 12% reduction in NEFA, 30% reduction in TG, 13% reduction in TC, and 17% reduction in LDL-C levels, particularly in the AS400 group. Metformin remarkably decreased hyperlipidemia, and lipid levels in the MT300 group were much lower than those in ASSB ethanol extract-treated groups. NEFA levels were unaffected, however.

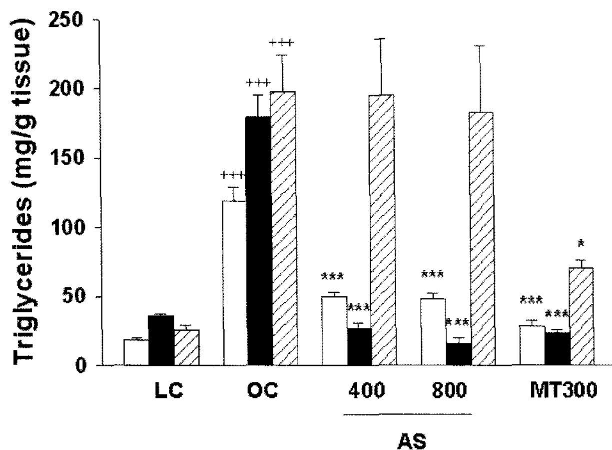

Fig. 3. Effect of the ethanol extract of ASSB on triglycerides in non-adipose tissues, liver (□), skeletal muscle (■), pancreas (▨) Values represent the mean ± SE (n=6). †††P < 0.001 vs. LC; *P < 0.05, ***P < 0.001 vs. OC

Effects of ASSB on triglycerides in non-adipose tissues

The accumulation of triglycerides (TG) was evaluated in non-adipose tissues, as it could be associated with lipotoxicity and insulin resistance (Manco *et al.*, 2004). OC mice showed five- to eight-fold greater increases in TG levels in non-adipose tissues than those levels in the LC mice (Fig. 3). TG levels in the livers of mice treated with ASSB were significantly decreased by about 60%, and those in skeletal muscles of these groups were reduced by 85% with AS400 and 91% with AS800. However, ASSB ethanol extract failed to prevent the accumulation of TG in pancreatic tissue.

Effects of ASSB on PEPCK and G6Pase expressions in liver

The quantitative analyses for phosphoenolpyruvate carboxykinase (PEPCK) and glucose 6-phosphatase

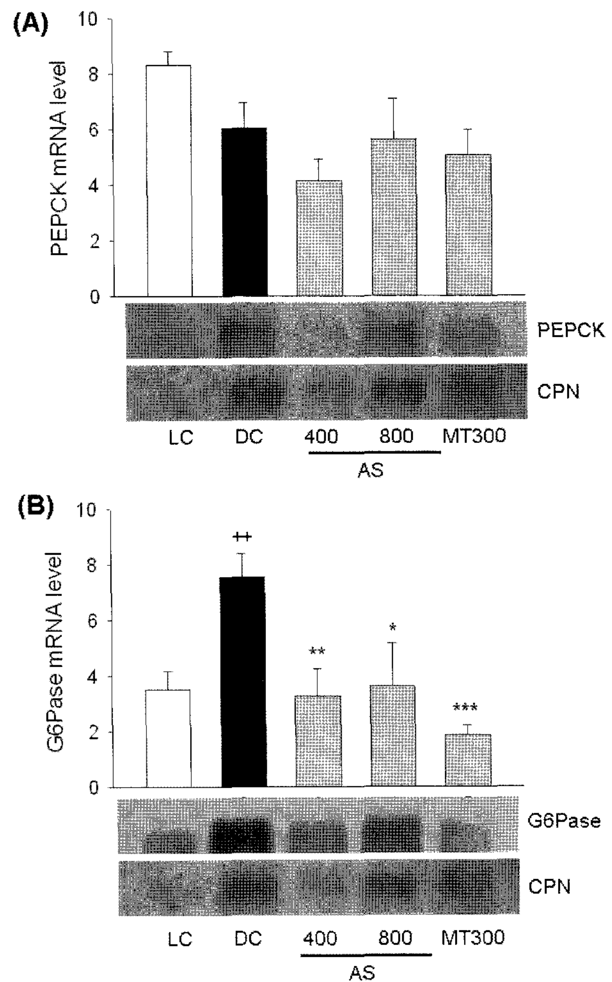

Fig. 4. Effect of the ethanol extract of ASSB on mRNA expressions of PEPCK (A) and G6Pase (B) in ob/ob mice livers. The amount of RNA loaded in each lane was confirmed by Northern blot of CPN mRNA. ††P < 0.01, †††P < 0.001 vs. LC; *P < 0.05, **P < 0.01, ***P < 0.001 vs. OC.

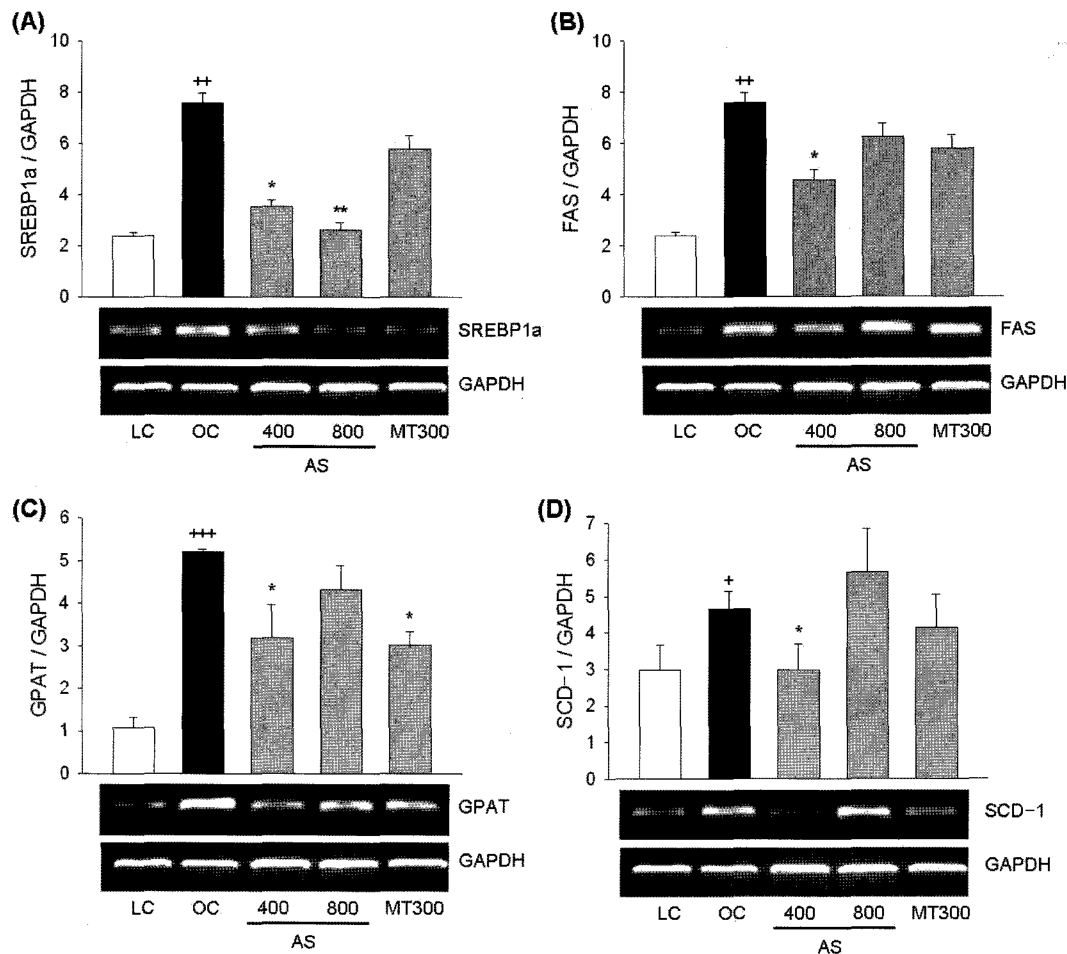

Fig. 5. Effect of the ethanol extract of ASSB and metformin on either SREBP1a (A), FAS (B), GPAT (C) or SCD-1 (D) mRNA levels in ob/ob mice livers. The amount of RNA loaded in each lane was confirmed by RT-PCR of GAPDH mRNA. Values represent the mean ± SE (n=6). $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$, $^{\dagger\dagger\dagger}P < 0.001$ vs. NC; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. DC.

(G6Pase), which are the rate-controlling gluconeogenic enzymes in the liver, were performed using Northern blotting (Mithieux, 1997; Davies *et al.*, 2001). Increases of 2.2-fold in the mRNA level of G6Pase were seen in the OC group when compared to that of the LC group (Fig. 4B), but there was no significant difference in PEPCK mRNA levels between the OC and LC groups (Fig. 4A). Conversely, mRNA expressions of G6Pase in the AS400 group were 57% lower than in the OC mice. The mRNA level of G6Pase in metformin-treated mice was also 75% lower than that observed in the OC mice.

Effects of ASSB on lipogenic genes in liver

To elucidate the molecular mechanism by which ASSB ethanol extract reverses hepatic steatosis, we probed for differential changes in gene expressions involved in hepatic lipogenesis by RT-PCR. In the obese control group, mRNA expressions of SREBP1a and its target enzymes such as FAS, GPAT, and SCD-1 were all significantly higher than those in the LC group (Fig. 5). On the other hand, AS400 decreased the mRNA expression of SREBP1a by 54%. AS400 subsequently suppressed the FAS, GPAT, and SCD-1 mRNA levels. In contrast, mRNA levels of the fatty acid transporter CD36, related to hepatic lipolysis, in the AS400 group was significantly increased 2.7 times that in the LC group (data not shown).

DISCUSSION

There is no established treatment for NAFLD, a condition which is strongly associated with obesity, and which can progress to cirrhosis, liver failure or hepatocellular carcinoma (Angulo, 2002; Hookman and Barkin, 2002). Since NAFLD is very common in obese diabetics, it is interesting that antidiabetic drugs have a beneficial effect of (Lin *et al.*, 2000; Marchesini *et al.*, 2001; Neuschwander-Tetri *et al.*, 2003). For example, metformin decreased hepatic steatosis and normalized aminotransferase levels in ob/ob mice, in parallel with suppression of FAS, uncoupling protein-2 (UCP-2), and tumor necrosis factor-α

(TNF-α) levels in liver tissue (Lin *et al.*, 2000). Recent studies in humans have also shown modest effects of metformin and insulin-sensitizing thiazolidinediones on NAFLD, although the mechanisms are unclear (Marchesini *et al.*, 2001; Neuschwander-Tetri *et al.*, 2003).

In this study, we evaluated the actions of an ethanol extract of ASSB in ob/ob mice. As a result of genetic leptin deficiency, this breed of mouse develops obesity, insulin resistance, late-onset diabetes, hyperlipidemia, and hepatic steatosis (Lin *et al.*, 2000; Cohen *et al.*, 2002). Hepatic steatosis in ob/ob mice is characterized by increased expression of lipogenic genes and suppression of genes that mediate hepatic lipolysis (Cohen *et al.*, 2002; Liang and Tall, 2001). Moreover, the presence of obesity, insulin resistance, hyperlipidemia, and diabetes in ob/ob mice can provide a useful model for assessing the contributions of these risk factors to hepatic steatosis.

In agreement with our previous study, ethanol extract of ASSB significantly decreased body weight and percent liver/body weight ratio in ob/ob mice (Table I and Fig. 1). Our finding that ethanol extract of ASSB also decreased hepatic triglyceride production is important (Fig. 3). It has been reported that de novo lipogenesis in the liver is elevated in insulin-resistant states and NAFLD (Schwarz *et al.*, 2003). Donnelly *et al.* found that de novo lipogenesis accounted for 26% of liver TG content in hyperinsulinemic subjects with NAFLD (Donnelly *et al.*, 2005). Insulin activates the membrane-bound transcription factor SREBP1a, which transcriptionally activates most genes required for lipogenesis. In mice, even insulin-resistant ones, insulin stimulates hepatic SREBP1a transcription and increases lipogenesis (Shimonura *et al.*, 1999). In our study, the expression of mRNAs for the SREBP1a's target enzymes FAS, GPAT, and SCD-1 were suppressed in ob/ob mice by the ethanol extract of ASSB. This suggests a regulation of hepatic lipogenesis at the level of transcription (Fig. 5). These findings suggest that the anti-steatosis action of ASSB ethanol extract is mediated mainly through inhibition of lipogenesis.

Donnelly *et al.* (2005) found that the plasma NEFA pool accounts for approximately 60% of TG contents in the livers of NAFLD patients, reflecting the importance of the NEFA pool in the pathogenesis of NAFLD. We also observed a strong association between hepatic steatosis and elevated insulin, glucose, and lipid levels in ob/ob mice. Conversely, the ethanol extract of ASSB reversed hepatic steatosis (Fig. 1b) and reduced levels of glucose and insulin, thus markedly improving insulin resistance (Table II). In agreement with this, ob/ob mice treated with the ethanol extract of ASSB were more sensitive to acute glucose challenge relative to vehicle alone (Fig. 2). Hence we reasoned that the ethanol extract of ASSB would enhance insulin response.

Another potential action of the ethanol extract of ASSB is the reduction in mRNA expression of G6Pase, a microsomal enzyme catalyzing the hydrolysis of glucose 6-phosphate (coming from gluconeogenic and glycogenolytic pathways) into glucose (Mithieux, 1997). Improvement of insulin resistance, as mentioned above, reduced the transcriptional levels of the rate-limiting gluconeogenic enzymes including PEPCK and G6Pase.

In summary, we have demonstrated several unique properties of the ethanol extract of ASSB in terms of reducing body weight, and reversing insulin resistance and hepatic steatosis in ob/ob mice. This medicinal plant acts as an insulin sensitizer and specifically decreases circulating glucose and lipids, in addition to suppressing hepatic lipogenesis. However, it is important to emphasize that these findings mainly relate to steatosis evident in ob/ob mice. Future studies will determine which active ingredient(s) is/are responsible for this action, and whether ethanol extract of Siberian ginseng stem bark is able to reverse other abnormalities in NAFLD, including inflammation, necrosis, and fibrosis.

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