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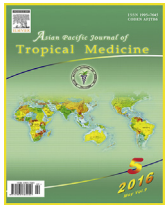

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# Effects of feeding a diet containing *Gymnema sylvestre* extract: Attenuating progression of obesity in C57BL/6J mice

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ABSTRACT

**Objective:** To investigate the effect of *Gymnema sylvestre* extract (GS) on initial anti-obesity, liver injury, and glucose homeostasis induced by a high-fat diet (HFD).

**Methods:** The dry powder of GS was extracted with methanol, and gymnemic acid was identified by high performance liquid chromatography as deacyl gymnemic acid. Male C57BL/6J mice that fed on either a normal diet, normal diet containing 1 g/kg GS (CON+GS), HFD, or HFD containing 1.0 g/kg GS (HFD + GS) for 4 weeks were used to test the initial anti-obesity effect of GS. Body weight gain and food intake, and serum levels about lipid and liver injury markers were measured. Histopathology of adipose tissue and liver stained with hematoxylin and eosin (H&E) and oil-red O were analyzed. After 4 weeks of GS extract feeding, intraperitoneal glucose tolerance test (IPGTT) was performed.

**Results:** The methanol extracts of GS exerted significant anti-obesity effects in HFD + GS group. They decreased body weight gain, a lower food and energy efficiency ratio, and showed lower serum levels of total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL)-cholesterol, very-low density lipoprotein (VLDL)-cholesterol and leptin compared with the HFD group. The decreases of abdominal as well as epididymal fat weight and adipocyte hypertrophy, lipid droplets in liver, and serum levels of aspartate aminotransferase (AST) and alanine transaminase (ALT) were also observed. The CON + GS group showed an effect of glucose homeostasis compared to the CON group.

**Conclusions:** This study shows that GS provide the possibility as a key role in an initial anti-obesity effects feeding with a HFD.

## 1. Introduction

Obesity, a major risk factor of various disorders, has greatly increased world widely. According to the World Health Organization, obesity has doubled since 1980, and in 2014, more than 1.9 billion people were overweight and over 600 million were obese [1]. Obesity is related to many health problems such as hypertension, type 2 diabetes, stroke, cardiovascular disease, osteoarthritis, asthma, and even certain types of cancer [2,3]. Therefore, the prevention of obesity is an important issue for public health. Major causes of obesity are an energy imbalance between calorie consumption and expenditure and increased intake of a high-fat diet (HFD) [4,5]. A HFD is known to induce a variety of metabolic disorders such as adipocyte hypertrophy, adipose chronic inflammation, hepatic steatosis of mature adipocytes, and insulin resistance [6]. As these metabolic disorders are related to the progression of obesity, many studies have been conducted to find anti-obesity agents. For a long time, several medicinal herbs have been

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used continuously for prevention or treatment obesity. *Gymnema sylvestre* extract (GS), a dicotyledonous medicinal herb belonging to the family Asclepiadaceae, is a woody climber found in tropical Africa, Australia, central and southern India and China [7]. GS is known to have antimicrobial and anti-hypercholesterolemic effects, hepatoprotective properties, and especially, effects on obesity and diabetic mellitus [8,9]. In many phytochemical analyses studies, GS is known to include gymnemic acids, sapoins, stigmasterol, quercitol and the amino acid derivative of choline, trim ethylamine and betaine. Above all, its main active compound is gymnemic acid, saponins and gymnemagenin [10]. In particular, several experimental studies of GS have been performed by using gymnemic acid properties and reported in the various fields of chemistry, pharmacology, and biotechnology since the 1930s [11]. In previous studies of obesity, oral administration of GS reduced the serum lipid concentration and the effect of atherosclerosis in albino rats fed a HFD [12], and after GS administration for 8 weeks had anti-obesity effects such as a decrease in body weight, food consumption, and total cholesterol (TC) and triglyceride (TG) levels in HFD-induced obese rats [13]. Oral administration of GS for 3 weeks also decreased serum TC and TG levels but did not significantly affect body weight gain in rats [14]. Moreover, antidiabetic effects have also been reported; administration of GS for 7 weeks decreased blood glucose levels but increased serum insulin levels in streptozotocin (STZ)-treated diabetic rats [2]. Many scientific studies reported anti-obesity and antidiabetic effects of GS, but most studies used a mutant mouse model such as *ob/ob* or *db/db* or STZ-treated diabetic mice or the HFD-induced obese mouse model. However, the initial preventive regulation of anti-obesity and antidiabetic effects when normal C57BL/6J mice are fed a normal diet or HFD containing GS from the beginning have yet to be confirmed. Therefore, the objective of this study was to evaluate whether gymnemic acid in GS extract had initial anti-obesity and antidiabetic effects in mice fed a normal diet or HFD containing GS and identify its role as a functional food additive.

## 2. Materials and methods

### 2.1. Chemicals and reagents

Paraformaldehyde 20% solution was purchased from Electron Microscopy Sciences (Hatfield, PA, USA). A total of 10% neutral buffered formalin solution, sucrose, D-(+)-Glucose, xylenes, Mayer's hematoxylin solution, eosin Y solution and 0.5% oil red O stock solution in propylene glycol were purchased from Sigma–Aldrich (St. Louis, MO, USA). ELISA kit for leptin assay was purchased from SHIBAYAGI (Gunma Prefecture, Japan) and hemoglobin A1c (HbA1c) assay from Crystal Chem (Downers Grove, IL, USA).

### 2.2. Plant material and extraction of GS

The methanol extract of GS was offered in All Season Herbs Pvt, Ltd. The obtained GS powder was stored at 4 °C until used. The specimen voucher of GS extract (SOP No: ASH/QC/MS/1012) was retained in the All-Season Herbs Pvt. Ltd., Bangalore-66, India. In this specification sheet, GS extract (Batch No: ASH/GYM/4858) was analyzed for physical, product, microbiological profiles and performed gravimetric assay. Additional specimen voucher of GS extract (Report No: NRPL/QCO/09037) was retained in the Natural Remedies Pvt. Ltd., Bangalore-560100, India. In this report, GS extract (Batch No: ASH/GYM/5856) was analyzed for gymnemic acid assay by using HPLC and total gymnemic acid assay by using gravimetry protocol.

### 2.3. Phytochemical analysis of GS

For identify of gymnemic acid in GS, gravimetry and HPLC method were performed. In brief, weigh accurately 2 g of GS into a 100 mL beaker and dissolve completely with 60 mL of water. Add 2 mL of 0.1N NaOH and few drops of 10% sulfuric acid with constant stirring till the pH of the solution reaches 2 to 2.5. Standing for 1 h and filtered, and after dry, the percentage of gymnemic acid was calculated. HPLC as Deacyl gymnemic acid (>95% Pure, Natural Remedies Pvt. Ltd., Bangalore-560100, India) was performed for quantification of gymnemic acid. The method of GS extraction and HPLC were performed based on previous methods [15].

### 2.4. Animals and diets

Eight-week-old C57BL/6J and *db/db* male mice were purchased from Central Lab Animal Co. (Seoul, Korea), and housed under a 12-h light/dark cycle in a laboratory animal facility with a temperature of (22 ± 1) °C and a relative humidity level of 41% ± 2%. They had free access to pelleted food, except when fasted before necropsy. The animal study methods were approved by the Seoul National University Animal Ethics Committee (SNU-141023-1-2). After 1 week of acclimation on a normal diet (D12450K; Research Diets, New Brunswick, NJ, USA), animals were randomly divided. First, C57BL/6J and *db/db* mice were divided into 3 groups (7 mice/group): 1) a control (CON) group, a single oral administration of water, 2) a single oral administration of GS 1.0 g/kg body weight, and 3) a single oral administration of GS 1.5 g/kg body weight. Fasting and postprandial glucose test were performed in these groups.

Second, C57BL/6J mice were divided into 4 groups (10 mice/group): 1) a control (CON) group fed a normal diet, 2) a group fed a normal diet plus GS 1.0 g/kg (CON + GS group), 3) a HFD group, and 4) a group fed a HFD plus GS 1.0 g/kg (HFD + GS group). HFD contains 60% kcal fat (D12492; Research Diets). These groups were fed the diets for 4 weeks, and body weight gain and food intake were assessed twice per week during the experimental period.

### 2.5. Measurement of serum glucose levels after a single administration of GS

A blood glucose test was performed in C57BL/6J and *db/db* mice after a single oral administration of GS. The CON groups were orally administration of water. Blood glucose concentrations were measured with an Accu-Chek glucometer (Roche, Basel, Switzerland) using Accu-Chek test strips.

### 2.6. Blood and tissue sample collection

After 4 weeks of feeding experimental diets, the mice were killed. Blood samples were gained from the abdominal vein, and organs were collected. Serum was obtained by centrifugation at

13 000 rpm for 35 min and stored −70 °C until used for analyses. The liver, epididymal fat, and peritoneal fat tissue were dissected and weighed. Randomly selected liver and epididymal fat tissue samples were fixed by 4% and 10% neutral buffered formalin, and the remaining samples were stored at −70 °C.

### 2.7. Hematoxylin and eosin (H&E) staining

The liver and adipose tissue were fixed in 10% neutral buffered formalin, paraffin processed, and sectioned at 5 μm. For histopathological analysis, the sections were placed in xylenes and rehydrated through serial alcohol gradients (100%, 95%, 90%, 80%, 70%, and 50%, 2 min each) and then stained with H&E [16]. For histological analyses of the tissue morphology, samples were examined under a light microscope (Nikon Eclipse Ti; Nikon, Tokyo, Japan). ImageJ software was used to quantify the adipocyte number and size (version 1.48; National Institutes of Health, Bethesda, MD, USA).

### 2.8. Oil red O staining

Liver tissues were fixed in 4% paraformaldehyde solution at 4 °C for 1 d; transferred to 10%, 20%, and 30% sucrose solutions for 1 d each; and then embedded with Tissue-Tek OCT (Sakura, Torrance, CA, USA). Frozen liver sections (8 μm) were cut with a microtome (Leica, Nussloch, Germany) and mounted on slides. After drying, the slides were placed in 100% propylene glycol for 3 min and stained with 0.5% oil red O stock solution in propylene glycol for 7 min at 56 °C. The slides were then placed in 85% propylene glycol solution for 3 min, rinsed in distilled water for 3 changes, counterstained with Mayer's hematoxylin, and mounted with an aqueous solution [17].

### 2.9. Biochemical analysis

Serum TC, TG, high-density lipoprotein (HDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol, very-low density lipoprotein (VLDL)-cholesterol, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) levels were analyzed by the Korea Animal Medical Science Institute (Gyeonggi-do, Korea). Atherogenic index was calculated by using the formula of Schulpis and Karikas; TC-HDL-cholesterol/HDL-cholesterol [18]. Serum leptin and HbA1c concentrations were measured by commercial ELISA kit. All procedures were performed following the manufacturer's instructions.

### 2.10. Measurement of serum glucose level and intraperitoneal glucose tolerance test (IPGTT)

An intraperitoneal glucose tolerance test was performed in C57BL/6J mice after an intraperitoneal injection of glucose (1 g/kg body weight) after a 16-h fast. At the time points indicated, blood glucose concentrations were measured with an Accu-Chek glucometer (Roche, Basel, Switzerland) using Accu-Chek test strips before (0 min) and after (15, 30, 60, 90, and 120 min) the glucose injection.

### 2.11. Statistical analyses

The results are expressed as mean ± SEM. One-way analysis of variance (ANOVA) with a post hoc Student–Newman–Keuls multiple comparison test was performed using GraphPad (San Diego, CA, USA). Body weight gain, postprandial glucose test, IPGTT, and glucose level curves were statistically compared using two-way repeated-measures ANOVA with a Bonferroni post hoc test (GraphPad). All results were considered statistically significant at $P < 0.05$.

## 3. Results

### 3.1. Effects on blood glucose levels after a single oral administration of GS in C57BL/6J and db/db mice

To evaluate the antidiabetic effects after a single oral administration of GS, fasting and postprandial blood glucose levels were measured in C57BL/6J and *db/db* mice. Figure 1A showed the fasting glucose levels in each group, and there were no significant differences. However, blood glucose levels after a single oral administration of GS at 1.0 g/kg body weight concentrations showed significantly decreased both C57BL/6J and *db/db* groups (Figure 1B). Furthermore, changes of glucose levels within 2 h in *db/db* mice also showed significantly decreased at 1.0 g/kg and 1.5 g/kg body weight groups (Figure 1C), and confirmed to area under the curve (AUC) (Figure 1D). Based on these results, further experiments of GS were performed at 1.0 g/kg body weight concentrations.

### 3.2. Effects of GS on body weight gain, food efficiency ratio and energy efficiency ratio in C57BL/6J mice

To investigate whether GS could regulate body weight gain and the food and energy efficiency ratio, 4 groups of mice: CON, CON + GS, HFD, and HFD + GS were compared. Figure 2A showed the change in body weight during the experimental period. The HFD group had a greater increase in body weight after 6 d than the CON and CON + GS groups. Interestingly, the difference in body weight between the HFD and HFD + GS groups also increased significantly after 21 d. The total body weight gain of the HFD + GS group was significantly reduced compared with the HFD group ($P < 0.001$, Figure 2B). Food intake values were measured, and no significant difference was shown between groups (Figure 2C). However, compared with the HFD group, the energy efficiency ratio was significantly reduced in the HFD + GS group ($P < 0.001$, Figure 2D). Total energy intake values of each experimental diet for 4 weeks were measured, and the value of the HFD and HFD + GS groups were significantly higher than the CON and CON + GS groups (Figure 2E). Interestingly, energy efficiency ratio values in the HFD + GS group were significantly lower compared with the HFD group ($P < 0.001$, Figure 2F). These results suggested that GS decreased body weight gain induced by HFD in association with decreases in food intake and energy efficiency ratio.

### 3.3. Effects of GS on serum levels of lipid parameters in C57BL/6J mice

Serum levels of lipid parameters are important for evaluation of obesity. As shown in Table 1, feeding a HFD increased serum TC, TG, LDL-cholesterol, VLDL-cholesterol and leptin levels compared to feeding a normal diet. Interestingly, GS significantly decreased serum TG ($P < 0.05$), LDL-cholesterol

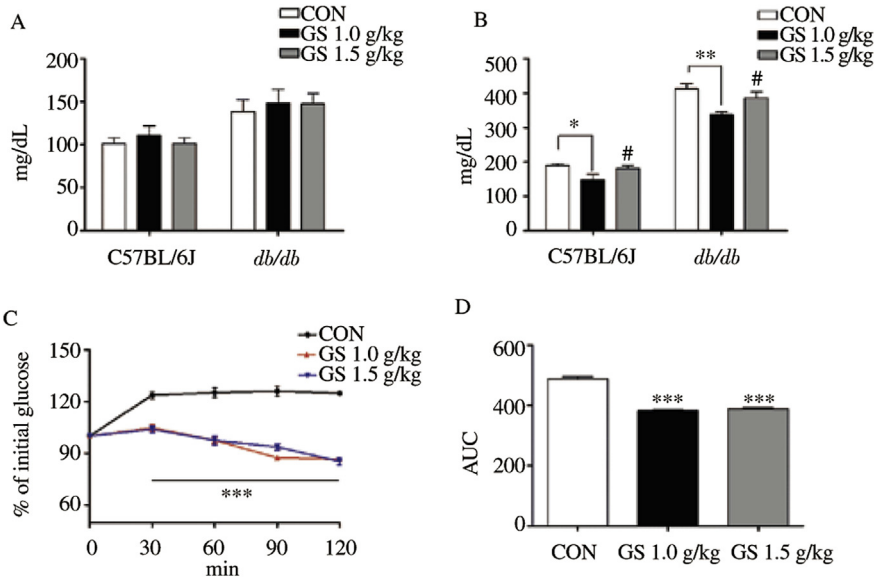


**Figure 1.** Effects of GS on blood glucose levels after a single oral administration of GS at different concentrations (1.0 g/kg and 1.5 g/kg body weight) in C57BL/6J and *db/db* mice.
(A) fasting glucose levels, (B) glucose levels after 2 h, (C) postprandial glucose test, (D) AUC of postprandial glucose test. $n = 7$. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ *vs.* CON group, $^{\#}P < 0.05$ *vs.* GS 1.0 g/kg group.

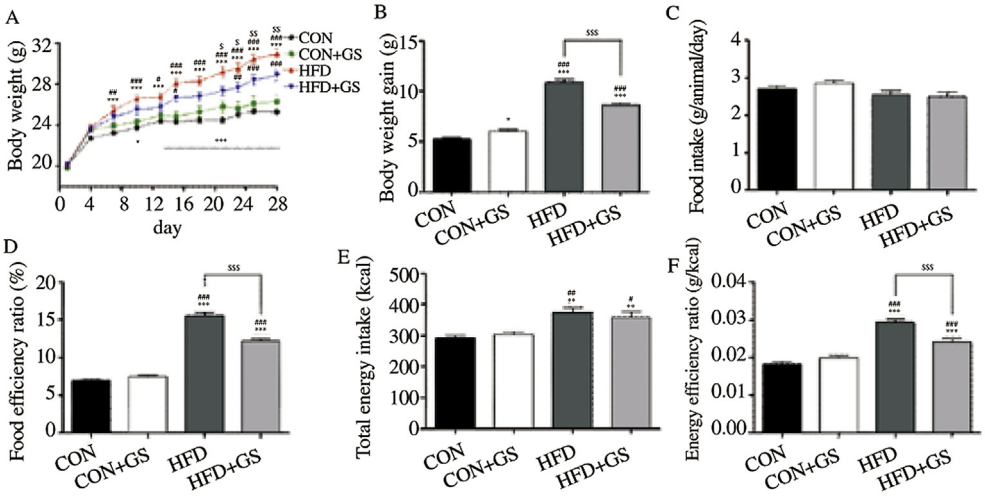


**Figure 2.** Effects of GS on body weight gain, food efficiency ratio and energy efficiency ratio in C57BL/6J mice.
(A) body weight changes, (B) total body weight gain, (C) food intake, (D) food efficiency ratio (ratio between total body weight gain and total food intake), (E) total energy intake, and (F) energy efficiency ratio (ratio between total body weight gain and total energy intake). $n = 10$. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ *vs.* CON group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ *vs.* CON containing GS (CON + GS) group, $^{\$\$\$}P < 0.001$ *vs.* HFD group.

($P < 0.05$), VLDL-cholesterol ($P < 0.01$) and leptin ($P < 0.01$) levels, and increased serum HDL-cholesterol levels ($P < 0.05$) when feeding with HFD. When feeding a normal diet, however, no significant differences between groups except for HDL-cholesterol levels ($P < 0.01$). Atherogenic index was also significantly increased in the HFD group compared to CON and CON + GS groups, but not in the HFD + GS group. These results indicated that the HFD for 4 weeks induced hypercholesterolemia, whereas GS could prevent the increases in serum lipid levels.

**Table 1**
Serum levels of lipid parameters ($n$ = 6–9).

| | CON | CON + GS | HFD | HFD + GS |
|---|---|---|---|---|
| TC (mg/dL) | 176.40 ± 14.40 | 176.40 ± 42.80 | 429.00 ± 176.20$^{***,\#\#}$ | 298.40 ± 20.80$^{**,\#\#}$ |
| TG (mg/dL) | 73.60 ± 27.20 | 90.40 ± 22.00 | 109.00 ± 21.40$^{**,\#}$ | 88.80 ± 31.60$^{\$}$ |
| HDL-cholesterol (mg/dL) | 110.16 ± 11.96 | 125.84 ± 11.76$^{**}$ | 119.84 ± 22.16$^{**}$ | 144.12 ± 7.44$^{***,\#\#,\$}$ |
| LDL-cholesterol(mg/dL) | 5.68 ± 2.48 | 5.44 ± 2.00 | 11.16 ± 3.52$^{**,\#\#}$ | 7.40 ± 1.72$^{\$}$ |
| VLDL-cholesterol (mg/dL) | 14.16 ± 6.00 | 18.20 ± 4.52 | 27.04 ± 15.44$^{**,\#}$ | 16.68 ± 9.40$^{\$\$}$ |
| Leptin (pg/mL) | 324.75 ± 100.00 | 313.50 ± 81.25 | 612.25 ± 190.00$^{***,\#\#\#}$ | 394.75 ± 42.50$^{\$\$}$ |
| Atherogenic index (mg/dL) | 0.59 ± 0.11 | 0.46 ± 0.46 | 2.22 ± 1.43$^{**,\#\#}$ | 1.07 ± 0.23 |

$^{**}P < 0.01$, $^{***}P < 0.001$ *vs.* CON group, $^{\#}P < 0.05$,$^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ *vs.* CON containing GS (CON + GS) group, $^{\$}P < 0.05$, $^{\$\$}P < 0.01$ *vs.* HFD group.

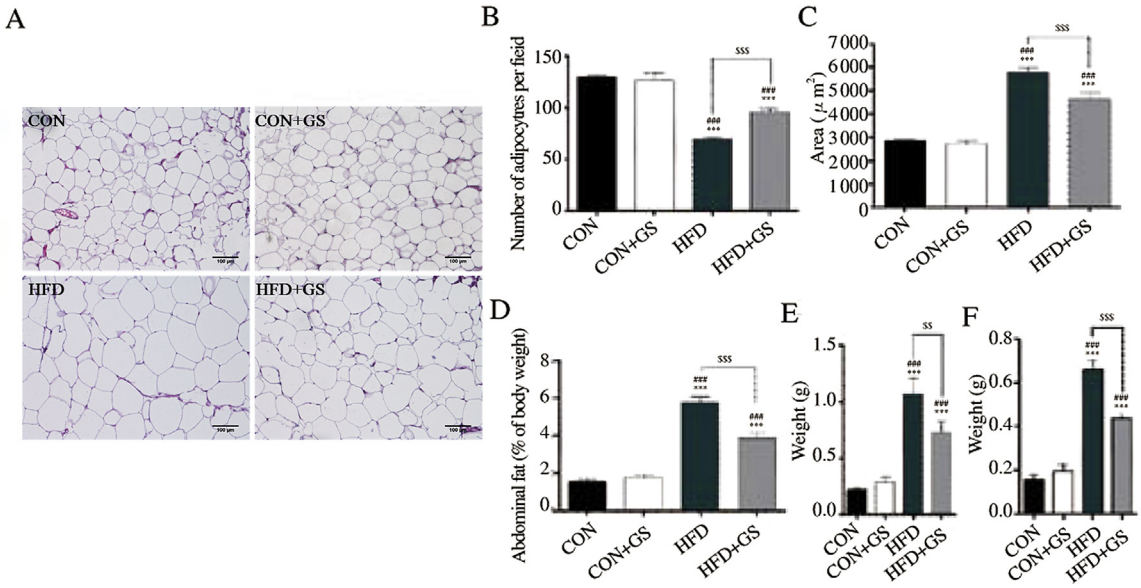


**Figure 3.** Effects of GS extract on adipocyte hypertrophy and hyperplasia.
(A) representative H&E stain of adipose tissue, (B) adipocyte density, (C) adipocyte mean area, (D) abdominal adipose tissue weight (percentage of body weight), (E) epididymal fat weight, and (F) peritoneal fat weight. Scale bars = 100 μm $n = 10$. $^{***}P < 0.001$ *vs*. CON group, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ *vs*. CON containing GS (CON + GS) group, $^{\$\$}P < 0.01$, $^{\$\$\$}P < 0.001$ *vs*. HFD group.

### *3.4. Effects of GS on adipocyte hypertrophy and hyperplasia in C57BL/6J mice*

Next, whether the decreased body weight gain in the HFD + GS group was related to adipocyte changes was questioned. Adipocyte hypertrophy and hyperplasia in abdominal fat was analyzed. Histologic examination of epididymal adipose tissue stained with H&E demonstrated that adipocyte size in the HFD group was significantly larger than that in the CON and CON + GS groups, but the HFD + GS group showed a marked decrease in adipocyte size (Figure 3A). We also quantified the number of adipocytes and mean area based on a normalized field. In the HFD group, the number of adipocytes was significantly lower ($P < 0.001$, Figure 3B), and the mean adipocyte area was significantly larger than in the HFD + GS group ($P < 0.001$, Figure 3C). In the adipocyte area distribution; the graph shape was distributed to forward in CON and CON + GS groups; but the HFD group to widespread. Interestingly, the shape of the distribution of the HFD + GS group was similar to that of the CON and CON + GS groups; therefore, these results indicated that GS acts to prevent adipocyte hypertrophy and hyperplasia. The percentage of total abdominal fat per body weight was significantly increased in the HFD and HFD + GS groups compared with the CON and CON + GS groups ($P < 0.001$, Figure 3D), but significantly decreased in the HFD + GS group compared with the HFD group ($P < 0.001$). The epididymal and peritoneal fat weights of the HFD + GS group were also markedly decreased ($P < 0.01$, $P < 0.001$, Figure 3E,F).

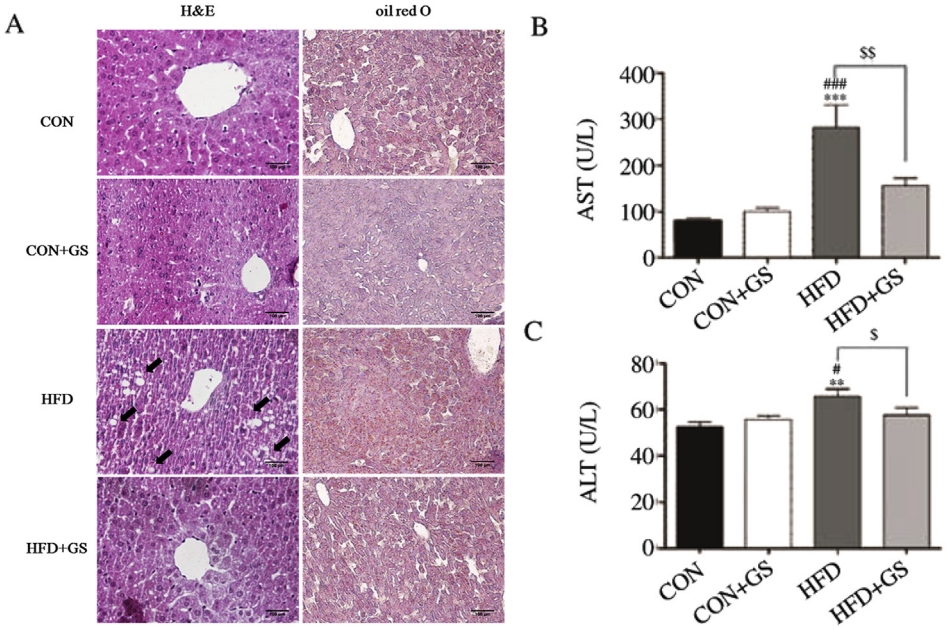


**Figure 4.** Effects of GS on hepatic steatosis and injury.
(A) Lipid droplets (black arrows) in liver tissue stained with H&E, (B) serum AST level, (C) serum ALT level. $n = 7–9$. Scale bars = 100 μm $^{**}P < 0.01$, $^{***}P < 0.001$ *vs*. CON group, $^{\#}P < 0.05$, $^{\#\#\#}P < 0.001$ *vs*. CON containing GS (CON + GS) group, $^{\$}P < 0.05$, $^{\$\$}P < 0.01$ *vs*. HFD group.

### 3.5. Effects of GS on hepatic steatosis and injury in C57BL/6J mice

Hepatic steatosis and injury are usually correlated with obesity, so liver sections from all groups stained with H&E was evaluated (Figure 4A). Representative liver sections of HFD group showed enlarged vacuoles (black arrows), suggesting hepatic lipid deposition, compared with the CON, CON + GS and HFD + GS groups. In addition, the serum AST and ALT levels were also evaluated (Figure 4B,C). AST and ALT levels in the HFD group was significantly increased compared with the CON and CON + GS groups, but significantly decreased in the HFD + GS group compared to HFD group. Consequently, these results indicated that HFD triggered hepatic steatosis and injury, whereas GS significantly suppressed this effect.

### 3.6. Effects of GS on blood glucose levels in C57BL/6J mice

Figure 5 shows the effect of GS on glucose homeostasis in C57BL/6J mice. After 4 weeks, an IPGTT was performed after 16 h of fasting (Figure 5A). The blood glucose levels were all higher in the HFD and HFD + GS groups compared with the CON and CON + GS groups during the 120-min test, indicating glucose intolerance. Interestingly, there was significant difference between the HFD and HFD + GS groups at 60 min ($P < 0.05$). The AUC of the IPGTT was also measured, which showed significant increase in the HFD and HFD + GS groups compared with CON and CON + GS groups. Interestingly, this results showed 11.3% decrease in the CON + GS group compared with CON group and 9.2% decrease in the HFD + GS group compared with the HFD group (Figure 5B). Additionally, HbA1c levels were also evaluated after 4 weeks, and the HFD + GS group was significantly decreased compared with the HFD group ($P < 0.05$, Figure 5C). These results indicated that there was an effect on glucose homeostasis in C57BL/6J mice fed the HFD containing GS for 4 weeks.

## 4. Discussion

Obesity is a major risk factor of various diseases such as type 2 diabetes, hypertension, cardiovascular disease, arthritis, and certain types of cancer [2,19]. Recently, the imbalance between energy consumption and expenditure has been known as a major cause of obesity and this imbalance could be facilitated by eating a HFD [20,21]. It is well known that HFD results in increased body weight, adipocyte hypertrophy, hepatic steatosis, and visceral adiposity [22]. Furthermore, HFD influences the serum levels of several biochemicals such as TC, TG, LDL-cholesterol, and VLDL-cholesterol [19]. For these reasons, the HFD-induced obesity model is widely used to investigate anti-obesity effects. GS is one of the traditional medicinal plants used to treat diverse diseases [23]. A number of reports demonstrated that GS has antidiabetic, anti-inflammatory, antioxidant, anti-atherosclerotic, anticancer, and anti-obesity activities [2,10]. Recently, the anti-obesity effect of GS has been evaluated in many studies using HFD-induced obesity or *ob/ob* mouse models. However, a limitation of these models is that the animals are already obese; making it difficult to evaluate an initial anti-obesity effect. Therefore, in the present study, we evaluated the initial anti-obesity effect by feeding a normal diet containing GS and HFD containing GS to determine whether GS could regulate the increase in body weight, adipocyte hypertrophy, and hepatic steatosis. To set the concentration of GS, a single oral administration of GS at 1.0 g/kg and 1.5 g/kg body weight concentration was performed in C57BL/6J and *db/db* mice. In these results, blood glucose levels were significantly decreased in both C57BL/6J and *db/db* mice, especially at 1.0 g/kg body weight concentration. At the concentration of 1.5 g/kg body weight showed marginal effect compared to 1.0 g/kg body weight concentration. Based on these results, the optimal concentration of further experiments was 1.0 g/kg body weight in this study. Our present study clearly showed that the HFD + GS group had significantly decreased body weight gain and food and energy efficiency ratio compared with the HFD

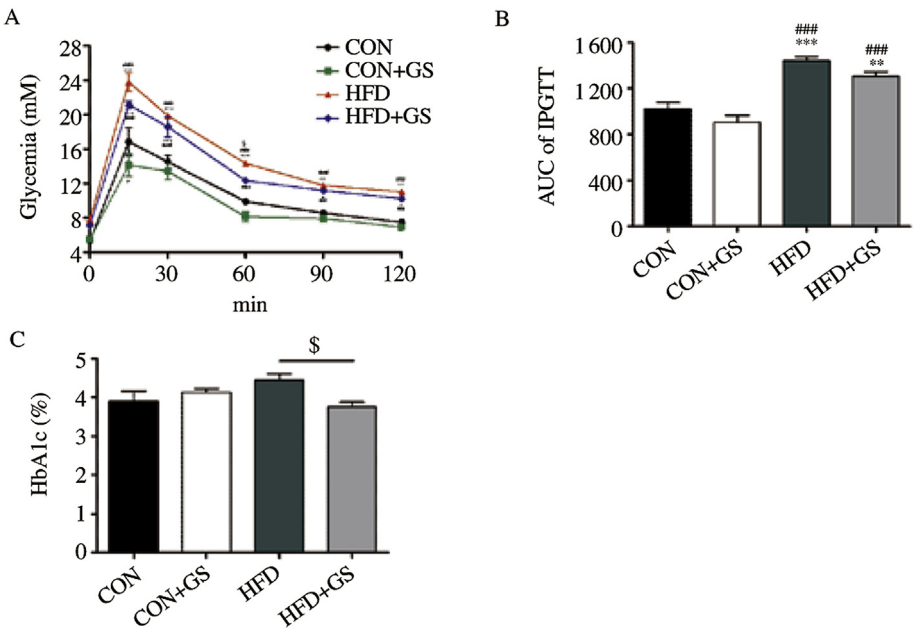


**Figure 5.** Effects of GS on glucose homeostasis.
(A) IPGTT after 4 weeks, (B) AUC of IPGTT, (C) HbA1c levels. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ *vs.* CON group, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ *vs.* CON containing GS (CON + GS) group, $^{\$}P < 0.05$ *vs.* HFD group.

group; however, there was no difference in food intake among all groups. According to the food efficiency ratio and energy efficiency ratio, the change in body weight was the most important factor, as there was no significant change in food and energy intake. Thus, food efficiency ratio and energy efficiency ratio could be effective parameters to predict an anti-obesity effect [24] and these results indicated that GS could attenuate body weight gain related to food efficiency ratio and energy efficiency ratio. Moreover, several studies have shown that GS affects the serum levels of lipid parameters; TG, TC, HDL-cholesterol, LDL-cholesterol and leptin that related to obesity. Oral administration of GS (100 mg/kg body weight daily) to STZ diabetic rats decreased TG, TC, and LDL-cholesterol but increased HDL-cholesterol levels [25], and GS (120 mg/kg, orally) fed to HFD rats for 21 d decreased serum lipid, apolipoprotein A, and leptin levels and increased HDL-cholesterol levels [10]. In the present study, serum levels of TC, TG, LDL-cholesterol and VLDL-cholesterol were markedly decreased and HDL-cholesterol was increased in the HFD + GS group compared with the HFD group. In particular, serum leptin levels are correlated with body weight changes, adiposity, and the proportion of body fat mass [26]. In this study, serum leptin levels in the HFD + GS group were also markedly decreased, indicating that GS may regulate the decrease of body weight gain and fat accumulation in adipose tissue. Atherogenic index is used for predictors for metabolic disturbances like dyslipidemia, hypertension, atherosclerosis and cardiovascular diseases [27]. In the HFD group, serum levels of atherogenic index was significantly increased compared to CON and CON + GS groups. This result confirmed that feeding a HFD for 4 weeks induce metabolic disturbances and GS could prevent these metabolic disturbances markedly. Adipocyte hypertrophy and hepatic steatosis are induced by HFD, and they play critical roles in the development of the metabolic syndrome, such as inflammation and insulin signaling problems [28]. Recent studies have shown that adipose tissue is not a simple energy store but is also an endocrine organ that secretes agents such as adipokines and growth factors, which have an important role in homeostasis regulation [25]. When adipose tissue is expanded by hypertrophy, hyperplasia, or both, it promotes inflammation and macrophage infiltration and can lead to the development of obesity [26]. Furthermore, imbalanced lipogenesis and interaction between adipose tissue and liver may cause hepatic steatosis [6], and the prevention of adipocyte hypertrophy and hepatic steatosis is an important factor for evaluating anti-obesity effects. In our histological examination of adipose tissue, adipocyte mean area and density were significantly decreased in the HFD + GS group. Epididymal and peritoneal as well as abdominal fat weight was also decreased compared with the HFD group. Furthermore, the number of fat droplets in liver tissue was significantly decreased in the HFD + GS group compared with the HFD group. To investigate whether GS could prevent liver injury induced by HFD, we measured serum levels of AST and ALT, representative biomarkers of liver injury and also indicators of liver function [25,29]. Both AST and ALT levels were decreased in the HFD + GS group compared with the HFD group; therefore, we suggest that GS plays a key role in suppression of adipocyte hypertrophy and hepatic steatosis induced by HFD. In addition, to evaluate whether GS could regulate the initial glucose homeostasis induced by HFD, glucose and HbA1c levels and performed IPGTT were measured. The hypoglycemic effects of GS were identified in many studies, especially those targeting type 2 diabetes. Oral administration of GS (20 mg/kg body weight) to STZ diabetic rats decreased plasma glucose levels by more than 50% and HbA1c by 40% [30], and GS (100 mg/kg) fed to STZ diabetic rats for 7 weeks decreased blood glucose levels and increased serum insulin levels [2]. In this study, GS decreased blood glucose level during IPGTT in the CON + GS and HFD + GS groups. Therefore, further mechanism studies that how to regulate the initial progression of obesity and glucose homeostasis are needed. In conclusion, GS had several initial anti-obesity effects when fed with a normal diet and HFD for 4 weeks; decreased body weight gain, serum levels of lipid parameters, epididymal and peritoneal fat weight, percentage of body weight that is abdominal fat, adipocyte hypertrophy and hyperplasia, and hepatic steatosis and injury. These results provide the possibility of GS as a food additive that could play a key role in an initial anti-obesity effect. Taken together, our results strongly suggest that GS seems to have initial anti-obesity as well as preventive effects against obesity.

## Conflict of interest statement

The authors declare that they have no competing interests.

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