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Abstract

Effect of *Gymnema sylvestre* extract in high-fat diet induced metabolic disorders

Hyeon-Jeong Kim

Department of Veterinary Pathobiology and Preventive

Medicine Major

The Graduate School

Seoul National University

Obesity rates has greatly increased in modern societies and this is related to various metabolic diseases such as diabetes, liver steatosis, chronic inflammation and adiposity. Currently, an energy imbalance between calorie consumption and expenditure by high-fat diet(HFD) is considered as a major cause of obesity. Being an important issue in the modern public health, many studies have been conducted to find its precaution.

In particular, natural products having biological activities have been used for anti-obesity and antidiabetic agents. *Gymnema sylvestre* extract (GS), a dicotyledonous medicinal herb belonging to the family of Asclepiadaceae, is a woody climber found in tropical Africa, India and China. Its main active compound is gymnemic acid, and several experimental studies of GS have been performed by using its properties. Many scientific studies of GS, however, have been performed using mutant mice or STZ-treated diabetic models, and the administration method was mostly oral. Therefore, the objective of this study is to evaluate anti-obesity and antidiabetic effects of GS through feeding mixed

food and correlated analysis of insulin resistance, dysfunction of the liver and adipose tissue in C57BL/6J mice.

Male C57BL/6J mice were divided into 5 groups, comprising a normal chow fed group, high-fat diet fed group and high-fat diet plus GS in different doses (100, 250 and 500 mg/kg body weight), and feeding food for 8 weeks. As a result, GS significantly decreased the body weight, liver and adipose tissue weight, and improved insulin resistance and impaired glucose level. In addition, the level steatosis and inflammation of the liver and adipose tissue were decreased.

In summary, this results support relationships among insulin resistance, liver steatosis,, adiposity and cytokine level such as leptin and adiponectin, and confirmed the mechanism by amylase activity of GS. Further mechanism studies are needed to investigate anti-obesity and antidiabetic effects for in vivo model.

Key words: *Gymnema sylvestre*, High-fat diet, Obesity, Type 2 diabetes, Non-alcoholic fatty liver disease, Adiposity

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1. Introduction

Obesity is associated with various metabolic disorders like insulin resistance, diabetes, hypertension, stroke, cardiovascular disease and nonalcoholic fatty liver disease (NAFLD) (Pothuraju *et al.*, 2013; van der Heijden *et al.*, 2015). Among many causes of obesity, nutrient overload due to increased intake of a high-fat diet (HFD) is regarded as a major factor and leads to an imbalance between energy intake and expenditure (Gao *et al.*, 2015; Li *et al.*, 2013; Strauss *et al.*, 2000). For this reason, HFD-induced obesity in rodents is considered a good model to study metabolic disorders including adiposity, hepatic lipid accumulation and insulin resistance (Jin *et al.*, 2013; Tanaka *et al.*, 2015; Bhutani *et al.*, 2007).

Over the last two decades, knowledge about obesity-induced insulin resistance has improved significantly, and the disorder is closely related to the pathogenesis of type 2 diabetes, adipocyte dysfunction and NAFLD (Begrache *et al.*, 2013). Dysfunction of adipose tissue causes metabolic disruption in energy homeostasis as well as an energy imbalance, and plays a role in NAFLD development and progression (Duval *et al.*, 2010; Gastaldelli *et al.*, 2009). In addition, chronic inflammation characterized by macrophage infiltration or excessive lipid accumulation in adipocytes contributes to hypertrophied adipocytes, and they lead to changes in the secretion levels of adipokines such as adiponectin and leptin. These pathological states are strongly linked to insulin resistance in obesity (Lumeng *et al.*, 2011; Jiang *et al.*, 2011).

Following the altered metabolic state with insulin resistance and inflammation of adipose tissue, the liver is also associated with hepatic dysfunction such as NAFLD. The liver is well known for the regulation of energy homeostasis and systemic metabolic balance. However, pro-inflammatory activation of Kuffer cells is induced by the consumption of a HFD, and it could lead to obesity-induced liver disease and insulin resistance (van der Heijden *et al.*, 2015; Asai *et al.*, 2014). Thus, insulin resistance, adipose dysfunction and NAFLD are correlated with obesity induced by a HFD (Asai *et al.*, 2014; García-Ruiz *et al.*, 2015).

At present, natural herbal resources with biological activities have been widely investigated as anti-obesity and antidiabetic agents. *Gymnema sylvestre* (GS) is a woody climber of the Asclepiadaceae family, and is mainly distributed in

tropical regions in the world (Leach *et al.*, 2007). GS is well known to have anti-hypercholesterolemia, anti-inflammatory and antimicrobial with the most studied property being the antidiabetic and anti-obesity effects (Vermaak *et al.*, 2011; Tiwaru *et al.*, 2014; Kishore *et al.*, 2014). In many phytochemical analyses, GS is known to include gymnemic acids, stigmasterol, quercitol and amino acids derivative of betaine, ethylamine and choline. One of the major active compounds is gymnemic acids and found in all parts of the plant. This mechanism is known to stimulate insulin secretion from pancreas and delay the glucose absorption in the blood. In addition, other compounds also have a similar effect; however, they showed lesser anti-sweet effect than those of gymnemic acids (Tiwari *et al.*, 2014; Kishore *et al.*, 2014).

In previous studies, administration of GS (120 mg/kg/day) for 7 days in streptozotocin (STZ)-treated diabetic mice increased the regeneration and function of β -cells in pancreatic islets, and resulted in higher serum levels of C-peptide (Shanmugasundaram *et al.*, 1990). Oral administration of GS also decreased the levels of blood glucose, glycated hemoglobin and free fatty acid while increasing insulin levels in STZ-treated diabetic rats (Daisy *et al.*, 2009; Kosaraju *et al.*, 2014). In addition, GS also decreased body weight gain and the serum levels of TC, TG, LDL-cholesterol exhibiting anti-obesity effects (Kumar *et al.*, 2013; Shigematsu *et al.*, 2001; Reddy *et al.*, 2012). Many scientific studies involving GS, however, have been performed using mutant mice or rats such as *db/db*, *ob/ob*, and STZ-treated diabetic models, and the administration method was mostly oral.

In our previous studies, we made a mixed-feed containing a HFD and GS and proceeded with feeding for 4 weeks. GS showed significant anti-obesity effects compared to the consumption of only a HFD, however, the hypoglycemic effect was insufficient (Kim *et al.*, 2016). Therefore, the objective of this study is to evaluate the acute and chronic anti-obesity as well as the antidiabetic effects of GS through correlated analyses of insulin resistance, dysfunction of adipose tissue and the liver.

2. Materials and Methods

2.1 Extraction and phytochemical analysis of GS

The extract of GS was obtained from All Season Herbs Pvt, Ltd in powder form and stored at 4°C until used. HPLC and gravimetric analysis were performed to identify the amount of gymnemic acid in GS. Extraction and HPLC were performed based on previous methods (Kusum *et al.*, 2014). The HPLC chromatogram for deacyl gymnemic acid is shown in Fig.S1, and the profile is described in Table S1.

2.2. Animals experiments

Male C57BL/6J mice were purchased from Central Lab Animal Co. (Seoul, Korea), and housed under a 12 h dark/light cycle. All animal experiments were performed in accordance with the Seoul National University Animal Ethics Committee protocol (SNU-150829-3). After 1 week of acclimation on a normal chow (D12450K; Research Diets, New Brunswick, NJ, USA), animals were randomly divided. First, for acute study, mice were divided into 4 groups (n=5, 7 wk old): 1) a control (CON) group that received a water, a group that received a single oral administration of GS 2) at 100 mg/kg body weight, 3) at 250 mg/kg body weight and 4) at 500 mg/kg body weight. Animals fed a normal chow in this experiment. Second, for evaluation of the chronic effects, mice were divided into 5 groups (n=10, 7 wk old): 1) a control (CON) group fed a normal chow, 2) a HFD group, a group fed a HFD plus GS 3) at 100 mg/kg body weight (HFD+GS 100 mg/kg group), 4) at 250 mg/kg body weight (HFD+GS 250 mg/kg group) and 5) at 500 mg/kg body weight (HFD+GS 500 mg/kg group). The HFD contained 60% kcal fat (D12492; Research Diets), and all groups fed a diet for 8 weeks. The detailed dietary composition is shown in Table S2. Body weight and food intake were assessed twice per week during the experimental period. Based on them, food efficiency ratio (increased body weight (g)/food intake (g)) and energy efficiency ratio (increased body weight (g)/energy intake (kcal)) were calculated.

2.3. Biochemical analysis

Serum TC, TG, creatine, total protein, AST, and ALT levels were analyzed by the Preclinical Research Center (ChemOn, Inc., Gyeonggi-do, Korea). HDL-cholesterol, LDL/VLDL-cholesterol, leptin, insulin, HbA1c, liver TG were measured with a commercial ELISA kit. Amylase activity concentrations were measured by serum after acute study of GS. All procedures were performed following the manufacturer's instructions.

2.4. Histological analysis

The adipose tissues and liver samples were fixed in 10% neutral buffered formalin, paraffin processed, and sectioned at 5 μ m. Paraffin-embedded sections were stained with hematoxylin and eosin and Picrosirius Red according to the manufacturer's protocols, and then examined under a light microscope (Nikon Eclipse Ti; Nikon, Tokyo, Japan). The number and size of adipocytes and collagen deposition in the liver tissue were measured by using Image J software (version 1.48; National Institutes of Health, Bethesda, MD, USA). The NAFLD activity score (NAS) data were analyzed based on the following histologic data: diagnosis rendered by the Pathology Committee (i.e. "not steatohepatitis", "borderline, zone 3 pattern", "definite steatohepatitis"); the aggregate NAS; the score of each component of the NAS (steatosis (0 - 3), lobular inflammation (0 - 3), ballooning (0 - 2)), and fibrosis scores (0,1a,1b,1c,2,3).

2.5. Oil red O staining

Liver tissues were fixed in 4% paraformaldehyde solution at 4°C for 1 day, transferred to 10%, 20%, and 30% sucrose solutions for 1 day each, and then embedded with Tissue-Tek OCT (Sakura, Torrance, CA, USA). The tissues were sectioned at 8 μ m and stained with 0.5% Oil red O stock solution in propylene glycol (O1516, Sigma-Aldrich) for 10 min at 56 °C, and then counterstained with Mayer's hematoxylin.

2.6. Glucose and insulin tolerance tests.

The intraperitoneal glucose tolerance test (IPGTT) and insulin tolerance test (ITT) were performed. For IPGTT, animals were fasted overnight and 1.5g/kg body weight of glucose was administered by intraperitoneal injection. An ITT was performed after fasting for 6 h, using insulin (human insulin HI-0310; Lilly) at 0.75 U/kg body weight administered by intraperitoneal injection. At the time points indicated, blood glucose concentrations were measured with an Accu-Chek glucometer (Roche, Basel, Switzerland).

2.7. Postprandial glucose test for GS

The postprandial glucose test was performed after single oral administration of GS. Animals were postprandial state with intraperitoneal injection of glucose (1.5g/kg body weight), and a single oral administration of GS (100, 250, 500 mg/kg body weight) was performed after 10 min. At the time points indicated, blood glucose concentrations were measured.

2.8. Western blotting

Western blotting was performed based on previous methods (Yu *et al.*, 2016), and membranes were immunoblotted with primary antibody, specifically α -SMA (ab5694), UCP1 (ab10983) antibodies (Abcam, Cambridge, MA), AMPK α (#2532) and pAMPK α (#2535) antibodies (Cell Signaling, Danvers, MA), NF- κ B (sc-372), p38 (sc-7942) and β -actin (sc-1616HRP) antibodies (Santa Cruz Biotechnology, CA). Secondary antibodies were conjugated to horseradish peroxidase for 2 hours at room temperature, rinsed and detected using an ATTO CS image analyzer 3.0 (ATTO, Tokyo, Japan).

2.9. Real Time Quantitative Polymerase Chain Reaction

Total RNA was extracted by using the QuickGene RNA tissue kit SII (Fujifilm, Tokyo, Japan), and cDNA was synthesized with the Suprime script RT premix (GeNet Bio, Daejeon, Korea) according to the manufacturer's

instructions. Quantitative polymerase chain reaction was performed with the CFX96 real time system (Bio-Rad, Hercules, CA), and each cDNA was amplified with the Q-master mix (Genet Bio, Daejeon, Korea). The designed primers are shown in Table S3. We used Manager Version 2.1 Software (Bio-Rad, Hercules, CA) to analyze the data.

2.10. Immunohistochemistry

Primary F4/80 antibody (PA5-32399, Thermo Fisher Scientific) and UCP-1 (ab10983, Abcam) were used for immunohistochemistry and performed according to previous method (Yu *et al.*, 2016). The slides were observed using a light microscope (Eclips Ti-S, Nikon, Tokyo, Japan). Counting the number of positive cells and measuring intensity in randomly selected field images were performed using the Image J software (version 1.48; National Institutes of Health, Bethesda, MD, USA).

2.11. Statistical analyses

Results are shown as mean \pm 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). IPGTT, ITT and a postprandial glucose test 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 of GS on body composition

The characteristics of the mice were determined in Table 1. Total body weight gain showed significant increases with a HFD, whereas GS significantly reduced the weight gain. Food intake was decreased in HFD+GS 500 mg/kg group, but it is due to bitter taste of GS. Food and energy efficiency ratios were significantly decreased in all concentrations of GS. Increased organ weight in the liver and white adipose tissue was also closely related to the body weight gain, and the HFD group showed significant increases in the organ weight whereas these values were significantly decreased in the GS groups. These results suggest that the consumption of GS influenced body weight gain induced by a HFD in association with decreased food and energy efficiency ratio.

3.2. Effects of GS on glucose homeostasis and insulin resistance

All HFD+GS groups showed significant decreases the level of final fasting glucose, serum insulin, HOMA-IR and glyated hemoglobin (HbA1c) compared to the HFD group (Table 2). To further examine metabolic changes, we performed an IPGTT and ITT and GS induced significantly lower glucose levels, confirmed by the area under the curve (Figure 1A and 1B). Additionally, to measure whether single oral administration of GS also affects hyperglycemia, a postprandial glucose test and ITT were performed as acute study. At all concentrations of GS, glucose levels were significantly decreased relative to the CON group at 30, 60 and 90min (Figure 1C).

ITT also showed significant decreases at 60, 90 and 120 min compared to the CON group, and confirmed by AUC (Figure 1D). In addition, GS significantly reduced amylase activity in all concentrations (Figure 1E) and these results indicated that GS could ameliorate glucose intolerance and insulin resistance, and decrease postprandial glucose level via inhibition of amylase activity.

3.3 Effect of GS on serum levels of lipid parameters

Consumption of a HFD for 8 weeks induced marked elevation of serum total cholesterol (TC), triglycerides (TG), low-density lipoproteins(LDL)/very low density lipoproteins(VLDL)-cholesterol, total protein and creatine, but reduced high density lipoprotein (HDL)-cholesterol levels (Table 3). However, GS significantly decreased serum TC, TG, LDL/VLDL-cholesterol levels compared to the HFD group. Compared to the CON group, serum HDL-cholesterol level was significantly decreased in the HFD and HFD+GS 100 mg/kg groups, however, there were no significant changes in other groups. Serum creatine levels showed no significant differences among all groups.

3.4 Effects of GS on adiposity and inflammation in white adipose tissue

Next, we questioned whether previous beneficial effects of GS were related to adipose tissue, and H&E staining and F4/80 immunohistochemistry were performed (Figure 2A). GS significantly increased the cell density (Figure 2B), and the adipocyte mean area also significantly increased compared to the HFD group (Figure 2C). The cells positive for F4/80 exhibited crown-like structures in white adipose tissue (black arrow; Figure 2A).

A HFD induced a significant increase in macrophage (F4/80⁺) infiltration relative to the CON group, however, GS significantly reduced it (Figure 2D). GS also effectively decreased NF- κ B and p38 protein levels (Figure 2E) and the mRNA expression of TNF- α and SREBP-1c compared to the HFD group (Figure 2F). Therefore, these results indicated that GS could prevent adiposity and inflammation induced by consuming a HFD.

3.5 Effects of GS on energy homeostasis in white and brown adipose tissue

To evaluate whether GS can regulate systemic energy homeostasis, UCP-1 IHC performed in white and brown adipose tissue (Figure 3A). In white adipose tissue, the expression level in the HFD+GS 250 mg/kg group was significantly higher compared to the HFD and HFD+GS 100 mg/kg groups (Figure 3B). In addition, GS showed marked up-regulation of protein levels of UCP-1 in white adipose tissue whereas significantly decreased in brown adipose tissue (Figure 3C). With reduced the serum leptin levels (Figure 3D), mRNA expression of leptin and adiponectin also showed significant effects in white adipose tissue (Figure 3E). These results suggest that GS could ameliorate energy imbalance by regulating leptin and adiponectin levels in white adipose tissue.

3.6 Effects of GS on lipid accumulation in the liver

After feeding a HFD for 8 weeks, microvesicular steatosis and inflammatory cell infiltration were observed with H&E and oil red O staining of liver slides (Figure 4A). In histopathological examination, GS showed significantly lower scores (Figure 4B), especially in steatosis (Figure 4C). With the changes of oil red O staining among the groups, the amount of neutral lipids in the liver was also significantly elevated in the HFD group whereas GS significantly reduced it (Figure 4D). In addition, GS significantly elevated the ratio of phosphorylation of AMPK α (Figure 4E), and the activities of the liver marker enzymes, AST and ALT, were also significantly decreased (Fig 4F and 4G). Consequently, these results indicated that HFD triggered hepatic steatosis, whereas GS significantly ameliorated these effects.

3.7 Effects of GS on collagen deposition and hepatic inflammation

We confirmed that HFD induced hepatic steatosis through collagen deposition and inflammation in the liver. Hepatic collagen deposition was evaluated with Sirius red staining (Figure 5A) and the intensity was quantified (Figure 5B). Macrophages in the liver were identified by F4/80 immunohistochemistry (Figure 5A) and the percent area was quantified (Figure 5C). Compared to the CON group, the HFD group showed significant increases, while GS showed significant decreases. Additionally, GS significantly decreased the protein levels of α -SMA, NF- κ B and p38 (Figure 5E) as well as mRNA levels of TNF- α , TGF- β , IL-6 and FAS (Figure 5F). These results indicated that GS could ameliorate liver inflammation and fibrosis.

4. Discussion

The aim of this study is to investigate the anti-obesity and antidiabetic effects of GS as a functional dietary supplement with a HFD. Many metabolic disorders induced by HFD are mainly related with insulin resistance, dysfunction of adipose tissue and liver. Our present study focused on these correlations and identified the potential and effectiveness of GS as a functional dietary supplement.

Analyzing the body composition such as body weight gain, food and energy efficiency ratios and organ weight, and serum levels of lipid such as TC, TG, HDL and LDL-cholesterol are the most fundamental to study metabolic disorders caused by HFD. In our present study, all groups fed with GS showed significant decreases of the body weight gain, food and energy efficiency ratios and the organ relative to the HFD group. The reason why food intake of HFD+GS 500 mg/kg group was decreased compared to other groups is expected to an anti-sweet property of GS. In addition, GS effectively reduced the serum levels lipid relative to the HFD group. In previous studies showed that hyperglycemia and insulin resistance are induced by a HFD, and the modest body weight loss and improved serum lipid levels are related to glucose homeostasis and insulin resistance (Choi et al., 2013), and GS could help to promote body weight loss by controlling serum lipid parameters such as TC, TG, in STZ diabetic or HFD induced models (Kishore et al., 2014; Potawale et al., 2008).

The exact mechanisms how to GS could improve these body composition and serum lipid parameters have not yet been revealed. Some researches investigated the mechanisms of GS; it may suppress the increase of blood glucose levels by inhibiting glucose uptake in the intestine and increasing the secretion of insulin in the pancreas (Kishore et al., 2014; Potawale et al., 2008). In our study, we confirmed that GS significantly reduced the level of fasting glucose, serum insulin, HOMA-IR and HbA1c after the consumption of a HFD. Further, the single oral administration of GS showed antidiabetic effects in postprandial state and this mechanism was based on the inhibition of amylase activity. Amylases

are enzymes that break down of disaccharide or oligo to monosaccharides, and the inhibition of amylase is known for causing a delayed digestion of carbohydrate (Srinivasulu et al., 2016), and the significant decreases of its activity indicated that GS could decrease the rate of glucose absorption and regulate lipid level.

Adipose tissue is known for an important site of inflammation, energy homeostasis and the maintenance of lipid flux (Tanaka et al., 2015; García-Ruiz et al., 2015), and dysfunction of it could lead to insulin resistance and NAFLD. Many reports have demonstrated that increased macrophages in adipose tissue is correlated with obesity (Lumeng et al., 2011; Luo et al., 2015). Here we show that consumption of a HFD led to an increase of white adipose tissue inflammation and adiposity, however, GS showed significantly reversed results. Forming crown-like structures because of macrophage infiltration in adipose tissue is known for the main inducer of inflammation, and we confirmed that GS significantly decrease the percentage of F4/80-positive cells per total cells. In addition, when adipocytes are stimulated with toll-like receptors, they activate NF- κ B and p38 (Jiang et al., 2011), and we confirmed GS significantly decreases their protein levels. These results indicated that GS could ameliorate the adipose tissue inflammation induced by a HFD.

Brown adipose tissue is also related to the modulation of energy balance and thermogenesis induction that are important for body weight maintenance and glucose homeostasis (García-Ruiz et al., 2015). UCP1 is a key component of thermogenesis and is used as the brown adipocyte-specific gene marker, and several studies showed that HFD increased the thermogenic capacity which can lead to the maintenance of body weight and the dissipation of excess energy intake. We confirmed that HFD significantly decreased UCP1 expression in white adipose tissue, but increased in brown adipose tissue relative to the CON group. Protein levels also showed opposite results in white and brown adipose tissue. This result is in agreement with previous studies, and indicates that HFD acts as stimuli for the activation of brown adipose tissue. White adipose tissue browning induction can also prevent the increase energy waste (García-Ruiz et al., 2015), and our data showed that GS could reverse browning in white adipose tissue.

Furthermore, we investigated the cytokines in white adipose tissue: leptin and adiponectin. Leptin is a key factor in regulating energy intake and expenditure, and it is well known that decreased leptin synthesis is associated with lipolysis (Mopuri et al., 2015; Qureshi et al., 2007). Adiponectin is an adipocyte-specific protein and is also well known to regulate insulin sensitivity and glucose homeostasis (Tolman et al., 2007). Our study demonstrated that GS significantly lowered the leptin levels and improved the mRNA expression levels of both. Taken together, GS could effectively improve adiposity and inflammation in adipose tissue as well as energy homeostasis.

As NAFLD has also been linked to obesity, we confirmed whether GS could improve liver steatosis induced by a HFD (Luo et al., 2015; Tarantino et al., 2010). GS effectively alleviated the NAS and TG levels in the liver, and serum AST and ALT levels used as markers of liver damage (Strauss et al., 2000; Kim et al., 2016) also. Sirius red staining and F4/80 immunohistochemistry of the liver, protein and mRNA levels indicated that GS could effectively improve the NAFLD induced by a HFD.

In conclusion, our data support the acute hyperglycemia effect of GS in postprandial state, and existence of a relationship among insulin resistance, dysfunction of adipose tissue and the liver induced by a HFD. GS demonstrated preventative effects against HFD-induced metabolic disorders when it used as a functional dietary supplement in this study, however, further studies are needed to investigate the relationship between anti-sweet property and food intake with pair-wise feeding study to investigate the control of food intake and its beneficial effect

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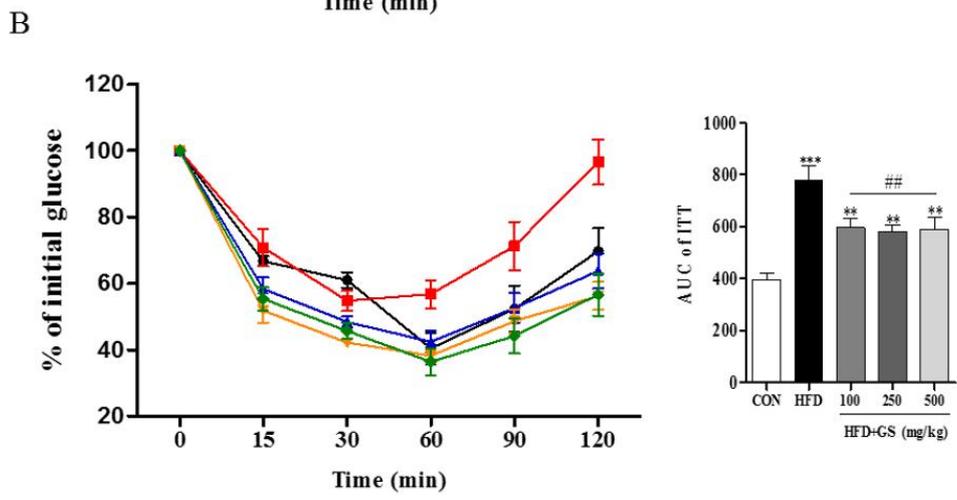
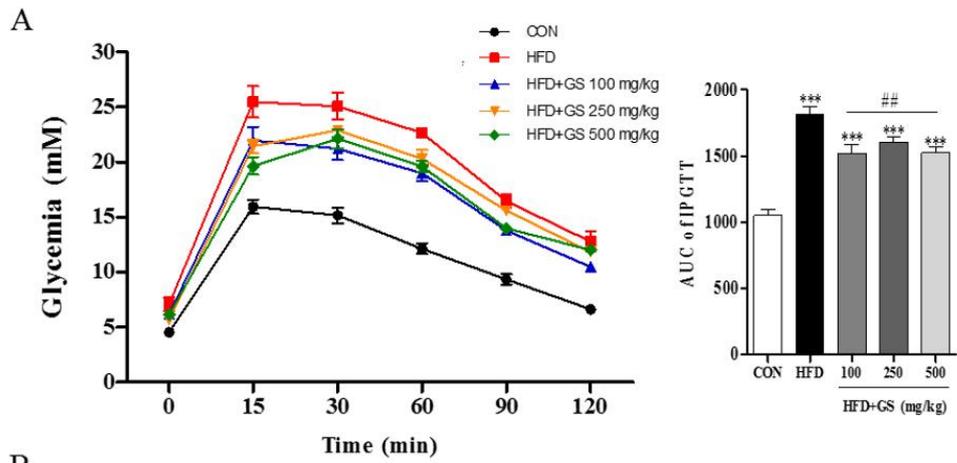
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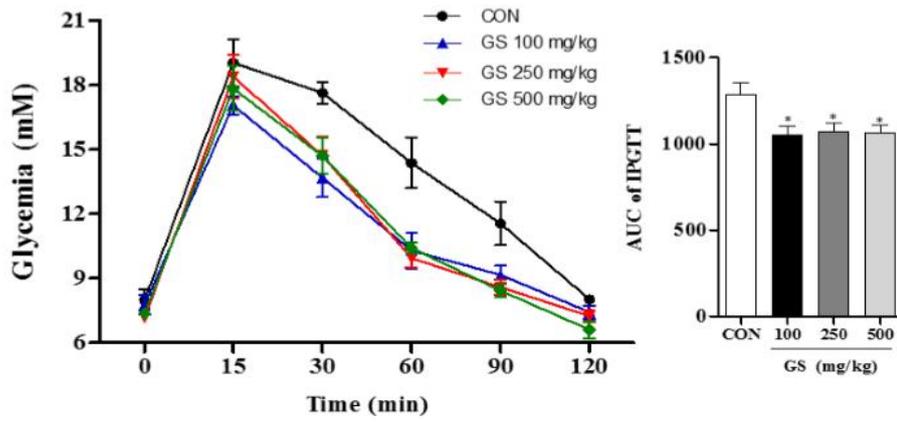
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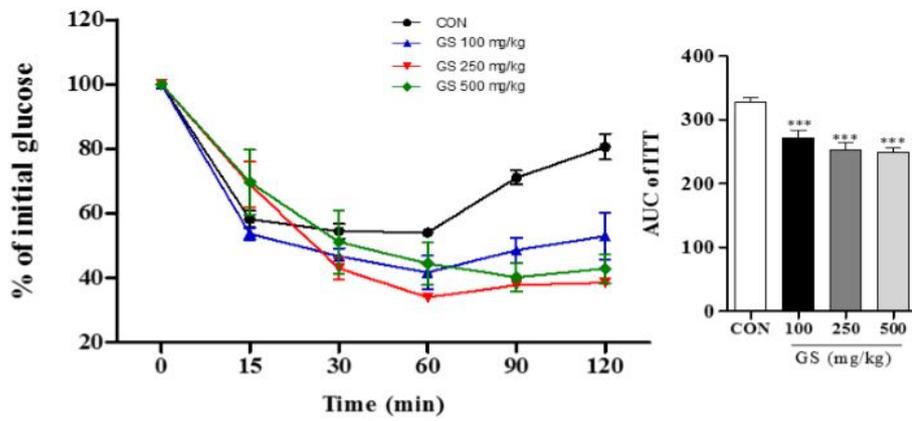
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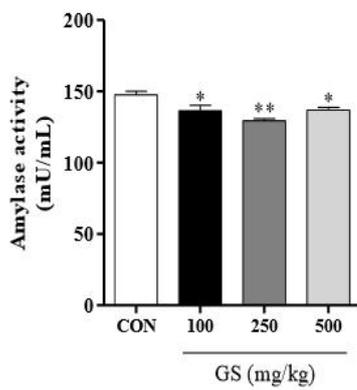
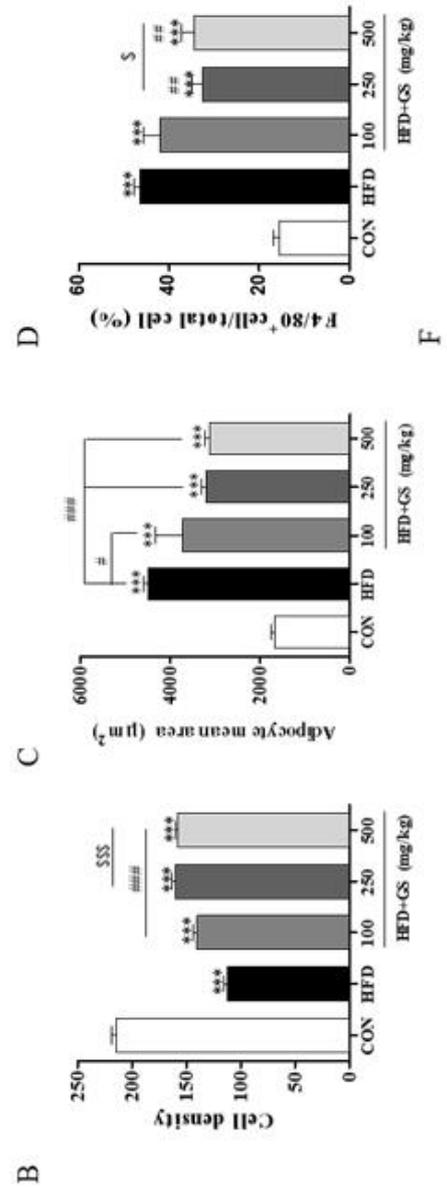
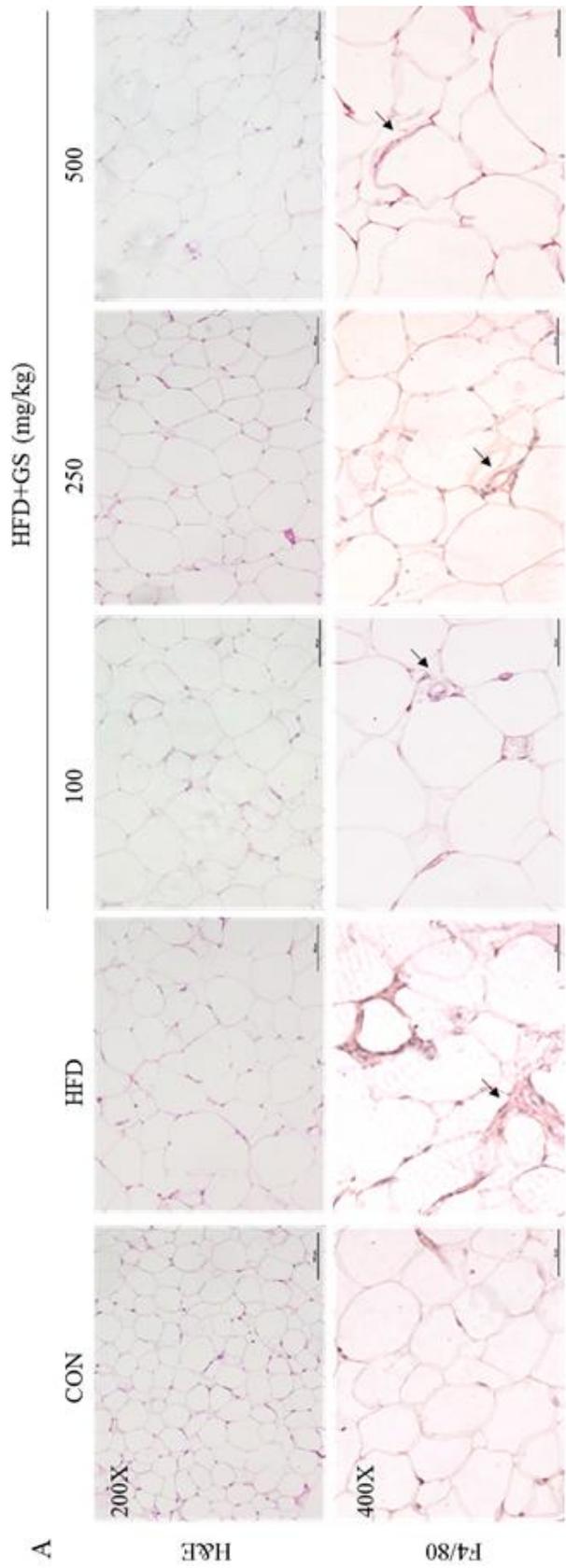


Figure 1. Effects of *Gymnema sylvest*re extract on glucose intolerance and insulin resistance. (A) Intraperitoneal glucose tolerance test (IPGTT) after 8 weeks and the area under the curve (AUC), (B) insulin tolerance test (ITT) after 8 weeks and the area under the curve (AUC), (C) postprandial glucose levels after single oral administration in normal C57BL6J mice and the area under the curve (AUC), (D) insulin tolerance test (ITT) after single oral administration in normal C57BL/6J mice and the area under the curve (AUC) and (E) amylase activity. Values are mean \pm SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. control (CON) group, # $p < 0.05$, ## $p < 0.01$ vs. high-fat diet (HFD) group.



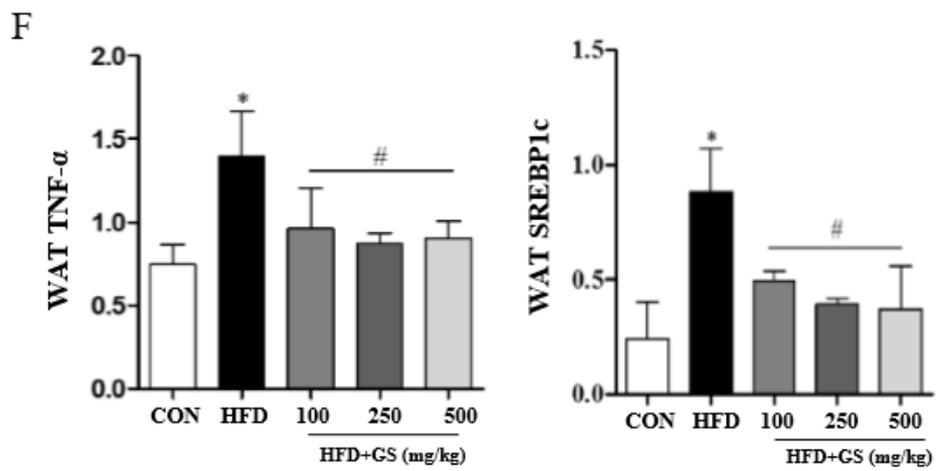
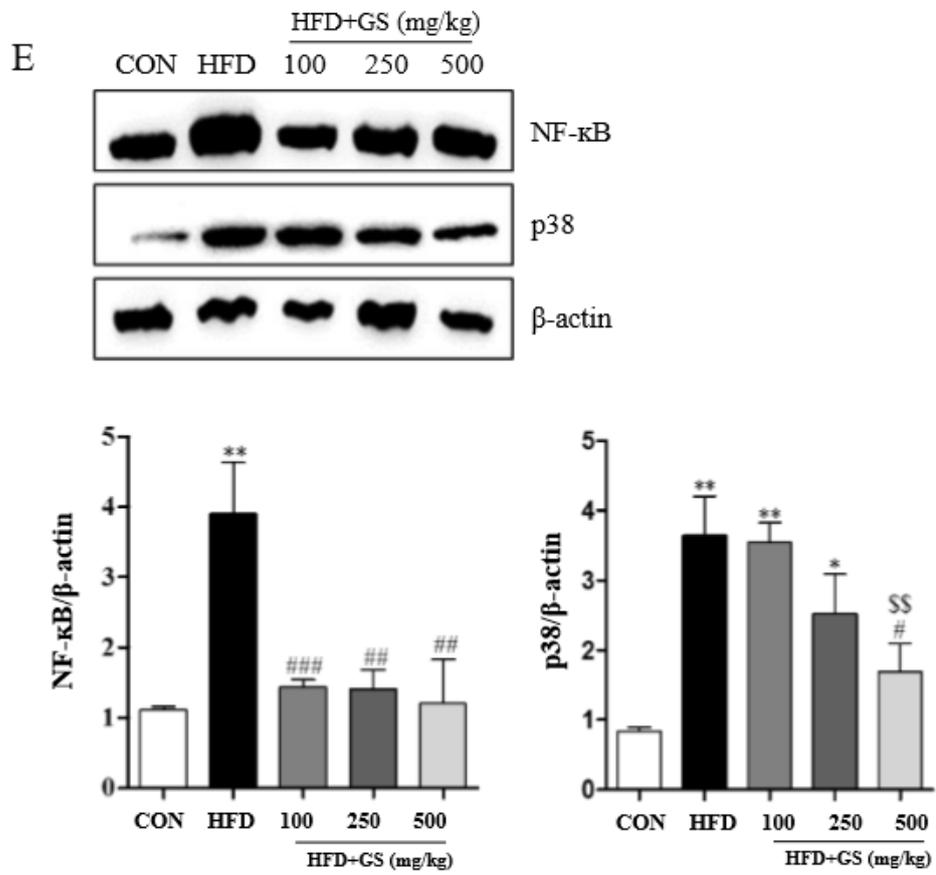
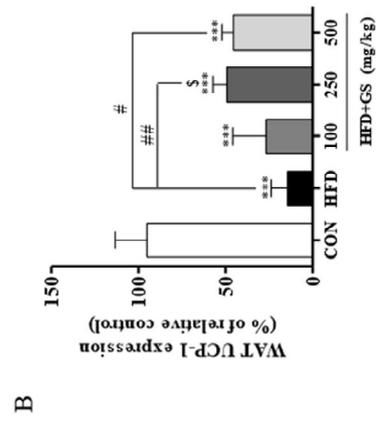
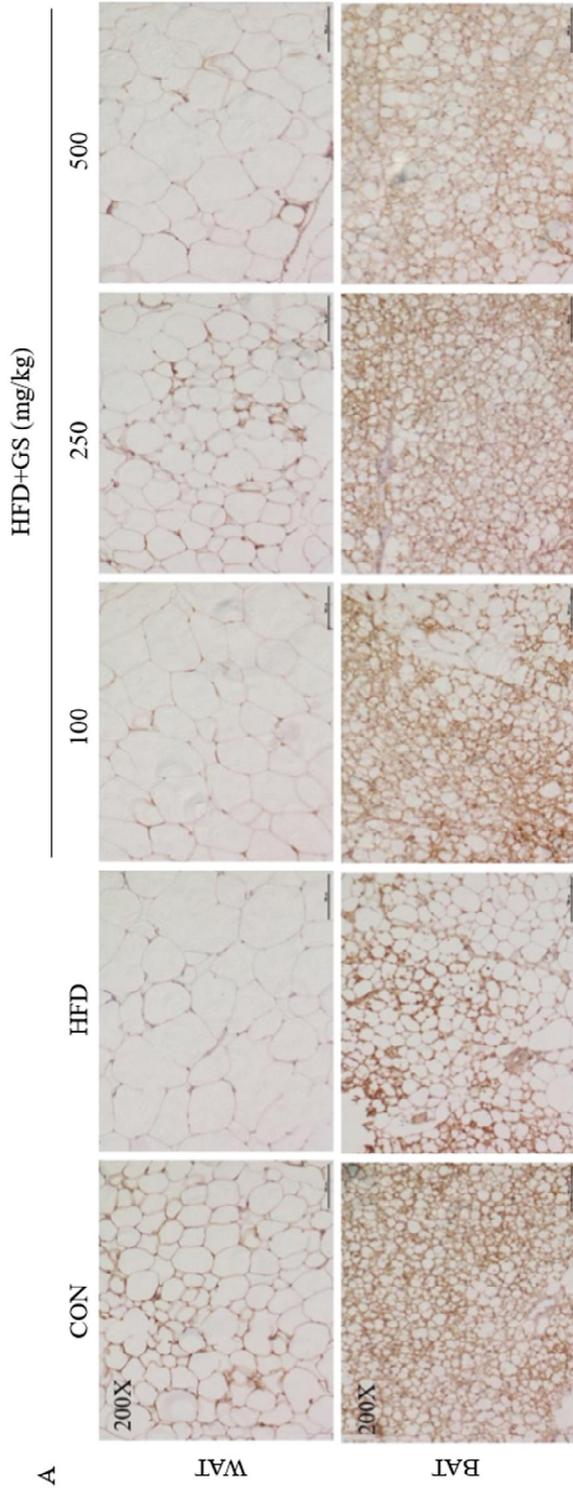


Figure 2. Effects of *Gymnema sylvestre* extract on adiposity and inflammation in white adipose tissue. (A) White adipose tissue stained with hematoxylin and eosin and F4/80 immunohistochemistry (black arrow: macrophage infiltration), (B) adipocyte density, (C) adipocyte mean area, (D) quantitative assessment of F4/80-positive cells per total cell, (E) relative protein levels of NF- κ B, p38 and β -actin by western blot and densitometry, and (F) mRNA expression levels of TNF- α and SREBP-1c by qRT-PCR. Scale bars = 50 μ m. Values are mean \pm SEM. * p < 0.05, ** p < 0.01, *** p < 0.001 vs. control (CON) group, # p < 0.05, ## p < 0.01, ### p < 0.001 vs. high-fat diet (HFD) group, \$ p < 0.05, \$\$ p < 0.01, \$\$\$ p < 0.001 vs. high-fat diet containing GS 100 mg/kg body weight (HFD+GS 100 mg/kg) group.



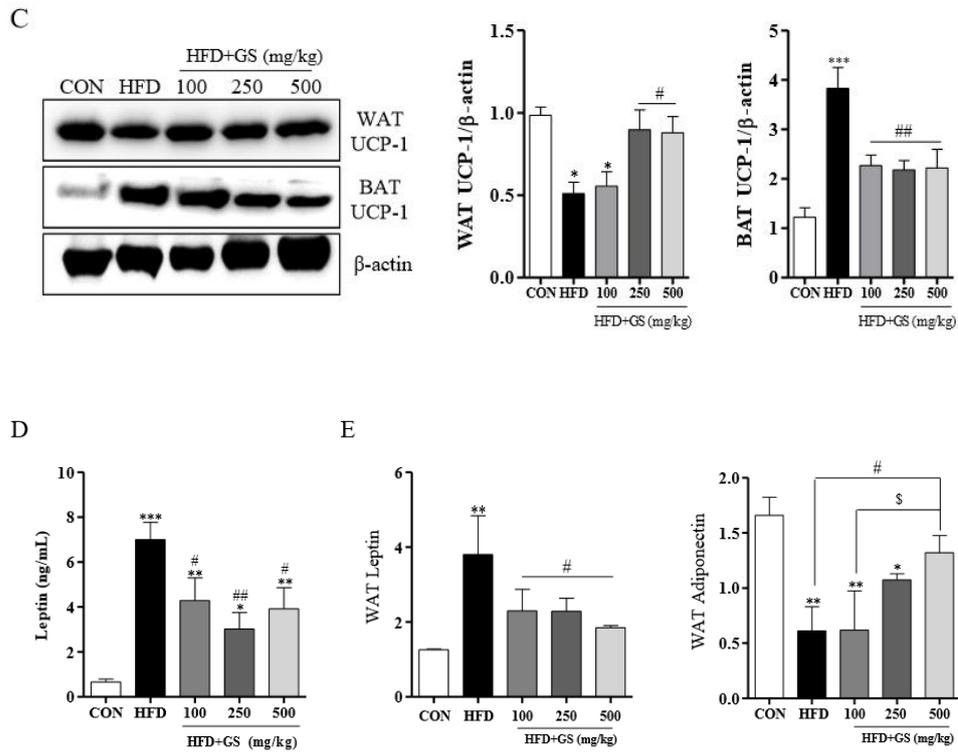
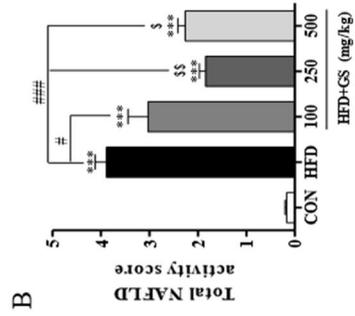
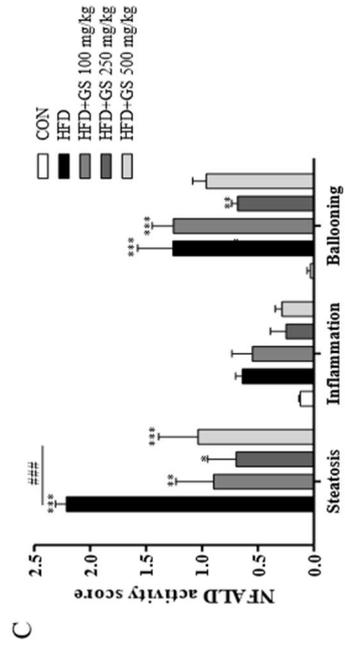
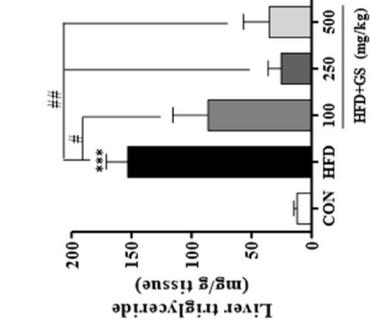
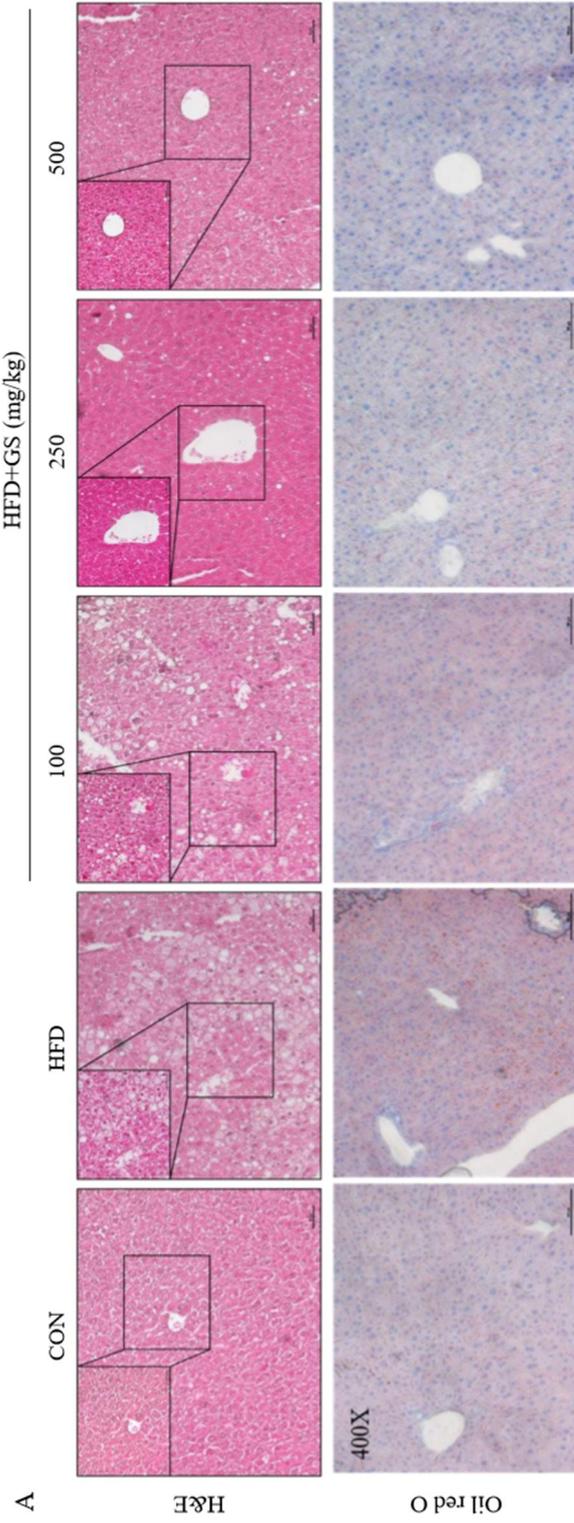


Figure 3. Effects of *Gymnema sylvestre* extract on energy homeostasis in white and brown adipose tissue. (A) Immunohistochemistry of UCP-1 in white and brown adipose tissue, (B) quantitative assessment of UCP-1-positive expression in white adipose tissue and (C) relative protein levels UCP-1 and β -actin in white and brown adipose tissue by western blot and densitometry, (D) serum leptin levels and (E) mRNA expression levels of leptin and adiponectin in white adipose tissue. Scale bars= 100 μ m. Values are mean \pm SEM. * p < 0.05, ** p < 0.01, *** p < 0.001 vs. control (CON) group, # p < 0.05, ## p < 0.01 vs. high-fat diet (HFD) group, \$ p < 0.05 vs. high-fat diet containing GS 100 mg/kg body weight (HFD+GS 100 mg/kg) group.



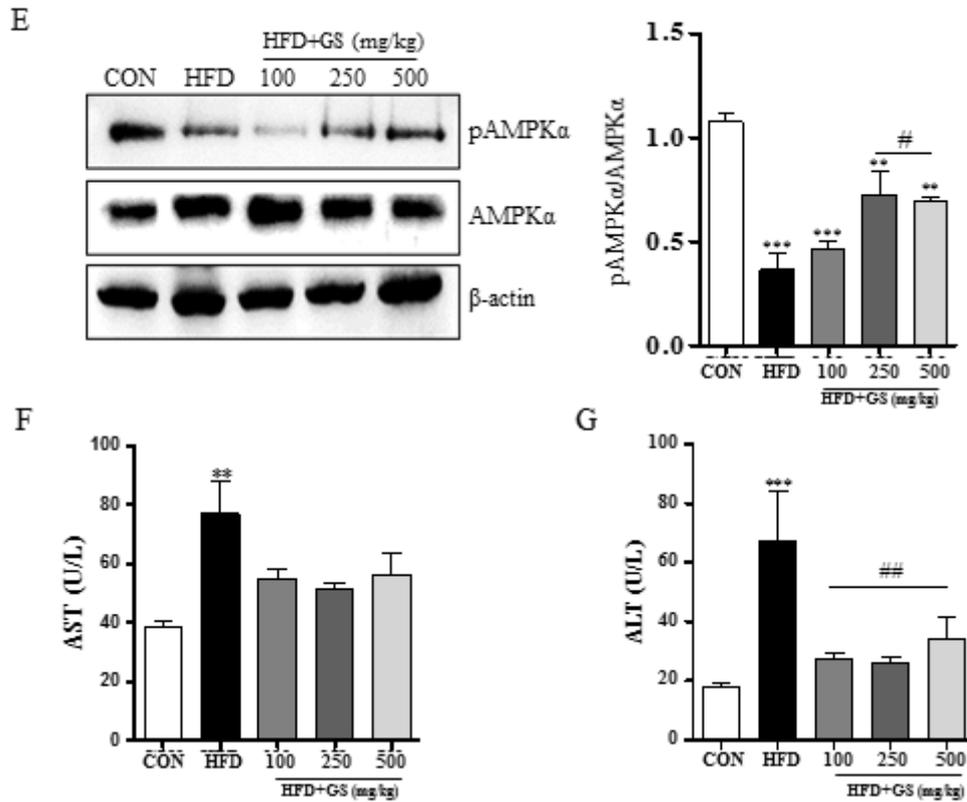
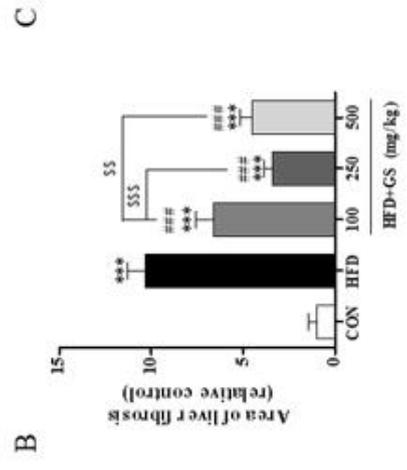
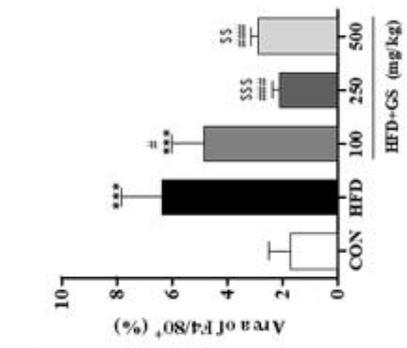
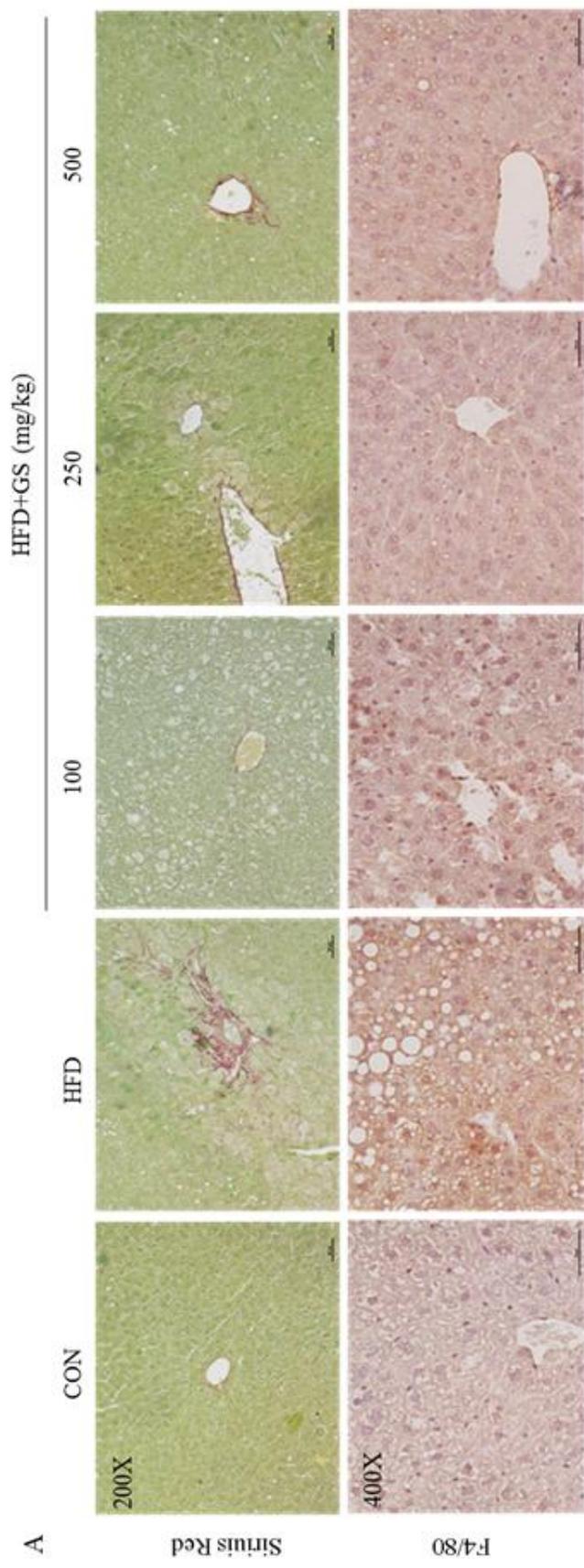


Figure 4. Effects of *Gymnema sylvestre* extract on lipid accumulation in the liver. (A) Liver tissue stained with hematoxylin and eosin and oil-red O, (B) the non-alcoholic fatty liver disease (NAFLD) score, (C) separate scores for steatosis, inflammation and ballooning, (D) total liver triglyceride (TG) content after 8 weeks, (E) relative protein levels of pAMPK α , AMPK α and β -actin by western blot and densitometry, (F) serum aspartate aminotransferase (AST) level and (G) serum alanine transaminase (ALT) level. Scale bars = 100 μ m. Values are mean \pm SEM. * p < 0.05, ** p < 0.01, *** p < 0.001 vs. control (CON) group, # p < 0.05, ## p < 0.01, ### p < 0.001 vs. high-fat diet (HFD) group.



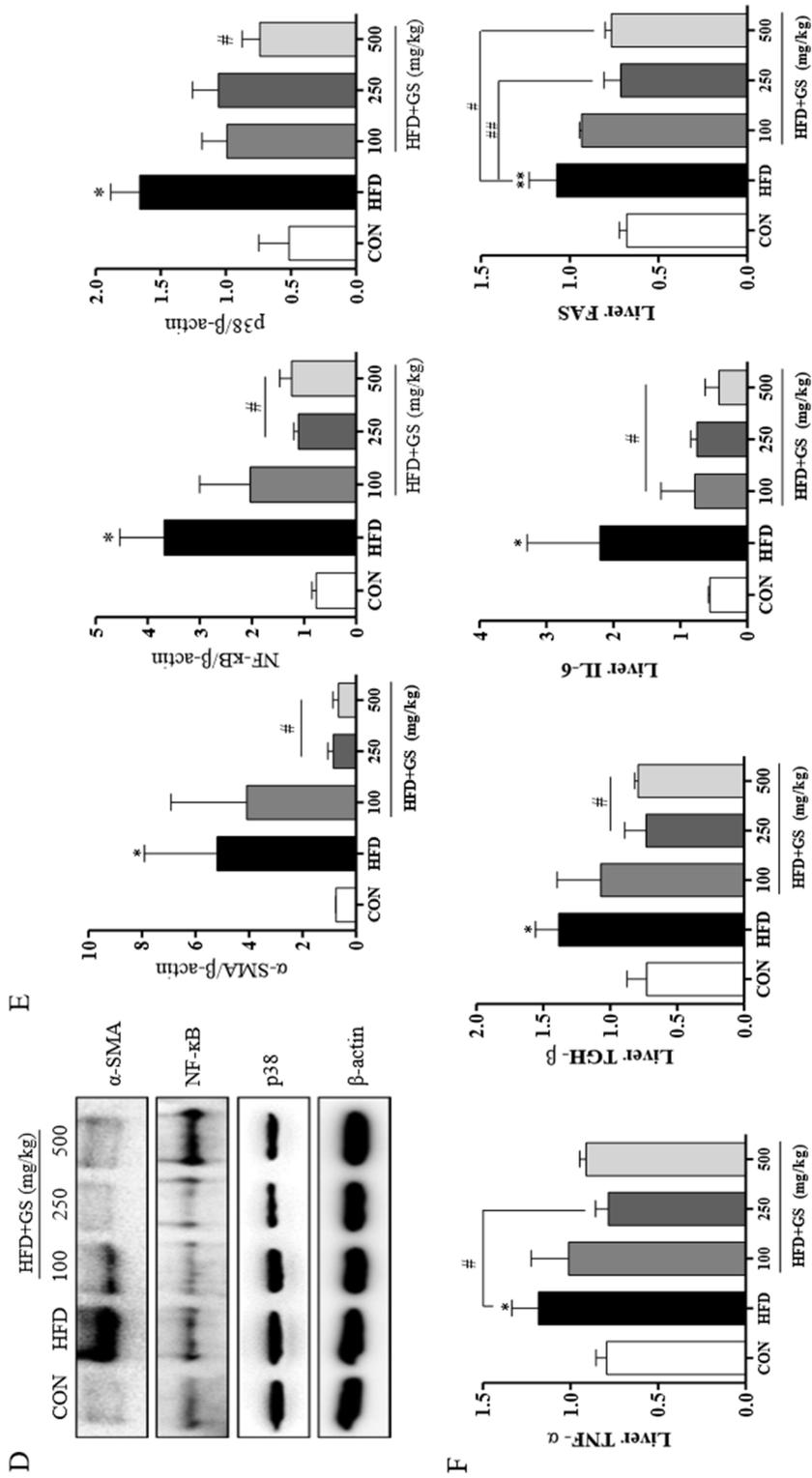


Figure 5. Effects of *Gymnema sylvestre* extract on hepatic inflammation and fibrosis. (A) Liver tissue stained with Sirius red and F4/80 immunohistochemistry, (B) intensity of Sirius red, (C) intensity of F4/80 immunohistochemistry, (D) relative protein levels of α -SMA, NF- κ B, p38 and β -actin by western blot, (E) western blot densitometry, and (F) mRNA expression levels of TNF- α , TGF- β , IL-6 and FAS by qRT-PCR.

Scale bars = 50 μ m. Values are mean \pm SEM. * p < 0.05, ** p < 0.01, *** p < 0.001 vs. control (CON) group, # p < 0.05, ## p < 0.01, ### p < 0.001 vs. high-fat diet (HFD) group, \$\$ p < 0.01, \$\$\$ p < 0.001 vs. high-fat diet containing GS 100 mg/kg body weight (HFD+GS 100 mg/kg) group.

Table 1. Characteristics of mice after 8-week experimental period

	CON	HFD	100	250	500
			HFD+GS (mg/kg)		
Initial BW (g)	25.38±0.42	26.08±0.44	24.85±0.28	24.63±0.53	24.88±0.35
Final BW (g)	31.60±0.57	41.79±1.70 ^{***}	37.49±1.08 ^{**,††}	37.45±0.66 ^{**,†}	37.35±1.09 ^{***,†}
△BW (g)	6.22±0.24	15.72±1.29 ^{***}	12.64±0.82 ^{***,†}	12.83±0.15 ^{***,†}	12.47±0.75 ^{***,†}
Food intake (kcal/day/mouse)	13.76±1.53	16.54±1.29 ^{**}	15.56±2.06	15.65±0.99	14.77±1.35 ^{††}
Total energy intake (kcal)	780.35±95.60	893.48±105.35	882.73±126.77	876.51±55.33	853.22±101.92
Food efficiency (%)	3.11±0.25	9.66±2.65 ^{***}	7.23±0.88 ^{***,††}	7.32±0.32 ^{***,†}	7.32±1.72 ^{***,†}
Energy efficiency (mg/kcal)	7.70±1.00	18.75±3.35 ^{***}	12.55±4.85 ^{***,†}	14.10±1.00 ^{***,††}	13.95±3.25 ^{***,††}
Organ weight					
Liver weight (g)	1.03±0.01	1.45±0.03 ^{***}	1.29±0.03 ^{***,†††}	1.27±0.04 ^{***,†††}	1.32±0.02 ^{***,††}
Epididymal fat weight (g)	0.79±0.23	3.49±0.67 ^{***}	2.80±0.45 ^{***,††}	2.59±0.44 ^{***,†††}	2.54±0.48 ^{***,†††}
Peritoneal fat weight (g)	0.41±0.12	1.18±0.14 ^{***}	1.09±0.15 ^{***,††}	1.03±0.18 ^{***,††}	1.03±0.17 ^{***,†}
Abdominal fat weight (g/g BW)	3.39±0.82	10.82±1.46 ^{***}	10.13±2.54 ^{***}	9.20±1.59 ^{***,†}	10.00±2.30 ^{***,†}

All values are mean ± SEM. (n=8-10) ^{**} $p < 0.01$, ^{***} $p < 0.001$ vs. control (CON) group, [†] $p < 0.05$, ^{††} $p < 0.01$,

^{†††} $p < 0.001$ vs. high-fat diet (HFD) group.

Table 2. Effect of GS on the glucose homeostasis and insulin resistance

	CON	HFD	100	250	500
			HFD+GS (mg/kg)		
Initial fasting glucose (mg/dl, 0wk)	85.56±1.94	85.30±2.07	86.50±1.70	86.80±2.18	85.50±1.55
Final fasting glucose (mg/dl, 8wk)	81.88±0.98	139.17±11.33 ***	114.00±4.62 **†	108.86±3.56 **†	112.86±5.30 **†
Serum insulin (ng/ml)	0.11±0.01	0.18±0.02 *	0.13±0.02 *	0.10±0.01†	0.11±0.01†
HOMA-IR ¹⁾	0.59±0.06	1.77±0.30 **	0.93±0.17††	0.70±0.11††	0.72±0.11††
Insulin sensitivity	1.74±0.17	0.60±0.10 *	1.19±0.23	1.55±0.25†	1.47±0.19†
HbA1c (%)	3.66±0.13	4.80±0.38 *	4.12±0.22	4.16±0.08	3.87±0.12†

All values are mean ± SEM (n=5-7). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. control (CON) group, † $p < 0.05$, †† $p < 0.01$ vs. high-fat diet (HFD) group.

¹⁾HOMA-IR was calculated as follows: glucose (mmol/L) x fasting insulin (μIU/mL)/22.5

Table 3. Serum levels of lipid

	CON	HFD	HFD+GS (mg/kg)		
			100	250	500
Total cholesterol (mg/dl)	67.00±3.00	142.00±2.00 ^{***}	121.00±11.00 ^{***,†}	107.00±23.00 ^{***,†}	122.00±20.00 ^{***}
Triglycerides (mg/dl)	41.00±7.00	62.00±20.00 ^{**}	41.00±9.00 ^{††}	40.00±10.00 ^{††}	48.00±14.00 ^{††}
HDL-cholesterol (mg/dl)	33.20±10.22	15.49±7.04 [*]	15.94±7.04 [*]	19.12±10.67	24.57±7.95
LDL/VLDL-cholesterol (mg/dl)	4.36±1.36	19.58±6.13 ^{***}	18.21±7.95 ^{**}	10.27±2.27 [†]	10.04±2.50 [†]
Creatine (mg/dl)	0.33±0.02	0.34±0.04	0.33±0.03	0.30±0.02	0.34±0.00
Total protein (g/dl)	4.09±0.30	4.71±0.23 ^{**}	4.62±0.34 [*]	4.31±0.31	4.45±0.29 [*]
Leptin (pg/ml)	0.82±0.38	7.06±1.67 ^{***}	3.67±2.28 ^{***,†}	3.36±2.02 ^{*,††}	3.51±2.22 ^{*,†}

All values are mean ± SEM (n=5-7). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. control (CON) group, † $p < 0.05$, †† $p < 0.01$, ††† $p < 0.001$ vs. high-fat diet (HFD) group.

국문초록

당살초의 고지방 식이에 의한 대사 질환 개선 효과 연구

김 현 정

서울대학교 수의과대학

수의학과 수의병인생물학 및 예방수의학 전공

현대 사회에서 비만 인구 비율이 높아지고, 그에 따른 당뇨병, 만성 염증, 간 손상, 지방과다증 등 다양한 대사 질환이 증가하고 있다. 비만의 가장 큰 원인으로 고지방식이 섭취에 따른 칼로리의 불균형이 꼽히고 있으며, 공중보건의 중요한 화두로서 전 세계적으로 예방책을 찾기 위해 많은 연구가 진행 중이다. 특히 생물학적 활성을 가지는 천연물을 이용한 항비만, 항당뇨제 연구가 활발하며, 본 연구에서는 당살초 추출물을 이용하여 고지방 식이에 의해 유도되는 여러 대사질환 개선 효과를 연구하고자 한다.

당살초는 아프리카, 인도, 중국에서 많이 발견되는 식물로서 주요 활성 성분인 김네마산을 이용한 연구가 많이 진행되고 있다. 하지만 대부분의 연구에서 유전 질환 모델을 또는 당뇨병 모델을 제작해 주로 경구 투여 형식으로 효과를 검증하였다. 따라서 본 연구에서는 당살초를 일반 식이와 고지방식에 일정 비율로 섞어 정상 설치류에 먹인 후 그 효과를 간, 지방조직 및 혈당 측정을 통해 항비만, 항당뇨 효과를 확인하였다.

정상 수컷 설치류를 정상식이 그룹, 고지방식이 그룹, 고지방 식이에 당당살초의 농도 (100, 250, 500 mg/kg b.w). 총 5그룹으로 세분화 하고 기간을 8주로 늘려 진행하였다. 그 결과 체중 및 간, 지방 무게가 유의적으로 감소했으며, 인슐린 저항성 개선 효과를 확인하였다. 또한 간과 지방의 염증을 완화 및 혈청에서의 콜레스테롤 수치에서도 유의적인 효과를 보였다.

이번 연구는 인슐린 저항성 및 비알콜성 지방간, 지방과다증 및 렙틴, 아디포넥틴 등 관련 사이토카인 분석을 통해 당살초의 항비만, 항당뇨 효과를 통합적 접근법으로 분석한 결과이며, 당살초가 아밀레이즈 효소 활성 감소 기전과 관련이 있다는 것을 확인하였다.

앞으로 당살초가 생체 내 어떠한 기전으로 항비만, 항당뇨 효과를 보이는지에 대해 연구가 더 필요하다고 생각된다.

핵심 단어: 당살초, 고지방식이, 비만, 당뇨, 비알콜성 지방간, 지방과다증

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