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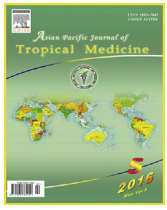

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Effects of feeding a diet containing *Gymnema sylvestre* extract: Attenuating progression of obesity in C57BL/6J mice

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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