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# Anti-diabetic and anti-obesitic effects of aqueous extracts of Yangkyuksanhwa-tang and its two major compositions on *db*/*db* mice

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ABSTRACT

Type 2 diabetes mellitus (T2DM) is a metabolic syndrome that results from target-tissue resistance to insulin. Obesity is the condition of excess body fat accumulation. T2DM and obesity are both associated with hypertension, hyperlipidemia, and abdominal obesity. In Korean medicine, Yangkyuksanhwa-tang (YKSHT) has been prescribed for patients with T2DM. Oral glucose tolerance tests (OGTT), multiplex assays and hemoglobin $A_{1C}$ (HbA1C) assessments were performed to determine the anti-diabetic effects of YKSHT and two major compositions of YKSHT, *Lonicera japonica* Thunb. (LJT) and *Rehmannia glutinosa* (RG) on *db/db* mice, a rodent model for T2DM. To study the anti-obesitic effects of LJT, RG or YKSHT, blood profiling including the triglycerides (TGs) and the total, LDL and HDL cholesterol levels were measured. In addition, body index measures such as the liver, retroperitoneal and epididymal fat tissues were collected and weighed. Mice treated with RG or YKSHT showed reduced blood glucose levels after stimulating the plasma GLP-1 levels. The multiplex assay results support the weight-controlling effects of the LJT, RG and YKSHT treatments, showing reducing levels of ghrelin and the induction of peptide YY (PYY) secretion. The YKSHT treatment reduced plasma TG levels and increased HDL cholesterol levels. The weights of the liver, retroperitoneal and epididymal fat tissues were reduced after the YKSHT treatment. Hence, we suggest that YKSHT can be utilized for the prevention and treatment of T2DM and obesity simultaneously.


## 1. Introduction

Currently, in the human population, diabetes is one of the principal causes of morbidity and mortality [1]. There are two main types of diabetes mellitus. A minor type is type 1 diabetes mellitus (T1DM), with patients experiencing pancreatic β-cell failure resulting from autoimmune destruction and leading to an absolute loss of insulin [2]. The far more common type, Type 2 diabetes mellitus (T2DM), is swiftly increasing in prevalence in modern societies [1]. T2DM is a metabolic disorder that is characterized by hyperglycemia resulting from target-tissue resistance to insulin that cannot be conquered by beta-cell hyper secretion [3,4]. The percentage of people who have both T2DM and obesity has increased throughout the whole of Asia, and the rates of increase in different countries show no signs of slowing. The international diabetes federation (IDF) found that in 2003, 194 million people had diabetes. They also hold that 333 million people will have this disease by 2025 [5]. The increased number of those with obesity, a medical condition in which excess body fat has accumulated to a great extent, has begun to attract attention a worldwide problem [6,7]. Obesity is associated with several conditions, and T2DM may be the most devastating among them [8].

T2DM is linked closely with obesity because both T2DM and obesity are associated with insulin resistance and have common characteristics such as hypertension, hyperlipidemia, and abdominal obesity [9]. Although obesity and T2DM are risk factors for cardiovascular disease, there have been no combined T2DM and obesity target treatment studies owing to side effects. Therefore, most treatments of metabolic syndrome only focus on the regulation of blood glucose levels or on weight loss in the patient.

Glucagon-like peptide-1 (GLP-1), a gastrointestinal hormone secreted from enteroendocrine L cells, has been well studied as a

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potential anti-hyperglycemic agent because its functions increase insulin secretion and sensitivity [10–12]. Peptide YY (PYY), another gastrointestinal hormone secreted from enteroendocrine L cells, acts to moderate appetite by inhibiting gastric motility and increasing water absorption in the colon [13]. On the other hand, ghrelin, known as the hunger hormone, is secreted form ghrelin cells in the gastrointestinal tract and increase gastrointestinal motility to prepare the body for food intake [14,15].

Yangkyuksanhwa-tang (YKSHT) is frequently prescribed in Korean traditional medicine regimens for the treatment of diabetes [16]. However, it lacks scientific evidence of its efficacy as a T2DM treatment. Therefore, in order to provide experimental evidence regarding the clinical use of YKSHT in T2DM, we studied the anti-diabetic and anti-obesitic effects of YKSHT and the major herbal medicine compositions of YKSHT, LJT and RG, in a *db*/*db* mice model, a mouse model of T2DM [17]. To determine the anti-diabetic effects, the oral glucose tolerance test (OGTT) and a multiplex assay were conducted with *Lonicera japonica* Thunb. (LJT), *Rehmannia glutinosa* (RG) and YKSHT to stimulate GLP-1 secretion in *db/db* mice, with the finding that these herbal medicines and decoctions were able to regulate blood glucose levels. Defining the anti-obesitic effects of LJT, RG and YKSHT, other gastrointestinal hormones secreted as a result of LJT, RG and YKSHT were investigated. Moreover, the weights of liver, epididymal and retroperitoneal fat tissues were measured on the last day.

## 2. Materials and methods

### 2.1. Animal

Seven weeks old male *db*/*db* mice (Daehan Bio Link Co., Ltd, Eumseong-gun, Chungcheonbuk-do, South Korea), genetically modified based on the C57BL/6 background, was used for this study, and all animal studies were performed according to protocols approved by the Institutional Animal Care and Use Committee (IACUC) from Kyung Hee University (confirmation number: KHUASP(SE)-14-046). Animals were housed in a specific-pathogen free (SPF) animal room in the condition of a 12 h light-dark cycle at moderate temperature (21–23 °C) and humidity levels (55–60%). All mice were acclimated for a week before the experiment. During the acclimation period, all mice were fed with standard rodent chow [28.507% of protein, 13.496% of fat and 57.996% of carbohydrates (LabDiet, St. Louis, MO, USA)] and water *ad libitum*.

### 2.2. Preparation of LJT, RG and YKSHT extracts

Two herbal medicines; LJT and RG, and YKSHT were obtained from KHMC Medical Science Research Institute (Seoul, Korea). The preparation of theses herbal medicines and decoction are described in references [18–20]. The procedure in brief is as follow: each medicinal plants was performed reflux extraction with distilled water (D.W) for 3 h at 100 °C. Filtration and evaporation were performed with rotary vacuum evaporator (N–N series, EYELA, Japan) at 60 °C. The solution was freeze dried for 24 h at −80 °C and lyophilized to yield. The composition and dosage of YKSHT are epitomized in Table 1.

### 2.3. Analysis of LJT, RG, and YKSHT extract using by LC/ESI–MS with SIM

LC/ESI–MS in positive and negative ion selected ion monitoring (SIM) mode was performed in the Korea Basic Science Institute (Seoul, Korea). Chromatographic separation of two herbal medicines and decoction by Agilent 1290 Infinity LC (Agilent Technologies, Santa Clara, CA, USA) was performed using a Zorbax Eclipse Plus $C_{18}$ column (50 mm × 2.1 mm i.d.,1.8 μm, Agilent). The mobile phase consisted of solvent A: water and solvent B: acetonitrile both containing 0.1% formic acid. The flow rate of mobile phase was 300 μl/min and the gradient program was: 0–15 min (5–90% B in A), 15–20 min (90% B), 20–23 min (90–50% B), and the column was then equilibrated with 5% B for 7 min. Sample of 2 μl was injected into the column using an auto sampler. The HPLC system was interfaced to the MS system, an Agilent 6550 Accurate-Mass Q-TOF (Agilent Technologies, Santa Clara, CA, USA) equipped with a Dual AJS ESI source operating in positive and negative ion mode. ESI spray voltage was set to 4000 V (Vcap). Mass spectra were acquired at a scan rate of 1.5 spectra/s with a mass range of 100–1700 *m*/*z*.

**Table 1**
Compositions and dosage of Yangkyuksanhwa-tang.

| Herbal medicine name | Dosage |
|---|---|
| *Lonicera Japonica* Thunb. | 7.5 g |
| *Rehmannia glutinosa* | 7.5 g |
| *Forsythiae Fructus* | 7.5 g |
| *Gardeniae Fructus* | 3.75 g |
| *Menthae Herba* | 3.75 g |
| *Anemarrhena Rhizoma* | 3.75 g |
| *Gypsm Fibrosum* | 3.75 g |
| *Schizonepetae Herba* | 3.75 g |
| *Ledebouriellae Radix* | 3.75 g |

### 2.4. Measurement of liver, retroperitoneal fat, and epididymal fat tissue weight

All mice were divided into 5 groups ($n$ = 6–7), matched for body weight over the week preceding the start of the experiment. Mouse were orally treated saline, 100 mg/kg of metformin, 100 mg/kg of LJT, 100 mg/kg of RG or 300 mg/kg of YKSHT extract every other day for 8 weeks. At the day of the experiment, all groups of mice were sacrificed then liver, retroperitoneal fat and epididymal fat tissue of each mouse were enucleated.

### 2.5. OGTT

OGTT was performed at the 7th week of the experiment. All mice were fasted 16 h before the experiment. On the experimental day, glucose gavage (5 g/kg) was carried out after an oral administration of each drug: saline, metformin, LJT, RG or YKSHT. The blood glucose levels were measured from the tail vein using an Accu-Chek Performa device (Roche Diagnostics, Mannheim, Germany); measurements were taken immediately before feeding (time point 0) and 10 (10 min after the glucose gavage) and at 20, 40, 90 and 120 min.

### 2.6. Blood sampling

All mice were allowed to rest for three days to prevent hypotensive or hemorrhage shock after OGTT. After three days, mice were re-fasted 16 h before the experiment. The first blood samples of each group (40 μl) were collected from tail vein and transferred into an EDTA-coated 1.5 ml micro centrifuge tube including dipeptidy1 peptidase-4 (DPP-4) inhibitor (EMD Millipore CO., Billerica, MA, USA), 4-(2-Aminoethyl)benzenesulfonyl fluoride hydrochloride (AEBSF; Sigma-Aldrich, St. Louis, MO, USA) and a protease inhibitor cocktail (Roche Diagnostics, Mannheim, Germany) to protect blood coagulation for determination of plasma hormone levels: GLP-1, insulin, PYY and ghrelin. After the first blood samples collection, each mouse group was orally administrated saline, metformin, LJT, RG or YKSHT just before the glucose gavage (2 g/kg). After each drug and glucose