Anti-obesitic Effect of *Orostachys japonicus* in Rats Model fed a Hyperlipidemic Diet†

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Abstract — We investigated the effect of *Orostachys japonicus* extracts on serum lipids, leptin and insulin level in hyperlipidemic rats. Also, diacylglycerol acyltransferase (DGAT) activity and thiobarbituric acid reactive substance (TBARS) were assessed. Inhibitory effect of DGAT related to triglyceride synthesis emerged approximately 96% in EtOAc fraction and showed 90% and 67%, respectively, in CHCl3 and BuOH fractions. Furthermore, the EtOAc and BuOH fractions inhibited 81% and 77%, respectively, in glycerol-3-phosphate acyl transferase (GPAT). Hyperlipidemia and obesity marker, contents of leptin and insulin on serum of hyperlipidemic rats, decreased 50% and 25%, respectively, compared with control group in treated EtOAc fraction. The oxidative stress marker, a concentration of TBARS, showed decrease of approximately 30% in treated EtOAc fraction. Moreover, high density lipoprotein (HDL)-cholesterol contents on serum of rats fed a hyperlipidemic diet were increased 10% and low density lipoprotein (LDL)-cholesterol decreased 50% as well as triglyceride amount of feces multiplied approximately two times more than control group in treated EtOAc fraction. The data suggest that the fractions of *O. japonicus* may be a potent biomaterial for treatment of hyperlipidemia or obesity.

Keywords — *Orostachys japonicus*, diacylglycerol acyltransferase (DGAT), glycerol-3-phosphate acyl transferase (GPAT), hyperlipidemia, obesity

Introduction

Obesity and overweight have been regarded as disease in association with various factors, such as genetic background, quantity of physical effort, fat, salt, and sugar etc. (Kopelman, 2000). They have caused many health problems, hypertension, type II diabetes, cardiovascular disease, dyslipidemia and metabolic disease. Moreover, according to recent research, overweight and obesity were causative of 44% of diabetes, 23% of ischemic heart disease, and 7–41% of cancer patients (Lee, 1992; WHO, 1997). *Orostachys japonicus* A. Berger (Crassulaceae), a perennial herbaceous plant has known as the plant growing on the rock of a remote mountain or the old filed roof. It has been used as a treatment for cancer in traditional medicine. Major compounds of *O. japonicus* have known as triterpene, sterols, and flavonoids. The *O. japonicus* has been reported to show antioxidant, anticancer, inhibition of HIV-1 Protease, and hepatoprotective effect (Park et al., 1991; Jung et al., 2007). For a long time, we have been trying to find potent antiobesitic herbal medicine. A search of non-toxic antiobesitic medicine without any side effect is of major importance. Among them methanol extract of *O. japonicus* and its fractions were reported for its antiobesity activity in high lipid diet-induced hyperlipidemic rat model (Kim et al., 2009). Continuously, we have tried to present a detailed study on *MeOH* extract along with different fractions of *O. japonicus* regarding its antiobesity activity.

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117
Materials and Methods

Plant material – Whole plants of *O. japonicus* were purchased from the Chunjil Yakupsa in Wonju. Plant was identified by professor Byung-Sun Min, College of Pharmacy, Catholic University of Daegu.

Extraction and Fractionation – The *O. japonicus* (3.6 kg) was extracted with MeOH three times for 3 hr under reflux. The extract was concentrated in vacuo and obtained solid MeOH extract (250 g). It was suspended in distilled water (2 l) and then partitioned with CHCl₃ (112 g), EtOAc (16 g), BuOH (251 g) and H₂O (24 g) fraction, respectively.

Diacylglycerol acyltransferase (DGAT) assay – According to the method of Oh et al. (2009), enzyme used DGAT, that is microsomal protein of rat [1-¹⁴C] oleoyl-CoA and sn-1, 2-diacyl-sn-glycerol were used as substrates. After a 10 min microsomal protein reaction, heptane and H₂O was added and it extracted in order to separate the [¹⁴C] triglyceride substance. After mixing the supernatant with the alkaline ethanol solution, radioactivity of supernatant was measured by liquid scintillation counter (LSC).

Glycerol-3-phosphate acyltransferase (GPAT) assay – According to the method of Lewin et al. (2004), mitochondrial protein (GPAT) separated from liver of rat served as enzyme. Palmitoyl-CoA and [¹⁴C] glycerol-3-phosphate were used as substrates. After enzymatic reaction, the radioactivity amount of [¹⁴C] lysophosphatidic acid (LPA) product was measured with LSC.

Animals and sample treatment – Four weeks old male rats (Sprague Dawley) were purchased from Biolink Co., Chingbuk, Korea and adapted them in the institutional animal facility (temperature: 22 ± 3°C, relative humidity: 50 ± 10%, light/dark cycle: 12 hr) for one week. Animals were fasted for 24 hr prior to the experiment with water randomly. Considering the change of enzyme activity, they were sacrificed at fixed time (10:00-12:00 A.M.). The MeOH extract and fractions (CHCl₃, EtOAc, BuOH, H₂O) of *O. japonicus* dissolved in 4% tween 80 each at 100 mg/kg body weight, were administered perorally using a oral sonde for four weeks. The whole procedure of animal experiments was authorized by Kyungsung Animal Care and Use Committee and carried out according to the “Guide for Care and Use of Laboratory Animals” published by the National Institutes of Health.

High fat diet induced hyperlipidemia – Hyperlipidemia was induced in the rats with high fat diet added beef tallow for six weeks (Table 1).

Contents of leptin and insulin in serum – Concentration of leptin was determined by immunno analyzer using an assay kit (Linco Research, St. Charles, MI. USA) and content of insulin was measured with γ-counter by radioimmuno assay using a RIA kit.

Measurement of total cholesterol (TC) content – According to the method of Richmond (1976), total cholesterol was determined with cholesterol assay kit (AM 202-K, Asan). Reagent (cholesterol esterase 20.5 U/l, cholesterol oxidase 10.7 U/l, sodium hydroxide 1.81 g/l) was dissolved in reagent solvent (potassium phosphate monobasic 13.6 g/l, phenol 1.88 g/l) in ice bath. Sample (20 µl) was added with 3.0 ml of prepared solution and incubated at 37°C for 5 min. Absorbance was measured at 500 nm with considering blank.

Measurement of high density lipoprotein-cholesterol (HDL-C) content – According to the method of Nomura et al. (1978), HDL-C was determined with assay kit (AM 203-K, Asan). Briefly, Reagent (0.2 ml-dextran sulfate 0.1%, magnesium chloride 0.1 M) was added with 20 µl of serum and centrifuged at 3,000 rpm for 10 min. Supernatant (0.1 ml) was taken and added with enzyme reagent (3 ml), vortexed and then incubated at 37°C for 5 min. Absorbance was measured at 500 nm with considering blank.

Measurement of Low density lipoprotein-cholesterol (LDL-C) content – According to the method of Fridewald...