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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 $CHCl_3$ 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 tiled 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.

†Dedicated to professor KiHwan Bae for his leading works on bioactive natural products.
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Materials and Methods

Plant material – Whole plants of *O. japonicus* were purchased from the Chunil 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 $CHCl_3$ (112 g), EtOAc (16 g), BuOH (251 g) and H_2O (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-^{14}C] oleoyl-CoA and *sn*-1, 2-dioloeoylglycerol were used as substrates. After a 10 min microsomal protein reaction, heptane and H_2O was added and it extracted in order to separate the [1-^{14}C] 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. Palmitotyl-CoA and [^{14}C] glycerol-3-phosphate were used as substrates. After enzymatic reaction, the radioactivity amount of [^{14}C] lysophosphatidic acid (LPA) product was measured with LSC.

Animals and sample treatment – Four weeks old male rats (Sprague Dawley) were purchased from Biolink Co., Chngbuk, 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 h 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 ($CHCl_3$, EtOAc, BuOH, H_2O) 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).

Table 1. Composition of basal and hyperlipidemic diet

Ingredient	Basal Diet (%)	Hyperlipidemic Diet (%)
Casein	20.0	20.0
DL-Methionine	0.3	0.3
Corn Starch	15.0	15.0
Sucrose	50.0	34.5
Fiber[1)]	5.0	5.0
Corn oil	5.0	–
AIN-mineral Mixture[2)]	3.5	3.5
AIN-vitamin Mixture[3)]	1.0	1.0
Choline Bitartate	0.2	0.2
Beef Tallow	–	20.5

[1)] Cellulose: Sigma Co. LTD., USA
[2)] Mineral mixture based on the pattern of Rogers and Haper (1965) contain the following (g/kg diet): calcium phosphate dibasic 500.0, sodium chloride 74.0, potassium citrate monohydrate 220.0, potassium sulfate 52.0, magnesium oxide 24.0, magnesium carbonate 3.5, ferric citrate 6.0, zinc carbonate 1.6, cupuric carbonate 0.3, potassium iodate 0.01, chromium potassium sulfate 0.55, sucrose, finely powered make 1,000.
[3)] Vitamin mixture (g/kg diet): thiamine HCl 0.6, biotin 0.02, riboflavin 0.6, cyanocobalamine 0.001, pyridoxine HCl 0.7, retinyl acetate 0.8, nicotinic acid 3.0, DL-tocopherol 3.8, Ca-pantothenate 1.6, 7-dehydrocholesterol 0.0025, folic acid 0.2, methionine 0.005, sucrose, finely powered make 1,000.

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

Measurement of total cholesterol (TC) content – According to the method of Richimond (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 Noma *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

et al. (1972), LDL-C content was calculated using following formula;

LDL-C = [(Total cholesterol – HDL-C) – triglyceride/5)]

Measurement of triglyceride (TG) content – According to the method of McGowan (1983), triglyceride was determined with assay kit (AM 157S-K, Asan). Reagent (lipoprotein lipase 10,800 U, glycerol kinase 5.4 U, peroxidase 135,000 U, L-α-glycerol phosphoxidase 160 U) was dissolved in reagent solvent [N,N-bis(2-hydroxyethyl)-2-aminomethane sulfonic acid 0.427 g/dl] in ice bath. Sample (20 μl) was added with 3.0 ml of prepared solution and incubated at 37 °C for 10 min. Absorbance was measured at 550 nm with considering blank.

Measurement of TBARS and protein carbonyl concentration of hepatic homogenate – According to the method of Ohkawa *et al*. (1979), liver tissue was homogenized in 0.1 M potassium phosphate buffer (pH 7.4) in ice bath using glass Teflon homogenizer and incubated at 37 °C for 3 hr. After then, homogenate was added with 8.1% sodium dodecyl sulfate, 20% acetate buffer (pH 3.5) and 0.8% thiobarbituric acid and incubated at 95 °C for 1 hr. Solution was added with *n*-BuOH : pyridine (15 : 1) and centrifuged at 15 min. Supernatant was taken and measured at 532 nm. 1,1,3,3-tetramethoxypropane (TMP) was used as standard and measured values was converted as quantity of malondialdehyde (MDA).

Mesurement of body weight and fat fad weight – Change of body weight was measured in one week. Weight of retroperitoneal and epididymal fat fad weighed after sacrificing rats.

Determination of fecal lipids – Contents of Fecal lipids (TC and TG) were determined with dry feces according to method of Folch *et al* (1957). Dry feces was extracted with $CHCl_3$: MeOH (2 : 1) at 4 °C for 24 hr. Supernatant was measured with TC and TG assay kit.

Statistical analysis – Values are represented as mean ± S.D. Statistical analysis was performed by Duncan's multiple range test using the SPSS (version 11.5, SPSS Institute, Chicago, IL). Significance decided on $p < 0.05$.

Results and Discussion

Enzyme activity of isolated compounds from *Orostachys japonicus* – DGAT and GPAT are the enzymes known to be concerned with triglyceride biosynthesis in obesity (Smith *et al*., 2000). GPAT is an enzyme a catalyst doing the first step of glycerol phosphate pathway, and DGAT is an enzyme that a catalyst did the last process in triglyceride synthesis (Coleman *et al*., 2004). They were carried out with different fractions of *O. japonicus* as an enzyme activity test *in vitro* related with obesity. Inhibitory effect on DGAT of EtOAc and $CHCl_3$ fractions were approximately 96% and 90% respectively. Furthermore, MeOH extract and BuOH fraction showed the inhibitory effect of approximately 67% but water fraction did not show any significant activity. In GPAT activity, EtOAc fraction had the highest inhibitory effect (81%) among the different fractions and BuOH fraction inhibited approximately 77%. Also, MeOH extract and $CHCl_3$ fraction inhibited 61% and 50% respectively but H_2O fraction showed the lower inhibitory effect (24%). From the active ethyl acetate-soluble fraction, three major compounds kaempferol, quercetin, isoquercitrin were isolated. The isolated compounds were identified by comparing with reference (Park *et al*., 1991). Only kaempferol (100 μg/ml) inhibited 46% in GPAT. But, in contrary with the fraction activity, the isolated compounds did not show any significant activity in DGAT and GPAT (Table 2).

Table 2. Inhibitory effect of fractions and isolated compounds from *O. japonicus* on DGAT and GPAT

Sample	Conc. (μg/ml)	DGAT	GPAT
		Inhibition (%)	
MeOH	125	67.0	61.5
$CHCl_3$	125	90.2	50.0
EtOAc	125	96.6	81.9
BuOH	125	67.2	77.7
H_2O	125	13.3	24.8
Kaempferol	30	3.9	16.9
	100	0.1	46.4
Quercetin	30	-12.5	13.4
	100	0.2	19.2
Isoquercitrin	30	-7.2	-9.4
	100	-9.0	16.0

The measured values were presented as the means of triplicate experiments.
Conc. : concentration

Concentration of leptin and insulin – When leptin produced from adipocyte along with insulin produced from pancreatic β-cells is high in blood, it stimulates POMC/CART neuron which inhibits NPY, AgRP and GABA neuron and it transmit signal of dietary suppression in secondary neuron (Shintani *et al*., 2001). Even though leptin and insulin causes dietary suppression, their high concentration in blood causes increase in lipid content in the body which is the major symptoms of hyperlipidemia and obesity. So, leptin and insulin are regarded as a factor

of obesity. In Table 3, the serum concentration of leptin and insulin in normal group was 8.79 ± 0.55 and 2.51 ± 0.14 ng/ml respectively which was observed in control group to be 26.3 ± 3.21 and 4.15 ± 0.31 ng/ml respectively. The EtOAc fraction of *O. japonicus* showed a decrease of serum leptin and insulin concentration to 13.2 ± 1.43 and 3.07 ± 0.17 ng/ml which is inhibition rate 74% and 65% compared with control group. Again a dramatic inhibition was showed by BuOH fraction of *O. japonicus* with leptin and insulin concentration of 15.6 ± 0.99 and 3.31 ± 0.26 ng/ml respectively, which is 61% and 50% inhibition compared with control group. Meanwhile, MeOH extract of the plant showed inhibitory effect on serum concentration of leptin and insulin by 40% and 32% respectively while $CHCl_3$ and H_2O fractions do not have any significant activity.

Measurement of serum cholesterol content – Serum lipid content in hyperlipidemic rats treated with samples was tabulated in Table 4. HDL-cholesterol content of EtOAc and BuOH fractions was increased by 10% while MeOH extract, $CHCl_3$ and H_2O fractions was similar with that of control. LDL-cholesterol content of EtOAc and BuOH fractions was decreased by 52% and 46% respectively. Specially, EtOAc fraction showed similar the HDL-cholesterol content with that of normal group. In contrary, MeOH extract, $CHCl_3$ and H_2O fractions did not show any significant change in HDL-cholesterol content. Increase of LDL-cholesterol content will cause deposition of lipid in blood vessels while HDL-cholesterol transport lipid from peripheral tissue and blood vessel to liver for degradation of lipid which act as defense for atherosclerosis (Fungwe *et al*., 1993). Atherosclerosis can be measured by atherosclerosis index (AI), the EtOAc and BuOH fractions inhibited 40% and 35% of AI comparing with control group. This shows the inhibitory effect of lipid content on serum.

Measurement of lipid and protein in liver tissue – Increased lipid level related with hyperlipidemia and obesity induces the production of variety of reactive oxidative species (ROS) leading to lipid peroxidation (Penn *et al*., 1994). In such situation, if lack of antioxidants is inside body, the ROS and their by products will cause various diseases related to oxidative damage. The lipid peroxidation was evaluated as measuring peroxidation by products malondialdehyde (MDA) and carbonyl concentration from liver tissue. From the experiment, the MeOH extract decreased MDA by 22% and carbonyl concentration by 33% in hyperlipidemic rat model. Specially, in the EtOAc fraction treated group, MDA concentration was found to be 25.8 ± 3.56 nM/mg which is 51% less than that of control group while 36% inhibition was observed in case of BuOH fraction treated group in comparison with control group. Furthermore, carbonyl concentration in case of EtOAc fraction treated group was found to be 3.55 ± 0.60 nM/mg which was

Table 3. Effect of fractions of *O. japonicus* on serum leptin and insulin levels

Treatment	Dose (mg/kg)	Leptin	Insulin
		ng/ml	
Normal		8.79±0.55^{d}	2.51±0.14^{e}
Control		26.3±3.21^{a}	4.15±0.31^{a}
MeOH	100	19.3±1.43^{b}	3.62±0.33^{bc}
$CHCl_3$	100	24.8±0.87^{a}	3.98±0.29^{ab}
EtOAc	100	13.2±1.43^{c}	3.07±0.17^{d}
BuOH	100	15.6±0.99^{c}	3.31±0.26^{cd}
H_2O	100	26.0±1.13^{a}	3.82±0.22^{ab}

Values are represent mean±S.D. (n = 6). Values sharing the same superscript letter are not significantly different each other ($p < 0.05$) by Duncan's multiple range test.

Table 4. Effect of *O. japonicus* extracts on serum cholesterol and AI in rats fed a hyperlipidemic diet

Treatment	Dose (mg/kg)	Cholesterol (mg/dl)			AI
		Total	HDL	LDL	
Normal		60.9±4.63^{c}	44.5±1.36^{a}	2.64±0.33^{c}	0.37±0.09^{d}
Control		75.8±3.22^{a}	37.4±1.88^{c}	7.62±0.85^{a}	1.03±0.13^{a}
MeOH	100	74.6±4.21^{a}	38.6±3.10^{bc}	7.43±0.41^{a}	0.93±0.07^{a}
$CHCl_3$	100	75.0±3.16^{a}	37.0±3.19^{c}	6.98±0.30^{a}	1.03±0.06^{a}
EtoAc	100	68.1±2.13^{b}	42.5±2.23^{ab}	3.62±0.27^{b}	0.60±0.08^{c}
BuOH	100	70.6±2.43^{ab}	40.6±2.98^{abc}	4.10±0.29^{b}	0.74±0.05^{b}
H_2O	100	73.4±3.17^{ab}	36.5±4.11^{c}	7.49±0.47^{a}	1.01±0.03^{a}

Values are represent mean±S.D. (n = 6). Values sharing the same superscript letter are not significantly different each other ($p < 0.05$) by Duncan's multiple range test.
AI (Atherosclerosis Index) = (total cholesterol – HDL cholesterol) / HDL cholesterol

Table 5. Effect of fractions of *O. japonicus* on TBARS (Thiobarbituric Acid Reactive Substances) and protein carbonyl concentration of hepatic homogenate of rats fed hyperlipidemic diet.

Treatment	Dose (mg/kg)	TBARS	Carbonyl concentration
		MDA nM/mg protein	nM/mg protein
Normal		13.7±4.87^{d}	2.56±0.46^{d}
Control		38.9±5.43^{a}	4.93±0.98^{a}
MeOH	100	33.2±3.12^{ab}	4.13±0.42^{abc}
$CHCl_3$	100	39.1±2.87^{a}	4.54±0.38^{ab}
EtOAc	100	25.8±3.56^{c}	3.55±0.60^{c}
BuOH	100	29.6±2.11^{bc}	3.87±0.31^{bc}
H_2O	100	36.2±2.76^{a}	4.61±0.46^{ab}

Values are represent mean±S.D. (n = 6). Values sharing the same superscript letter are not significantly different each other ($p < 0.05$) by Duncan's multiple range test.

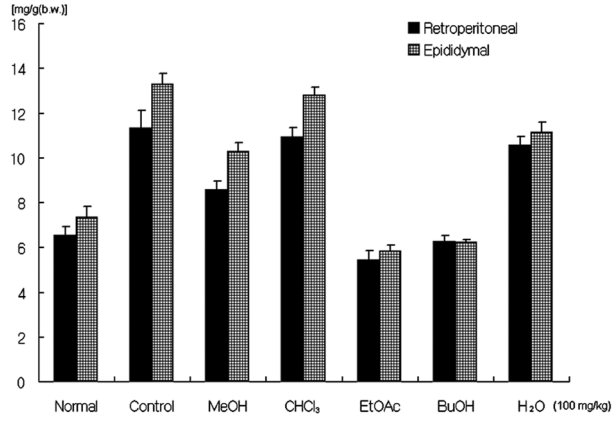

Fig. 1. Abdominal fat pad weight in the normal and high fat diet-induced obesity rats fed *O. japonicus* extracts for 4 weeks. Values are represent mean±S.D. (n = 6). Significance decided on $p < 0.05$ by Duncan's multiple range test. b.w.: body weight.

58% less than control and BuOH fraction treated group inhibited 44% as compared with control group. In contrast, $CHCl_3$ aqnd H_2O fractions did not show any significant activity (Table 5).

Measurement of change in body weight and fat pad weight – The major indication of obesity is deposition of adipose tissue especially in abdomen and other internal organs which is known to be serious threat for human body. We evaluated the retroperitoneal and epididymal fat mass of hyperlipidemic rat when they were treated with different fractions of *O. japonicus*. From the experiment, the MeOH extract dramatically reduced the retroperitoneal and epididymal fat mass by 57% and 50% respectively. Especially in case of EtOAc and BuOH fractions, the inhibitory effect was more strongly in comparison with normal group. The EtOAc fraction showed 123% and 125% decrease of retroperitoneal and epididymal fat mass and the BuOH fraction showed 105% and 118% decrease rate (Fig. 1). We had reported the non toxicity of the MeOH fraction of *O. japonicus* on previous report (Kim *et al.*, 2009).

Measurement of fecal lipid content and serum lipid content – The dry fecal content of EtOAc fraction of *O. japonicus* treated group was 0.72 ± 0.18 g/day while comparing with 2.57 ± 0.18 g/day of normal rats group, which shows the increase of water and lipid content. BuOH and H_2O fractions also showed 0.83 ± 0.13, 0.89 ± 0.16 g/day of dry fecal lipid content (Fig. 2A). Fecal triglyceride content in EtOAc fraction treated group was 48.9±4.17 mg/g which is double as that of control group. Fecal lipid content in BuOH fraction treated group was 40.7 ± 3.98 mg/g which is more than that of control group. Total-cholesterol content in EtOAc fraction treated group was 30.7 ± 1.43 mg/g and BuOH fraction treated

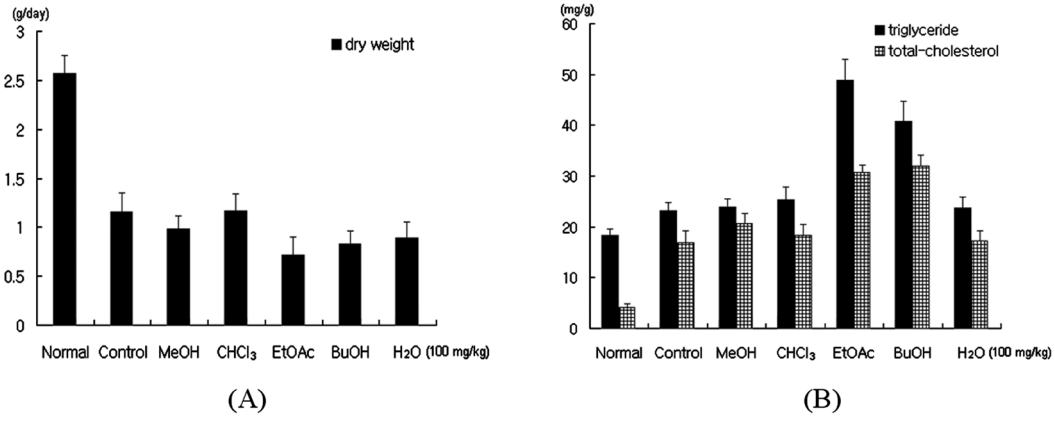

Fig. 2. Feces lipid contents of the normal and high fat diet-induced obesity rats fed *O. japonicus* extracts for 4 weeks. Values are represent mean±S.D. (n = 6). Significance decided on $p < 0.05$ by Duncan's multiple range test. (A) dry weight of feces, (B) feces lipid contents.

group was 31.9 ± 2.25 mg/g, which is seven times more than that of normal group and approximately two times more than that of control group. The increase in discharge of triglyceride content from body could be suggested as a contribution to antiobesitic activity (Fig. 2B).

Conclusion

In this study, the EtOAc fraction of *O. japonicus* in hyperlipidemic rats decreased serum lipids level and abdominal fat pad and showed inhibitory effect of DGAT and GPAT enzymes related to lipids metabolism such as fat storage in adipocytes. Also, it increased triglyceride content in feces and decreased concentration of insulin and leptin. Moreover, it lowered the content of TBARS related with lipid peroxidation. The effect of BuOH fraction was proved along with EtOAc fraction but should be more studied with EtOAc fraction in order to explain the exact mechanism.

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