Anti-obesity Activity of Peucedanum Japonicum Thunb Extract in Obese Diabetic Animal Model C57BL/6J Ham Slc-ob/ob Mice

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***Abstract*- Aim of the study is to evaluate the anti-diabetic activity of PJT. This study examined its anti-obesity activity in obese-diabetic animal model of C57BL/6J Ham Slc-ob/ob mice. By dividing animals into control and ethanol extract of PJT groups, body weight gain, tissue weight, biochemical parameters in serum, liver, fecal lipid concentrations and gene expression in the tissues were compared between control and PJT extract groups after been fed with the basal low-fat diet supplemented with or without PJT extract for four weeks. The results show that supplementation of the diet with ethanol extract of PJT significantly reduced white adipose tissue (WAT), serum triglyceride (TG), total cholesterol (TC) and adipocyte size. The ethanol extracts also significantly increase insulin sensitivity, fecal excretion of TG, TC, and decreased that of bile acid. The ethanol extract of PJT modulated the obesity related genes in liver, adipose tissues of ob/ob mice: upregulation of PBEF1, PPARα, and downregulation of FAS genes in the liver; upregulation of RORC, DGAT1, FXRα, PPARγ genes in the adipose tissue; increased expression of CPT1α, UCP2 and GLUT4 genes in muscle. In conclusion, it is suggested that ethanol extract of PJT exerted anti-diabetic activity through the modulation of obesity-related lipid parameters in obese-diabetic animal model.**

Keywords- Anti-obesity; C57BL/6J Ham Slc-ob/ob Mice; Lipid Metabolism; Gene Expression; Ethanol Extract of Peucedanum Japonicum Thunb

I. INTRODUCTION

Diabetes mellitus is a heterogeneous disorder characterized by deficiency in both insulin secretion and tissue sensitivity (Prato et al., 2002). The ability of insulin in glucose homeostasis is to maintain the blood glucose level constant by promoting the postprandial glucose clearance by facilitating the uptake by the peripheral tissues (Weyer et al., 1999). Excess body fat in obesity frequently causes insulin resistance, which is a major factor in the pathogenesis of type 2 diabetes mellitus (Bonadonna et al., 1990). Adipose tissue is not only an energy reservoir but also an endocrine and secretary organ, and the functionality of this tissue greatly influences the entire lipid metabolism via sensitization to insulin signaling (Zhao et al., 2006). Thus, the regulation of obesity is an important issue for the prevention of diabetes mellitus.

Peucedanum japonicum Thunb (PJT), a medicinal plant belonging to the family of Umbelliferae, grows on the cliffs of Okinawa Island, Japan. PJT leaves are traditionally consumed on Okinawa Island as a medicinal herb for the treatment of cough. The roots of this plant have been used as a folk medicine for cold and neuralgic diseases in Taiwan (Chen et al., 1996). A number of studies have reported on the physiological activities of PJT including antioxidant activity (Hisamoto et al., 2003), tyrosinase inhibitory effect (Hisamoto et al., 2004), and anti-platelet aggregation activity (Chen et al., 1996) *in vitro*. Furthermore, several lines of *in vitro* study have demonstrated the occurrence of hypolipidaemic compounds in the leaves of PJT (Hsu and Yen, 2007; Li et al., 2006). Our previous study demonstrated that dietary intake of PJT powder inhibited the high-fat diet-induced obesity in C57BL/6 mice (Okabe et al., 2011). This study explored the lipid lowering mechanism of PJT, and found that inhibition of lipid absorption and modulation of the obesity related gene expression was involved in the anti-obesity activity of PJT (Nukitrangsan et al., 2011). Furthermore, the occurrence of anti-obesity phytochemicals in PJT was confirmed by examining the *in vivo* anti-obesity activity of PJT extracts: water-, 50% ethanol- or ethanol-extract. Of the extract, ethanol extracts showed the highest anti-obesity activity with largely similar changes as shown for PJT powder with the diet-induced obesity animal model. Our foregoing studies thus suggested that ethanol extract of PJT might ameliorate the onset of diabetes by increasing the insulin sensitivity in liver, adipose and muscle tissues (Nukitrangsan et al., 2012).

This study thus extended these previous observations, and examined the anti-obesity activity of ethanol extract of PJT with obese diabetic animal model C57BL/6J Ham Slc-*ob/ob* mice. This mutant strain lacks leptin gene, and the deficiency of leptin signal in the hypothalamus causes obesity due to hyperphagia (Coleman, 1978). The *ob/ob* mice become resistant to insulin due to lack of leptin gene, and exhibit morbid obesity and metabolic abnormalities such as hyperglycemia, hyperinsulinemia and glucose tolerance (Zhang et al., 1994). These pathophysiological characteristics of *ob/ob* mice resemble those of human non-insulin dependent diabetes mellitus (Zhang et al., 1994). This study therefore investigated the anti-diabetic activity of PJT extract in this obese diabetes animal model for the first time.

II. MATERIALS AND METHODS

A. Materials

1) PJT:

PJT used in this study was cultivated and harvested in Yonaguni Island of Okinawa, Japan. PJT leaves and stems were freeze-dried and ground at Yonagunijima Yakusoen Co. (Okinawa, Japan). The dried powder of PJT was analyzed by Japan Food Research Laboratories (Tokyo, Japan), and its nutrient composition was found to be as follows: carbohydrate, 46.7%; fiber, 31.6%; mineral, 9.8%; protein, 7.6%; and fat, 4.4% (w/w).

2) Diet:

Casein, β-corn starch, cellulose, and mineral mix and vitamin mixes for AIN-76 rodent diets were purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan). DL-methionine and choline bitartrate were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).The composition of the experimental diets (AIN-76 purified diet) was as follows: carbohydrate, 66.4%; protein, 18.7%; fat; 5.0%; fiber, 5.0%; AIN-76 mineral mix, 3.5%; AIN-76 vitamin mix, 1.0%; DL-methionine, 0.3%; choline bitartrate, 0.2%; and energy, 385.0 kcal/100 g.

3) Preparation of Extract:

Dried powder (200 g) of PJT was extracted with 1 L of ethanol for 6 h at ambient temperature. This extract was centrifuged at 3000 x g for 10 min to remove insoluble matter. The supernatant was further filtered with filter paper No. 2 (Toyo Roshi Kaisha, Tokyo, Japan) evaporated under vacuum, freeze-dried and stored at -80^{0}℃.

4) Experimental Animals:

Five-week-old male C57BL/6 J Ham Slc-ob/ob (SPF Mouse) mice were purchased from Japan SLC, Inc. (Shizuoka, Japan). The animals were housed in a temperature-, humidity-, and light-controlled room (12-h light/dark cycle) and were allowed free access to water. The mice were maintained on commercial chow for 7 days for acclimatization.

B. Methods

1) Experimental Design and Animal Grouping:

Tatal 12 ob/ob mice were divided into control group (n=6) and ethanol extract group (n=6). The mice were fed control (0% PJT) and 0.4% ethanol extract of PJT with the semi-synthetic diet (AIN-76 purified diet) for four weeks. All animals were pair-fed on the diets and allowed free access to water.

2) Tissue Samples:

Mice were sacrificed after 6 h of fasting. Samples of serum, liver tissue, WAT and soleus muscle tissue samples were collected, and stored at -80°C. The weights of the liver tissue and WAT were measured immediately after collection.

3) Lipid Concentrations and Biochemical Parameters in the Serum and Liver:

Serum levels of total cholesterol (TC), triglyceride (TG), and glucose were determined using a commercial enzymatic kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Serum adiponectin, and insulin levels were measured using ELISA kits (insulin: Morinaga Institute of Biological Science, Inc., Yokohama, Japan; adiponectin: Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan). Total lipids were extracted from the liver tissues by a previously described method (Folch et al., 1957). Liver levels of TC and TG were determined by the Shoenheimer-Sperry method (Sperry and Webb, 1950) and Fletcher's method (Fletcher, 1986), respectively.

4) Lipid Concentration in Feces:

Feces were collected during the last 3 days of the feeding period and dried. The TC and TG contents in the fecal samples were extracted by a previously described method (Folch et al., 1957), and their levels were determined using commercial enzymatic assay kits (Wako Pure Chemical, Osaka, Japan), as described previously (Attie et al., 2002). Total bile acids were extracted from the feces with ethanol and were measured using the total bile acid test Wako kit (Wako Pure Chemical, Osaka, Japan).

5) Adipocyte Size:

Epididymal adipose tissue was fixed in 10% buffered formalin, embedded in paraffin, and stained with hematoxylin and eosin for microscopic measurement of cell sizes, as described elsewhere (Chen and Farese, 2002). The stained sections were viewed at 20× magnification, and photographed with a digital camera (Olympus BX41, NY, USA). The images were converted into a binary format by using Adobe Photoshop 5.0.1 software (Adobe Systems, CA, USA), and incorporated into Scion Image for measurement of the cross-sectional area. The total cross-sectional areas of at least 200 adipocytes were manually measured in four microscopic fields, and were used to calculate the average cell surface area (μm2).

6) Isolation of Total RNA:

Total RNA was extracted from 30 mg of liver tissue, epididymal adipose tissue, and soleus muscle tissue by using the RNeasy Lipid Tissue Mini kit (Qiagen, CA, USA). RNA purity was checked using agarose gel electrophoresis, and quantified with a UV spectrophotometer. Total RNA (5μg) was reverse transcribed to produce cDNA using the PrimeScriptTM 1st Strand cDNA Synthesis kit (Takara BIO Inc., Shiga, Japan). cDNA samples were stored at -80°C as 50 μL aliquots.

7) Quantitative Real-time RT-PCR:

Quantitative real-time RT-PCR was performed on an icycler (Bio-Rad Laboratory, Tokyo, Japan) using SYBR Green I (Takara Bio Inc., Shiga, Japan) and TaKaRa TaqTM Hot Start Version (Takara Bio Inc., Shiga, Japan), according to the manufacturer's instructions. The primer sequences are listed in Table Ⅰ. Amplification of the target cDNA was checked by agarose gel electrophoresis and by identification of its DNA sequence as described previously (Matsumoto et al., 2009). A standard curve for each gene was constructed by performing PCR with serially diluted known concentrations of the template, and used for the estimation of target mRNA, as described elsewhere (Wang et al., 2006). All analyses were performed in duplicate and normalized to the expression of GAPDH expression in the hepatic tissue, β-actin in the adipose tissues, and Cypa in the soleus muscle tissue.

C. Statistical Analysis

Statistical significance of the mean values of the two

groups was evaluated using Student's t test and the SAS statistical software program (SAS Institute, Tokyo, Japan).

TABLE Ⅰ NDA SEQUENCES OF PRIMERS USED FOR QUANTITATIVE REAL-TIME PCR

Gene	Description	Primer sequence	Product	Accession no.
CYP7A1	cytochrome P450, family 7, subfamily a, polypeptide 1	5'-AGC AAC TAA ACA ACC TGC CAG TAC TA-3' (forward primer, F) 5'-GTC CGG ATA TTC AAG GAT GCA-3' (reverse primer, R)	82	NM_007824
PBEF1 (Visfatin)	Pre-β-cell colony-enhancing factor 1	5'-GCT GAT CCC AAC AAA AGG TC-3' (forward primer, F) 5'-CTG CTC GAT GTT CAG CTG TG-3' (reverse primer, R)	204	NM_021524
PPP1R10	Protein phosphatase 1, regulatory subunit 10	5'-TGA TGG AGG CCA CAG TCA TG-3' (forward primer, F) 5'-GGT AGA AGG CAC AGT TGT TC-3' (reverse primer, R)	104	NM_00116381
RORC	RAR-related orphan receptor gamma	5'-CAA TTT GGA ACT GGC TTT CC-3' (forward primer, F) 5'- CTG AAG AGT TCC TTA TAG AG -3' (reverse primer, R)	189	NM_011281
DUSP1	Dual specificity phosphatase 1	5'-TGA CAT GCG CGT ATG AGA G-3' (forward primer, F) 5'- CTG GCT TTG TCT GTC AGT GC-3' (reverse primer, R)	182	NM_013642
INSIG2	Insulin induced gene 2	5'-CAG AGG TGG TTA TCA ACA TC-3' (forward primer, F) 5'-CTG TGA GCT TCA GTA GCC A -3' (reverse primer, R)	209	NM_133748
SERPINA12 (Vastin)	Serine (or cysteine) peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 12	5'-GCT CCG CAT CTC GTC TAC TT -3' (forward primer, F) 5'-CTT CCA TGC CTT TCT CAT CC -3' (reverse primer, R)	151	NM_026535
PPARα	Peroxisome proliferator activated receptor alpha	5'-CCT CAG GGT ACC ACT ACG GAG T-3' (forward primer, F) 5'-GCC GAA TAG TTC GCC GAA-3' (reverse primer, R)	69	NM_011144
PPARγ	Peroxisome proliferator activated receptor gamma	5'-AGG CCG AGA AGG AGA AGC TGT TG-3' (forward primer, F) 5'-TGG CCA CCT CTT TGC TGT GCT C-3' (reverse primer, R)	276	NM_011146
FAS	Fatty acid synthase	5'-TGC TCC CAG CTG CAG GC-3' (forward primer, F) 5'-GCC CGG TAG CTC TGG GTG TA-3' (reverse primer, R)	91	AF_127033
FXRα	Farnesoid X receptor alpha	5'-TGG GCT CCG AAT CCT CTT AGA-3' (forward primer, F) 5'-TGG TCC TCA AAT AAG ATC CTT GG-3' (reverse primer, R)	72	NM_00116350
SREBP-1c	Sterol regulatory element-binding protein-1	5'-GCG CCA TGG ACG AGC TG-3' (forward primer, F) 5'-TTG GCA CCT GGG CTG CT-3' (reverse primer, R)	205	NM_011480
C/EBPα	CCAAT/enhancer binding protein alpha	5'-TGG ACA AGA ACA GCA ACG AGT AC-3' (forward primer, F) 5'-GCA GTT GCC CAT GGC CTT GAC-3' (reverse primer, R)	257	NM_007678
PEG1/MEST	paternally expressed gene 1(Peg1)/mesoderm specific transcript (Mest)	5'-GTT TTT CAC CTA CAA AGG CCT ACG-3' (forward primer, F) 5'-CAC ACC GAC AGA ATC TTG GTA GAA-3' (reverse primer, R)	52	NM_008590
DGAT1	Diacylglycerol acyltransferase 1	5'-TCC GCC TCT GGG CAT TC-3' (forward primer, F) 5'-GAA TCG GCC CAC AAT CCA-3' (reverse primer, R)	65	NM_010046
ATGL	Adipose triglyceride lipase	5'-AGG ACA GCT CCA CCA ACA TC-3' (forward primer, F) 5'-TGG TTC AGT AGG CCA TTC CT-3' (reverse primer, R)	165	AY894805
LPL	Lipoprotein lipase	5'-AGG GCT CTG CCT GAG TTG TA-3' (forward primer, F) 5'-AGA AAT CTC GAA GGC CTG GT-3' (reverse primer, R)	199	NM_008509
UCP2	Uncoupling protein 2	5'-CAG GTC ACT GTG CCC TTA CCA T-3' (forward primer, F) 5'-CAC TAC GTT CCA GGA TCC CAA G-3' (reverse primer, R)	101	NM_011671
UCP3	Uncoupling protein 3	5'-CCA CCT TAG GGC AAG AAC GA-3' (forward primer, F) 5'-GGT ACG ACC CTG AGA TGA GAA AA-3' (reverse primer, R)	76	AB010742
GLUT4	Glucose transporter type 4	5'-CTG CAA AGC GTA GGT ACC AA-3' (forward primer,F) 5'-CCT CCC GCC CTT AGT TG-3' (reverse primer, R)	87	BC014282
PGC1α	Peroxisome proliferator-activated receptor gamma coactivator 1-α	5'-GGC ACG CAG CCC TAT TC-3' (forward primer,F) 5'-TCG ACA CGG AGA GTT AAA GGA-3' (reverse primer, R)	66	NM_008904
CPT1α	Carnitine palmitoytransferase 1 alpha	5'-AAA GAT CAA TCG GAC CCT AGA CA-3' (forward primer,F) 5'-CAG CGA GTA GCG CAT AGT CA-3' (reverse primer, R)	124	NM_013495
CPT1β	Carnitine palmitoytransferase 1 beta	5'-CCA AAC GTC ACT GCC TAA GCT-3' (forward primer,F) 5'-GGC CGC ACA GAA TCC AAG TA-3' (reverse primer, R)	75	NM_009948
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	5'-ACC CAG AAG ACT GTG GAT GG-3' (forward primer, F) 5'-ACA CAT TGG GGG TAG GAA CA-3' (reverse primer, R)	172	NM_008084
ACTB	Actin, beta, cytoplasmic	5'-CAG AAG GAG ATT ACT GCT CTG GCT-3' (forward primer, F) 5'-GGA GCC ACC GAT CCA CAC A-3' (reverse primer, R)	93	NM_007393
Cyp a	Cyclophilin a	5'-GGG TTC CTC CTT TCA CAG AAT TAT T-3' (forward primer, F) 5'-CCG CCA GTG CCA TTA TGG-3' (reverse primer, R)	78	AF171073

III. RESULT

A. Growth Parameter and Weight of the Liver and Adipose Tissues

A significant decrease in the mean weight of WAT was noted for the ethanol extract of PJT diet group in ob/ob mice. The mean final body weight, abdominal fat and subcutaneous fat weight tended to decrease in the ethanol extract group, compared with the control diet group. No significant differences were noted for food intake, energy intake and liver weight between treatments (Table Ⅱ).

B. Lipid Metabolism-related Parameters

Table Ⅲ lists the serum levels of TC, TG, glucose, insulin and adiponectin; levels of TC and TG in the liver; and fecal excretion of TC, TG and bile acid. Ob/ob mice fed the

ethanol extract of PJT diet showed significantly lower serum TC, TG levels than those fed the control diet. The liver TG level of the ethanol extract of PJT group was significantly lower than that of the control group. Increased fecal TC, TG and decreased bile acid excretion were also noted for the ethanol extract of PJT group.

TABLE Ⅱ EFFECT OF PEUCEDANUM JAPONICUM THUNB (PJT) EXTRACT ON GROWTH PARAMETERS, LIVER AND ADIPOSE TISSUE WEIGHTS

Parameters	Diet group[a]	
	Control	Ethanol extract
Food intake (g/day)	4.76 ±0.11	4.74 ±0.12
Energy intake (kcal/day)	18.27 ±0.43	18.18 ±0.45
Initial body weight (g)	31.8 ±0.8	31.8 ±1.1
Final body weight (g)	39.4 ±1.2	37.4 ±1.5
Liver weight (g/100g body weight)	9.97 ±0.57	8.44 ±0.61
White adipose tissue (g/100 g body weight)	22.08 ±0.53	19.96 ±0.63*
Abdominal fat	13.83 ±0.39	12.72 ±0.47
Epididymal fat	7.14 ±0.22	6.6 ±0.35
Perirenal fat	3.74 ±0.10	3.58 ±0.14
Omental fat	2.95 ±0.26	2.54 ±0.14
Subcutanous fat	8.25 ±0.46	7.42 ±0.41
Brown adipose tissue (g/100 g body weight)	0.22 ±0.02	0.21 ±0.03

a: C57BL/6 J Ham Slc-*ob/ob* mice were fed basal AIN76 diet containing 5% corn oil with 0% (control) or 0.4% of PJT ethanol extract for 4 weeks. Values are mean ± SE of six mice. Asterisk shows significant difference from the control (0% PJT) diet group by Student's *t* test (*: $p < 0.05$).

TABLE Ⅲ EFFECT OF PEUCEDANUM JAPONICUM THUNB (PJT) EXTRACT ON LIPID METABOLISM RELATED PARAMETERS

Parameters	Diet group[a]	
	Control	Ethanol extract
Serum		
Total cholesterol (mg/dL)	329.4 ±20.7	255.0 ±25.4*
Triglyceride (mg/dL)	129.1 ±5.3	79.1 ±10.8**
Adiponectin (μg/mL)	7.61 ±0.21	8.11 ±0.45
Glucose (mg/dL)	308.1 ±34.9	256.6 ±61.1
Insulin (ng/mL)	2.79 ±0.96	1.34 ±0.63
Liver tissue		
Total cholesterol (mg/g liver)	21.6 ±0.9	20.0 ±1.5
Triglyceride (mg/g liver)	404.4 ±25.3	304.9 ±37.2*
Fecal excretion		
Total cholesterol (mg/day)	1.14 ±0.07	1.45 ±0.08*
Triglyceride (mg/day)	0.23 ±0.05	0.57 ±0.03**
Bile acid (mg/day)	0.72 ±0.09	0.39 ±0.04*

a: C57BL/6 J Ham Slc-ob/ob mice were fed basal AIN76 diet containing 5% corn oil with 0% (control) or 0.4% of PJT ethanol extract for 4 weeks. Values are mean ± SE of six mice. Asterisk shows significant difference from the control (0% PJT) diet group by Student's t test (*: $p < 0.05$, **: $p < 0.01$)

Moreover, there was a tendency for serum adiponectin level to increase, and glucose and insulin level to decrease by the feeding of ethanol extract. In this study, the insulin resistance status was determined by the homeostatic metabolic assessment (HOMA), which was assessed by the formula: fasting insulin (μU/mL) × fasting glucose (mg/dL)/405 (Keskin et al., 2005). The animal group fed ethanol extract of PJT diet showed a significantly decreased HOMA index compared to control group (Fig. 1).

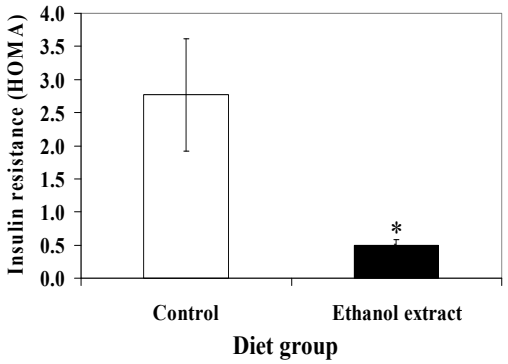
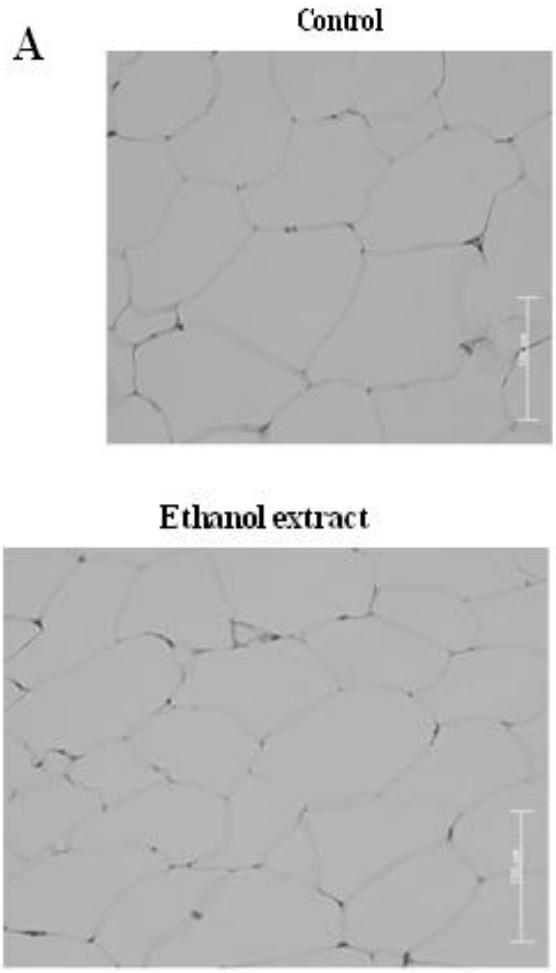


Fig. 1 Effect of ethanol extracts of Peucedanum japonicum Thunb (PJT) on insulin resistance as expressed by HOMA index. C57BL/6 J Ham Slc-ob/ob mice were fed basal AIN76 diet containing 5% corn oil with 0% (control) or 0.4% of PJT ethanol extract for 4 weeks. Values are mean ± SE of six mice. HOMA index: fasting insulin (μU/mL) × fasting glucose (mg/dL)/405. Asterisks denote a statistically significant difference from the control (0% PJT) diet group (Student's t test; *: $p < 0.05$)

C. Adipocyte Size

The mean cell size of epididymal adipocytes for the ob/ob mice fed with the ethanol extract of PJT diet was significantly smaller than that for the 0% control diet (Fig. 2). The average adipocyte sizes were 11116 μm2 and 6854 μm2 for control and PJT ethanol extract group, respectively (Fig. 2).



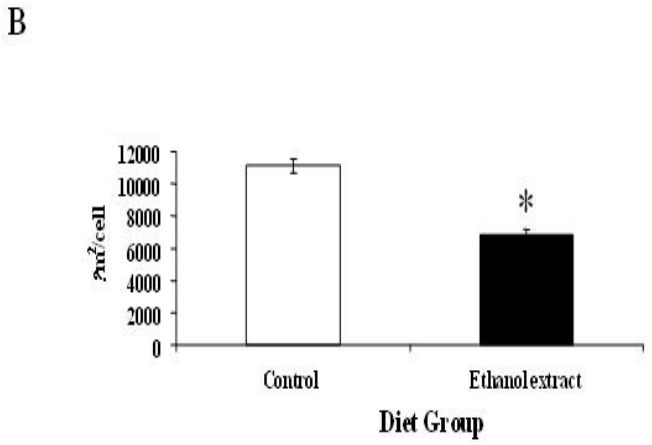


Fig. 2 Effect of ethanol extracts of Peucedanum japonicum Thunb (PJT) on adipocyte size C57BL/6 J Ham Slc-ob/ob mice were fed basal AIN76 diet containing 5% corn oil with 0% (control) or 0.4% of PJT ethanol extract for 4 weeks: microscopic observation (A) and cell size of epididymal adipocytes (B). Values are mean ± SE of 6 mice. Asterisks denote a statistically significant difference from the control (0% PJT) diet group (Student's t test; *: $p < 0.05$)

D. Analysis of Gene Expression in the Liver, Adipose Tissue, and Soleus Muscle by Real-time RT-PCR

The ethanol extract of PJT significantly increased the expression of PBEF1 and PPARα genes in the liver (Fig. 3). An increasing tendency was noted for RORC, PPP1R10 and FXRα. In contrast, a significant decrease in the expression of FAS gene, and a decreasing tendency in the expression of INSIG2, DUSP1 and SREBP-1c genes were observed with the ethanol extract group.

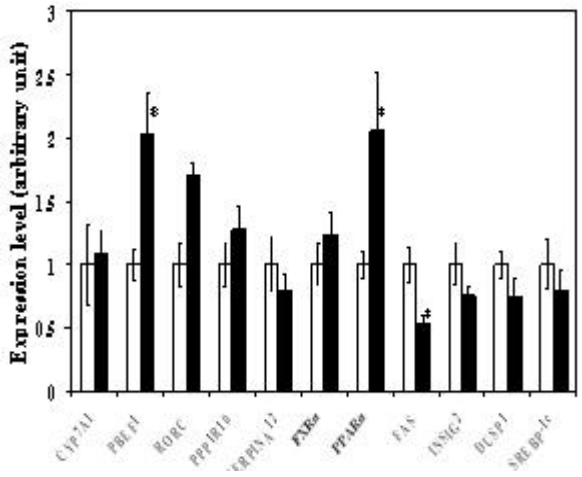


Fig. 3 Effect of ethanol extracts of Peucedanum japonicum Thunb (PJT) on gene expression in liver tissue. For descriptions of genes, see Table Ⅰ. C57BL/6 J Ham Slc-ob/ob mice were fed basal AIN76 diet containing 5% corn oil with 0% (control) or 0.4% of PJT ethanol extract for 4 weeks. Closed and open columns, respectively, show the value for 0% (control) and 0.4% ethanol extract group. Values are in arbitrary units, and expressed as mean ± SE of 6 mice. Asterisk denotes a statistically significant difference from the control (0% PJT) diet group (Student's t test; *: $p < 0.05$)

Supplementation of the diet with ethanol extract of PJT significantly upregulated the expression of RORC, PPARγ, FXRα and DGAT1genes in adipose tissue compared with the control group (Fig. 4). An increasing tendency in the expression of C/EBP, ATGL and UCP2 gene was noted for ethanol extract group. The expression of PEG1/MEST gene, marker of adipocyte size, tended to decrease with feeding of ethanol extract diet.

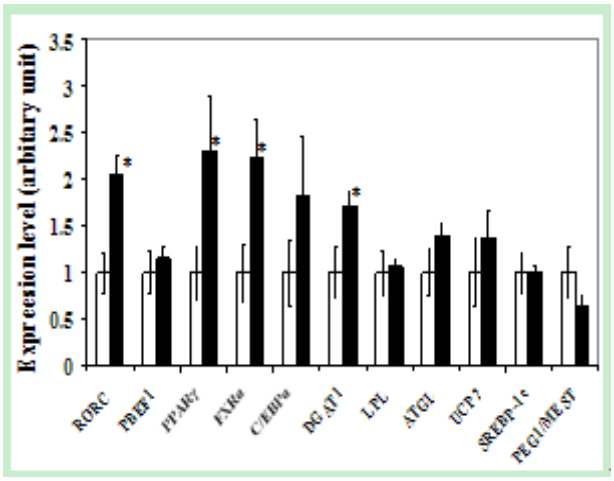


Fig. 4 Effect of ethanol extracts Peucedanum japonicum Thunb (PJT) on gene expression in adipose. For descriptions of genes, see Table 1. C57BL/6 J Ham Slc-ob/ob mice were fed basal AIN76 diet containing 5% corn oil with 0% (control) or 0.4% of PJT ethanol extract for 4 weeks. Closed and open columns, respectively, show the value for 0% (control) and 0.4% ethanol extract group. Values are in arbitrary units, and expressed as mean ± SE of 6 mice. Asterisk denotes a statistically significant difference from the control (0% PJT) diet group (Student's t test; *: $p < 0.05$)

Ethanol extract of PJT significantly increased the expression of CPTα, UCP2, GLUT4 genes in soleus muscle. An increasing tendency was seen for the gene expression of PGC1α, UCP3, LPL, and C/EBPα in the ethanol extract group compared to the control. No significant difference was noted for other genes studied (Fig. 5).

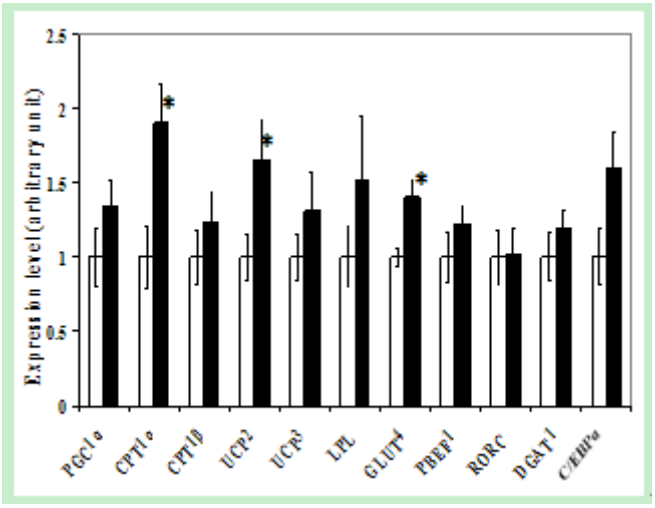


Fig. 5 Effect of ethanol extracts Peucedanum japonicum Thunb (PJT) on gene expression in soleus muscle. For descriptions of genes, see Table 1. C57BL/6 J Ham Slc-ob/ob mice were fed basal AIN76 diet containing 5% corn oil with 0% (control) or 0.4% of PJT ethanol extract for 4 weeks. Closed and open columns, respectively, show the value for 0% (control) and 0.4% ethanol extract group. Values are in arbitrary units, and expressed as mean ± SE of 6 mice. Asterisk denotes a statistically significant difference from the control (0% PJT) diet group (Student's t test; *: $p < 0.05$)

IV. DISCUSSION

Ethanol extract of PJT ameliorated the diabetic symptom in obese-diabetes animal model ob/ob mice. These observations were largely in agreement with our previous observation on high-fat induced obesity in normal mice (Okabe et al., 2011, Nukitrangsan et al., 2011). This animal model of ob/ob mice lacks leptin gene, and hence the deficiency of leptin signaling in the hypothalamus causes obesity due to hyperphagia (Coleman, 1978). Blood glucose

and insulin levels in the high-fat induced obesity animals were respectively 68.4±16.3 mg/dl and 197± 35 pg/ml in our previous study (Nukitrangsan et al., 2011). Thus, the levels of glucose and insulin were 4.5 and 15 times of those for normal mice on high-fat diet, respectively. The elevated levels of glucose and insulin in this obese diabetic animal model were lowered by the PJT extract, resulting in the significant decrease in the HOMA index that is the hallmark for insulin sensitivity (Fig. 1). These findings clearly demonstrated that PJT contained anti-obesity or anti-diabetic components, and was useful for the development of natural agent to combat with diabetes-mellitus.

The modulation of gene expressions and biochemical parameters were largely similar to those observed in normal high-fat obese mouse (Nukitrangsan et al., 2011). Our previous study using microarray and RT-PCR analyses revealed modulation of obesity-related gene expression in PJT-fed mice: RORC and PBEF1 were up-regulated and DUSP1, INSIG2 and SERPINA12 were down-regulated in the liver; FXRα and PPARγ were up-regulated and PEG1/MEST, the size-marker of adipocytes, was down-regulated in the adipose tissue. Furthermore, PJT ethanol extract up-regulated the expression of UCP2, UCP3 and CPT1α. in this study, which appeared to prefer more heat generation rather than energy production as discussed for obese-normal mice on high-fat diet in our previous work (Okabe et al., 2011). Thus the trends seen in the normal mice appeared to be largely reproduced in the obese-diabetic animal model *ob/ob* mice.

Of the genes modulated by PJT, FXRα appeared to play a pivotal role in the prevention of obesity or diabetes. FXRα is a member of the nuclear receptor subfamily, and an important regulator of lipid metabolism (Fiorucci et al., 2007). In FXR deficient mice, serum high-density lipoprotein cholesterol and TG levels were elevated (Stedman et al., 2006). Hepatic TG levels are controlled by a balance between oxidation and biosynthesis of fatty acid, with the former regulated by PPARα. and the latter by SREBF-1. FXRα. down-regulates CYP7A1 and FAS, and up-regulates PPARα. Ethanol extract of PJT consistently increased the expression of FXRα and PPARα in our previous study as well as to some extent in this study. PPARα agonism increases fatty acid oxidation, lipolysis, energy uncoupling and expenditure, while decreasing lipogenesis and TG secretion. This view may be applicable to the liver of PJT fed mice, and may explain the low adiposity as shown by the low TG levels in serum and liver (Table Ⅲ).

Similarly, the increased expression of FXRα may well explain the up-regulation of PPARγ expression in adipose tissue. PPARγ promotes the differentiation of adipocytes, and enhance the generation of small adipocytes rather than the fat-loaded large cells. This feature is considered beneficial for the normalization of the functionality of endocrine adipose tissues to produce adipokins (Rizzo et al., 2006). FXR agonism has reported to increase the expression of several genes including C/EBPα, PPARγ, fatty acid binding protein, syndecan-1, SREBF-1 and AdipoQ, and to decrease the expression of TNFα (Kim et al., 2003). The increased expression of FXRα therefore seems to be associated with the release of adipokins present in normal conditions, and simultaneously reduce the liberation of other adipokins predominant in situation of obesity or diabetes. This scenario appeared to explain well our current findings mediated by PJT extract.

The uptake of glucose by muscle is also affected by the expression of FXRα. FXR expression is decreased in rodent models of diabetes (Lefebvre et al., 2009). The expression of glucose transporter GLUT4 has been shown to be FXRα-dependent (Shen et al., 2008). Thus, up-regulation of FXRα may increase the uptake of glucose from circulation; this may reasonably explain the normalization of serum glucose levels and insulin sensitivity (Fig. 1). In accordance with the increased glucose uptake, up-regulation of UCP2 and CPT1α may further boost up the energy expenditure, which also may contribute to the anti-diabetic activity of PJT.

Bile acids are signaling molecules and play important roles in glucose and lipid metabolism: ligands for FXRα and G-protein-coupled receptors TGR5 (Fiorucci et al., 2009). The activated FXR-SHP pathway regulates enterohepatic cycling of bile acid via the down-regulation of CYP7A1 that catalyze the production of bile acid from cholesterol (John and Chiang et al., 1966). The response of CYP7A1 gene expression to PJT diet or extract was somewhat inconsistent between our studies although the fecal bile acid excretions were consistently reduced. FXRα has been considered to sense the hepatic bile acid levels, and regulate the bile acid recycling by repressing its biosynthesis. However, the increased expression of FXRα did not necessarily cause the inhibition of CYP7A1 gene expression in our study. Therefore, some uncertainty still exists whether PJT reduced the bile acid excretion into small intestine via the inhibition of biosynthesis, or PJT enhanced the re-absorption of bile acid in the ileum resulting in the decreased fecal bile acid excretion. The authors first postulated that the decreased fecal excretion of bile acid in PJT fed mice might be due to increased reabsorption, and the increased return flux, consequently, might more strengthen the beneficial feature of PJT because bile acid is a natural ligand for FXRα. This postulate, however, appeared to need further testing.

V. CONCLUSIONS

This study demonstrated that the ethanol extract of PJT ameliorated the symptom of diabetes in animal model. The overall action of mechanisms for anti-obesity activity of PJT appeared to be similar to that observed in normal mice model. Of the genes studied, PJT may prevent the obesity via the up-regulation of FXRα that plays a pivotal role in the obesity related lipid metabolism.

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