Anti-obesity Effect of *Pinellia ternata* Extract in Zucker Rats

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**Notes**

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**MATERIALS AND METHODS**

**Preparation of PE** Crude drugs (*Pinellia ternata* Tenore *et breit.*) originated in North Korea were identified by Prof. Ho-Chul Kim (Department of General Herbal Pharmacology, College of Oriental Medicine, Kyung Hee University). *P. ternata* (157 g) was decocted in a round flask with distilled water (3000 ml) at 100 °C for 2 h and filtered. Distilled water (1000 ml) was then added and decocted at 100 °C for 1 h and filtered again. The filtrate was reduced and freeze-dried as a powder with 24.2% yield (38 g).

**Animals** Male obese (HsdHlr:Zucker-fa, *n* = 12) and lean Zucker (HsdHlr:Zucker-lean, *n* = 6) rats were purchased.
from the femoral artery, centrifuged at 900 g and then removed.

Tissue (BAT) and white visceral adipose tissue (WAT) were ﬁshed distilled water daily. After the experiment period, animals were anesthetized and the interscapular brown adipose tissue (BAT) and white visceral adipose tissue (WAT) were removed.

**Plasma Biochemical Analysis** Blood was withdrawn from the femoral artery, centrifuged at 900 g for 15 min and the plasma was obtained. Alamine aminotransferase (ALT), aspartate aminotransferase (AST), total cholesterol (TC) and triglyceride (TG) were measured using Roche Diagnostic reagents on Cobas Mira analyzer (Roche Diagnostic Pty Ltd.). Free fatty acids (FFA) were measured using an enzymatic assay kit (NEFA C kit; Wako Chem., Japan).

**Real Time PCR** Total RNA was isolated using RNAzol Bee (Tel-Test, U.S.A.). First-strand cDNA was generated from 2 μg of total RNA using a Ready-To-Go T-Primed First-Strand kit (Amersham Biosciences). Primer and probes were designed using Primer Express software package version 2.0 (Applied Biosystems, Foster City, CA, U.S.A.) from gene sequences obtained from GenBank (UCP1 NM_012682; PGC1α, NM_031347; PPARα, NM_013196).

PCR was performed using the ABI PRISM 7900HT sequence detection system (Applied Biosystems). To compensate for variations in input RNA amounts and efficiency of reverse transcription, GAPDH (NM_017008) mRNA was quantiﬁed, and results were normalized to these values. The relative expression of the gene of interest was calculated and reported as arbitrary units.

**Quantification of Ephedrine from PE** Approximately 1.0 g of the water extract was added to a round-bottomed ﬂask containing 20 ml of distilled water and the mixture was shaken at room temperature for 6 h. After centrifugation for 30 min at 1000 g, the supernatant was freeze-dried and the dried material was dissolved in 2 ml of water. Twenty microliters of the sample was loaded onto a Hitachi L-6000 instrument equipped with a UV detector (Spectra–Physics Spectra 100) and an Agilent Zorbax SB-Aq C18 column (4.6 mm I.D.×150 mm, particle size 5 μm). HPLC conditions were as follows: eluent A, 10% acetonitrile in water containing 0.4% SDS; eluent B, 35% acetonitrile in water containing 0.4% SDS; gradient, 0—20 min (0—100% B), 20—40 min (100% B), and then equilibrated with 0% B for 10 min. The ﬂow rate was 1 ml/min and the efﬂuent was monitored at 210 nm.

**Statistical Analysis** All data were presented as the mean±S.E. Statistical comparisons were made using analysis of variance (ANOVA) by Bonferroni’s post hoc comparison. A value of p<0.05 was considered statistically significant.

**RESULTS AND DISCUSSION**

The beneﬁcial effect of PE is clearly visible from the blood biochemical proﬁles. The data in Table 1 show that PE significantly improves plasma TG level (p<0.01) and FFA level (p<0.05). There was no signiﬁcant difference in body weight between the obese control group and the PE treated group. After the 6 week experiment, the effect of genotype (lean vs. obese) on daily food intake was as follows: 25.12±0.70 g/d in lean rats vs. 38.80±1.05 g/d in obese rats (p<0.001), vs. 37.0±1.05 g/d in PE-treated rats (p<0.001).

There was no signiﬁcant difference in daily food intake between obese and PE-treated rats, even though the intake showed a 4.64% (p=0.599) greater downward trend in PE-treated groups than in obese control; this was similar to body weight changes.

The thermogenic effect of PE has gained strong interest because of expectations of its possible contribution to anti-obesity. The PE treatment induced the expression of UCP1 mRNA in BAT to increase by 3.78-fold, but no differences were noted in the expression of mRNA for PPARα and PGC1α (Fig. 1); in WAT the PE treatment group showed a drastic increase in mRNA expression of PPARα (9.42-fold) and PGC1α (6.57-fold) (Fig. 2).

UCP1 expression is an important factor in regulating energy balance and it is modulated by diet and metabolic hormones such as leptin and glucocorticoids. UCP1 is found exclusively in brown adipose tissue, composed of multilocular lipid storage cells that play a crucial role in non-shivering thermogenesis. Brown adipose tissues comprise a very large number of mitochondria packed with cristae, densely innervated by the sympathetic nervous system (SNS). Nora-drenaline (NA) from sympathetic nerve endings is released in the proximity of brown fat cells. NA binds β3-adrenergic receptors on the fat cell surface, which initiates a cascade
of metabolic events including the activation of UCP1.20) The agonist of \( \beta \)-adrenergic receptors prevents or reverses obesity.6) In our results, the PE treatment induced a significant 3.78-fold increase in the expression of UCP1 mRNA in BAT, but the level of UCP1 mRNA in WAT was negligible due to its low sensitivity. These results were confirmed in the expression of protein level through Western blot analysis. The protein expression was very small and not different from the control (data not shown). This result is consistent with the previous report that the UCP protein is not present in WAT.20)

Our observations suggest that supplementation of PE induced an increase thermogenesis in BAT and fatty acid oxidation in WAT. This result would create a negative energy balance and subsequent loss of fat content within these tissues.

PPAR\( \alpha \) and PGC1\( \alpha \), transcription factors that regulate genes involved in fatty acid oxidation and mitochondrial biogenesis were observed in BAT and WAT. The increased expression of PPAR\( \alpha \) and PGC1\( \alpha \) genes in WAT was observed, while no difference was noticed in the expression of mRNA for PPAR\( \alpha \) and PGC1\( \alpha \) in BAT (Fig. 1). Within BAT the PE treatment group showed a significant increase in mRNA expression of PPAR\( \alpha \) (0.70\( \pm \)0.25 vs. 0.07\( \pm \)0.01: 9.49-fold, \( p<0.05 \)), PGC1\( \alpha \) (2.42\( \pm \)0.23 vs. 0.37\( \pm \)0.03: 6.57-fold, \( p<0.001 \)) compared with control (Fig. 2). The PGC1\( \alpha \) highly expressed in WAT was capable of preventing fat from storing and it affected the UCP1 expression in BAT. As a result, PPAR\( \alpha \) and PGC1\( \alpha \) were down-regulated in the BAT of obese rats. Following our experiment, we recognized that the alteration of energy balance through an increased utilization of WAT may be conceivable for the treatment of obesity.

Koza et al. reported that PPAR\( \alpha \) and PGC1\( \alpha \) were more highly expressed in WAT \( \beta \)-adrenoceptor agonist CL-fed NZO mice than in NZO control animals.17) Their observations suggest that inguinal and retroperitoneal fat depots have at least converted into thermogenically active BAT, thus being capable of utilizing fatty acids production via uncoupling of oxidation phosphorylation. These changes would create a negative energy balance and subsequent loss of fat content within these tissues.

Pinellia sp. is one of the plants used as a source containing ephedrine,21,22) including \( \beta \)-sitosterol, and choline.22) It has been suggested that ephedrine may have a sedative effect rather than stimulant property, which might be quite opposite to expectation on ephedrine,21,23) however the content of this component is very small (0.002%).21) Ephedrine is used for obesity treatment, which is known to have a thermogenic effect via stimulation of \( \beta \)-adrenergic receptors and release of noradrenaline from sympathetic nerve endings.24) We treated 1.2 \( \mu \)g ephedrine in 1 mg PE (0.012%), and which is equivalent to the amount of 0.48 \( \mu \)g ephedrine fed to the obese Zucker rats (kg). There is no effect of PE on animal behavior during experimental period.

To ascertain whether PE induced hepatic damage, serum catalytic concentrations of ALT and AST levels were also measured. A significant \( (p<0.05) \) increase in AST levels was observed in plasma from the obese rats (obese, PE) compared with lean control rats. The levels of ALT and AST did not change with PE treatment. This data suggests that PE does not exert any appreciable hepatic toxicity. As previously reported, the repeated-dose toxicity of PE in male and female Sprague–Dawley rats also did not exert considerable toxic effects.25)

In conclusion, PE leads the white fat depots to convert into thermogenically active BAT, at least partially, which is capable of utilizing fatty acids for production of heat via uncoupling of oxidative phosphorylation. These changes would create a negative energy balance and subsequent loss of fat content within these tissues. It suggests that PE also protect, against fat storage and may have an anti-obesity effect.

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REFERENCES


