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Effect of dietary *Platycodon grandiflorum* on plasma glucose and lipid metabolism in KK-A^y mice and streptozotocin-induced diabetic rats

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SUMMARY

This study was designed to investigate the effect of dietary *Platycodon grandiflorum* on plasma glucose and lipid metabolism in KK-A^y mice and STZ-induced diabetic rats. Both plasma triglyceride and plasma cholesterol levels in STZ-induced diabetic rats were significantly decreased by dietary *Platycodon grandiflorum* feeding for 4 weeks compared to those of control rats, but there were no marked differences in KK-A^y mice. However, for plasma glucose values, *Platycodon grandiflorum* feeding resulted in a significant decrease in both STZ-induced diabetic rats and KK-A^y mice. Also, dietary *Platycodon grandiflorum* slightly decreased the postprandial glucose level at 30 and 60 mins during oral glucose tolerance test in KK-A^y mice. Although there was no statistical significance, the fasting plasma insulin levels of *Platycodon grandiflorum* dieted KK-A^y mice tended to decrease when compared to that of control mice. Therefore, the present results suggested that dietary *Platycodon grandiflorum* may have a beneficial effect on preventing hypercholesterolemia and hyperlipidemia.

INTRODUCTION

The root of *Platycodon grandiflorum* A. DC (Chinese name, 'Jiegeng'; Korean name, 'Doraji'; and Japanese name, 'Kikyo') has been used as an expectorant in traditional Oriental medicine. In Korea, the root of *Platycodon grandiflorum* (*P. g.*) grown for 4 years has generally been used as food, and the root of *P. g.* grown for 22 years has been employed in folk remedies for diseases of adulthood such as hyperlipidemia, hypertension and diabetes. Some studies on chemical (Akiyama *et al.*, 1972; Tada *et al.*, 1975; Ishii *et al.*, 1984) and immunopharmacological effects (Kubo *et al.*, 1986; Nagao *et al.*, 1986) of *P. g.* have been done, but little is known about

Key words: Platycodon grandiflorum; KK-A^y mice; Streptozotocin-induced diabetic rats; Lipid metabolism

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its clinical and dietary effects. In view of this point, our research have focused on elucidating pharmacological effects of the root of *P. g.* grown for 22 years on hyperlipidemia, hypertension and diabetes. As the results, recently, we reported that effects of dietary *P. g.* grown for 22 years on serum and liver lipid concentrations in rats with diet-induced hyperlipidemia (Kim *et al.*, 1995). The *P. g.* feeding markedly decreased both serum and liver lipid levels in hyperlipidemic rats. Furthermore, in the very recent report, we demonstrated that dietary *P. g.* grown for 22 years markedly decreased both plasma cholesterol and fasting plasma insulin levels, and significantly decreased the postprandial glucose level at 30 min during oral glucose tolerance test in obese Zucker rats (Kim *et al.*, 2000).

As a subsequent work, the present study was performed to examine the effects of dietary *P. g.* grown for 22 years on plasma glucose and lipid metabolism in KK-A^y mice and streptozotocin (STZ)-induced diabetic rats.

MATERIALS AND METHODS

P. g.

The root of *P. g.* (grown for 22 years) was obtained from Jangsang Doraji Farm (Gyeong Nam, South Korea). For the purposes of this experiment it was freeze-dried, milled and sifted through a 0.59 mm screen. The composition of the root of *P. g.* is shown in our previous study (Kim *et al.*, 1995).

Animals and diets

The genetically obese-diabetic KK-A^y mice (Iwatsuka *et al.*, 1970) and STZ-induced diabetic rats were used in this study. STZ-induced diabetic rats were produced by intravenous injection in the tail vein of 65 mg/kg streptozotocin (freshly reconstituted with 0.1M citrate buffer, pH 4.5) using Sprague-Dawley rats in accordance with a previously described method (Ramlal *et al.*, 1989). These animals were purchased from Tokyo Experimental Animals (Tokyo, Japan) at three months of age and maintained on a laboratory chow diet consisting of 52.7% carbohydrate, 23.6% protein, 4.4% fat, 4.9% fiber, 6.6% minerals and vitamins (CE-2, Clea Japan Incorp., Tokyo) for 1 week, and water. They were individually housed in stainless steel cages and kept in an isolated room at a controlled temperature (23-24°C) and ambient humidity (50-60%). Lights were mantained on a reversed 12-h light/dark cycle. KK-A^y mice (12 weeks-old, male) and STZ-induced diabetic rats (12 weeks-old, male) were divided into two groups (*P. g.* diet and control diet groups, each with 5 rats, respectively). Table 1 shows composition of the control and experimental diets. In the previous study, a supplement diet of 5% *P. g.* has been shown to be more effective than that of 10% *P. g.* in reducing the cholesterol and triglyceride concentra-tion in serum and liver (Kim *et al.*, 1995). In this study, the control group received a diet with 5% cellulose, and the experimental group had diet containing 5% (wt/wt) *P. g.* powder. Except for the oral glucose tolerance test (OGTT), the animals were always allowed free access to the diet for 4 weeks. At 18 weeks of age, the animals were anesthetized via intraperitoneal injection of pentobarbital sodium (5 mg/100 g of body weight) after an overnight fast, followed by OGTT.

Table 1. Composition of control and experimental diets

Ingredients	Diets (g/100g diet)	
	Control	Experiment
Casein	20.0	20.0
Sucrose	10.0	10.0
Corn starch	50.0	50.0
Lard	5.0	5.0
Corn oil	5.0	5.0
Mineral mixture[a]	3.5	3.5
Vitamin mixture[b]	1.0	1.0
DL-methionine	0.5	0.5
Cellulose	5.0	-
P.g.powder[2]	-	5.0

[a]This is identical with AIN-76 mixture.
[b]P. g. (grown for 22 years)

Oral glucose tolerance test (OGTT)
After an overnight fast, D-glucose (1g/kg body weight) was given by oral tube. Blood samples were obtained by cutting the tail end before glucose loading, and at 30, 60 and 120 min after glucose loading, respectively. Blood glucose levels were determined with TIDEX glucose analyzer (Miles, Slough, U.K.).

Plasma measurement
After anesthesia, blood was drawn from the inferior vena cava and centrifuged at 4°C. Plasma was used for measurements of glucose, immunoreactive insulin, triglyceride and total cholesterol. The concentrations of triglyceride and total cholesterol were measured by enzyme assay with Determiner TG and Determiner TC555 (Kyowa Medics, Tokyo, Japan), respectively.

Statistical analysis
Data were expressed as mean ± SE for five rats in each group. Statistical analysis was performed using the paired Student' *t*-test. Values of $P<0.05$ were considered to be significant.

RESULTS

Final body weight and plasma concentrations of glucose, triglyceride and total cholesterol in KK-A^y mice and STZ-induced diabetic rats are summarized in Table 2. Dietary *P. g.* feeding had no statistically significant effects on body weight between these animals. In the fasting plasma glucose values, *P. g.* feeding caused a 26% reduction (188 ± 20 vs. 253 ± 42 mg/dl) in KK-A^y mice and a 35% decrease (267 ± 31 vs. 411 ± 82 mg/dl) in STZ-induced diabetic rats. However, in KK-A^y mice, *P. g.* feeding had no significant effects on plasma triglyceride concentration (237 ± 25 vs. 255 ± 42 mg/dl) and plasma cholesterol concentration (112 ± 7 vs. 116 ± 8 mg/dl). On the contrary, in STZ-induced diabetic rats, *P.*

g. feeding resulted in a 61% reduction (66 ± 30 vs. 169 ± 66 mg/dl) in plasma triglyceride concentration and a 62% decrease (60 ± 12 vs. 159 ± 62 mg/dl) in plasma cholesterol concentration. Fig. 1 shows the effect of dietary P. g. on plasma insulin level in KK-A^y mice. The insulin level in P. g. feeding group was decreased by 24% (76.8 ± 4.2 vs. 58.2 ± 5.6 μU/mℓ) compared to control group. To determine the functional effects of dietary P. g., OGTTs were performed. As shown in Fig. 2, dietary P. g. feeding caused a slight decrease in plasma glucose level at 30 and 60 mins after glucose loading in KK-A^y mice, but there was no significant difference.

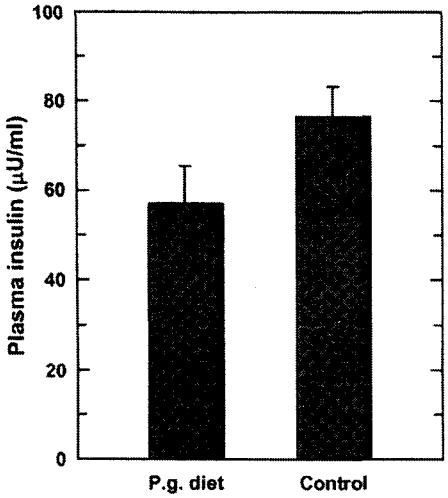

Fig 1. The effect of P. g. diet on plasma insulin levels in KK-A^y mice. P. g. diet; KK-A^y mice fed P. g. diet, Control; KK-A^y mice fed control diet. Values are mean ± SE for the five samples.

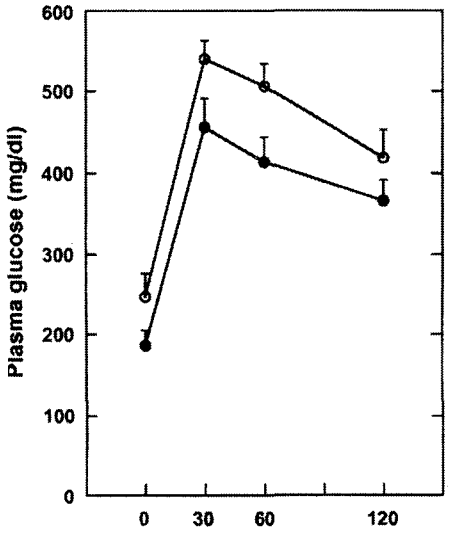

Fig 2. The effect of dietary P. g. feeding on oral glucose tolerance tests in KK-A^y mice. (•); KK-A^y mice fed control diet, (O); KK-A^y mice fed P. g. diet. Values are mean ± SE for the five samples.

Table 2. Effect of dietary P.g. on body weight, plasma glucose, triglyceride, and cholesterol concentrations

Animal	Body weight (g)	Plasma glucose (mg/dl)	Plasma triglyceride (mg/dl)	Plasma cholesterol (mg/dl)
KK-A^y mice				
P.g.diet	46.2±1.2[a]	188±15[a]	237±25[a]	112±7[a]
Control diet	45.9±0.6[a]	253±32[b]	255±42[a]	116±8[a]
STZ-induced rats				
P.g.diet	196±32[a]	267±27[a]	66±15[a]	60±12[a]
Control diet	204±17[a]	411±58[b]	169±36[b]	159±38[b]

[a]Values are mean±SE; N=5. [b]Values in a column not sharing a common superscript are significantly different at $p<0.05$

DISCUSSION

Obese Zucker rat and KK-A^y mouse which are genetic models of obesity have been extensively used as a representative model with NIDDM (non-insulin-dependent diabetes mellitus) for human obesity (Iwatsuka *et al.*, 1970; Stern and Johnson, 1977; Shafrir, 1992). In the previous report, we showed that dietary *P. g.* feeding significantly reduced plasma triglyceride and cholesterol levels in obese Zucker rats, whereas there were no significant differences in the fasting plasma glucose values (Kim *et al.*, 2000). However, in this study, *P. g.* feeding had no significant effects on plasma triglyceride and cholesterol levels in KK-A^y mice, whereas the fasting plasma glucose values were reduced by *P. g.* feeding. In addition, the fasting plasma insulin level of obese Zucker rats was significantly decreased by 78% (357 ± 58 vs. 77 ± 23 μU/mℓ) in *P. g.* diet group when compared to control group (Kim *et al.*, 2000), whereas that of KK-A^y mice was decreased by 24% (76.8 ± 4.2 vs. 58.2 ± 5.6 μU/mℓ). However, because obese Zucker rats exhibited an excessive degree of hyperinsulinemia (357 ± 58 μU/mℓ), whereas KK-A^y mice showed normal insulin level (76.8 ± 4.2 μU/mℓ), the reduction of insulin level by *P. g.* feeding in KK-A^y mice is not likely to be significant.

Dietary *P. g.* feeding resulted in significant decrease in plasma glucose level at 30 min after glucose loading in obese Zucker rats (Kim *et al.*, 2000), whereas in KK-A^y mice it did not show a significant effect on improvement of glucose tolerance, although fasting glucose level was prominently increased compared to that of obese Zucker rats.

Considering the above results, it is difficult to explain the reson for these differences between obese Zucker rats and KK-A^y mice at this time. However, our findings that while obese Zucker rats showed hyperinsulinemia and normal plasma glucose level, KK-A^y mice exhibited hyperglycemia and normal insulin level, though both are genetically obese animal models, are noteworthy and may have a significant contribution to further diabetic studies using these animals. Also, these results suggest that diabetic syndromes, such as hyperglycemia, glucose intolerance, hypertriglyceridemia and hyperinsulinemia, in these two kinds of obese animal models might be caused by different metabolic mechanisms.

On the other hand, as another animal model of diabetes mellitus, STZ-induced rat have been used to study both pathophysiological mechanisms of diabetes mellitus and hypoglycemic activity of plants (Kedar and Chakrabarti, 1982; Obatomi *et al.*, 1994; El Fiky *et al.*, 1996; Jouad *et al.*, 2000). Because STZ treatment causes the insulin deficiency and the decrease of glucose upake by a defect of pancreaic β-cells, STZ-induced animals with IDDM (insulin-dependent diabetes mellitus) exhibit hypoinsulinemia and hyperglycemia (Like and Rossini, 1976). Very recently, Ohno et al. (2000) reported that STZ-treated shrews exhibited hyperlipidemia with severe IDDM: plasma triglyceride and cholesterol concentrations in STZ-treated shrews were significantly higher than those of controls.

Our results presented in this study showed that dietary *P. g.* feeding significantly reduced plasma glucose level, and especially caused a remarkable decrease in plasma triglyceride and cholesterol levels in STZ-induced rats. However, plasma insulin levels in STZ-induce rats were less than 0.2 μU/mℓ, and dietary *P. g.* feeding did not alter plasma insulin level (data not

shown), suggesting that *P. g.* feeding did not affect the impaired glucose tolerance in insulin depleted state.

In conclusion, the present results indicated that dietary *P. g.* is effective on abnormal glucose and lipid metabolisms associated with diabetic syndromes, and may be useful in reducing the incidence of metabolic disorders characterized by hyperglycemia and hyperlipidemia.

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