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Hypolipemic and Hypoglycemic Activities of *Prunus davidiana* in High Fat-Fed Rats

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Abstract □ Blood lipid levels in rats with high fat-fed hyperlipemia were determined after intraperitoneal administration of a methanolic extract of *Prunus davidiana* Fr. stems. Administration of the methanolic extract for 6 days produced a significant decrease of blood triglyceride and total cholesterol, and the atherogenic index was also improved. In addition to the hypolipemic effect, the methanolic extract was also shown to be effective in reducing an elevated level of glucose in rats with hyperlipemia resulting from high-fat feeding. On the other hand, blood triglyceride and total cholesterol in rats fed with stock diet were not affected by administration of the methanolic extract even if there was a tendency to decrease. No significant change was also found in the level of glucose. Thus, it is suggested that this methanolic extract probably may increase the metabolic utilization only when fed with excess fat.

Keywords □ *Prunus davidiana*, hypolipemic, atherogenic index, triglyceride, cholesterol

It has been noted that there is a causal relationship between increased plasma lipid levels and the development of atherosclerotic disease.[1] Hence, many new classes of hypolipemic agents have been widely used for the improvement of hyperlipemia associated with atherosclerosis during the past decade.[2-4] In the course of screening for hypolipemic drugs among Korean folk medicines, we found that intraperitoneal administration of a methanolic extract of *Prunus davidiana Franch* (Rosaceae) stems resulted in a significant improvement in hypercholesterolemic mice.[5] In a recent communication[6], we also have reported the hypoglycemic and hypolipemic activities of the methanolic extract of *P. davidiana* stems and its main component, prunin in rats with streptozotocin-induced diabetes. In these rats, hypotriglyceridemic effect was pronounced. In the light of the above reports, studies were conducted on the effect of a methanolic extract of *P. davidiana* stems on serum parameters of rats fed on a high-fat diet. This paper describes the results obtained, and also the effect of methanolic extract on normal rats.

MATERIALS AND METHODS

Animals and diets

Male rats of the JCL: Wistar strain (SLC Ltd., Hamamatsu, Japan), initially weighing 150 g, were maintained in an air-conditioned room with lighting from 06:00 to 18: 00 h. The room temperature (about 25°) and humidity (about 60%) were controlled automatically. The animals were fed on commercial feeds (CLEA Japan Inc., Tokyo, type CE-2) for 1 week after arrival. They were then placed on a experimental diets consisting of 10 g of soybean oil, 4g of salt mixture, 1g of vitamin mixture, 2g of cellulose powder, 0.1g of choline chloride, and 18g of casein. The weight was made up to 100g by addition of an appropriate amount of α-cornstarch (Table I). The animals were allowed access to the experimental diet and tapwater *ad libitum* for 6 days, and then pair matched for blood triglyceride level and body weight for use in the experiments. During the experimental period, there were no statistically significant differences between the control and each of the methanolic extract treated rats with regard to changes in body weight.

Table I. Composition of experimental diet

Ingredients	High fat diet (%)
Casein	18
α-cornstarch	49.9
Sucrose	15
Salt mixture[a]	4
Vitamin mixture[b]	1
Cellulose powder	2
Choline chloride	0.1
Oil mixture[c]	10

[a] Salt mixture obtained from Oriental Yeast Co., Tokyo, contains 29.29% $CaCO_3$, 0.43% $CaHPO_4 \cdot 2H_2O$, 34.31% KH_2PO_4 25.06% NaCl, 9.98% $MgSO_4 \cdot 7H_2O$, 0.623% Fe-citrate, 0.156% $CuSO_4 \cdot 5H_2O$, 0.121% $MnSO_4 \cdot 5H_2O$, 0.02% $ZnCl_2$, 0.0005% KI, 0.0025% $(NH_4)_6 Mo_7O_{24} \cdot 4H_2O$.
[b] Vitamin mixture obtained from Oriental Yeast Co., Tokyo, contains 0.059% thiamine-HCl, 0.059% riboflavin, 0.294% nicotinic acid, 0.235% calcium pantothenate, 0.029 % pyridoxine-HCl, 0.006% menadione, 0.001% D-biotin, 0.002% folic acid, 0.0002% vitamine B_{12}, 1.176% inositol, 0.588% ascorbic acid, 0.0932% vitamin A-acetate, 0.000 5825% vitamine D_3, 96.257% lactose.
[c] Oil mixture obtained from CLEA Japan Inc., contains 80% soybean oil, and 20% cod liver oil.

No case of diarrheal symptom was found.

Plant material

P. davidiana used was purchased from the Chinese herb medicine shop at the Pyongwha market, Pusan, Korea. The plant was identified by the botanist Professor J. H. Park and a voucher specimen deposited in the Herbarium of College of Pharmacy, Pusan National University, Pusan, Korea.

Preparation of methanolic extract

Dried stems (2.2 kg) of commercially available *P. davidiana* were extracted with methanol under reflux. The extracts were concentrated to dryness *in vacuo* at 40° to give the methanolic extract (140g, yield: 6.4%).

Experimental procedure

The methanolic extract suspended in 5% ethanol-saline was administered intraperitoneally once a day for 6 days to rats at the indicated dose, while control rats were treated with an equal volume of 5% ethanol-saline. Rats were killed at 2 p.m. 4 hours after the last dose. Blood was collected and allowed to stand for several hours in a cold room at 4°. Serum was separated by centrifugation (1,000×g, 10 min, 4°).

Determination of triglyceride, total cholesterol, low-density lipoprotein (LDL)-cholesterol, and high-density lipoprotein (HDL)-cholesterol in serum

Triglyceride and total cholesterol were determined using commerical reagents (TG-Five Kainos, Kainos Laboratories, Inc., Tokyo, Japan; Cholesterol E-Test Wako, Wako Pure Chemical Industries, Ltd., Osaka, Japan). LDL- and HDL-cholesterol were determined by the method of Noma *et al.*[7,8]

Chemicals

Heparin sodium salt was purchased from Wako Pure Chemical Industries, Ltd. Amberlite® IRA-400 was purchased from Organo Ltd., Tokyo, Japan. All other reagents were of the highest grade commercially available.

Statistics

The significance of differences between the control and methanolic extract-treated groups was tested using Student's *t* test.

RESULTS

Effect of methanolic extract on serum levels in rats with hyperlipemia

Table II shows the effects on serum constituents after intraperitoneal administration of the methanolic extract at 40 and 80 mg/kg. Rats in the methanolic extract-treated group showed a significant decrease of serum triglyceride and total cholesterol. The serum triglyceride level in the group given an 80 mg dose was significantly decreased by 43%. Total cholesterol was significantly lowered in the groups given doses of 40 and 80 mg by as much as 17% and 15%, respectively. Table II also shows the changes in serum lipoprotein concentrations and the atherogenic index (AI=total cholesterol-HDL-cholesterol/HDL-cholesterol). The effect of a methanolic extract on serum HDL-cholesterol was insignificant, while the AI was significantly reduced in the 80 mg dose group as compared with the control group. In particular, a significant decrease was observed in the level of LDL-cholesterol. Thus, hyperlipemia induced with a high fat diet was improved in the group given an 80 mg dose. The rats treated with the methanolic extract

Table II. Effect of a methanolic extract of *P. davidiana* (Stem) on serum levels in rats fed on a high fat diet

Treatment	Dose (mg/kg body Wt)	Triglyceride (mg/dl)	TC[a] (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	A.I.	Glucose (mg/dl)
Control		127.16± 19.89 (100)	85.22± 4.15 (100)	10.64± 0.44 (100)	18.72± 0.88 (100)	7.04± 0.36 (100)	165.80± 4.22 (100)
MeOH ext.	40	132.14± 10.79 (104)	70.95± 4.63 (83)*	10.38± 0.30 (98)	16.44± 1.22 (88)	5.82± 0.35 (83)*	161.53± 3.53 (97)
MeOH ext.	80	73.38± 8.38 (57)*	72.78± 3.55 (85)*	11.50± 0.49 (108)	15.11± 1.19 (81)*	5.56± 0.15 (79)**	145.90± 7.05 (88)*

[a] TC=total cholesterol; HDL-C=high density lipoprotein-cholesterol; LDL-C=low density lipoprotein-cholesterol; A.I. =*atherogenic index*

* Significantly different from the control value, $p<0.05$, **$p<0.001$

Table III. Effect of methanolic extract of *Prunus davidiana* (ST) on serum levels in normal rats

Treatment	Dose (mg/kg)	Triglyceride (mg/dl)	Total cholesterol (mg/dl)	Glucose (mg/dl)
Control	-	61.44± 6.92 (100)	82.02± 1.64 (100)	129.23± 1.59 (100)
MeOH ext.	40	53.80± 2.77 (88)	79.99± 1.65 (98)	131.54± 3.56 (102)
MeOH ext.	80	54.77± 5.96 (89)	82.55± 3.49 (101)	132.12± 3.36 (102)

Values are mean± S.E for seven rats. Figures in parenthesis are percentages of the control value.

also showed a significant decrease of glucose from 165.8 to 145.9 mg/dl.

Effect of methanolic extract on serum levels in normal rats

The effects of methanolic extract treatment on serum levels in rats fed with stock diet and control rats are shown in Table III. Total cholesterol and the level of triglyceride were not affected by administration of the methanolic extract, although there was a decreasing tendency. Furthermore, no significant changes were found in the level of glucose.

DISCUSSION

Rats fed with the experimental diet increased almost twice as much triglyceride level as those fed with stock diet (Table II vs III). A high fat diet has been known to cause hyperlipemia and there is a close relationship between atherosclerosis with an increase of serum lipids. In particular, very-low-density lipoprotein (VLDL) and LDL may be risk factors, and HDL may be a preventive factor.[9]

In the previous reports, we found that the methanolic extract of *Prunus davidiana* stems as well as prunin isolated from it showed significant hypoglycemic and hypolipemic effects in rats with streptozotocin-induced diabetes.[6] In this work, we obtained evidence that repeated administration of the methanolic extract improved the hyperlipemia induced by a high fat diet in rats. In the present experiment employing high fat-fed hyperlipemic rats, the serum triglyceride level showed a less marked change after 6 day intraperitoneal administration of the methanolic extract at 40 mg/kg, whereas in the rats given with a dose of 80 mg the decrease was significant. The present study demonstrated that the repeated administrations of the extract at 80 mg/kg had a more pronounced hypolipemic action. The rats in the extract-treated group caused a decrease of total cholesterol and LDL-cholesterol with a concomitant slight increase in the level of HDL-cholesterol, although these were less marked than the significant decrease of serum triglyceride. Since Apo B-containing lipoprotein fractions are thought to be responsible for cholesterol deposition into atherosclerotic plaques,[10] a reduction

in LDL would be advantageous clinically. It was thus shown clearly that the methanolic extract had an improving effect on the hyperlipemia induced by a high fat diet. In addition to the hypolipemic effect, the methanolic extract (80 mg/kg) was also shown to be effective in reducing an elevated level of glucose in the rats with hyperlipemia resulting from high-fat feeding.

This is perhaps the first report on the hypotriglyceridemic, hypocholesterolemic and hypoglycemic activities of the extracts from *Prunus* species in rats with high fat-fed hyperlipemia.

Compared with the significant decrease in serum triglyceride and total cholesterol levels in rats with high-fat fed hyperlipemia, the levels of such parameters in rats fed with stock diet were not affected.

We also demonstrated that the methanolic extract did not exert hypolipemic activity in normal rats and additionally point out that this methanolic extract may probably increase the metabolic utilization only when fed with excess fat.

The findings of the present work indicate that the methanolic extract of *Prunus davidiana* stems may be useful for treatment of hyperlipemic disease. Further comprehensive chemical and pharmacological investigations will be needed to elucidate the exact mechanism of these effect and to isolate the active principles responsible.

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