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ANTIHYPERLIPIDEMIC EFFECT OF FLAVONOIDS FROM *PRUNUS DAVIDIANA*

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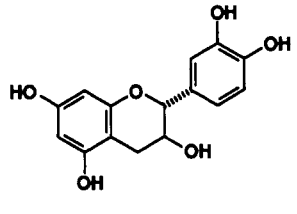
ABSTRACT.—Blood lipid levels in rats with hyperlipidemia resulting from high-fat feeding were determined after ip administration of an MeOH extract of *Prunus davidiana* stems and its flavonoid components, (+)-catechin [**1**], prunin [**2**] (=naringenin 7-*O*-glucoside), and hesperetin 5-*O*-glucoside [**3**]. Administration of the MeOH extract for 3 days produced a significant decrease of blood triglyceride and total cholesterol, and the atherogenic index was also improved. (+)-Catechin [**1**] was shown to be effective in reducing the elevated level of triglyceride. Prunin [**2**] and hesperetin 5-*O*-glucoside [**3**] did not show such an effect in high-fat-fed hypertriglyceridemic rats, but they did exhibit a significant hypocholesterolemic effect.

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 hypolipidemic agents have been widely used for the improvement of hyperlipidemia associated with atherosclerosis during the past decade (2–4). In the course of screening for hypolipidemic drugs among Korean folk medicines, we found that ip administration of an MeOH extract of *Prunus davidiana* Fr. (Rosaceae) stems resulted in a significant improvement of hypercholesterolemia in mice (5). In a recent communication (6), we also reported hypoglycemic and hypolipidemic activities of this MeOH extract of *P. davidiana* stems and its main component, prunin, in rats with streptozotocin-induced diabetes. This included a pronounced hypotriglyceridemic effect. In the light of the above reports, studies were conducted on the effect of an MeOH extract of *P. davidiana* stems and its flavonoid components, (+)-catechin [**1**], prunin [**2**], and hesperetin 5-*O*-glucoside [**3**], on serum parameters of rats fed on a high-fat diet.

MATERIALS AND METHODS

ANIMALS AND DIETS.—Male rats of the JCL:Wistar strain (SLC Ltd., Hamamatsu, Japan), weighing 150 g, were maintained under uniform room temperature (about 25°) and humidity (about 60%).



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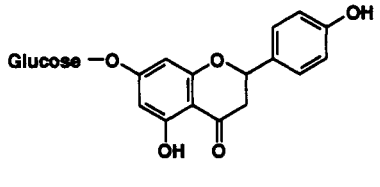

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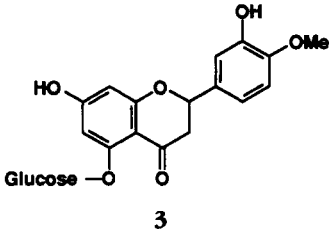

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Room light from 0600 to 1800 h was controlled automatically. The animals were fed on a commercial feed (CLEA Japan Inc., Tokyo, Japan, type CE-2) for 1 week after arrival. They were then placed on an experimental diet consisting of 10 g of soybean oil, 4 g of salt mixture (7), 1 g of vitamin mixture (7), 2 g of cellulose powder, 0.1 g of choline chloride, and 18 g of casein. The weight was made up to 100 g by addition of an appropriate amount of α-cornstarch. The animals were allowed access to the experimental diet and tap-H_2O 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 the sample-treated rats with regard to changes in body weight. No case of diarrheal symptoms was found.

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

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

ISOLATION OF (+)-CATECHIN, PRUNIN AND HESPERETIN 5-*O*-GLUCOSIDE.—(+)-Catechin [**1**], prunin [**2**], and hesperetin 5-*O*-glucoside [**3**] were isolated according to the procedure of Choi *et al.* (8) and identified by direct comparison with authentic samples (mp, ir, ^1H nmr, ^{13}C nmr).

EXPERIMENTAL PROCEDURE.—The MeOH extract, (+)-catechin, prunin, and hesperetin 5-*O*-glucoside suspended in 5% EtOH/saline were each administered ip once a day for 3 days to rats at the doses indicated in the tables, while control rats were treated with an equal volume of 5% EtOH/saline. Four hours after the last dose, the rats were sacrificed by decapitation and exsanguinated. Rats were killed between 2 and 3 PM to avoid the effect of circadian variation. Blood was collected and allowed to stand for several hours in a cold room at 4°. Serum was separated by centrifugation (1000 *g*, 10 min, 4°). The liver and epididymal adipose tissue were immediately removed, washed, blotted on filter paper, and weighed.

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 commercial reagents (TG-Five Kainos, Kainos Laboratories, Tokyo, Japan; Cholesterol E-Test Wako, Wako Pure Chemical Industries, Osaka, Japan). LDL- and HDL-cholesterol were determined by the method of Noma and co-workers (9,10).

DETERMINATION OF TOTAL LIPID AND TRIGLYCERIDE IN LIVER AND ADIPOSE TISSUE.—A portion of the liver was homogenized with 3 volumes of ice-cold 0.9% NaCl solution in a Potter-Elvehjem glass homogenizer with a Teflon pestle. The homogenate was filtered through 4 layers of gauze, and 1 ml of the filtrate was mixed with 20 ml of $CHCl_3$-MeOH (2:1). Adipose tissue was placed in 20 ml of $CHCl_3$/MeOH mixture. Total lipid was extracted by shaking. The residual tissue was then removed, and the $CHCl_3$/MeOH solution was partitioned and washed by the method of Folch *et al.* (11). The organic solution was evaporated and the residue was dried over P_2O_5 overnight. The concentration of total lipid was determined by gravimetry. A portion of the $CHCl_3$/MeOH solution extracted from both tissues was used for the determination of triglyceride, employing a commercial reagent as described above.

DETERMINATION OF TOTAL AND FREE CHOLESTEROL IN LIVER.—Cholesterol was extracted by a modification of the method reported by Ichida (12). A portion of the liver was homogenized with 15 volumes of EtOH. The homogenate was treated at 50–60° in an H_2O bath for 30 min and filtered through defatted filter paper (No. 7, Toyo Roshi). The residue was further extracted with about 10 ml of EtOH-Et_2O (3:1) at 50–60° for 30 min. The volume of the combined filtrates was adjusted to 10 ml. The concentrations of total and free cholesterol in this extract were determined using a commercial reagent as described above.

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

STATISTICS.—The significance of differences between the control and MeOH-extract-treated or flavonoid-component-treated groups was tested using Student's *t*-test.

RESULTS

EFFECT OF MeOH EXTRACT ON LIPID CONSTITUENTS IN SERUM.—The serum

triglyceride level showed a reduced tendency in the group given a dose of 40 mg, and that in the group given a dose of 80 mg was significantly decreased by 39% (Table 1). Total cholesterol was significantly lowered by 10% at both doses. The effect of an MeOH extract on serum lipoprotein concentrations was insignificant, while the atherogenic index (total cholesterol − HDL-cholesterol/HDL-cholesterol) was significantly reduced in the 80-mg-dose group as compared with the control group. Thus, hyperlipidemia induced with a high-fat diet was improved in the group given an 80-mg dose.

TABLE 1. Effect of an MeOH Extract of *Prunus davidiana* (stem) on Serum Levels in Rats Fed on a High-Fat Diet.[a]

Treatment	Dose (mg/kg body wt)	Triglyceride (mg/dl)	TC (mg/dl)	LDL-C (mg/dl)	HDL-C (mg/dl)	A.I.
Control	—	213.9 ± 20.7 (100)	126.2 ± 4.2 (100)	23.0 ± 2.0 (100)	18.5 ± 1.3 (100)	5.61 ± 0.21 (100)
MeOH extract . .	40	166.8 ± 22.0 (78)	113.2 ± 3.4 (90)[b]	19.4 ± 2.2 (84)	17.0 ± 0.5 (92)	5.66 ± 0.19 (101)
MeOH extract . .	80	129.6 ± 16.1 (61)[c]	114.1 ± 3.3 (90)[b]	17.5 ± 2.1 (76)	21.2 ± 1.0 (115)	4.40 ± 0.13 (78)[d]

[a]TC = total cholesterol; LDL-C = low-density lipoprotein-cholesterol; HDL-C = high-density lipoprotein-cholesterol; A.I. = atherogenic index. Values are mean ± SE for six rats. Figures in parentheses are percentages of the control value.

[b]Significantly different from the control value, $p < 0.05$.

[c]Significantly different from the control value, $p < 0.01$.

[d]Significantly different from the control value, $p < 0.001$.

EFFECT OF MeOH EXTRACT ON LIPID CONSTITUENTS IN LIVER.—Total lipid content and the level of triglyceride were not affected by administration of the MeOH extract, although there was a decreasing tendency (Table 2). Furthermore, no significant changes were found in the levels of total and free cholesterol.

TABLE 2. Effect of an MeOH Extract of *Prunus davidiana* (stem) on Lipid Contents in the Liver in Rats Fed on a High-Fat Diet.[a]

Treatment	Dose (mg/kg body wt)	Total lipid (mg/g tissue)	Triglyceride (mg/g tissue)	Total cholesterol (mg/g tissue)	Free cholesterol (mg/g tissue)
Control	—	21.65 ± 1.48 (100)	9.87 ± 0.68 (100)	3.88 ± 0.10 (100)	1.39 ± 0.04 (100)
MeOH extract . .	40	20.82 ± 0.67 (96)	9.51 ± 0.56 (96)	4.18 ± 0.27 (108)	1.46 ± 0.05 (105)
MeOH extract . .	80	20.20 ± 0.88 (93)	8.47 ± 0.92 (86)	4.08 ± 0.13 (105)	1.42 ± 0.07 (102)

[a]Values are mean ± SE for six rats. Figures in parentheses are percentages of the control value.

EFFECT OF MeOH EXTRACT ON LIPID CONSTITUENTS IN ADIPOSE TISSUE.—A striking increase in the level of triglyceride in adipose tissue was observed after ip administration of the MeOH extract at both 40 and 80 mg/kg (Table 3). The wet wt (% body wt) also increased significantly in the rats given 80 mg of the MeOH extract.

TABLE 3. Effect of an MeOH Extract of *Prunus davidiana* (stem) on Triglyceride Content in Adipose Tissue and Adipose Wet Wt in Rats Fed on a High-Fat Diet.[a]

Treatment	Dose (mg/kg body wt)	Triglyceride (mg/g tissue)	Wet wt (% of body wt)
Control	—	558.0 ± 14.0 (100)	1.24 ± 0.05 (100)
MeOH extract	40	633.5 ± 14.1 (114)[b]	1.28 ± 0.05 (103)
MeOH extract	80	653.7 ± 13.3 (117)[d]	1.56 ± 0.08 (126)[c]

[a]Values are mean ± SE for six rats. Figures in parentheses are percentages of the control value.
[b]Significantly different from the control value, $p<0.05$.
[c]Significantly different from the control value, $p<0.01$.
[d]Significantly different from the control value, $p<0.001$.

EFFECTS OF (+)-CATECHIN, PRUNIN, AND HESPERETIN 5-*O*-GLUCOSIDE ON LIPID CONSTITUENTS IN SERUM.—The effects on serum constituents after ip administration of flavonoid components purified from *P. davidiana* are shown in Table 4. In rats given (+)-catechin [**1**] (20 mg/kg), the serum triglyceride level fell to 141.0 mg/dl (a significant decrease of 27% from the control value). On the other hand, ip administration of prunin [**2**] (20 mg/kg) and hesperetin 5-*O*-glucoside [**3**] (10 mg/kg) to rats had no effect on serum triglyceride level, although the total cholesterol level was reduced significantly as compared with the control group.

TABLE 4. Effect of Flavonoid Compounds Isolated from *Prunus davidiana* (stem) on Serum Levels in Rats Fed on a High-Fat Diet.[a]

Treatment	Dose (mg/kg body wt)	Triglyceride (mg/dl)	Total cholesterol (mg/dl)
Control	—	192.9 ± 8.6 (100)	106.4 ± 2.6 (100)
(+)-Catechin	10	145.5 ± 24.2 (75)	100.8 ± 6.4 (95)
(+)-Catechin	20	141.0 ± 9.9 (73)[c]	105.8 ± 5.8 (99)
Prunin	10	173.0 ± 38.4 (90)	108.6 ± 4.1 (102)
Prunin	20	206.7 ± 35.4 (107)	96.7 ± 1.6 (91)[b]
Hesperetin 5-*O*-glucoside	10	192.4 ± 29.0 (100)	93.5 ± 4.3 (88)[b]
Hesperetin 5-*O*-glucoside	20	174.5 ± 37.9 (90)	107.1 ± 6.2 (101)

[a]Values are mean ± SE for six rats. Figures in parentheses are percentages of the control value.
[b]Significantly different from the control value, $p<0.05$.
[c]Significantly different from the control value, $p<0.01$.

DISCUSSION

A high-fat diet is known to cause hyperlipidemia and there is a close relationship between atherosclerosis and an increase or decrease of serum lipids. In particular, very-low-density lipoprotein and LDL may be risk factors, and HDL may be a preventive factor (13).

In the previous study, we found that an MeOH extract of *P. davidiana* stems as well as prunin isolated from it showed significant hypoglycemic and hypolipidemic effects in rats with streptozotocin-induced diabetes (6). In this work, we obtained evidence that repeated administration of the MeOH extract improved the hyperlipidemia induced by a high-fat diet in rats. Rats in the 80-mg dose group showed 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. Because apo-B-containing lipoprotein fractions are thought to be responsible for cholesterol deposition in atherosclerotic plaques (14), a reduction in LDL would be advantageous clinically, and in fact it was shown clearly that the present MeOH extract had an improving effect on the hyperlipidemia induced by a high-fat diet. This is thought to be the first report on the hypotriglyceridemic and hypocholesterolemic activities of an extract from *Prunus* species in rats with hyperlipidemia due to high-fat feeding.

Compared with the significant decrease in serum triglyceride and cholesterol levels, the levels of liver metabolites were not affected. On the other hand, the wet wt and the level of triglyceride in epididymal adipose tissue were increased following administration of the extract. These results suggest that the MeOH extract may accelerate the accumulation of lipid in adipose tissue as a result of its stimulating action on the uptake of circulating chylomicron and lipoprotein triglyceride. However, other mechanisms for this anti-hyperlipidemic action, such as inhibition of lipid absorption in the small intestine, inhibition of cholesterol biosynthesis in the liver, and acceleration of lipid utilization in muscles, cannot be ruled out.

The present study was also carried out to investigate whether the flavonoid components isolated from *P. davidiana* would be useful for the treatment of hyperlipidemia. Treatment of hyperlipidemic rats with 20 mg/kg (+)-catechin decreased the level of triglyceride. This result was more marked than that obtained with the MeOH extract. Thus, it is suggested that (+)-catechin is one of the active principles of this plant extract in high-fat-fed rats. (+)-Catechin has been isolated from many plants, e.g., *Polygonum bistorta* (15), *Chamaecyparis pisifera* (16), *Eucalyptus* spp. (17), *Prunus* spp. (18,19), *Sanguisorba* spp. (20), *Hypericum* spp. (21,22), *Ephedra helvetica* (23), *Rheum palmatum* (24), and pharmacological studies on inhibition of prostaglandin synthesis (25), mutagenicity (26), antiviral (27), anti-inflammatory (28), antioxidative (29), and anticoagulative activities (30) of (+)-catechin have been reported. Although Bonati and Mustich (31) have reported that oral administration of catechin (100 mg/kg) reduced the level of triglyceride by 30% compared with the control (not statistically significant) in rats with olive-oil-induced hyperlipidemia, no report on the hypotriglyceridemic activity of (+)-catechin has appeared. It is also evident that (+)-catechin does not produce hypocholesterolemic action in rats maintained on a high-fat diet. Hara and Oya (32) observed a hypocholesterolemic action of tea leaf catechin in cholesterol-fed rats but not in rats fed a stock diet. We also confirmed that (+)-catechin did not exert hypocholesterolemic activity in normal rats (data not shown), and additionally that this compound probably increases the utilization of cholesterol only when given to rats fed with excess cholesterol. In addition to the hypotriglyceridemic effect of (+)-catechin [**1**], prunin [**2**] (20 mg/kg) and hesperetin 5-*O*-glucoside [**3**] (10 mg/kg) exhibited a lowering action on the serum level of total cholesterol in high-fat-fed rats, indicating the possible hypocholesterolemic principles of this plant material. Flavonoids have been shown to possess a variety of biochemical and pharmacological activities, including hypolipidemic effects. Isoflavones such as formononetin, biochanin A, and pratensein, isolated from some commonly used legumes, showed hypolipidemic activity in both Triton WR 1339-induced hyperlipidemic and hypercholesterolemic

rats when administered individually (33,34). The effect of oral administration (10 mg/rat/day) of quercetin in rats fed a stock diet and a 1% cholesterol diet for a period of 6 and 12 weeks was studied by Basarkar and Nath (35). They demonstrated that quercetin exhibited hypolipidemic activity in cholesterol-fed rats but had no such effect in rats fed the stock diet at the end of both periods. On the other hand, Kato *et al.* (36) reported that dietary addition of 0.5% quercetin decreased the serum triglyceride level in both mice and rats fed a commercial stock diet. These differences in the results concerning the stock diet may have been due to dietary conditions and the animals used. Administration of rutin, *O*-ethylrutoside and methylchalcone from hesperidin through a stomach tube in rats under conditions of experimental cobalt-induced hyperlipidemia decreased the levels of free fatty acids, esterified fatty acids, total cholesterol, β-lipoproteins and total lipids distinctly (37). However, no report on the hypocholesterolemic activity of prunin and hesperetin 5-*O*-glucoside has appeared.

The findings of the present work indicate that an MeOH extract of *P. davidiana* stems and its flavonoids, (+)-catechin, prunin, and hesperetin 5-*O*-glucoside, may be useful for the treatment of hyperlipidemic disease. However, production of hypolipidemia by some other compounds entirely different from these cannot be ruled out at present. Further comprehensive chemical and pharmacological investigations will be needed to elucidate the exact mechanism of these effects and to isolate the active principles responsible.

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