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Effect of *Platycodon Grandiflorum* Aqueous Extract on Lipid Levels in Rats

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This study investigated the effects of *Platycodon grandiflorum* aqueous extract on lipid concentration of serum and liver in rats fed high cholesterol diet. Male Sprague-Dawley rats were assigned to three groups (control, low dose of extract, high dose of extract) for four weeks. The serum total cholesterol concentration was significantly lower in the low and high doses of extract groups than in the control group. There was a significant decrease in the free cholesterol, cholesterol ester, LDL-cholesterol, and triglycerides concentrations in serum, and the total cholesterol and the triglycerides contentin liver in the low and high doses of extract groups compared to the control group. When the serum phospholipid concentration was compared among the groups, it was significantly lower in high dose of extract group than in control and low dose of extract group. It can be postulated that *Platycodon grandiflorum* aqueous extract may possess substantial hypolipidemic properties in rats.

Key words: *Platycodon grandiflorum*, Lipids, Serum, Liver, Rats

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INTRODUCTION

Platycodon grandiflorum is a perennial grass that grows autogenously in Korea, the northeastern region of China and Japan, and in grasslands of fields and mountains as well exposed to the sun. The root of *Platycodon grandiflorum* is used for a medicinal herb for patients with hemoptysis or for food.[1] When the root is washed with water and dried or peeled and then dried, it is called as the dried root of Platycodon and used for medicinal purposes. The root contains triterpenoid saponin of 2% and sterol of 0.03% of dry weight. In addition, it contains inulin and platycodinin (polysaccharide composed of 10 molecules of fructose) as saccharide.[1]

It has been used more often for food than for medicinal purposes from old times. In the Korean traditional medicine, it has been prescribed that *Platycodon grandiflorum* is good for cough, phlegm, sore throat having a close relation to a pulmonary disorder, amusia, dysuria, diarrhea, tenesmus and the like.[2,3] In some studies, it was reported that *Platycodon grandiflorum* had effects on the protection of the liver against acetaminophen in mice[4] and further an effect on hyperlipidemia induced by a diet with high-fat in rats.[5]

In this study, we examined the effect of aqueous extract of *Platycodon grandiflorum* on lipid levels when rats were fed a high cholesterol diet.

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MATERIALS AND NETHODS

1. Materials

1) Experimental Animals

Male Sprague-Dawley rats, which were 4 weeks old, were preliminarily fed AIN-76A diet for 2 weeks and adapted to the diet. Then, they were divided into three groups by 6 rats according to the randomized complete block design, and each rat was put into a cage and experimentally bred for 4 weeks. For the experimental period, they were let to take a diet and water freely, and the temperature and the humidity were maintained at 20± 2℃ and 50±10%, respectively. For light and darkness, it was illuminated in a cycle of 12 hours (07:00~19:00).

2) Preparation of Diet

A diet was prepared in accordance with Table 1 as follows: 1 kg of *Platycodon grandiflorum* was put into 3 liter of water, which was boiled at 100℃ for 24 hours.

Then, it was decompressed and extracted at 60℃. For low and high doses of extract groups, the extract from *Platycodon grandiflorum* was orally administered respectively by 1 ml and 2 ml per 100 g of the weight at a given time every day, and for a control group, 1 ml of physiological saline was orally administered as a sham component.

Table 1. Composition of diets (%)

Ingredient	
Casein	20.0
Mineral mixture[1)]	3.5
Vitamin mixture[2)]	1.0
Choline bitartrate	0.2
Cholesterol	0.75
Sodium Cholate	0.25
Sucrose	59.3
Cellulose powder	3.0
Lard	12.0

[1)] Mineral mixture (per 1 kg): Calcium carbonate, 357 g; monopotassium phosphate, 196 g; Potassium citrate, 70.78 g; Sodium chloride, 74 g; Magnesium oxide, 24 g; Ferric citrate, 6.06 g; Zinc carbonate, 1.65 g; Manganous carbonate, 0.63 g; Cupric carbonate, 0.30 g; Potassium iodate, 0.01 g; Ammonium paramolybdate, 0.00785 g

[2)] Vitamin mixture(per 1 kg): Nicotinic acid, 3.0 g; Ca Pantothenate, 1.6 g; Pyridoxine HCl 0.7 g; Thiamin HCl, 0.6 g; Riboflavin 0.6 g; Folic acid, 0.2 g; D-Biotin, 0.02 g; Vitamin B12, 2.5 g; Vitamin E, 15.0 g; Vitamin A, 0.8 g; Vitamin D3, 0.25 g; Vitamin K, 0.075 g; Powdered sucrose, 974.655 g

3) Works with Experiment Animals

The weight of each experiment animal was measured in the morning every other day during the experimental period, and the diet consumption was calculated by measuring the residual amount. The blood was collected from heart and left in ice water for 1 hour or so, and then centrifuged at 3,000 rpm for 15 minutes to collect serum, which was used in the experiment. The liver was extracted from the experimental animals and washed with saline; and then water was removed from these organs with gauze before they were placed in a deep freezer at -70℃ until further analysis.

2. Methods

1) Isolation of Lipid Component

One milliliter of serum and 1.0 g of the liver tissue were collected, and about 25 ml of the mixed liquid of chloroform: methanol (C:M=2:1, v/v) was added. Lipid was extracted and dried with nitrogen gas. Then, it was dissolved in a proper quantity of hexane, with which a thin layer using 60 g of Kieselgel was spotted. Then, it was developed with a developing liquid (petroleum ether : ethyl ether: acetic acid=82:18:1, v/v) and dried. Further, it was color-developed with iodine vapor. Phospholipids, triglycerides and cholesterol ester were isolated.

2) Measurement of Cholesterol Concentration

Total cholesterol concentration and free cholesterol concentration in serum were measured by a spectrophotometer (Hitachi, 4020), by using a kit for measuring total cholesterol and free cholesterol concentrations. Cholesterol ester concentration was calculated by subtracting the free cholesterol concentration from the total cholesterol concentration. Serum LDL concentration was measured by using a kit (Boehringer Mannheim) and LDL-cholesterol concentration was indicated by multiplying the LDL concentration by 0.35. HDL-cholesterol concentration was measured by a biochemical automatic analyzer (Hitachi 736-20), after letting the serum to react on the reagent included in the kit (Boehringer Mannheim). Lipid was extracted from 0.5 g of the liver tissue with the mixed liquid of chloroform: methanol, which was made to be a constant volume of 50 ml.

3) Measurement of Triglycerides and Phospholipids

Triglycerides concentration and phospholipids concentration in the serum were measured by using a biochemical automatic analyzer (Hitachi, 736-20) and a spectrophotomete (Hitachi 4020) respectively. Triglycerides concentration and phospholipids concentration in the liver were measured and calculated by the same method as mentioned above, after a given quantity of the lipid extract dissolved in the mixed solution of chloroform: methanol was collected, dried and solidified.

4) Statistical Analysis

The results were statistically processed using the SPSS software packages and mean values and standard errors per each experimental group were calculated. At the level of $\alpha = 0.05$, significance between respective experiment groups was examined by the Duncan's multiple test.

RESULTS AND DISCUSSION

1. Serum Lipid Profiles

Total Cholesterol, HDL-Cholesterol, Free Cholesterol and Cholesterol Ester Concentrations

Concentrations of total cholesterol, HDL-cholesterol, free cholesterol and cholesterol ester were shown in Fig. 1. The total cholesterol concentration in the serum was significantly lower in the low and high doses of extract feeding groups than in the control group (control 180.1±7.4 mg/dl, low dose of extract 141.5±2.8 mg/dl, high dose of extract 137.8±8.4 mg/dl). The HDL-cholesterol concentration was not statistically different among the three groups.

The free cholesterol (control 27.2±1.2 mg/dl, low dose

of extract 21.3±0.8 mg/dl, high dose of extract 18.9±1.2 mg/dl) and the cholesterol ester concentration (control 154.9±6.5 mg/dl, low dose of extract 119.7±2.5 mg/dl, high dose of extract 118.9±7.3 mg/dl) were significantly lower in the low and high doses of extract groups than in the control group.

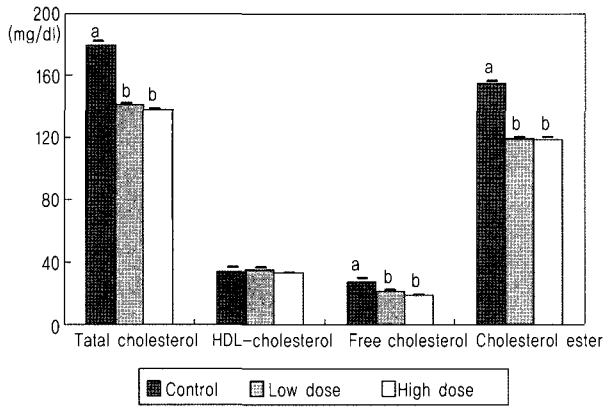

Fig. 1. Concentration of total cholesterol, HDL-cholesterol, free cholesterol, and cholesterol ester in serum of rats fed.

n=6 each group; low dose group: 1 ml aqueous extract of 100 g body weight; high lose group: 2 ml aqueous extract of 100 g body weight; Means not sharing common letters are significantly different at $\alpha<0.05$.

In the experiment of feeding monkeys with a diet containing saponin extracted from alfalfa leaves, extracts were added 0.6% of total dry weight and the results showed that extracts lowered the serum cholesterol concentration.[6] Saponin of alfalfa might be combined with cholesterol in the small intestine so that the absorption of cholesterol was prevented and thus alfalfa had an action to lower the serum cholesterol concentration.[7] Further the refined saponin lowered the blood cholesterol concentration by inhibiting re-absorption of bile acids in the small intestine and increasing excretion through feces.[8] It was reported that saponin promoted the hydrolysis of chylomicron by accelerating the activity of lipoprotein lipase, inhibited the increase of the blood cholesterol concentration by accelerating an enzyme associated with lipid metabolism and prevented atheriosclerosis.[9]

It has been known that HDL-cholesterol carries cholesterol from peripheral tissuesto the liver, prevents LDL-cholesterol from being accumulated in the vessel wall and further prevents arteriosclerosis by removing cholesterol accumulated in the vessel wall.[9,10] When water soluble fiber of psyllium was added to a low fat diet, the serum cholesterol concentration and the LDL-cholesterol concentration of hypercholesterolemic patient went down and the HDL-cholesterol concentration went up.[11] However, the group to which a low fat diet with grain was given showed no significant changes in the HDL-cholesterol concentration.[12] When dietary cholesterol was absorbed in the small intestine, it entered mucosal cells in the form of cholesterol ester.13) When dietary cholesterol was administered to rats, free cholesterol concentration and cholesterol ester concentration in the blood went up.[14]

LDL-Cholesterol, Triglycerides and Phospholipids Concentration

Serum concentrations of LDL-cholesterol, triglycerides and phospholipidswere shown in Fig. 2. The LDL-cholesterol concentration was significantly lower in the low and high doses of extract group than in the control group (control 86.1±1.1 mg/dl, low dose of extract 66.5±1.3 mg/dl, high dose of extract 60.4±1.5 mg/dl).

The triglycerides concentration in the serum was significantly lower in the low and high doses of extract groups than in the control group (control 131.3±10.7 mg/dl, low dose of extract 55.7±2.0 mg/dl, high dose of extract 31.3±2.5 mg/dl). When the phospholipids concentration was compared among the groups, it was significantly lower in high dose of extract group than in the low dose of extract group and control group (control 138.7±4.3 mg/dl, low dose of extract 124.9±2.9 mg/dl, high dose of extract 84.1±4.4 mg/dl).

It is known that LDL-cholesterol is a main carrier of blood cholesterol, which is a factor promoting arteriosclerosis by carrying and accumulating cholesterol into the arterial vessel wall or the peripheral tissue.[15] The serum LDL is combined with a receptor, a specific combination region of the cell surface, and it is removed by the liver and

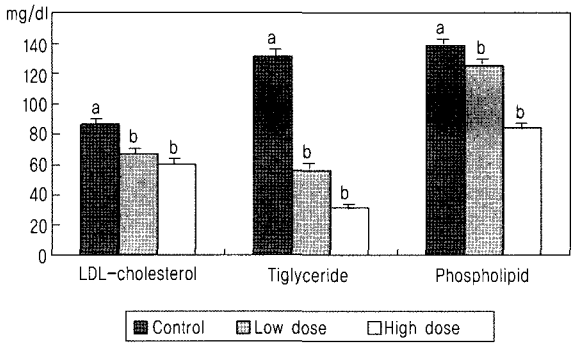

Fig. 2. Concentration of LDL-cholesterol, triglyceride, and phospholipid in serum of rats

n=6 each group; low dose group: 1 ml aqueous extract of 100 g body weight; high lose group: 2 ml aqueous extract of 100 g body weight; Means not sharing common letters are significantly different at $\alpha<0.05$.

other tissues.[16,17] Meanwhile, if this LDL-receptor has any defect due to a genetic factor, or this LDL-receptor's activity is deteriorated due to dietary cholesterol, LDL fails to be combined with the LDL-receptor, and the serum LDL concentration goes up.[18]

It was reported that a mixed diet of rice bran with fish oil had positive effect on lipid metabolism by increasing the LDL-receptor's activity and deteriorating the synthesis of fatty acids.[19] Also, when 3 kinds of bran, wheat bran, rice bran and oat bran were respectively administered to hypercholesterolemic patients, only oat bran decreased the LDL-cholesterol concentration significantly.[20] Water soluble fiber of oat bran lowered the plasma cholesterol level and the portion of water soluble fiber of total plant fiber was 38.7%.[21] Meanwhile, water soluble pectin lowered the LDL-cholesterol concentration more than oat bran and wheat bran did.[22]

The blood triglycerides concentration was lowered since lipoprotein lipase existing in capillary wall acts as a catalyst to decompose chylomicron and VLDL which are major carriers of triglycerides in the blood.[23] As a result of feeding chickens with alfalfa for 3~6 weeks, the serum triglycerides concentration went down considerably. Cellulose, such as rice bran and peanut hull also lowered serum phospholipids concentration compared to the diet without fiber.[24] When a mixture of rice bran with fish oil was administered, the plasma triglycerides concentration and the liver triglycerides concentration were significantly decreased.[25]

The hypolipidemic properties of Platycodon grandiflorum aqueous extract may be due to saponin or water-soluble fiber. The results of this present study are in agreement with previous results[7-9,19-25] which showed that the diets supplemented with saponin or water-soluble fiber promoted the excretion by curtailing internal resorption of triglycerides and cholesterol. Platycodon grandiflorum aqueous extract may inhibit internal resorption of triglycerides and cholesterol by combining directly with, and increasing the excretion of, intestinal triglycerides and cholesterol.

Total Cholesterol, Triglycerides and Phospholipids Contents in Liver

Contents of total cholesterol, triglycerides and phospholipidsin the liver are shown in Fig. 3. The total cholesterol (control 14.8±0.3 mg/g, low dose of extract 10.8±3.5 mg/g, high dose of extract 10.3±3.0 mg/g) and the triglycerides contents (control 47.2±6.1 mg/g, low dose of extract 31.1±2.7 mg/g, high dose of extract 32.7±2.6 mg/g) were lower in the low and high doses of extract groups than in the control group. However, there was no significant difference in the phospholipids content among the three groups.

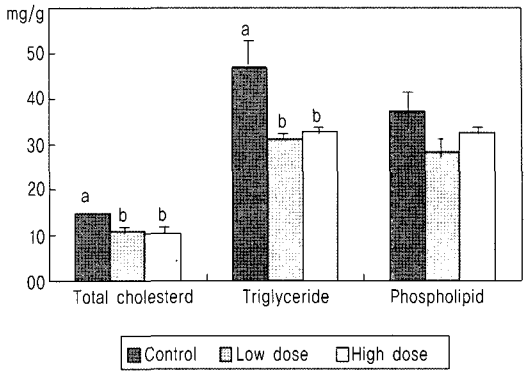

Fig. 3. Concentration of total cholesterol, triglyceride and phospholipid in liver of rats

n=6 each group; low dose group: 1 ml' aqueous extract of 100 g body weight; high lose group: 2 ml aqueous extract of 100 g body weight; Means not sharing common letters are significantly different at $\alpha<0.05$.

In the liver tissue of rats fed dietary fiber extracts of black gram, the cholesterol content was decreased, because the conversion of cholesterol into bile acids in the liver was more increased.[26] Some reports showed that cellulose increased the cholesterol level slightly in the serum and the liver,[27,28] while there is even a report showing that unless refined cellulose is added to a diet by more than 15%, it failed to have an effect on the lowering of cholesterol.[26] In the group to which cellulose was administered, the cholesterol content in the liver was significantly increased, and in the group to which hemicelluloses was administered, it was not changed. But in the group to which pectin and lignin were respectively administered, it was slightly increased, and further in the group to which pectin and hemicelluloses were respectively administered, the triglycerides content was the highest.[29] Meanwhile, as a result of feeding rats with wheat flour and corn flour respectively for 8 weeks, the cholesterol content in the liver was decreased.[30] When feeding rats with 15% cellulose for 6 weeks, the triglycerides content in the liver was considerably decreased.[31]

Future investigations may produce different results when either using different ways of extracts of the same plant or implementing the same experimental procedure to other animal species. Additional studies regarding the mechanism of action of *Platycodon grandiflorum* remains to be done to aim at purifying and characterizing the specific biochemical components.

CONCLUSION

In groups administered with *Platycodon grandiflorum* aqueous extract, the following significant effects were observed: the serum total cholesterol concentration was significantly lower in the low and high doses of extract groups than in the control group; the free cholesterol, cholesterol ester, LDL-cholesterol, triglycerides concentrations in serum and the total cholesterol and the triglycerides contentsin liver were lower in the low and high doses of extract groups than in the control group. It may eventually be possible to recommend consuming *Platycodon grandiflorum* aqueous extract for treating and preventing hyperlipidemia.

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