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Effects of Red Ginseng-Crude Saponin on Plasma Lipid Levels in Rats Fed on a Diet High in Cholesterol and Triglyceride

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Abstract ☐ The effect of Red ginseng saponin on plasma lipid levels in Wistar rats fed on a diet high in cholesterol and triglyceride was determined. A dose of Red ginseng-crude saponin (150 mg/kg/day) was administered orally for 4 weeks to Wistar rats fed on a diet containing 2% cholesterol and 10% olive oil. Plasma levels of total cholesterol, HDL-cholesterol and triglyceride were measured and lipoproteins were analyzed by using electrophoretic technique. Red ginseng saponin showed no significant changes of HDL-cholesterol level but it lowered plasma levels of total cholesterol and elevate those of triglyceride intensively.

Keywords ☐ Red ginseng saponin, Lipoprotein, Hypocholesterolemic effect, Hypertriglyceridemic effect, HDL-cholesterol.

It has been reported that plasma lipid levels and lipoprotein pattern such as total cholesterol, triglyceride, very low density lipoprotein (VLDL) and low density lipoprotein (LDL) have been associated with the risk of atherosclerosis [1-5]. The higher the concentration of any one of these blood lipids, the greater the risk of atherosclerosis.

Barr *et al.*[6] reported that healthy men showed higher level of high density lipoprotein (HDL) than men with coronary heart disease. Though the mechanism for a possible antiatherogenic effect of HDL has been unknown, the following hypothesis have been suggested as the functions of plasma HDL[8-11].

1) The transport of cholesterol from peripheral tissues to the liver for subsequent catabolism and excretion.

2) The inhibition of uptake of LDL by intimal cell.

It was found that ginseng saponin, one of major component of *Panax ginseng* had the influences on lipid metabolism.[14-20] Saponin stimulated the absorption, metabolism and transport of lipids[14-15]. It has been also reported that ginseng saponin decreased plasma cholesterol and triglyceride level and inhibited aortic atheroma formation in animals with hypercholesterolemia caused by long administration of high cholesterol or feeding on a diet containing high cholesterol[16-20]. Administration of ginseng sapoin was found to inhibit increase in LDL and VLDL level and decrease in HDL level induced by feeding rats on a high cholesteroldiet[20].

But, the effect of ginseng saponin on lipid metabolism in a high neutral fat and cholesterol diet-fed animals has been not yet reported.

The present report describes the influence of crude ginseng saponin on plasma lipid levels observed in Wistar male rats fed on a diet high in cholesterol and triglyceride.

EXPERIMENTAL METHODS

Materials

Acrylamide, bisacrylamide, tetramethylethyle-

nediamine were perchased from E.Merck Co. Tris, glycine riboflavin and triglyceride standard were obtained from Shinyo Pure Chemicals Co., and ammonium persulfate was the product of Sigma Chemical Co. Cholesterol E Kit (Enzymatic method) was gifted by Young-Dong Pharmaceutical Co.. All other reagents used were of special reagent grade.

Red ginseng-crude saponin from the fine roots of red ginseng was obtained as a gift sample from Korea Ginseng & Tobacco Research Institute, which was further purified by using the filtration technique on the deactivated neutral Al_2O_3 column (2×3 cm bed for 500 mg crude saponin, elution solvent: methanol) followed by the treatment with charcoal (saponin: charcoal=2 : 1, stirred overnight at room temperature).

Animals

Sixteen healthy Wistar male rats weighing 200-250 g were divided into 3 groups.

Control group (I)-fed on normal chow diet and administered saline 1 ml/day orally.

Fat group (II)-fed on chow diet containing 2% cholesterol plus 10% olive oil and administered saline 1 ml/day orally.

Treated group(III)-fed on chow diet containing 2% cholesterol plus 10% olive oil and administered ginseng crude saponin 150 mg/kg/day orally.

All experimental rats were obtained from the Breeding Center of Seoul National University and were allowed free access to food and water. They were maintained on the above mentioned conditions for 4 weeks, and blood was drawn by cardiac puncture under ethyl ether anesthesia after a 12 hour-fast. Plasma was prepared from blood treated with EDTA 1 mg/ml.

Determination of Plasma Lipid Levels

Triglyceride was analyzed by a modification of the method of V.M.Sardesci *et al.*[21] and E.V.Handel[22]. This procedure involved the extraction and saponification of triglyceride, the oxidation of the glycerol moiety to formaldehyde, and the conversion of formaldehyde to a yellow-colored compound, 3, 5-diacetyl-1, 4-dihydrolulidine, the intensity of which would be determined spectrophotometrically. One ml of chloroform was added to 1 g zeolite ground to 80-100 mesh in a glass stoppered 15 ml-centrifuge tube, and shaken. 0.5 ml plasma was placed on the zeolite and mixed thoroughly. Nine ml of chloroform was added to the test tube. The tube was stoppered, shaken vigorously and kept overnight. Through coarse, chloform-washed filter paper, the mixture was filtered and 1 ml of filtrate was pipetted into other test tube. After removal of the solvent, 0.5 ml of alcoholic potassium hydroxide was added to the test tube and incubated in 60°C water bath for 15 min. At the end of the incubation period, 0.5 ml of 0.2 N sulfuric acid was added to the test tube, followed by 0.1 ml of sodium metaperiodate (0.05N). After exactly 10 min., the oxidation was stopped by the addition of sodium arsenite 0.1 ml. Several minutes later, 1 ml of water was added to the test tube, followed by 2 ml of acetyl acetone reagent, the contents of the test tube were mixed well. The mixture was incubated in a water bath at 58°C for 10 min., cooled to room temperature and the absorbance of the mixture was determined at 412 nm.

Total cholesterol was determined by the method of C.A.Charles *et al.*[23] and HDL-cholesterol by the methods of M.Burstein *et al.*[24] and C.A.Charles *et al.*[23] Cholesterol esters are hydrolyzed to free cholesterol by cholesterol hydrolase. The free cholesterol produced is oxidized by cholesterol oxidase to cholest-4-en-3-one with the simultaneous production of hydrogen

peroxide, which oxidatively couples with 4-aminoantipyrine and phenol in the presence of peroxidase to yield a chromogen with maximum absorption at 500 nm. 20 μl of plasma was pipetted into the test tube. One ml of reaction mixture (Young-Dong Pharmaceutical Co.) was added to the test tube and mixed thoroughly. The mixture was incubated in a water bath at 37°C for 15 min. The absorbance of mixture was measured at 412 nm.

Chylomicrons, VLDL and LDL are precipitated by adding phosphotungstic acid and magnesium ions to the sample. Centrifugation leaves only the HDL in the supernatant. HDL-cholesterol level is determined enzymatically. One ml of plasma was pipetted into the test tube. 100 μl of precipitant solution (phosphotungstic acid 4.8 g/100 ml: $MgCl_2$ 3.0 mol/l=5 : 1) was added to the plasma and mixed thoroughly. The mixture was allowed to stand for 10 min. and centrifuged at 12,000 rpm for 2 min. HDL-cholesterol level was determined enzymatically in the supernatant.

All measured values were expressed as mean ±standard deviation, and statiscally analyzed by student's t-test. P-value above 0.05 was considered as not significant.

Polyacrylamide-Gel Electrophoresis of Plasma Lipoprotein

Lipoprotein electrophoresis on 3.75% polyacrylamide gel was run according to the method of C.C. Frings *et al*[25]. 1.5 ml of freshly prepared separating gel solution was added to each tube. Enough water was carfully added on top of the gel solution and the separating gel solution allowed to polymerize for 30 minutes at room temperature. After photoplymerization was complete, inverted the tubes and blotted on absorbent paper. 0.1 ml of concentrating gel solution was added to each tube. The gel solution was layered with water and allowed the tubes to remain undisturbed for 10 minutes under intense light. After photopolymerization, the tubes were inverted and blotted on absorbent paper. 20 μl of plasma gel solution and 0.2ml of Sudan black B-gel solution were added to each tube. Each tube was mixed by inversion and each tube was layered with reservoir buffer. The tubes were allowed to stand for 30 minutes under intense light. After photopolymerization was completed, the gel tubes were ready to be inserted into the electrophoretic cell. Reservoir buffer was poured into the upper (200ml) and lower parts (800ml) of electrophoretic cell. The electrophoresis was performed for 35 minutes at 5mA per gel tube. Densitograms were measured with a 625nm interference filter without removing the gel from the glass tube[26] and the glass tubes were photographed with Fuji micrfilm HR_2.

RESULTS AND DISCUSSION

The plasma levels of total cholesterol, triglyceride and HDL-cholesterol obtained for each group are given in Table I. Fat group (II) showed significantly higher levels of plasma cholesterol (172%) and triglyceride (185%), but a significantly lower level of HDL-cholesterol (76%) than control group (I). Comparing treated group (III) with fat group (II), we could easily realize that the adminstration of ginseng crude saponin showed a highly significant restriction (84%) in the rise of total cholesterol level, and produced a significantly higher level (167%) in plasma triglyceride than fat group (II). It was also found that the HDL-cholesterol levels were not significantly changed in ginseng crude saponin-treated group (III).

Effects of ginseng saponin on plasma lipid

Table I: Effects of ginseng crude saponin on total cholesterol, triglyceride and HDL-cholesterol level in plasma. (mg/dl. Mean±S.D.)

		Total cholesterol	Triglyceride*	HDL cholesterol	HDL-total cholesterol ratio
Control group(I)	5	57.16± 4.00	91.47± 8.46	46.18±3.47	0.79±0.04
Fat group(II)	5	98.17±17.92[a]	169.34±17.85[a]	35.22±3.32[a]	0.37±0.07[a]
Treated group(III)	6	82.43±18.65[a]	283.46±70.35[a]	36.30±2.96[b]	0.47±0.12[c]

* Triglyceride levels are expressed as triolein-equivalents.

a: $p<0.001$ b: NS(not significant) (Control Group vs Fat Group, Fat Group vs. Treated Group) c: $p<0.02$

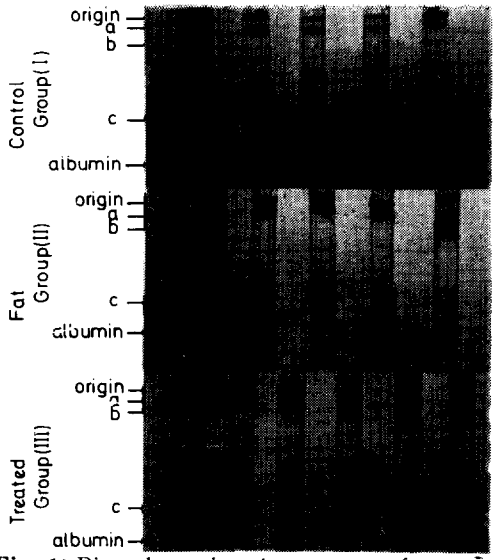

Fig. 1: Disc electrophoretic patterns of rat-plasma lipoproteins stained with Sudan black-B
a: VLDL band, b: LDL-band, c: HDL-band

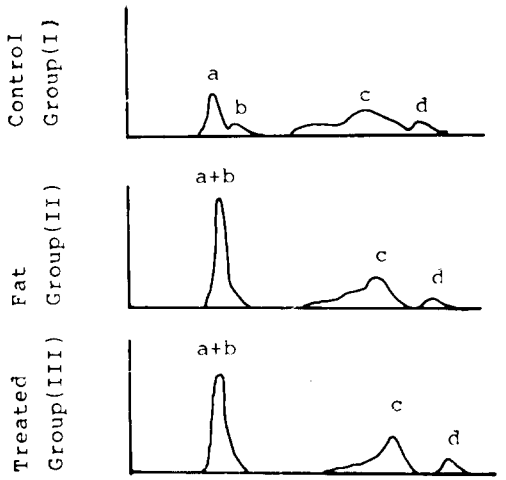

Fig. 2: Densitometric scans of rat plasma-lipoprotein electrophoresed on polyacrylamide-gel
a: Very low density lipoprotein
b: Low density lipoprotein
c: High density lipoprotein
d: Albumin

levels have been reported by many investigators[14-20]. Park[16] and Im[19] *et al.* reported that administration of ginseng saponin to rats fed on normal chow diet produced hypocholesterolemic and hypotriglyceridemic effect, and the same effects were observed in rats treated with ginseng saponin and cholesterol suspension. But our results are dfferent from those of these investigators. Ginseng crude saponin-treated group (III) showed unexpectedly a significantly higher triglyceride level (167%) than fat group (II). Park[16] reported that ginseng saponin-administration to normalipidemic rats produced an increase in plasma triglyceride level at the 4th week and its decrease at the 8th week. Considering the results of Park's study, further studies are necessary to confirm the influence of the adminstration period of ginseng saponin on the plasma trigycerlae level.

The electrophoregrams and their densitometric scans of lipoprotein pattern are shown in Fig. 2 and 3. Obvious isolation of VLDL-and LDL-band was not observed on polyacrylamide electrophoregrams of the plasma lipoproteins of fat group (II). This pattern indicates hypercholesterolemic and hypertriglyceridemic state, which could be comparable with the lipoprot-

ein pattern of patients with hyperlipoproteinemia type III on the same supporting medium[27].

Densitometric quantitation of lipoproteins corresponds nearly to the results of direct measured values of lipoproteins. Data obtained from the direct measured total cholesterol and HDL-cholesterol levels and the relative peak intensities of lipoprotein densitograms indicate that Ca. 473% increase in the sum of LDL- and VLDL-cholesterol level and Ca. 53% decrease in the HDL-/total-cholesterol ratio were produced in the plasma of rats treated with the high fat diet. Administration of ginseng crude saponin produced a marked decrease (74%) in the sum of LDL-and VLDL-cholesterol level as seen in the treated group. The HDL-/total-cholesterol ratio was significantly increased (127%), but HDL-cholesterol level was not changed in treated in treated group (III). This means that the administration of ginseng crude saponin stimulate a decrease in plasma total cholesterol level. In conclusion, ginseng crude saponin from the fine roots of red Ginseng showed hypertriglyceridemic effect and hypocholesterolemic effect. Because of these opposite effects, it is very difficult to evaluate ginseng crude saponin as a positive antiatherosclerotic and hypolipidemic effect.

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