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Changes of Plasma and Hepatic Lipids, Hydroxy-Methyl-Glutaryl CoA Reductase Activity and Acyl-CoA:Cholesterol Acyltransferase Activity by Supplementation of Hot Water Extracts from *Rosa rugosa*, *Crataegus pinnatifida* and *Polygonum cuspidatum* in High-Cholesterol Fed Rats

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Abstract

Lipid lowering properties from three plant water extracts, *Rosa rugosa*, *Crataegus pinnatifida* and *Polygonum cuspidatum*, were tested by supplementing a 1% high-cholesterol diet with them in rats. Plasma triglyceride levels in *Rosa rugosa*, *Crataegus pinnatifida* and *Polygonum cuspidatum* groups were significantly lower compared to that of the control, by 29%, 24% and 47% respectively. Hepatic triglyceride levels in *Rosa rugosa* and *Crataegus pinnatifida* groups were significantly lower compared to the control by 11% and 15% respectively. Hepatic HMG-CoA reductase activity in *Rosa rugosa* group was significantly greater compared to the control by 406%. Hepatic ACAT activity was significantly lower in *Polygonum cuspidatum* group compared to the control by 28%. By multiple regression results, only plasma cholesterol was associated significantly ($p<0.05$) with liver HMG-CoA reductase activity. Plasma cholesterol explained 12% of the variance of the liver HMG-CoA reductase activity. In conclusion, we have shown that hot water extracts from *Rosa rugosa*, *Crataegus pinnatifida* and *Polygonum cuspidatum* lowered plasma triglycerides in rats fed on a high-cholesterol diet. Data suggests that these extracts could potentially prevent or treat hypertriglyceridemia induced by a high fat diet and fatty liver.

Key words: triglycerides, cholesterol, HMG-CoA reductase, ACAT

INTRODUCTION

According to 1995 Korean annual report on the cause of death statistic, the death rate from diseases of the circulatory system (26.3%) was greater than that of neoplasm (21.3%) (1). With an increase of high-fat fast food (hamburger, pizza, fried chickens etc.) and meat consumption, the prevalence of circulatory system disease has been increasing every year. As a result, there is an increased awareness for the need to lower elevated plasma lipids in order to decrease the risk of developing coronary heart disease (CHD). Considering the increment of high-fat food intake in modern lifestyle, lipid lowering agents can be used as tools for removing extra cholesterol and triglyceride from body, thus the health food market for lipid lowering functional foods is expected to be greatly increased. In Korea as well as western societies, a variety of different diseases associated with high blood cholesterol have become a hot issue. To solve this problem, much effort to screen and identify lipid lowering components and to develop bioactive compound as potential enzyme inhibitors has been made.

In the current study, we have investigated changes of plasma and hepatic lipids, hydroxy-methyl-glutaryl CoA (HMG-CoA) reductase activity and acyl-CoA:cholesterol acyltransferase (ACAT) activity by supplementation of *Rosa rugosa*, *Crataegus pinnatifida* and *Polygonum cuspidatum* extracts in high-cholesterol fed rats.

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MATERIALS AND METHODS

Preparations of plant extracts

Rosa rugosa (dried root), *Crataegus pinnatifida* (dried fruit) and *Polygonum cuspidatum* (dried root) purchased in a Korean herb shop were minced after a washing and drying process in the shade. These materials were extracted with boiled distilled water (3 kg/10 L H_2O) in a pressure cooker twice. These extracts were filtered and lyophilized before adding into the animal diet.

Animals and diets

Seventy-one 3-week-old male Sprague-Dawley (Korean chemical institutes Daejun, Korea) were obtained and individually housed in stainless steel cages in a temperature (22±1°C), humidity (50±5%) and light controlled room with a 12-hr light-dark cycle. The animals were fed normal chow (Jeiljedang Suwon, Korea) for 3 weeks, then randomly divided into 4 groups; one control group and three experimental groups, *Rosa rugosa*, *Crataegus pinnatifida* and *Polygonum cuspidatum* group. Experimental diets containing 1% cholesterol plus 2% herb extracts, as shown in Table 1, were fed for 47 days. The water and food were freely provided.

Food intake was measured daily and body weight was measured weekly. Weight gain was represented as weight increments for the experimental period. Food efficiency rate was calculated by weight gain/mean food intake.

Table 1. Composition of experimental diets (unit: % of diet)

	Control (n=11)	*Rosa rugosa* (n=10)	*Crataegus pinnatifida* (n=10)	*Polygonum cuspidatum* (n=10)
Casein	20	20	20	20
D,L-methionine	0.3	0.3	0.3	0.3
Sucrose	49	47	47	47
Corn starch	15	15	15	15
Cellulose powder	5	5	5	5
Corn oil	5	5	5	5
Choline bitartrate	0.2	0.2	0.2	0.2
Mineral mixture[1)]	3.5	3.5	3.5	3.5
Vitamin mixture[2)]	1	1	1	1
Cholesterol	1	1	1	1
Freeze-dried extract[3)]				
Rosa rugosa		2		
Crataegus pinnatifida			2	
Polygonum cuspidatum				2
Total	100	100	100	100

[1)]AIN-76 Mineral mixture
[2)]AIN-76 Vitamin mixture
[3)]Dried powder from plant extract with boiling water

Prior to sacrificing the animals, food was withheld from their cages for about 15 hours. Each animal was anesthetized by injection of Ketamin-HCl. Blood was collected from the inferior vena cava, centrifuged (4°C, 3000 rpm, 20 min), plasma was divided into vials and kept frozen at -70°C. Liver, heart and kidney were rinsed with phosphate buffered saline solution, wiped with a paper towel, weighed and frozen at -70°C. Two grams of liver tissue were taken separately for measurement of enzyme activity. Small portions of liver tissue were separately fixed in 10% formalin solution for preparation of light microscopic observation.

Measurements of plasma lipids, hepatic lipids, and plasma GOT and GPT activities

Plasma total cholesterol levels were determined by a commercial modification of the cholesterol oxidase method (2) using kits from Asan pharm. Co. (Korea). The HDL fraction was prepared by heparin-manganese precipitation method (3) and HDL cholesterol levels were determined by the same enzymatic method. Plasma triglyceride levels were measured by the lipase-glycerol phosphate method (4) using commercial kits (Asan pharm. Co., Korea).

Lipid extraction of liver tissues were carried out according to Folch et al. (5) with a slight modification. Five hundred mg of liver tissues from each rat was minced, extracted in 10 ml of solvent mixture of chloroform : methanol (2 : 1, v/v) for 48 hours at 4°C, filtered, and dried under nitrogen gas. One ml of solvent mixture of chloroform : methanol (2 : 1, v/v) was then added and a 100 μl aliquot of the solution was dried under nitrogen gas, and dissolved in 2 ml of ethanol. After appropriate dilution, 0.2 ml of lipid solution was mixed with reagent mixture. Hepatic total cholesterol and triglycerides were determined with the same enzymatic assay kit but slightly modified as previously described (2,4). The levels of lipid were expressed as mg/g liver tissue.

Plasma glutamate-oxaloacetate transaminse (GOT) and glutamate-pyruvate transaminse (GPT) levels were determined using commercial kits (Asan pharm. Co., Korea).

Preparation of liver microsomes

Microsomes were prepared according to Hulcher & Oleson (6) and Shapiro et al. (7) with a slight modification. Two grams of liver tissues were homogenized in 4 ml of ice-cold buffer containing 0.1 M triethanolamine, 0.02 M ethylenediamine tetraacetic acid (EDTA) (pH 7.4), and 2 mM dithiothreitol. The homogenate was centrifuged at 10,000 rpm and 12,000 rpm consecutively to sediment nuclei, cell debris and mitochondria. The supernatant was centrifuged at 32,500 rpm for 60 min at 4°C and the microsomal pellet obtained was suspended in 1 ml buffer solution. Aliquots of this suspension were used for the assay of HMG-CoA reductase and ACAT activity, and determination of protein. Protein was determined by the method of Bradford (8).

Assay of hydroxy-methyl-glutaryl CoA (HMG-CoA) reductase activity

The method of Shapiro et al. (7) was slightly modified. The incubation mixture contained 500 nmole of NADPH, 50 nmole of HMG-CoA, microsome, and reaction buffer. The incubation was carried out at 37°C for more than 15 minutes. The reaction was terminated by the addition of HCl, and the mixture was reincubated at 37°C for 15 minutes to insure the lactonization of mevalonic acid. The mixture was then centrifuged to sediment the denatured protein. An aliquot of the supernatant (15 μl) was used for thin layer chromatography (silica gel 60 FW_{254} TLC plate), and benzene : acetone (1 : 1, v/v) was used as a developing solvent. The area corresponding to mevalonolactone (Rf>0.6) was directly cut into the scintillation vial and its radioactivity was determined by a scintillation counter (Packard Tricarb 1600 TR, packard). HMG-CoA reductase activity was expressed as *p*moles of mevalonate formed per min per mg of microsomal protein.

Assay of Acyl-CoA:cholesterol acyltransferase (ACAT) activity

ACAT activity in liver tissue was determined by the method of Gillies et al. (9) with a slight modification. Cholesterol solution was dissolved in Triton WR-1339. Cholesterol (6 mg) was dissolved in 6 ml acetone, added to 600 mg of Triton WR-1339, and dried under nitrogen gas. This cholesterol mixture was dissolved in 20 ml of distilled water. The resultant cholesterol solution was prepared in a final concentration of 300 μg/ml. Oleoyl-CoA used as a substrate was diluted to be 200 μg/ml, divided into several vials, and stored at -70°C.

Twenty μl of cholesterol solution was added to the mixture of 20 μl of 1 M K-phosphate (pH 7.4), 10 μl of 0.6 mM BSA, 10 μl microsome, and 120 μl of distilled water and mixed well. The enzyme reaction was initated by the addition of 20 μl (5.62 nmole) of [^{14}C]oleoyl-CoA (specific activity; 15,000 cpm/nmole)

to the above mixture in a final volume of 200 μl. After a 30 minute incubation period at 37°C, the reaction was stopped by the addition of 500 μl of isopropanol : heptane (4 : 1, v/v) solution, 300 μl of heptane, and 200 μl of 0.1 M K-phosphate (pH 7.4). Then the phases were separated after few minutes, and an aliquot of the upper phase (200 μl of the supernant) was obtained and counted by scintilation counting technique (Packard Tricarb 1600 TR, packard). Corrections were made by multiplying 2 times the calculated value. ACAT activity was expressed as pmoles of cholesteryl oleoate formed per min per mg of microsomal protein.

Assay of plasma lipid peroxides

Plasma lipid peroxides were determined by high performance liquid chromatography (HPLC) (10). Plasma was centrifuged after mixing with methanol. Five hundred μl of supernatants was obtained, 0.7 ml of 1% phosphoric acid solution, 0.3 ml of thiobarbituric acid (TBA) solution were added into each tube, and vortex-mixed. The tubes were tightly capped and placed in boiling water bath (100°C) for 1 hour, then in a cold bath until HPLC analyses were performed. Preformed adduct was measured at 532 nm. The levels of lipid peroxides as malondialdehyde (MDA) from the calibration curve was determined. TEP (1,1,3,3- tetraethoxypropane) reagent was used as a standard. HPLC system used in this study was HP 1050 series with a auto injector, the column was RP-C_{18} column (Merck Co. 5 μm 30 cm), the mobile phase was 50 mM KH_2PO_4 : 100% acetonitrile (60 : 40, v/v) solution, the flow rate of the mobile phase was 0.6 ml/min, attenuation was 16, and the detector was a UV visible detector.

Morphologies of liver tissues

For microscopic view of liver tissues, liver sections were stored in 10% formalin solution, exchanged with fresh solution daily until staining. Specimens (4 μm thickness) stained with hematoxylin-eosin were observed under 200 times magnification through a light microscope.

Statistical analysis

Data are presented as mean±S.D. The differences in the mean values were evaluated by analysis of variance (one-way ANOVA), and followed by Duncan' multiple range test. The significant relationships between variables were calculated by Pearson's correlation analysis, partial correlation analysis, and multiple linear regression analysis (stepwise method). Statistical evaluation of the data were performed with SPSS statistical package program. Differences associated with $p<0.05$ were considered significant.

RESULTS

Food intake, weight gain and food efficiency

As shown in Table 2, the differences of daily food intake, body weight and weight gain between control and experimental groups were not statistically significant. Food efficiency of *Rosa rugosa* group was significantly lower compared to control (Table 2).

Organ weights

The differences of liver and heart weight among groups were not statistically significant. Kidney weight in *Crataegus pinnatifida* group was significantly lower compared to control. These results were the same when expressed as organ weights/body weights (Table 3).

Plasma lipid levels

Plasma triglyceride levels in *Rosa rugosa*, *Crataegus pinnatifida and Polygonum cuspidatum* groups were significantly lower compared to control, by 29%, 24% and 47%, respectively. Plasma total cholesterol levels were not different among groups. HDL cholesterol level in *Polygonum cuspidatum* group (16.50 mg/dl) was significantly lower compared to control (24.79 mg/dl) (Table 4).

Hepatic lipid levels

Hepatic triglyceride levels in *Rosa rugosa* and *Crataegus*

Table 2. Daily food intake, body weight, weight gain and food efficiency ratio in rats fed high-cholesterol diet with plant extract for 47 days

	Control (n=11)	*Rosa rugosa* (n=10)	*Crataegus pinnatifida* (n=10)	*Polygonum cuspidatum* (n=10)
Daily food intake (g/day)	24.26^{ab} ±0.55[1)]	24.56^{a} ±0.15	23.75^{b} ±1.21	24.49^{b} ±0.18
Body weight (g)	483.82^{NS} ±27.57	466.20 ±23.08	476.40 ±23.06	468.90 ±18.64
Weight gain (g/day)	4.69^{NS} ±0.46	4.31 ±0.40	4.53 ±0.56	4.42 ±0.35
Food efficiency (%)	19.33^{a} ±1.84	17.57^{b} ±1.66	19.02^{ab} ±1.51	18.05^{ab} ±1.42

Food efficiency ratio = weight gain/food intake
[1)]Mean±SD
Values in a row not sharing a common superscript letters are significantly different by Duncan's multiple range test at $p<0.05$.
[NS]Not significant

Table 3. Organ weight in rats fed high-cholesterol diet with plant extract for 47 days

	Control	*Rosa rugosa*	*Crataegus pinnatifida*	*Polygonum cuspidatum*
Liver (g)	19.36^{NS} ±3.18[1)]	18.16 ±1.74	18.71 ±2.03	18.24 ±1.47
Liver/wt (%)	3.99^{NS} ±0.51	3.89 ±0.31	3.92 ±0.34	3.89 ±0.20
Heart (g)	1.38^{NS} ±0.11	1.31 ±0.13	1.32 ±0.13	1.32 ±0.07
Heart/wt (%)	0.29^{NS} ±0.02	0.28 ±0.03	0.28 ±0.02	0.28 ±0.01
Kidney (g)	3.37^{a} ±0.20	3.29^{a} ±0.29	3.01^{b} ±0.35	3.42^{a} ±0.31
Kidney/wt (%)	0.70^{a} ±0.05	0.71^{a} ±0.05	0.63^{b} ±0.06	0.73^{a} ±0.05

wt: weight
[1)]Mean±SD
Values in a row not sharing a common superscript letters are significantly different by Duncan's multiple range test at $p<0.05$.
[NS]Not significant

Table 4. Plasma lipid levels in rats fed high-cholesterol diet with plant extract for 47 days

	Control	*Rosa rugosa*	*Crataegus pinnatifida*	*Polygonum cuspidatum*
Triglyceride (mg/dl)	95.75[a] ±33.33[1)]	68.29[b] ±20.79	73.17[b] ±28.11	51.11[b] ±9.91
Total cholesterol (mg/dl)	80.64[NS] ±36.62	62.04 ±30.17	80.55 ±30.29	73.06 ±25.99
HDL-cholesterol (mg/dl)	24.79[a] ±7.15	21.17[ab] ±8.88	23.61[ab] ±7.79	16.50[b] ±5.68

[1)]Mean±SD
Values in a row not sharing a common superscript letters are significantly different by Duncan's multiple range test at $p<0.05$.
[NS]Not significant

pinnatifida groups were significantly lower compared to control by 11%, 15%, respectively. Hepatic cholesterol levels in three experimental groups were not significantly different compared to that of control (Table 5).

Hepatic enzyme activities

Hepatic HMG-CoA reductase activities were greater in *Rosa rugosa* group (976.39 pmole/min/mg) compared to control (192.86 pmole/min/mg) by 406% (Table 6). Hepatic ACAT activity was significantly lower in *Polygonum cuspidatum* group (777.09 pmole/min/mg) compared to control (1078.14 pmole/min/mg) by 28% (Table 6).

Table 5. Hepatic lipid levels in rats fed high-cholesterol diet with plant extract for 47 days

	Control	*Rosa rugosa*	*Crataegus pinnatifida*	*Polygonum cuspidatum*
Triglyceride (mg/g tissue)	107.44[a] ±14.02[1)]	95.28[b] ±8.62	90.98[b] ±10.94	97.02[ab] ±13.17
Cholesterol (mg/g tissue)	20.73[ab] ±2.92	21.43[a] ±1.94	19.24[b] ±1.27	19.73[ab] ±0.90

[1)]Mean±SD
Values in a row not sharing a common superscript letters are significantly different by Duncan's multiple range test at $p<0.05$.

Plasma GOT and GPT activities

Plasma GOT and GPT levels were not significantly different among the groups (Table 7).

Light microscopic observations of liver tissues

As shown in Fig. 1, intracellular lipid accumulations and histopathological changes in hepatic tissue were observed

Table 6. *Hepatic HMG-CoA reductase and ACAT activities in* rats fed high-cholesterol diet with plant extract for 47 days

	Control	*Rosa rugosa*	*Crataegus pinnatifida*	*Polygonum cuspidatum*
HMG-CoA reductase activities (*pmole*/min/mg protein)	192.86[a] ±126.31	976.39[b] ±709.56	260.44[a] ±270.01	455.12[a] ±459.00
ACAT activities (*pmole*/min/mg protein)	1078.14[a] ±178.23[1)]	1138.16[a] ±143.74	1065.46[a] ±185.90	777.09[b] ±311.90

[1)]Mean±SD
Values in a row not sharing a common superscript letters are significantly different by Duncan's multiple range test at $p<0.05$.

Table 7. Plasma GOT and GPT levels in rats fed high-cholesterol diet with plant extract for 47 days

	Control	*Rosa rugosa*	*Crataegus pinnatifida*	*Polygonum cuspidatum*
GOT (IU/l)	39.96[ab] ±10.00[1)]	29.69[a] ±9.26	42.70[b] ±9.61	48.56[b] ±17.77
GPT (IU/l)	16.19[NS] ±8.65	11.51 ±7.92	16.36 ±7.54	19.57 ±11.92

[1)]Mean±SD
Values in a row not sharing a common superscript letters are significantly different by Duncan's multiple range test at $p<0.05$.
[NS]Not significant

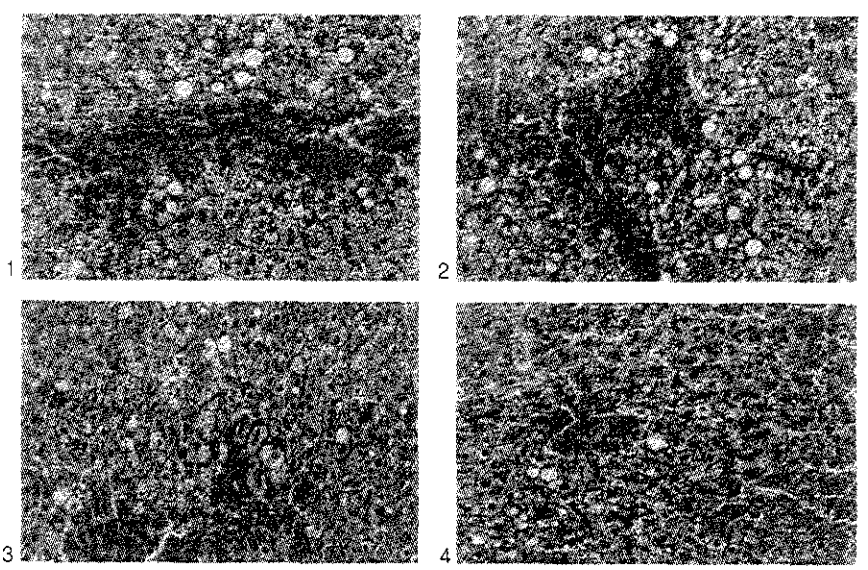

Fig. 1. Comparison of liver tissue morphology among various dietary groups. 1: Control, 2: *Rosa rugosa*, 3: *Crataegus pinnatifida*, 4: *Polygonum cuspidatum*

in all groups by using light microscope. Intracellular lipid depositions were observed less in the *Crataegus pinnatifida and Polygonum cuspidatum* groups than those of control.

Lipid peroxide levels

Lipid peroxide levels were not different among the groups (Table 8).

Relationships between variables

Multiple regression results for associations among various variables are given in Table 9. When the results of all animals were pooled together, highly significant correlations were obtained between body weight and plasma triglycerides ($r=0.59$, $p<0.001$) and total cholesterol ($r=0.36$, $p<0.05$). Plasma triglycerides correlated significantly with total cholesterol ($r=0.50$, $p<0.001$), but the correlation was slightly lower after adjusting for body weight ($r=0.35$, $p<0.05$). HDL cholesterol correlated significantly with plasma triglycerides ($r=0.64$, $p<0.001$) and total cholesterol ($r=0.49$, $p<0.001$). But the correlations tended to be somewhat lower when adjusted for body weight ($r=0.58$, $p<0.001$, $r=0.35$, $p<0.05$). Liver cholesterol correlated significantly with plasma triglycerides ($r=0.31$, $p<0.05$), but this interaction was not statistically significant when adjusted for body weight. Hepatic HMG-CoA reductase activity correlated significantly with plasma total cholesterol ($r=-0.37$, $p<0.05$). This was still consistent when adjusted for body weight ($r=-0.36$, $p<0.05$). Plasma lipid peroxide correlated negatively with hepatic cholesterol ($r=-0.31$, $p<0.05$). But this interaction was not statistically significant when adjusted for body weight (Table 10).

Only plasma cholesterol was associated significantly ($p<0.05$) with liver HMG-CoA reductase activity. Plasma cholesterol explained 12% of the variance of liver HMG-CoA reductase activity. The regression equation was: HMG-CoA reductase activity $=-5.9025\times$ plasma cholesterol $+892.6905$ ($p<0.05$).

Table 8. Plasma lipid peroxide levels in rats fed high cholesterol diet with plant extract for 47 days

	Control	*Rosa rugosa*	*Crataegus pinnatifida*	*Polygonum cuspidatum*
Lipid peroxide (μmol/L)	1.41[NS] ±0.46[1)]	1.05 ±0.33	1.40 ±0.43	1.37 ±0.53

[1)]Mean±SD
[NS]Not significantly different by Duncan's multiple range test at $p<0.05$

Table 9. Multiple linear regression models for HMG-CoA reductase activity

Independent variable	B	SE B	Beta	Sig T	R^2
Total cholesterol	-5.9025	2.8596	-0.3428	0.047	0.12
Constant	892.6905	230.1014			

DISCUSSION

We have investigated lipid lowering properties of some Korean herbs. Three tested materials, *Rosa rugosa*, *Crataegus*

Table 10. Unadjusted and adjusted correlation coefficients between plasma lipids, hepatic lipids, hepatic enzyme activities and lipid peroxide level

	Plasma lipids			Hepatic lipids		Hepatic enzymes		Lipid peroxide
Variable	TG	Total Chol	HDL-Chol	TG	Total Chol	ACAT	HMG-CoA reductase	Lipid-peroxide
Weight	.59***	.36*	.27	.06	.20	-.04	-.03	.15
Plasma lipids								
TG	1 (1)							
Total Chol	.50*** (.35*)	1 (1)						
HDL-Chol	.64*** (.58***)	.49*** (.35*)	1 (1)					
Hepatic lipids								
TG	.25 (.25)	-.01 (-.09)	.27 (.23)	1 (1)				
Chol	.31* (.33)	-.03 (.11)	.14 (.23)	.25 (.28)				
Hepatic enzyme activities								
ACAT	.19 (.29)	.01 (.04)	.24 (.28)	-.12 (.14)	.16 (.21)	1 (1)		
HMG-CoA reductase	-.16 (-.16)	-.37* (-.36*)	-.30 (-.30)	-.03 (-.04)	.24 (.21)	-.14 (-.15)	1 (1)	
Lipid peroxide	.08 (-.11)	.10 (-.29)	.036 (-.13)	.02 (-.03)	-.31* (-.23)	.04 (.07)	-.05 (.00)	1 (1)

Weight: body weight
( ): Partial correlations, adjusted for body weight
*$p<0.05$ **$p<0.01$ ***$p<0.001$

pinnatifida and *Polygonum cuspidatum*, are regarded to possess lipid lowering properties (11). Since Korean have traditionally enjoyed herbal teas, which are hot water extract, we have investigated the efficacy of three plant extracts on lipid profiles of plasma and liver, lipid peroxide levels, HMG-CoA reductase and ACAT activities *in vivo*. It is confirmed that the extracts in this study significantly lowered plasma triglycerides by 24~47%, and hepatic triglycerides by 11~15% in high cholesterol-fed rats. Hepatic ACAT activity in addition to plasma and liver triglycerides was lower only in *Polygonum cuspidatum* group compared to control.

It is now well established that inhibitors of HMG-CoA reductase, the rate limiting enzyme in cholesterol biosynthesis, lower plasma triglycerides but not plasma cholesterol in normal, chow-fed rats by inhibiting hepatic triglyceride secretion (12-18). This is a different situation in other species such as rabbits (19), miniature pigs (20) and guinea pigs (21), in which there is no change in plasma triglycerides and lowering of cholesterol represents the major drug action. The present findings are consistent with other reports that show that the rat is resistant to hypercholesterolemia when fed a high cholesterol diet (17,18).

We also identified that a very good correlation exists between hepatic HMG-CoA reductase and plasma total cholesterol in these animal models. Multiple regression analysis results for associations of liver HMG-CoA reductase activity and variables have shown that plasma cholesterol explained 12% of the variance of liver HMG-CoA reductase activity. These results suggest that hepatic HMG-CoA reductase is associated with total plasma cholesterol as well as triglyceride under this experimental condition.

ACAT catalyzes the esterification of cholesterol (22). ACAT activity has been identified in a variety of tissues such as intestine, liver and artery wall, which are the major organs involved in cholesterol metabolism and atherosclerosis (18, 23-27). Thus accumulation of esterified cholesterol is a major metabolic change in atherosclerotic lesions (28-30). Therefore inhibition of ACAT would be expected to decrease plasma cholesterol concentrations through the suppression of intestinal cholesterol absorption and hepatic VLDL secretion, thus delaying or preventing atherosclerosis. Since hot water extraction of *Polygonum cuspidatum* is apparently great in inhibition of ACAT, the extract can be expected to contribute to inhibition of cholesterol esterification in tissue.

Increased lipid peroxidation has been found to be associated with many well known pathologies including diabetes (31), atherosclerosis (32), cancer (33) and Parkinson's disease (34). But differences in lipid peroxidation levels were not observed in our study.

In conclusion, our study demonstrated that hot water extracts of *Rosa rugosa*, *Crataegus pinnatifida* and *Polygonum cuspidatum* lowered plasma triglyceride levels in rats fed a high cholesterol diet. These data suggest that these plant extracts may be potent agents in preventing or treating diet induced-hypertriglyceridemia and fatty liver disease. But further studies are necessary to elucidate the mechanisms by which these extracts decrease plasma triglyceride as well as hepatic triglyceride concentrations.

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