

## Antihyperlipidemic and Antioxidant Effects of the Mixture of Ginseng Radix and Crataegi Fructus: Experimental Study and Preliminary Clinical Results

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The mixture of Ginseng Radix and Crataegi Fructus (Gen-CF) was developed to increase the pharmacological effect of ginseng in the treatment of hypercholesterolemia and prevention of cardiovascular disease. This study evaluated the effects of Gen-CF on serum lipids of hypercholesterolemic rats *in vivo*, as well as its antioxidant activities *in vitro*, and explored its clinical effects on patients with hypercholesterolemia. *In vitro*, Gen-CF displayed 1,1-diphenyl-2-picrylhydrazyl and superoxide radical scavenging activities, and inhibited hemolysis induced by 2,2'-azobis-2-amidinopropane dihydrochloride in a dose-dependent manner. *In vivo*, Gen-CF significantly inhibited the increases of total cholesterol, low-density lipoprotein cholesterol and triglyceride in high cholesterol-diet and Triton WR-1339 models. It also significantly inhibited the decrease of high-density lipoprotein cholesterol in these models. In the clinical trial, Gen-CF significantly lowered total cholesterol, low-density lipoprotein cholesterol, triglyceride, total lipid and phospholipid, with no adverse events, including hepatic or renal toxicity. The data suggest that Gen-CF has the potential to treat hypercholesterolemia and prevent cardiovascular disease.

**Keywords:** *Panax ginseng*, Antioxidants, Cardiovascular diseases, Hypercholesterolemia

### INTRODUCTION

Hypercholesterolemia is a major cause of cardiovascular disease (CVD), such as atherosclerosis and coronary heart disease [1]. CVD is the most common cause of mortality and morbidity worldwide [2]. Although several factors, such as cigarette smoking, high-fat diet, high blood pressure, physical inactivity, age, and heredity have significant roles in causing CVD, high blood cholesterol is mainly responsible for the onset of CVD [2,3]. Lowering serum cholesterol levels by drug or dietary interventions could reduce the risk of CVD. Therefore, it

is worthwhile to develop new safe and effective cholesterol-lowering agents from natural products.

In arteries of hypercholesterolemic animals and patients, vascular superoxide production and oxidative stress are increased [4]. Oxidation of low-density lipoprotein cholesterol (LDL) is considered as an important step in the development of atherosclerosis. Oxidized LDL (ox-LDL) is cytotoxic to a variety of vascular cells [5], induces the synthesis of monocyte chemoattractant protein-1 [6], recruits inflammatory cells [7], and stimulates

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the production of autoantibodies [8]. Antioxidants such as vitamin E that is supplied in the diet can prevent oxidation of LDL [9]. In addition to inhibition of LDL oxidation, antioxidant therapy produces beneficial effects on atherosclerosis and prevents the progression of atherosclerosis in animal models by limiting vascular oxidative stress and superoxide production [10-13].

The mixture of Ginseng Radix and Crataegi Fructus (Gen-CF) was developed to increase the pharmacological effect of ginseng in the treatment of hypercholesterolemia and prevention of CVD. Many reports suggested that ginseng and hawthorn fruit may have an antihyperlipidemic effect [14-18] and the ability to prevent oxidation of LDL [19-27]. The present study evaluated the effects of Gen-CF on serum lipids of hypercholesterolemic rats *in vivo*, as well as its antioxidant activities *in vitro*, and explored its clinical effects on patients with hypercholesterolemia.

## MATERIALS AND METHODS

### Materials

Gen-CF was prepared as a capsulated water extract (300 mg per capsule) of Gen-CF (Table 1). Each preparation was extracted twice with boiling water for 2 h. These extracts were filtered and evaporated in a rotary vacuum evaporator and lyophilized. To standardize the quality of Gen-CF, ginseng radix ginsenoside Rg<sub>1</sub> was quantitatively assayed as previously described [28].

### Subjects for clinical trial

Inclusion criterion was that serum total cholesterol was more than 240 mg/dL. Exclusion criteria included 1) diabetes mellitus, 2) hepatic or renal diseases, 3) cardio or cerebral vascular diseases within 3 months, 4) patients who had taken anti-hyperlipidemic agents, or steroids within 6 months, and 5) alcoholic abusers. Informed consents were obtained from all subjects after being given a full explanation of the study.

### Clinical trial design

Subjects were administered Gen-CF (two capsules three times each day for 4 weeks). No dietary or exercise advice were provided, so that all subjects could maintain their normal life-style concerning diet and exercise. Serum lipids including total cholesterol (TC), triglyceride (TG), total lipid (TL), phospholipid (PL), high density lipoprotein cholesterol (HDL), and LDL were measured at baseline and after 4 weeks of medication. For each subject, 10 to 15 mL of blood was collected in blood col-

**Table 1.** Composition of the mixture of Ginseng Radix and Crataegi Fructus

Constituent herbs	Scientific name	Mass (g/capsule)
Ginseng Radix	<i>Panax ginseng</i> Meyer	1
Crataegi Fructus	<i>Crataegus pinnatifida</i> BGE	3
Total		4

lection tubes containing heparin after overnight fasting, and the analysis of serum lipids was performed using an enzymatic method with a model 7600-110 apparatus (Hitachi, Tokyo, Japan). Any possible adverse effect was monitored by physical examination during the treatment period. Hepatic and renal toxicity were assessed by aspartate transaminase (AST), alanine transaminase (ALT), blood urea nitrogen (BUN), and creatinine (Cr).

### Animals and diets

Sprague-Dawley male rats (200-250 g) were acclimatized for a week in colony cages. The animals were kept at a constant temperature (22-26°C) and humidity (50-55%), and were fed with commercial diet (Samyang, Seoul, Korea). Water was allowed *ad libitum*.

### 1,1-Diphenyl-2-picrylhydrazyl radical scavenging activity

Different concentrations of the extract were measured for hydrogen donating or radical scavenging ability, using the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH), as previously described [29]. The reaction mixture containing 1 mL of a DPPH methanolic solution (0.1 mM) plus 4 mL of the extract at different concentrations was incubated at room temperature for 30 min and the absorbance was measured spectrophotometrically at 520 nm. The percent of DPPH discoloration of the sample was then calculated. The results were expressed as percent inhibition.

### Superoxide anion scavenging activity

Superoxide anion scavenging activity of the Gen-CF extract was determined as previously described by measuring the superoxide radicals generated by the xanthine/xanthine oxidase system [30]. A 0.02 mL volume of different concentrations of the extract, 3 mM xanthine, 3 mM EDTA, 0.75 mM nitro blue tetrazolium and 0.15% bovine serum albumin were added to 0.48 mL of 0.05 M Na<sub>2</sub>CO<sub>3</sub> buffer and incubated for 10 min at room temperature. The reaction was initiated by the addition of 6 mU xanthine oxidase and carried out at 25°C for 20 min. After this period, the reaction was stopped by the addition of 6 mM CuCl<sub>2</sub>, and absorbance was measured at

560 nm. The results were expressed as percent inhibition.

### Induced hemolysis in erythrocytes

Hemolysis of erythrocytes mediated by 2,2'-azobis-2-amidinopropane dihydrochloride (AAPH) was determined using a modification of a method described elsewhere [31]. Blood samples were obtained by cardiac puncture in heparinized tubes and centrifuged (1,500 rpm, 10 min). After removing the supernatant, the pellet was washed three times with 0.15 M NaCl. During the last washing, the erythrocytes were centrifuged (1,500 rpm, 10 min). A 10% suspension of erythrocytes was prepared by adding phosphate buffered saline (PBS, pH 7.4). Test samples (0.2 mL) at different concentrations and 0.2 mL of 100 mM AAPH were added in succession to 0.2 mL of erythrocyte suspension. The reaction mixtures were incubated at 37°C for 3 h with gentle shaking. After incubation, an aliquot of the reaction mixture was diluted 20 times with PBS and centrifuged at 1000 ×g for 10 min. The absorbance of the supernatant (*A*) at 540 nm was read. Similarly, another aliquot of the reaction mixture was diluted with distilled water to yield complete hemolysis and the absorbance of the supernatant (*B*) after centrifugation was measured at 540 nm. Inhibition percentage of hemolysis exhibited by each sample was calculated by the equation  $(1-A/B) \times 100\%$ .

### High cholesterol diet model

Rats were given 1% cholesterol, 0.25% cholic acid, and 2.5% olive oil with a standard equilibrated diet (Samyang Seoul, Korea) for 2 wk. Then, 24 hypercholesterolemic rats were selected and randomly divided into four groups. The first group was given a high cholesterol diet and administered orally with Gen-CF extract (220 mg/kg) once a day for a wk (study group 1). The second group was similarly administered Gen-CF extract (440 mg/kg, study group 2). The third group was similarly administered lovastatin (50 mg/kg, positive control group). The fourth group was administered only normal saline (untreated control group). The fifth group was given only standard equilibrated diet during the whole study period and received normal saline for a wk (normal group). After a week of medication, animals were kept for overnight fasting and anesthetized with ether. Blood samples were obtained by cardiac puncture.

### Triton WR-1339 model

Thirty rats were randomly assigned to the aforementioned five groups. After a week of acclimation, all groups except group five received an intraperitoneal

injection of a 10% aqueous solution of Triton WR-1339 (Sigma-Aldrich, St. Louis, MO, USA) at a dose of 200 mg/kg. Food was withdrawn 16 h prior to Triton WR-1339 injection. In study group 1, Gen-CF extract (220 mg/kg) was administered orally once a day for 3 days; the last administration was 1 h before the injection. Using the same method, Gen-CF extract (440 mg/kg) was administered to the second group (study group 2) and lovastatin (50 mg/kg) to the third group (positive control group). The fourth group (untreated control group) was only injected with Triton WR-1339. The fifth group was injected with only normal saline (normal group). Blood samples were obtained by cardiac puncture 18 h after the injection of Triton WR-1339.

### Determination of serum lipoproteins

Blood samples were allowed to clot for 30 to 40 min. Serum was separated after centrifugation (3,000 rpm, 30 min) and used for biochemical analysis. Analysis of blood serum for TG, TC, and HDL was performed using standard enzymatic assay kits (Asan Pharmacy, Seoul, Korea). LDL was determined using a LDL-cholesterol kit (bioMerieux, Marcy l'Etoile, France).

### Statistical analyses

All results were expressed as mean±SD unless otherwise stated. Data were analyzed by one-way ANOVA followed by the Student-Newman-Keuls test for experimental study and paired t-test for clinical trial. A value of  $p < 0.05$  was considered significant. All calculations were performed using SPSS ver. 11.5 (SPSS, Chicago, IL, USA).

## RESULTS

### Antioxidant activity of Ginseng Radix and Crataegi Fructus *in vitro*

To explore antioxidant activity of Gen-CF, we determined inhibitory effects of Gen-CF on DPPH and superoxide radical generation, and AAPH-induced hemolysis. Gen-CF displayed DPPH and superoxide radical scavenging activities in a dose-dependent manner (Fig. 1A). Gen-CF also inhibited hemolysis induced by AAPH in a dose-dependent manner (Fig. 1B).

### Hypolipidemic activity of Ginseng Radix and Crataegi Fructus *in vivo* (high cholesterol diet model)

The TC, LDL, and TG values of the high cholesterol diet group were significantly higher than those of the normal diet group ( $p < 0.001$ ) (Table 2). Gen-CF (440 mg/

**Table 2.** Effects of the mixture of Ginseng Radix and Crataegi Fructus on serum lipid levels in high cholesterol diet induced hyperlipidemic rats

Group	No.	TC (mg/dL)	LDL (mg/dL)	HDL (mg/dL)	TG (mg/dL)
Normal	6	82.0±2.81	21.2±1.59	36.5±1.97	75.0±3.71
Control	6	241.0±9.36 <sup>##</sup>	67.3±3.39 <sup>##</sup>	23.8±2.82 <sup>#</sup>	135.5±5.90 <sup>##</sup>
Gen-CF 220 mg/kg	6	193.5±6.05 <sup>***</sup>	60.7±3.13	29.8±2.46	117.2±8.53
Gen-CF 440 mg/kg	6	166.5±5.19 <sup>***</sup>	41.2±3.35 <sup>***</sup>	31.7±2.75 <sup>*</sup>	94.5±5.62 <sup>***</sup>
Lovastatin 50 mg/kg	6	150.0±6.37 <sup>***</sup>	36.8±2.99 <sup>***</sup>	32.5±0.92 <sup>**</sup>	88.7±7.88 <sup>***</sup>

TC, total cholesterol; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol; TG, triglyceride; Gen-CF, Ginseng Radix and Crataegi Fructus.

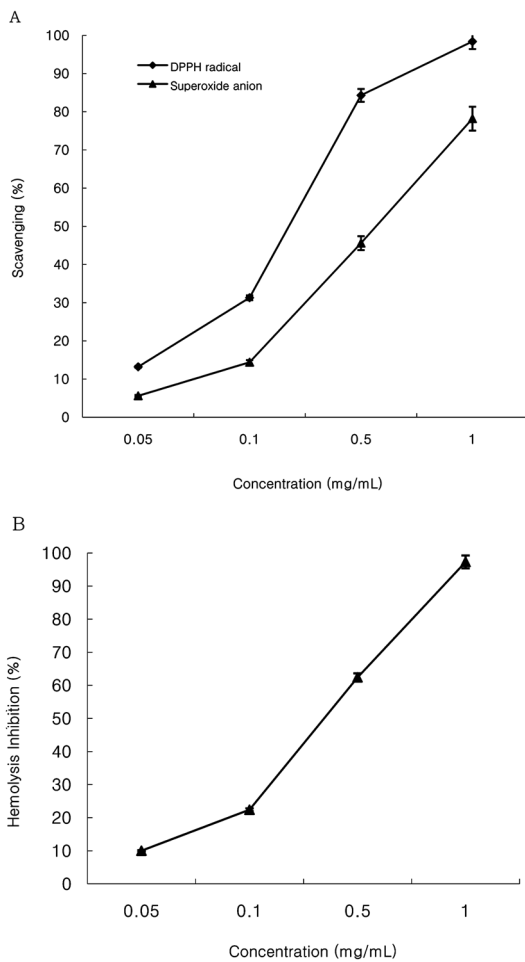
<sup>#</sup>Significantly different, compared with normal group ( $p < 0.01$ ).

<sup>##</sup>Significantly different, compared with normal group ( $p < 0.001$ ).

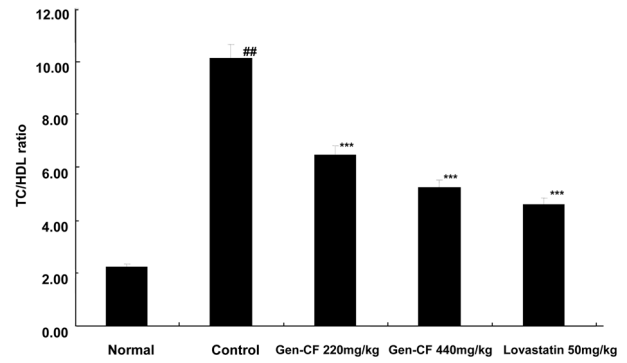
<sup>\*</sup>Significantly different, compared with control group ( $p < 0.05$ ).

<sup>\*\*</sup>Significantly different, compared with control group ( $p < 0.01$ ).

<sup>\*\*\*</sup>Significantly different, compared with control group ( $p < 0.001$ ).



**Fig. 1.** Antioxidant activities of the mixture of Ginseng Radix and Crataegi Fructus (Gen-CF) *in vitro*. (A) Concentration-response curves for the scavenging of free radicals by Gen-CF. Data are expressed as percentage of scavenging of 1,1-diphenyl-2-picrylhydrazyl (DPPH), and superoxide radicals generated by the xantine/xantine oxidase system. (B) Concentration-response curve for the inhibition of hemolysis by Gen-CF. Data are expressed as percentage of inhibition of 2,2'-azobis-2-amidinopropane dihydrochloride-induced hemolysis in erythrocytes. Values are mean±SD of three different assays.



**Fig. 2.** Effects of the mixture of Ginseng Radix and Crataegi Fructus (Gen-CF) on TC/HDL ratio in high cholesterol diet induced hyperlipidemic rats. TC and HDL are total cholesterol and high-density lipoprotein cholesterol, respectively. <sup>##</sup> Significantly different, compared with normal group ( $p < 0.001$ ); <sup>\*\*\*</sup>Significantly different, compared with control group ( $p < 0.001$ ).

kg) and lovastatin (50 mg/kg) significantly inhibited the increases of TC, LDL and TG values ( $p < 0.001$ ). HDL was significantly lowered in the high cholesterol diet group, compared to the normal diet group ( $p < 0.01$ ). Gen-CF (440 mg/kg) and lovastatin (50 mg/kg) significantly inhibited HDL decrease ( $p < 0.05$  and  $p < 0.01$ , respectively) (Table 2). The TC/HDL ratio was significantly higher in the control group than that in the normal diet group (Fig. 2). Gen-CF (220 mg/kg), Gen-CF (440 mg/kg) and lovastatin (50 mg/kg) significantly inhibited the increase of TC/HDL ratio (Fig. 2).

### Hypolipidemic activity of Ginseng Radix and Crataegi Fructus *in vivo* (Triton WR-1339 model)

The TC, LDL, and TG values in the Triton WR-1339 administered group were significantly higher than those in the normal diet group ( $p < 0.001$ ) (Table 3). Gen-CF (440 mg/kg) and lovastatin (50 mg/kg) significantly inhibited the increases of TC, LDL, and TG ( $p < 0.05$  and

**Table 3.** Effects of the mixture of Gen-CF on serum lipid levels in Triton WR-1339 induced hyperlipidemic rats

Group	No.	TC (mg/dL)	LDL (mg/dL)	HDL (mg/dL)	TG (mg/dL)
Normal	6	78.7±5.92	11.7±1.43	26.5±1.87	102.5±7.61
Control	6	249.2±22.6 <sup>###</sup>	81.2±5.16 <sup>###</sup>	15.8±1.98 <sup>#</sup>	323.7±7.61 <sup>###</sup>
Gen-CF 220 mg/kg	6	231.0±18.2	75.2±7.74	18.8±2.46	296.5±19.2
Gen-CF 440 mg/kg	6	182.0±13.0 <sup>*</sup>	56.7±5.45 <sup>*</sup>	22.7±1.95 <sup>*</sup>	277.7±12.8 <sup>*</sup>
Lovastatin 50 mg/kg	6	170.3±7.46 <sup>**</sup>	52.3±2.63 <sup>**</sup>	25.8±1.98 <sup>**</sup>	268.2±16.1 <sup>**</sup>

Gen-CF, Ginseng Radix and Crataegi Fructus; TC, total cholesterol; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol; TG, triglyceride.

<sup>#</sup>Significantly different, compared with normal group ( $p < 0.01$ ).

<sup>###</sup>Significantly different, compared with normal group ( $p < 0.001$ ).

<sup>\*</sup>Significantly different, compared with control group ( $p < 0.05$ ).

<sup>\*\*</sup>Significantly different, compared with control group ( $p < 0.01$ ).

**Table 4.** General characteristics of the study subjects

	(n=20)
Male, n (%)	9 (45)
Age, yr (range)	47±13.2 (28-69)
Coronary artery disease, n (%)	0 (0)
Hypertension, n (%)	5 (25)
Total cholesterol (mg/dL)	267.7±13.8

**Table 5.** Effects of the mixture of Ginseng Radix and Crataegi Fructus on serum lipid levels in patients with hypercholesterolemia

(mg/dL)	Baseline	4 wk
TC	267.7±13.8	238.8±23.1 <sup>**</sup>
TL	722.9±95.0	681.6±110.8 <sup>**</sup>
TG	154.6±43.7	134.1±34.8 <sup>*</sup>
PL	290.9±26.0	271.6±26.5 <sup>**</sup>
HDL	62.4±11.1	61.8±9.5
LDL	163.8±42.0	150.2±20.0 <sup>*</sup>

Values are mean±SD.

TC, total cholesterol; TL, total lipid; TG, triglyceride; PL, phospholipids; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol.

<sup>\*</sup>Significantly different, compared with baseline ( $p < 0.05$ ).

<sup>\*\*</sup>Significantly different, compared with baseline ( $p < 0.01$ ).

$p < 0.01$ , respectively). HDL was significantly lowered in the Triton WR-1339 administered group, compared to the normal diet group ( $p < 0.01$ ). Gen-CF (440 mg/kg) and lovastatin (50 mg/kg) significantly inhibited HDL decrease ( $p < 0.05$  and  $p < 0.01$ , respectively) (Table 3).

### Clinical effects of Gen-CF on serum lipid levels in patients with hypercholesterolemia

The initial characteristics of the subjects are summarized in Table 4. Twenty-four subjects were recruited, but four dropped-out during the study. Therefore, 20 subjects were included in the final analysis. Gen-CF significantly

**Table 6.** Safety assessment data

	Medication		p-value*
	Before	After	
AST (U/L)	19.0±4.7	16.7±3.9	NS
ALT (U/L)	19.3±9.0	17.8±9.1	NS
BUN (mg/dL)	14.8±5.2	12.4±2.5	NS
Cr (mg/dL)	0.9±0.3	0.9±0.2	NS

Values are mean±SD.

AST, aspartate transaminase; ALT, alanine transaminase; BUN, blood urea nitrogen; Cr, creatinine; NS, not significantly different.

lowered TC, TL, TG, PL, and LDL. But, HDL was not affected by Gen-CF administration (Table 5). There were no clinical adverse events during the 4 weeks of medication. There was no significant elevation of ALT, AST, BUN, and Cr (Table 6).

## DISCUSSION

It is well-established that elevated blood lipid is a major risk factor for atherosclerosis. Lipids are very susceptible to attack by free radicals, and ox-LDL species appear to contribute to the atherosclerosis pathobiology within the artery wall [5,32]. *In vitro* studies have demonstrated that ox-LDL activates endothelial cells to increase the expression of chemoattractant molecules leading to stimulated transendothelial migration of monocytes and formation of foam cells. Ox-LDL also increases the production of growth factors, including platelet-derived growth factor, stimulating the migration and proliferation of smooth muscle cells [33,34]. Furthermore, ox-LDL impairs endothelium-derived nitric oxide production [35,36].

Gen-CF was developed to increase the pharmacological effect of ginseng in the treatment of hypercholesterolemia and the prevention of CVD. The results of the present *in vitro* study show that Gen-CF has anti-oxidative

activities, as demonstrated by radical scavenging activity and inhibition of AAPH-induced hemolysis. It appears likely that ginseng has antioxidant activities, based on the scavenging of DPPH and superoxide radicals, inhibition of AAPH-induced hemolysis and metal ion-induced lipid peroxidation, and increased superoxide dismutase activity and nitric oxide synthesis [22,25,37-39]. Hawthorn fruit was also reported to scavenge hydrogen peroxidase and superoxidase, and inhibit Cu<sup>2+</sup>-mediated lipid peroxidation [26,27]. Therefore, Gen-CF is thought to work by integrating the actions of both herbs, as most traditional herbal formulations do.

In addition to the antioxidant activity, Gen-CF displayed potent hypolipidemic activity, decreasing serum TC, TG, and LDL in both hypercholesterolemic models. The TC/HDL ratio is a better indicator of coronary heart disease risk than individual lipoprotein concentration [40,41]. In this study, the TC/HDL ratio of the Gen-CF-treated group was significantly lower than that of the control group in the high cholesterol diet model. Gen-CF also significantly lowered TC, TG, PL, and LDL after 4 weeks of treatment in the patients. There were no adverse events, including hepatic or renal toxicity.

The mechanism by which Gen-CF decreases serum cholesterol remains unclear. Serum cholesterol can be lowered at several metabolic points including decreased synthesis, activation of LDL receptors, inhibition of the absorption of dietary cholesterol, and conversion of cholesterol to bile acids. It was previously reported that ginseng decreases blood cholesterol levels by increasing cholesterol excretion through bile acid formation [42,43], and may increase LDL receptors by promoting the synthesis of LDL receptors in rats [44]. In one study, 3-hydroxy-3-methylglutaryl coenzyme A reductase activity was significantly lowered by ginseng, which shows that the mechanism of the hypocholesterolemic action of ginseng involves the suppression of cholesterol biosynthesis [14]. Hawthorn fruit was reported to increase LDL-receptor activity of hepatic membrane in rats [45] to increase excretion of bile acids through the up-regulation of hepatic cholesterol 7 $\alpha$ -hydroxylase activity, and inhibit cholesterol absorption through down-regulation of intestinal acyl CoA:cholesterol acyltransferase activity [16]. These actions may have also occurred in the present study.

To summarize, Gen-CF was able to reduce serum lipid levels *in vivo*, including in patients. Gen-CF also showed antioxidant activity through radical scavenging activity and inhibition of AAPH induced hemolysis. The data suggest that Gen-CF has the potential to treat hypercho-

lesterolemia and prevent CVD.

The conclusions come with some caveats. The clinical trial had a small sample size and was not case-controlled. A large randomized controlled trial is needed. Secondly, there was no study on the mechanisms by which Gen-CF decreases serum cholesterol.

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