Hypocholesterolemic Effect of Hot-Water Extract from Mycelia of *Cordyceps sinensis*

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Received July 15, 2002; accepted September 18, 2002

This study was conducted to investigate the hypocholesterolemic effect of the hot-water fraction (HW) from cultured mycelia of *Cordyceps sinensis* in a 5 l fermenter. The composition of HW was mainly carbohydrate (83.9%) and protein (11.8%) on a dry basis, and the carbohydrate of HW consisted of glucose, mannose, galactose, and arabinose in the molecular ratio of 1.0 : 0.8 : 0.5 : 0.1, respectively. In mice fed a cholesterol-free diet and those fed a cholesterol-enriched diet, body and liver weights were not significantly different from those of the controls. The serum total cholesterol (TC) of all mice groups administered HW (150 and 300 mg/kg/d, respectively) with the cholesterol-enriched diet decreased more than in the control group. Among the mice fed the cholesterol-enriched diet, HW also increased the high-density lipoprotein (HDL) cholesterol level, but decreased the very low-density lipoprotein plus low-density lipoprotein (VLDL+LDL) cholesterol level. The changes in HDL- and VLDL+LDL-cholesterol levels consequently decreased the atherogenic value. The results indicate that HW in rats administered a cholesterol-enriched diet decreased the plasma cholesterol level. The 300 mg/kg dose had a significant effect on the serum TC level.

Key words *Cordyceps sinensis*; cholesterol; mycelia

It has been discovered that the medicinal properties of cultured mycelia are equally effectively as those of *Cordyceps* sp. found in the wild.[1)] Studies suggest that *Cordyceps sinensis*, which forms a fruiting body on the larvae of moths, may promote natural vitality and have diverse bioactivities.[2,3)] In addition, *Cordyceps militaris*, a species that is a parasite fungus on the larvae of *Lepidoptera*, has received considerable attention as a potential anti-hyperlipidemia agent.[4)]

However, since these physiologically active substances contained in various *Cordyceps* sp. were extracted from natural or the solid cultured stromata, only a few active substances from them have been developed for commercial use. Therefore, to provide opportunities for increased economic efficiency and practical use, artificial media for high-volume production have recently been developed in Korea. Koh *et al*. (2001) found that the hot-water fraction (HW) from submerged cultured mycelia of *C. sinensis* stimulates bone marrow cell proliferation through Peyer's patch cells, activates macrophages, and has some bioactivities *in vitro*.[5)]

Recently, hyperlipidemia has received research attention. It is well documented that lowering circulating cholesterol, especially low-density lipoprotein (LDL) cholesterol, levels can prevent, arrest, and even reverse coronary atherosclerosis.[6)] Various edible mushrooms have already proven to be an important natural regimen for controlling hyperlipidemia due to their high content of fiber, proteins, and microelements and low fat content.[7,8)] Although the hypolipidemic effect of mushrooms has been studied, no information is so far available on the hypocholesterolemic effect of the fungus *C. sinensis*. Therefore, the present paper investigated the potential cholesterol-lowering effect of the HW extract from mycelia of *C. sinensis* in mice.

MATERIALS AND METHODS

Microorganism and Materials *C. sinensis*, which was a gift from Sanming Microbiological Institute in China, were used in this experiment. The medium used for liquid culture contained, per liter, the following: 100 g of molasses, 1 g of yeast extract, and 3 g of K_2HPO_4. The submerged cultivation of *C. sinensis* was carried out at 150 rpm, 25°C, pH 5, and airflow rate of 1.0 vvm for 7 d in a 5 l jar fermenter using the liquid culture medium. The enzymatic kits for total cholesterol (TC), triacylglycerol (TG), and high-density lipoprotein (HDL) and very low-density lipoprotein (VLDL) cholesterol were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Animals Male ICR mice (6—8 weeks old) were obtained from Daihan-Biolink Co. (Korea). After an adaptation period, the mice were allowed free access to standard laboratory cholesterol-free and -enriched diets. Water was given *ad libitum* during the experimental period. They were housed under temperature (about 21°C) and humidity (about 60%)-controlled conventional clean conditions.

Preparation of HW Extraction Samples were prepared to investigate the physiological effects of the HW extract from cultured mycelia of *C. sinensis in vivo*. The freeze-dried mycelia (50 g) were blanched at 100°C for 5 min and homogenized with an Ultra-turrax T-50 at 7000 rpm for 20 min (Janke & Kunkel IKA-Labortechniker, Germany). After centrifugation (8000×***g***, 30 min), the residue was initially fractionated with ethyl acetate (1 l), which is a solvent with a low polarity, followed by fractionation with MeOH (1 l) and then hot water (1 l). The final fraction was centrifuged to remove insoluble material, and supernatant was lyophilized to give the HW extract (yield 29.3%).

Analysis of Chemical Components The carbohydrate composition of the mycelia was analyzed *via* gas liquid chromatography (GLC) using an SP-2380 capillary column (0.20 μm film, 0.25 mm i.d.×30 m, Supelco, U.S.A.) according to the method of Zhao *et al*.,[9)] and the molecular weight was determined by HPLC equipped with a refractive index (RI) de-

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tector (RI; Waters 410) and photodiarray (PDA) detector on a Shodex GS520+GS320+GS220 packed column (7.6 mm i.d.×30 mm, Asahi Chemical Industry Co. Ltd., Japan), using standard pullurans.[10]

Hypocholesterolemic Effects An experiment was carried to investigate changes in serum and liver lipid levels. After adaptation to the cholesterol-free diet, 24 ICR mice were divided into 6 groups (4 animals/group) fed HW (0, 150, 300 mg/kg/d, respectively) with either the cholesterol-free or -enriched diet for 7 consecutive days, as shown in Table 1. HW from cultured mycelia of *C. sinensis* was dissolved in distilled water, and each aliquot of the sample solution was administered orally to the mice once every day at each dose of 150 and 300 mg/kg body weight, respectively. As the controls, mice received distilled water alone instead of the sample solution. Food was withheld for 4 h before death. Blood was collected from the arota ventralis into tubes containing EDTA and was separated by centrifugation at 1100×***g*** for 20 min at 4°C. The livers were perfused *in situ* with cold saline (8.5 g NaCl/l), removed, weighed, and then stored in plastic bags at −20°C. Liver lipids were extracted by the gravimetric method of Folch *et al.*[11] and redissolved in ethanol. Serum levels of TC, HDL cholesterol and TG were measured using enzymatic kits. The liver TC and TG levels were assayed using the same method as for the serum after treatment with Triton X-100.

Statistical Analysis The data were subjected to analysis of variance and expressed as mean±S.E. The significance of differences was compared using Student's *t*-test and Duncan's multitest. Values of $p<0.05$ were considered to indicate statistical significance.

RESULTS AND DISCUSSION

Mycelial Growth in the Jar Fermenter Figure 1 shows the time profiles of mycelial growth and exo-polymer production in the 5 l jar fermenter under optimal culture conditions. The growth of mycelia gradually increased with fermentation time to 120 h, but thereafter the growth slightly decreased to 29.4 g/l. The exo-polymer concentration increased with the fermentation time and reached a maximum level of 15.27 g/l after 144 h, with corresponding depletion of the sugar concentration. The residual sugar concentration decreased during the entire fermentation period. The pH value during fermentation slowly increased from 4.8 to 6.2.

Lee *et al.*[12] reported that maximum polysaccharide production and mycelial biomass of *Ganoderma lucidum* were 7.51 g/l and 13.9 g/l, respectively. Kim *et al.*[13] reported that maximum polysaccharide production and mycelial biomass of *Pleurotus linteus* were 3.5 g/l and 14.2 g/l, respectively. Our results showed that the maximum polysaccharide production and mycelial biomass were 15.3 g/l and 29.4 g/l, respectively. The mycelia were lyophilized for further studies.

Chemical Components of Mycelia of *C. sinensis* The cultured mycelia of *C. sinensis* contained 67.1% carbohydrate, 9.2% protein, 17.3% lipid, and 6.6% ash, and the molecular ratio of carbohydrate was composed mainly of glucose and mannose (1.0 : 0.9), in addition to a small amount of galactose and arabinose (0.2 : 0.1), according to the results of Koh *et al.*[5] But the HW fraction of the cultured *C. sinensis* mycelia consists of 83.9% carbohydrate, 11.8% protein,

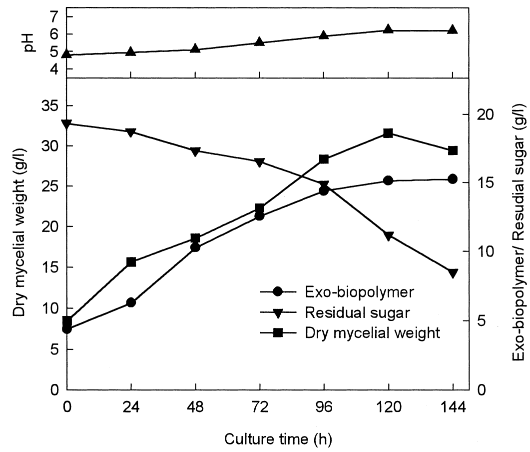

Fig. 1. Mycelial Growth and Exo-Biopolymer Production from *Cordyceps sinensis* in a 5 l Fermenter

The submerged culture was carried out at 150 rpm, 25°C, pH 5, and air flow rate of 1.0 vvm.

1.9% lipid, and 2.4% ash, and the carbohydrate of HW mainly consisted of glucose, mannose, galactose, and arabinose (molecular ratio 1.0 : 0.8 : 0.5 : 0.1), as shown in Table 2. The molecular weight of the HW was between 10 and 30 KD, and the main molecular weight was 29 KD.

Although the sugar component of *C. sinensis* mycelia was not previously known, the present results showed that the HW of the mycelia are mainly composed of carbohydrate, and the carbohydrate consisted of glucose, mannose, galactose, and arabinose (Table 2). Kiho[14] reported that CS-F30, the alkali extract fraction of the cultured *C. sinensis* mycelia, was mainly composed of glucose, galactose, and mannose molecular ratio 1.00 : 2.21 : 0.36 and had a molecular weight of 45 KD.

Hypocholesterolemic Effects Changes in the body, liver, and other internal organ weights of the mice after 7-d administration of both HW and control are presented in Table 3. No significant differences in food intake (data not shown) were found between the HW and control groups. Body and liver weights of the mice were not significantly different from those of the controls. The body weight gains were slightly lower in the mice fed HW (300 mg/kg) than in the control mice that received the cholesterol-enriched diets, but there was no significant difference. There were significant changes in the perirenal fat pad in the HW groups compared with the control group.

The changes in serum and liver lipid levels in the HW group are summarized in Table 4. There were no effects of HW on serum TC levels in the mice fed the cholesterol-free diet, although the mice fed the cholesterol-enriched diet with HW (150 and 300 mg/kg/d) showed significant decreases in serum TC levels ($p<0.05$). In contrast, the HW group did not have a lower plasma TG level than that of the control groups with both the cholesterol-free and -enriched diets. Among the mice fed the cholesterol-enriched diet, HW increased the HDL cholesterol level, but decreased the VLDL+LDL cholesterol level ($p<0.05$). Changes in cholesterol level consequently decreased the atherogenic value. The liver cholesterol concentration in the HW group fed on the cholesterol-enriched diet was lower than that in the control group. The results presented here show that HW in a cholesterol-enriched diet decreased the plasma cholesterol level in mice. Particularly, HW 300 mg/kg had a significant effect on serum TC level. Consequently, HW affected the serum lipid metabolism in mice, and this effect appeared to be greater on the cholesterol-enriched diet than on the cholesterol-free diet.

The serum atherogenic value in mice fed HW with the cholesterol-enriched diet was significantly decreased in this experiment. The atherogenic value is an indicator of cardiovascular risk. Several studies have demonstrated the protective effect of HDL in atherosclerosis and cardiovascular disease, while high levels of LDL constitute a risk factor. The addition of HW to cholesterol-free and cholesterol-added diets tended to decrease atherogenic values (Table 4), which may be beneficial in the prevention and treatment of cardiovascular disease. Also, the liver TC levels were significantly lower with the HW diet, showing that hepatic biosynthesis of cholesterol was suppressed. This could be due to a reduction in the activity of the liver enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase.[15] Therefore it is suggested that the hypocholesterolemic effect of HW extract from cultured mycelia of *C. sinensis* might be mediated by several mechanisms. A substantial reduction in LDL cholesterol and TC levels in serum could also be achieved by reduced production of TC by liver tissue and/or efficient removal of the LDL cholesterol by various tissues without subsequent renewal.[16]

Table 1. Composition of the Experimental Diet

	Cholesterol-free diet (%)	Cholesterol-enriched diet (%)
Casein	25.0	25.0
Soybean oil	10.0	10.0
Lard	7.0	7.0
Cellulose	5.0	5.0
Vitamin mixture[a)]	1.0	1.0
Mineral mixture[b)]	3.5	3.5
Cholesterol	–	0.35
Sucrose	25.0	25.0
Corn starch	23.5	23.15

a) Vitamin mixture: ICN Vit. mixture (No. 904654, 1999). *b*) Composition of mineral mixture was as follows (g/kg): $CaPO_4 \cdot 2H_2O$, 145.6; KH_2PO_4, 257.2; NaH_2PO_4, 93.5; NaCl, 46.6; calcium lactate, 350.9; ferric citrate, 31.8; $MgSO_4$, 71.7; $ZnCO_3$, 1.1; $MnSO_4 \cdot 4H_2O$, 1.2; $CuSO_4 \cdot 5H_2O$, 0.3; KI, 0.1.

Table 2. Chemical Composition of Mycelia and Mycelial Hot-Water (HW) Fraction of Cultured *Cordyceps sinensis*

Component	Content (%)	
	Mycelia	HW
Carbohydrate	67.1	83.9
Protein	9.2	11.8
Lipid	17.3	1.9
Ash	6.6	2.4
Component of sugar[a)]	Content (mol ratio)	
Glucose	1.0	1.0
Mannose	0.9	0.8
Galactose	0.2	0.5
Arabinose	0.1	0.1

a) Component sugar was determined by GC as alditol acetate derivatives and analyzed by GLC using an SP-2380 capillary column equipped with an FID.

Table 3. Effect of Orally Administered Hot-Water Fraction (HW) of Cultured *Cordyceps sinensis* Mycelia on the Relative Organ Weights of Mice fed Cholesterol-Free or Cholesterol-Enriched Diets

Group	HW dose (mg/kg BW)	Net weight gain (g)	Liver (g/100 g BW)	Kidney	Spleen	Perirenal fat pad
Cholesterol-free diet	0	2.3 ± 0.3^a	4.62 ± 0.05^a	1.21 ± 0.01^a	0.48 ± 0.02^a	0.31 ± 0.01^b
	150	1.8 ± 0.3^a	4.65 ± 0.18^a	1.31 ± 0.02^b	0.45 ± 0.01^a	0.20 ± 0.04^a
	300	2.0 ± 0.2^a	5.11 ± 0.13^a	1.38 ± 0.06^b	0.48 ± 0.03^a	0.23 ± 0.03^a
Cholesterol-enriched diet	0	2.0 ± 0.2^a	5.16 ± 0.17^a	1.35 ± 0.02^b	0.47 ± 0.02^a	0.39 ± 0.05^b
	150	2.1 ± 0.1^a	5.53 ± 0.17^a	1.27 ± 0.04^a	0.49 ± 0.02^a	0.20 ± 0.02^a
	300	1.9 ± 0.2^a	5.68 ± 0.33^b	1.34 ± 0.02^b	0.57 ± 0.02^b	0.10 ± 0.08^a

Mice were orally administered HW (0, 150, and 300 mg/kg body weight, respectively) for 7 consecutive days. Values are mean±S.E. for 4 mice. [a,b]Values followed by the same letter are not significantly different at the $p<0.05$ level.

Table 4. Effect of Orally Administered Hot-Water Fraction (HW) of Cultured *Cordyceps sinensis* Mycelia on Serum and Liver Lipid Concentrations of Mice Fed Cholesterol-Free or Cholesterol-Enriched Diets

Group	HW dose (mg/kg BW)	Serum lipid (mg/dl)					Liver lipid (mg/dry wt)	
		TC	TG	HDL-cholesterol	VLDL+LDL-cholesterol	Atherogenic index[a)]	TC	TG
Cholesterol-free diet	0	131.8 ± 2.9^a	115.7 ± 10.4^a	89.2 ± 15.7^a	42.6 ± 12.7^a	0.48 ± 0.06^a	18.4 ± 2.2^a	42.3 ± 4.2^a
(mg/kg BW)	150	134.0 ± 18.7^a	116.6 ± 8.5^a	91.8 ± 7.2^a	42.3 ± 11.6^a	0.46 ± 0.09^a	18.4 ± 3.7^a	44.4 ± 5.2^a
	300	113.2 ± 5.5^a	118.5 ± 3.0^a	83.4 ± 3.2^a	29.8 ± 3.6^a	0.36 ± 0.03^a	15.5 ± 1.2^a	43.1 ± 3.2^a
Cholesterol-enriched diet	0	204.6 ± 1.6^c	127.3 ± 7.9^a	73.5 ± 2.1^a	131.5 ± 10.1^c	1.83 ± 0.28^c	29.7 ± 2.3^b	48.7 ± 3.3^a
(mg/kg BW)	150	155.5 ± 4.0^b	132.7 ± 6.6^a	85.3 ± 3.1^b	70.2 ± 6.8^b	0.82 ± 0.04^b	25.2 ± 2.3^a	49.7 ± 6.3^a
	300	135.4 ± 12.2^a	139.1 ± 12.0^a	87.6 ± 4.9^b	47.8 ± 10.9^a	0.55 ± 0.008^a	21.7 ± 3.1^a	50.7 ± 7.3^a

Mice were orally administered HW (0, 150, and 300 mg/kg body weight, respectively) for 7 consecutive days. Values are mean±S.E. for 4 mice. [a,b,c]Values followed by the same letter are not significantly different at the $p<0.05$ level. *a*) Atherogenic index means (VLDL+LDL)/HDL.

Since Mann and Spoerry[17] reported a decrease in plasma cholesterol after the consumption of fermented Maasi, there has been strong interest in the effect on blood lipids. The polysaccharide CS-F30 (alkali extract fraction of the cultured *C. sinensis* mycelia) played a role in the 17% reduction in plasma TG level and 10% reduction in plasma cholesterol level at a dose of 24 mg/kg, i.p.[14] *Volvariella volvacea*, a cultivated tropical mushroom called straw mushroom, is not only a popular ingredient in traditional Chinese cuisine but also has a folk history concerning its medicinal applications in China. It had been demonstrated that both the fruiting bodies and the mycelia of straw mushrooms exert hypolipidemic effects in rats.[18] The fruiting bodies and mycelia of straw mushrooms contain a large amount of cell wall polysaccharides, of which about two-thirds are the β-glucan type.[8] The hypocholesterolemic effect observed in mycelial extracellular polysaccharide could be mainly due to the β-glucans present in it. It was reported that TC (44% reduction), TG (35% reduction), LDL cholesterol (27% reduction), and the atherogenic index (14% reduction) were significantly decreased in rats fed a cholesterol diet with 3% mycelia of *C. sinensis* compared with controls.[19] Zhao-Long *et al*.[20] indicated that both *C. sinensis* and *C. militaries* inhibited human glomerular mesengial cell proliferation induced by native LDL to a certain degree, but the inhibition by *C. sinensis* was greater than that by *C. militaris*. The present investigation demonstrated the potential of HW from *C. sinensis* in reducing the level of LDL (which is quantitatively the most significant) lipoprotein. All of the above effects would help to reduce the risk of atherosclerosis.[21]

Although the exact mechanism by which the HW of *C. sinensis* exerts a hypolipidemic effect is not clear, the possibility that combined effects on hyperlipidemia cannot be ruled out. These are: 1) the inhibition of cholesterol absorption and/or biosynthesis; 2) inhibition of synthesis of VLDL, the precursor of LDL, and acceleration of fractional turnover of LDL;[22] and 3) increased excretion of bile acids. Further studies are needed to elucidate the mechanism underlying the blood lipid-lowering effect of the HW extract from cultured mycelia of *C. sinensis*. In conclusion, the results of the present study demonstrate that the hot-water extract from cultured mycelium of *C. sinensis* lowered the plasma total cholesterol level in mice.

From these results, HW has hypocholesterolemic effects. For the evaluations on the effects of HW *in vivo*, we need a more detailed understanding of the factors that enable HW to play a role in this effect. Therefore more research on the characteristics of HW and the biological mechanism of the hypolipidemic effects will be done.

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