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Fruiting Body Extracts of *Paecilomyces tenuipes* Ameliorate Lipid and Antioxidant Metabolism in Rats Fed a High Fat-Cholesterol Diet

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Abstract The present study examined the lipid lowering and antioxidant activity of *Paecilomyces tenuipes*. Eight week-old male Sprague-Dawley rats were fed one of the three diets, a reference diet without cholesterol addition (NC), a high fat (17 g/100 g)-high cholesterol (1 g/100 g) diet (HC) and a HC diet supplemented with 3% *P. tenuipes* (PT) for 30 days. Total lipid and total cholesterol were reduced significantly by 33 and 37%, respectively, in the PT diet group compared with controls. A similar reduction was found for low density lipoprotein (LDL) cholesterol levels, while plasma high density lipoprotein (HDL) cholesterol and triglyceride (TG) concentrations were not significantly different among groups. Hepatic total lipid and total cholesterol levels, but not hepatic TG levels, were significantly decreased in the PT group compared to the HC group. The administration of *P. tenuipes* increased the plasma total antioxidant potential and decreased the levels of lipid peroxidation. These results suggest that *P. tenuipes* exerts significant health benefits through the modulation of physiological functions including a variety of atherogenic lipid profiles and antioxidants in hypercholesterolemia.

Keywords: cholesterol lowering, lipid peroxidation, antioxidant, *Paecilomyces tenuipes*

Introduction

Paecilomyces sp. is a common entomogenous fungus that parasitizes lepidopteran larvae, pupae, and adults and can be easily found in many mountainous areas in Korea (1). The fruiting body of this fungus is called the snow-flake dongchunghacho in Korea because of its appearance (2) and its entomogenous mycelium is widely used in traditional medicines. In spite of the complexities of the life cycles of the fungi impergecti, artificial cultivation techniques have been recently developed for these fungi and large-scale production of the fruiting bodies of *P. tenuipes* became possible in Korea (3).

Recently, its methanol extract has been shown to have hypoglycemic, immuno-stimulating, and anti-fatigue activities in a rat model (4). Koh and Choi (5) found that 3% mycelium or fruiting body of *P. tenuipes* in diet improves lipid profiles in rats fed a high fat diet. In addition, *P. tenuipes* has also been reported to contain novel ingredients that induce cellular differentiation and inhibit cell growth in various malignant cell lines (6, 7), and to induce apoptosis in a human leukemic cell line (8). Moreover, an extract obtained from the mycelium of *P. tenuipes* showed moderate monoamine oxidase inhibitory activity, and thus reduces the contribution to oxidant stress made by this reactive species. The extract was then hypothesized to be a possible means of controlling the aging process (9).

Hypercholesterolemia is regarded as a major risk factor of cardiovascular diseases, such as atherosclerosis, myocardial infarction, heart attacks, and cerebrovascular diseases, which are leading causes of death in advanced countries (10). Moreover, it has been well documented that lowering circulating cholesterol levels can reduce the risk of these diseases (11). Recently, hypercholesterolemia was reported to be related to increased levels of oxidative stress and lipid peroxidation (12). Also, up-regulated oxidized low density lipoprotein (LDL) generation was identified as a major contributor to the vascular damage induced by high cholesterol levels (13). Therefore, the inhibition of oxidative stress in the hypercholesterolemic state is considered to be an important therapeutic approach, and accordingly, much effort has gone into the characterization of the antioxidative functions of various materials, and this has included the potential uses of medicinal plants in food. However, no papers have yet reported the effect of *P. tenuipes* supplementation on lipid metabolism together with antioxidant status which involved in the etiology of cardiovascular diseases.

In this study, we evaluated the fruiting bodies of *P. tenuipes* as a potential agent for reducing plasma and hepatic cholesterol levels, and for improving antioxidative activity in rats fed a high fat/high cholesterol diet.

Materials and Methods

Reagents Casein, mineral mixture, and vitamin mixture were ICN Pharmaceuticals Inc. (Costa Mesa, CA, USA). Corn starch was obtained from Daesang Co. (Seoul, Korea) and sucrose and corn oil were from Cheiljedang Co. (Seoul, Korea). All other chemicals used in this study were purchased from Sigma Chemicals Co. (St. Louis, MO, USA).

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Experimental organism *Bombyx mori*, baegokjam, F_1 hybrid between the Japanese parental line Jam123 and the Chinese parental line Jam124 was used in this study, and reared with fresh mulberry leaves as described on the guide book of the silkworm rearing of National Institute of Agricultural Science and Technology, Rural Development Administration (RDA), Korea.

Inoculation of the entomophathogenic fungi The 4th day-old pupae were immersed for about 2 min in 70% ethanol solution for disinfection in the clean bench, and then the pupae were taken out from the fluid and dried in the same clean bench. The species of entomophatogenic fungi used in the study was *P. tenuipes*, which was kindly provided by National Institute of Agricultural Science and Technology, RDA, Korea. The Conidia in potato dextrose (PD) medium were inoculated by immersion of the pupae into the medium for about 1 min and the inoculation concentration of the fungi was 10^8 spores/mL.

Induction of endosclerotium and photoperiods To induce the formation of endosclerotium, the pupae that *P. tenuipes* was inoculated by immersion were cultivated at 28°C, 95% R.H. for 10 days. After the fungus completed development of the endosclerotium (hyphal bodies) in the host, the host pupae were continuously cultivated at 19-20°C, 95% R.H. under a dark photoperiod of 24 hr until the completion of fruiting bodies (Fig. 1).

Harvesting and drying of fruiting bodies The fruiting bodies were harvested by separating them from host medium and lyophilized for further experimentation.

Animal and diets Eight-week-old male Sprague Dawley rats (n=30) were purchased from Samtako Inc. (Osan, Korea) and were bred in the Department of Sericultural and Entomological Biology, Miryang University. This experiment was approved by the Miryang University Animal Use Committee and the animals were housed and cared for in accordance with the *Guide for Care and Use of Laboratory Animals* (Department of Health, Education and Welfare, 1985). They were allowed free access to water and fed a commercially-prepared pellet diet for 1 week for acclimation. The rats were fed one of the three diets, a reference diet without cholesterol addition (NC), a high fat (17 g/100 g)-high cholesterol (1 g/100 g) diet (HC), and a HC diet supplemented with 3% *P. tenuipes* (PT) for 30 days. The composition of the diets is shown in Table 1. Animals were monitored daily for general health, and body weights were recorded every week for the duration of the study. At the end of experimental period, the rats were anesthetized with ethyl ether and blood was collected from the abdominal artery into a heparin-coated sterile tube. Plasma was obtained from the blood samples by centrifugation (400×g for 30 min) and stored at -80°C until further analysis. The livers were removed and washed with ice-cold saline and then stored at -80°C before analyzed.

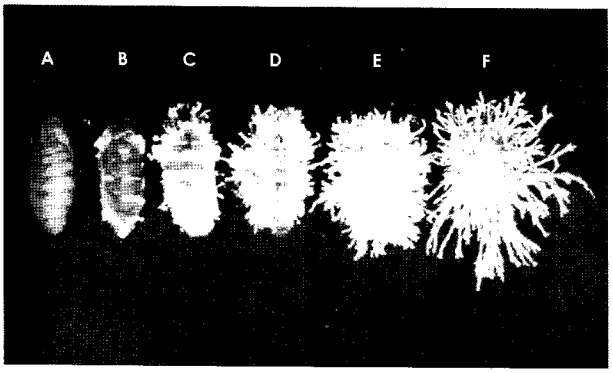

Fig. 1. The development of *Paecilomyces tenuipes*. The fungus proliferated inside the pupae and was cultivated with forming fruiting bodies of *P. tenuipes*. A, pupae before the inoculation; B, 7 days after the inoculation; C, 9 days after the inoculation; D, 11 days after the inoculation; E, 13 days after the inoculation; F, 15 days after the inoculation.

Plasma lipid profiles Plasma total lipids were measured using total lipid assay kit from Kokusai Pharm. Co. (Kobe, Japan) and other lipid profiles (total cholesterol, high density lipoprotein (HDL) cholesterol, and triglycerides) were measured using assay kits from Sigma Chemical Co. and a photometric autoanalyzer (SEAC, CH-100 plus, Italy). Plasma LDL cholesterol levels were calculated using the formula developed by Friedewald *et al.* (14).

Table 1. Composition of experimental diets[1)]

Ingredients	NC	HC	PT
Casein	20	20	20
Corn starch	55.949	42.949	39.949
Sucrose	10	10	10
Corn oil	5	5	5
Lard	-	12	12
Cholesterol	-	1	1
Cellulose	4	4	4
Vitamin mixture[2)]	1	1	1
Mineral mixture[3)]	3.5	3.5	3.5
Choline bitartarate	0.25	0.25	0.25
DL-Methionine	0.3	0.3	0.3
Butylated hydroxy toluene	0.001	0.001	0.001
P. tenuipes	-	-	3
Total	100	100	100

[1)]NC, normal control diet; HC, high-fat and high-cholesterol control diet (12 g lard + 1 g cholesterol/100 g diet); PT, high fat-high cholesterol diet supplemented with 3% of *P. tenuipes*.
[2)]AIN 93 vitamin mixture contained (in g/kg of mixture): thiamine HCl 0.6; riboflavin 0.6; pyridoxine HCl 0.7; niacin 3; *d*-calcium pantothenate 1.6; folic acid 0.2; d-biotin 0.02; cyanocobalamin (vitamin B_{12}) 0.001; dry vitamin A palmitate (500,000 U/d) 0.8; dry vitamin E acetate (500 U/d) 10; vitamin D_3 trituration (400,000 U/g) 0.25; menadione sodium bisulfite complex 0.15; sucrose finely powdered 981.08.
[3)]AIN 93 mineral mixture contained (in g/kg of mixture): calcium phosphate, dibasic 500; sodium chloride 74; potassium citrate, monohydrate 220; potassium sulfate 52; magnesium oxide 24; manganous carbonate (43-48% Mn) 3.5; ferric citrate (16-17% Fe) 6; zinc carbonate (70% ZnO) 1.6; cupric carbonate (53-55% Cu) 0.3; potassium iodate 0.01; sodium selenite 0.01; chromium potassium sulfate 0.55; sucrose, finely powdered 118.03.

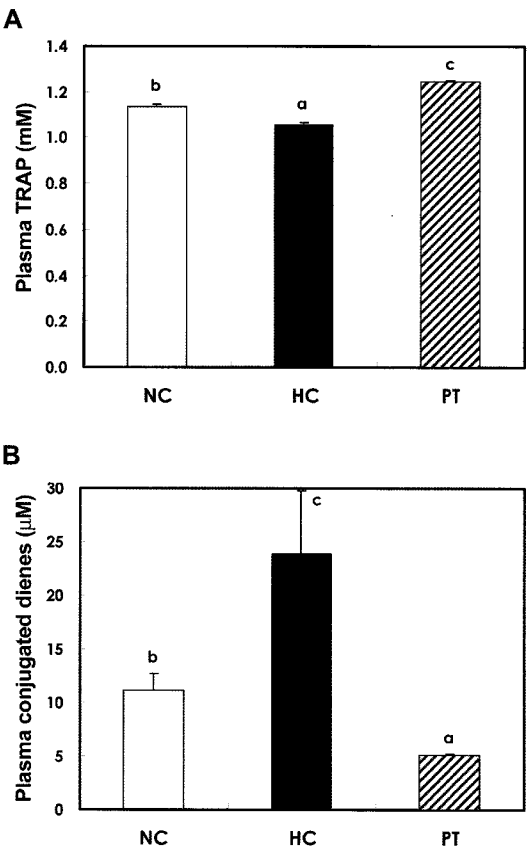

Fig. 2. Effect of *P. tenuipes* on the plasma total radical trapping anitioxidant potential (TRAP) (A) and LDL lipid peroxidation (conjugated dienes) (B). NC, normal control diet; HC, high-fat and high-cholesterol control diet (12 g lard + 1 g cholesterol/100 g diet); PT, high fat-high cholesterol diet supplemented with 3% of *P. tenuipes*. Each bar represents mean±SEM for 10 animals in each group. Bars with different superscripts are significantly different at the $p<0.05$ level by Duncan's multiple range test.

Liver lipid profiles The hepatic lipids were extracted using the procedure developed by Folch *et al.* (15). Hepatic cholesterol and triglycerides were analyzed with the same enzymatic kit used in the plasma analysis.

Plasma total radical trapping antioxidant potential (TRAP) TRAP was measured by a modification of photometric method according to Rice-Evans and Miller (16). The method for measuring antioxidant activity is based on the inhibition by antioxidants of the absorbance of the radical cation of 2,2-azinobis (3-ethylbenzothiazoline 6-sulfonate) ($ABTS^+$). The $ABTS^+$ radical cation was formed by the interaction of $ABTS^+$ (150 μM) with the ferrylmyoglobin radical species, generated by the activation of metmyoglobin (2.5 μM) with H_2O_2 (75 μM). Ten μL of sample/buffer/Trolox -standard were added into the tubes that contained 400 μL of PBS buffer, 20 μL of metmyoglobin, and 400 μL of ABTS and mixed by vortexing. The reaction was started by addition of 170 μL of H_2O_2. Absorbance was measured at 734 nm after 6 min of incubation using a spectrophotometer (state which model and manufacturer of spectrophotometer). Values were expressed as TEAC (Trolox equivalent antioxidant capacity), defined as the mM concentration of the Trolox antioxidant capacity of a calibration curve.

Baseline conjugated dienes in LDL Baseline LDL conjugated dienes were determined according to Ahotupa *et al.* (17) with little modification. One hundred μL of plasma was added to 700 μL of heparin citrate buffer (0.064 M trisodium citrate, 50,000 IU/L heparin, pH 5.05) and the suspension was allowed to stand for 10 min at room temperature. The insoluble lipoproteins were then sedimented by centrifugation at 1,000×g for 10 min. The pellet was resuspended in 100 μL of 0.1 M Na-phosphate buffer containing 0.9% NaCl (pH 7.4). Lipids were extracted from the 100 μL of LDL suspension using chloroform-methanol (2:1), dried under nitrogen, then redissolved in cyclohexane and analyzed spectrophotometrically at 234 nm. Oxidation during the sample preparation was prevented by adding ethylenediamine tetra acetic acid (EDTA).

Statistical analysis Data were analyzed using the SPSS package for Windows (Version 10). Values were expressed as mean standard error (SE). The data was evaluated by one-way analysis of variance (ANOVA) and the differences between the means were assessed using Duncan's multiple-range test. The differences were considered significant at $p<0.05$.

Results and Discussion

Effect of *P. tenuipes* supplementation on lipid metabolism In the present study we investigated the effects of powder extract prepared from the fruiting bodies of *P. tenuipes* on the lipid and antioxidant metabolism of rats fed a high fat and high cholesterol diet. Body weight gain, food intake, and food efficiency ratio were not influenced by *P. tenuipes* supplementation (data not shown). The plasma lipid profile results are summarized in Table 2. The rats fed high fat and high cholesterol diet had significantly higher total lipid, total cholesterol, and LDL cholesterol levels and significantly lower HDL cholesterol level than

Table 2. Effect of *P. tenuipes* supplementation on lipid profiles in plasma in male SD rats fed a high-fat and high-cholesterol diet[1)]

Group	NC	HC	PT
Total lipid (mg/dL)	264.3±5.0[2)a3)]	397.1±60.5[b]	266.5±12.2[a]
Total-cholesterol (mg/dL)	93.6±6.8[a]	125.7±24.7[b]	87.2±5.9[a]
HDL-cholesterol (mg/dL)	37.1±1.3[ab]	18.8±0.3[a]	20.7±1.9[a]
LDL-cholesterol (mg/dL)	35.9±6.8[a]	94.5±20.6[b]	56.0±5.7[a]
Triglyceride (mg/dL)	75.1±11.0[ns4)]	61.6±21.3	47.1±2.7

[1)]NC, normal control diet; HC, high-fat and high-cholesterol control diet (12 g lard + 1 g cholesterol/100 g diet); PT, high fat-high cholesterol diet supplemented with 3% of *P. tenuipes*.
[2)]Values are the mean±SEM for 10 animals in each group.
[3)]Values within a row with different superscripts are significantly different at $p<0.05$ by Duncan's multiple range test.
[4)]ns: not significant.

rats that were fed the basal diet. Total lipid and total cholesterol were reduced significantly by 33 and 37%, respectively, in the PT diet group compared with controls. A similar trend was found for LDL cholesterol levels, which were reduced by 41% in the PT groups. Plasma HDL cholesterol and triglyceride concentrations were not significantly different among the groups. The high fat and high cholesterol diet also induced fatty liver in rats where their livers showed increased levels of total lipid, total cholesterol, and triglyceride compared with the normal control group (Table 3). The liver weight per 100 g body weight appeared to decrease when rats were fed *P. tenuipes* extracts ($p>0.05$). Hepatic total lipid was significantly lower in the 3% PT group and hepatic total cholesterol levels were significantly reduced by 20% in the PT groups compared to the HC group. However, *P. tenuipes* supplementation did not affect the hepatic triglyceride concentration.

These results suggest that the plasma lipid-lowering effect of the *P. tenuipes* dietary supplementation was potent in hypercholesterolemic rats. *P. tenuipes* supplementation also significantly reduced hepatic cholesterol in hypercholesterolemic rats, suggesting that the hepatic biosynthesis of cholesterol had been suppressed. These results are consistent with the findings of Koh and Choi (5), where they found that a 3%(w/w) fruiting body extract of artificially cultivated *P. tenuipes* in diet reduced atherogenic index and hepatic cholesterol levels in rats fed a high fat diet. It is well known that plasma cholesterol concentrations can be regulated by cholesterol biosynthesis, cholesterol removal from the circulatory system, the absorption of dietary cholesterol, and the excretion of cholesterol via bile and feces (18). Although the mechanism by which *P. tenuipes* reduces plasma total cholesterol levels in hypercholesterolemic rats is not fully understood, the hypolipidemic effect of the fruiting bodies of *P. tenuipes* could be due to a reduced activity of the liver enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme of cholesterol biosynthesis (19). It is also possible that total cholesterol level suppression in plasma by *P. tenuipes* could be related to increased 7-α-hydroxylase activity (a key enzyme of cholesterol catabolism) and bile acid secretion, and the subsequent increased excretion of cholesterol through feces (20).

Table 3. Effects of *P. tenuipes* supplementation on lipid profiles in liver in male SD rats fed a high-fat and high-cholesterol diet

Group	NC[1)]	HC	PT
Liver weight (g/100 g bw)	2.9±0.1[2)a3)]	4.0±0.4[b]	3.5±0.3[ab]
Total lipid (mg/g wet liver)	14.1±1.5[a]	90.1±8.8[c]	56.2±5.2[b]
Total cholesterol (mg/g wet liver)	1.9±0.1[a]	9.3±0.8[c]	7.4±0.4[b]
Triglycerides (mg/g wet liver)	5.1±0.9[a]	12.8±0.9[b]	11.2±0.8[b]

[1)]NC, normal control diet; HC, high-fat and high-cholesterol control diet (12 g lard + 1 g cholesterol/100 g diet); PT, high fat-high cholesterol diet supplemented with 3% of *P. tenuipes*.
[2)]Values are the mean±SEM for 10 animals in each group.
[3)]Values within a row with different superscripts are significantly different at $p<0.05$ by Duncan's multiple range test.

Effect of *P. tenuipes* supplementation on antioxidant metabolism The TRAP value, an indicator of total antioxidant defense in plasma, was significantly affected by *P. tenuipes* supplementation (Fig. 2A). The administration of PT increased the plasma TRAP level, which was reduced by high fat and high cholesterol diet. Figure 2B shows the levels of conjugated dienes in plasma, a parameter of lipid peroxidation. *P. tenuipes* supplementation markedly inhibited conjugated diene levels by 78% in the *P. tenuipes* group.

Oxidative stress, the disturbed balance between oxidative and antioxidative processes, plays an important role in the pathogenesis of atherosclerosis (21). A cholesterol-rich diet resulted in increased lipid peroxidation by free radicals, and this was followed by hypercholesterolemia, a major risk factor for atherosclerosis (22, 23). The relationship between oxidative stress and cholesterol levels was confirmed in the present study, showing that high cholesterol diet induced a reduced plasma antioxidant potential and elevated plasma conjugated dienes (a primary product of lipid peroxidation). However, the plasma TRAP was significantly higher in the PT group, while the conjugated diene level was lower in the PT supplemented group. Our results are consistent with those of Kwon *et al.* (24) who found that diets supplemented with 2%(w/w) powder extracts of *P. tenuipes* fruiting bodies, cultivated in silkworm pupae, lowered plasma and liver thiobarbituric acid reactive substances and liver superoxide dismutase (SOD) activity in rats. An extract obtained from the mycelium of *P. tenuipes* showed moderated monoamine oxidase (MAO) inhibitory activity, which reduces oxidative stress by limiting the formation of this radical species (9). However, no published report to date explains the mechanisms of the antioxidant effect of the fruiting bodies of *P. tenuipes*. It may be possible that partially polymerized water-soluble β-glucans, which are major physiologically active substances in most mushrooms, are able to quench free radicals (25, 26), and might therefore account partially for the antioxidant activity we observed here.

Our results suggest *P. tenuipes* has significant health benefits as it modulates physiological functions, e.g., various atherogenic lipid profiles and antioxidants in hypercholesterolemia. Therefore, it may be a potential candidate for use as a functional food, where it may act as a prophylactic against hypercholesterolemia and provide health benefits in terms of counteracting hyperlipidemia and its related complications. Further studies should be conducted to elucidate the exact mechanism underlying the lipid-lowering and antioxidant effects of *P. tenuipes* and the possible hypolipidemic and antioxidant compounds contained in *P. tenuipes*.

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