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Antihyperlipidemic and Antioxidant Effects of Extracts from *Pleurotus citrinopileatus*

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Pleurotus citrinopileatus is a popular edible mushroom which is physiologically active in both humans and animals. In the study we investigate the effects of this mushroom on hyperlipidemic hamster rats. Four dietary forms of the mushroom were created as follows. The powdered dry fruiting body, hot-water extract, and two kinds of elutes were obtained, from ethyl acetate extract and methanol extract, respectively, in different mixed proportion solvents over silica gel column chromatography (referred to as EAE and MOE, respectively). They were tested at different dosages as a supplement to a high-fat diet in hyperlipidemic rats. Serum triglycerides and total cholesterol levels were significantly lower in groups supplemented with the highest dosages of EAE and MOE (0.5 g/kg, body weight daily) as compared with the control groups that received no mushroom additive. High-density lipoprotein levels in these same two experimental groups were also significantly higher than those in the negative control group. The tested rats that were fed with EAE had the highest serum glutathione peroxidase and superoxide dismutase activity, and those with the MOE and EAE had the highest DPPH free radical scavenging activities and ferric-reducing abilities, tested in vitro. The major constituents of MOE and EAE were identified as ergosterol and nicotinic acid, respectively. *P. citrinopileatus* extracts may have a significant antihyperlipidemia effect. Furthermore, antioxidant activities and antihyperlipidemic effects of MOE and EAE seemed to display similar tendencies.

KEYWORDS: Antihyperlipidemic; antioxidant; extracts; *Pleurotus citrinopileatus*

INTRODUCTION

Improper lipid intake is known to be related to a range of serious diseases and disabilities in many animals (*1*, *2*), including mammals (*3*). Excessive quantities or improper types of lipids may cause hyperlipidemia (*4*). Peroxidation of lipids is also related to coronary heart disease (*5*). Treatment of hyperlipidemia may be with therapeutic medicines or through natural edible materials which help lower serum lipid levels. Edible materials have the advantage in that they avoid side effects often associated with medications, while still improving or healing the hyperlipidemia (*6*). Many natural edible materials (or their components) have been identified as having antihyperlipidemic effects. Included in these items are fish liver (*1*), soybean (*2*), ginseng (*6*), carrots (*7*), oysters (*8*), tea (*9*), chitosan (*10*), pectin (*11*), and dietary fiber (*11*, *12*).

Due to significant benefits such as low contamination and high economic value, many studies were conducted to investigate the biofunction and application of edible mushrooms. Edible mushrooms are rich in dietary fiber, nutrients, and some particular compounds known to bring physiological benefit to mammals, including humans, as well as to other animals (*13*). Health benefits, including the lowering of serum total cholesterol levels, have been demonstrated with *Agaricus blazei* (*14*), *Hericium* spp. (*15*, *16*), *Pleurotus* spp. (*17*), *Grifola frondosa*, *Lentinus* edodes, and *Flammulina velutipes* (*18*, *19*). A multitude of studies of *Pleurotus* spp. focused specifically on the oyster mushroom, *Pleurotus ostreatus*. The oyster mushroom fruiting body, or extracts from the fruiting body, has been found to effectively enhance the rate with which cholesterol is totally cleared. It has also been shown to prevent the progress of hypercholesterolemia (*20*−*23*). Similar results were also found by Hossain et al. (*24*). The *P. citrinopileatus* is a flavorful edible mushroom rich in nutrients (*25*). Based on recent research on animal models, including our own research, it is suggested that

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the mushroom may have some physiological effects, including antitumor, immunoenhancement, and antihyperglycemia (*26*, *27*). In the research presented in this paper, we tested various forms from *P. citrinopileatus* (powdered whole fruiting body, water extract, and partial purified residues from crude solvent extracts) on animal models to explore their antihyperlipidemic effect. Metabolites of lipids in serum and feces were measured during the feeding period. The antioxidant activities were also assayed in vivo and in vitro to survey the relationship between lipid metabolism and antioxidant activity.

MATERIALS AND METHODS

Preparation and Analysis of Extracts from *P. citrinopileatus*. The mushroom was cultivated in a sawdust-based medium for this study. Dry sawdust was weighed and then mixed with the relative weights of wheat bran (8%), rice bran (6%), glucose (2%), and calcium carbonate (2%). Water content of the final mixture was adjusted to 65% (w/w). The mixture was filled into polyethylene bags and sterilized (121 °C, 60 min), then inoculated with *P. citrinopileatus* (You-Hao Mushroom Research Institute, China), and incubated for 22 days (26 ± 2 °C, equilibrium relative humidity 80 ± 5%). As fresh fruiting bodies developed, they were harvested and lyophilized. Freeze-dried fruiting bodies were processed in one of four ways. The water extract was prepared by boiling the mushroom in water for 30 min and then filtering it through filter paper (Whatman No. 1); the filtrate was then lyophilized. The dried fruiting bodies were milled into 60-mesh powder. The dried fruiting bodies were then successively macerated into methanol and ethyl acetate and then filtered. The filtrate was further concentrated in a rotary evaporator. The yield of methanol extract was 2.6% (w/v) and that of ethyl acetate extract was 2.2% (w/v). The methanol extract was chromatographed over silica gel (Merck, Kiesegel 60, 230−400 mesh, 1200 g) and eluted with mixtures of MeOH:EtOAc:$CHCl_3$ of increasing polarities (1:0:0 to 0:0:1, v/v) to yield 120 fractions (10 mL each). The fractions from 35th to 60th were combined and then concentrated. The resulting residue was named MOE. The ethyl acetate extract was chromatographed over silica gel and eluted with mixtures of MeOH:EtOAc:$CHCl_3$ (10:0:0 to 0:0:10, v/v) to yield 86 fractions. The fractions from 20th to 45th were combined and then concentrated. The resulting residue was named EAE. The MOE and EAE were further purified by silica gel column chromatography (5 cm × 40 cm) with MeOH:EtOAc:$CHCl_3$ (25:1:1, v/v) for MOE to give compound **1** (48.9 mg/kg, dry weight of raw material) and MeOH:EtOAc:$CHCl_3$ (50:1:1, v/v) for EAE to give compound **2** (21.2 mg/kg, dry weight of raw material), respectively. Compounds **1** and **2** were identified as nicotinic acid and ergosterol, respectively, by a polarimeter (JASCO DIP-370), mass spectrometer (JEOL JMS-SX1SX 102A, Japan), and ^{1}H NMR and/or ^{13}C NMR (Varian Gemini NMR Spectrometer, USA) data and by comparison with reports from related literature (*28*, *29*). The total dietary fiber of the powder was determined by following AOAC (*30*). The compositions of fatty acid of the MOE and EAE were also analyzed. To do this, samples were methylated by adding tetramethylammonium hydroxide to 25% (w/w) methanol (Sigma Chemical Co., USA). Gas chromatography (HP 5890C, column, Supelco-2560, 100 m × 0.25 mm i.d., 0.20 μm film) was used to analyze the components (oven temperature of 140 °C for 5 min, to 240 °C at 4 °C/min, carrier gas helium, 20 mL/min, FID detector, 260 °C, inject 1 μL, split 100:1) (*30*).

Animals. Experiments with female hamster rats (76 ± 3 g, 5 weeks old, purchased from National Laboratory Animal Center, Taiwan) were carried out at the qualified animal breeding room of the Animal Center in our institute. The protocol complied with guidelines described in the "Animal Protection Law ", (Amended January 17, 2001 Hua-Zong-(1)-Yi-Tzi-9000007530, Council of Agriculture, Executive Yuan, Taiwan). Hamster rats were housed under standard environmental conditions (23 ± 1 °C, humidity 55 ± 5%) under cycles of 12 h light/12 h dark. The rats had free access to water and a semipurified diet (AIN-76, ICN Biochemicals Inc., CA) in the 7 days prior to the experiment.

Antihyperlipidemic Effect. The rats were divided into 12 groups of 10 rats each. The control group received a regular AIN-76 diet. The other 11 groups were fed with a high-fat diet containing 10% lard and 0.3% cholesterol (Merck) in a basal diet AIN-76. When the total serum cholesterol in the treated animals reached at least 220 mg/dL, rats were considered hypercholesterolemic. Then, one group of hypercholesterolemic rats was fed continuously with high-fat AIN-76 diet, which was used as negative control. The other hypercholesterolemic rats were fed with the high-fat diet together with different levels of supplements derived from *P. citrinopileatus* for 42 days, followed with another 7 days of high-fat diet without the mushroom supplement. The mushroom supplement was added in the form of smashed powder, MOE, EAE, and water-soluble portion. The doses of supplements were 0.08, 0.2, or 0.5 g/kg of body weight for MOE, EAE, and water-soluble portion and 2 g/kg of body weight for the smashed mushroom powder group. At the end of the 49 day experiment, all of the rats were humanely killed and their livers immersed in 10% formalin for 24 h for further analysis. Livers were then rinsed, dehydrated, embedded in paraffin wax, mounted on slides, and stained to make them into tissue slices. The pathomorphological changes in the hepatic cells were observed microscopically.

Antioxidant Activity. DPPH free radical scavenging activities and ferric-reducing abilities of MOE, EAE, and water extract were determined as described by Schlesier et al. (*31*). An aliquot (0.1 mL) of a DPPH (2,2-diphenyl-1-picrylhydrazyl) solution (6×10^{-5} M) prepared in methanol containing different concentrations of each tested sample or authentic antioxidant was added to 3.9 mL of DPPH solution. The decrease in absorbance at 515 nm was measured at each predetermined checkpoint. The DPPH disappearance rate was calculated by fitting each curve of plotted values of DPPH remaining (%) against time to a logistic equation.

The ferric-reducing ability was determined. First, 150 μL of different concentrations of extract were added to an equal volume of 0.2 M sodium phosphate buffer solution (pH 8.6) and 1% $K_3Fe(CN)_6$. This was mixed well and then allowed to stand for 20 min at 50 °C. Then a series of materials were added: first, 150 μL of TCA (1%), then 0.6 mL of deionized water, and finally 120 μL of $FeCl_3$ (0.1%). The mixture was left to stand for 14 min and was then analyzed by a spectrophotometer at 700 nm (*31*).

Blood and Feces Analysis. Blood samples were collected, without anesthesia, from the socket of the eyeball into heparinized tubes and analyzed immediately. Throughout the study, fasting blood cholesterol, triglyceride, low-density lipoprotein (LDL) cholesterol, and high-density lipoprotein (HDL) cholesterol were determined every 7 days, following 18 h of food deprivation. Cholesterol, triglycerides, LDL cholesterol, and HDL cholesterol were determined by a Merck VitaLAB Selectra Bioanalyzer (Vital Scientific N.V., The Netherlands) (*8*, *24*). The activities of glutathion peroxidase and superoxide dismutase in the blood from the socket of the eyeball were determined in the 6th week by using Randox kits (RANDOX Labs Ltd., Antrim, UK) (*32*, *33*).

Neutral steroids in the feces were determined as follows. Feces were collected for 7 days before the end of 42 days, lyophilized, and then milled into powder. A fixed amount of powdered feces was macerated in 20-fold volume of Folch solution (chloroform:methanol, 2:1, v/v) for 12 h. The mixture was filtered, and the filtrate characteristics were determined by a spectrophotometer at 500 nm. The cholesterol content was expressed as mg of cholesterol/day g of feces (*34*).

Statistical Analysis. Analysis of variance and Duncan's multiple range test was used to determine significant differences ($p < 0.05$) among treatments. Statistical data analysis was run on SAS (version 6.08). The data are shown as mean ±SD.

RESULTS

Changes in Body Weight. In the MOE group, rats had an average increase in body weight over 42 days of nearly 36.5% (**Figure 1**). In the EAE, water extract, and powder groups the increases were 33.5%, 37.3%, and 37.5%, respectively. The weight increment rate of the EAE groups was significantly lower than those of the other three experimental groups ($p < 0.05$). In all four experimental groups the weight increment rate was significantly higher than the normal control group ($p < 0.05$),

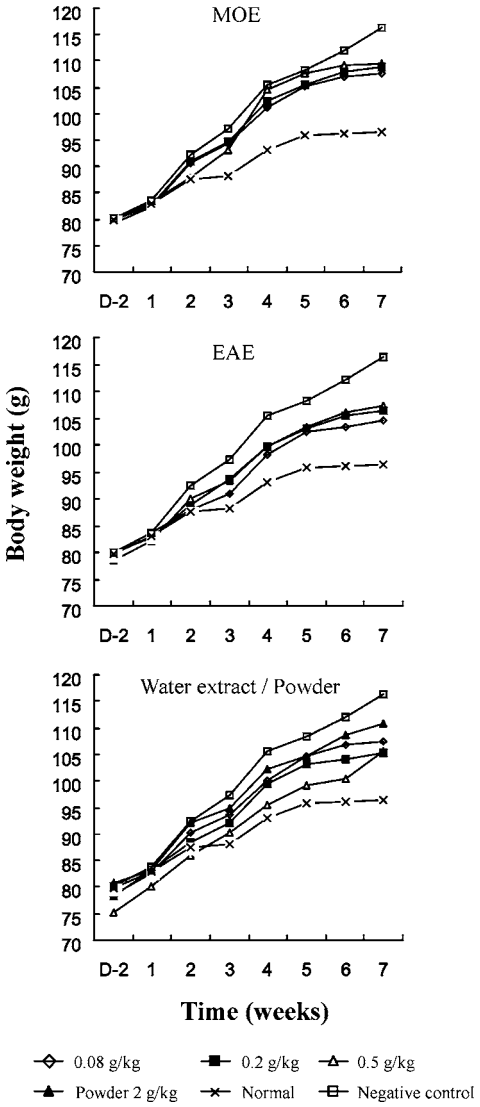

Figure 1. Effect of *P. citrinopileatus* on body weight in hyperlipidemic rats. Solvent extracts from mushroom was partially purified with silica gel chromatography. A part of elute from methanol extract was named MOE, and that from the ethyl acetate extract was named EAE. The hyperlipidemic rats were fed with regular AIN-76 combined with a high fat constitutes a different level of mushroom supplements, such as MOE, EAE, water extract, or powder from *P. citrinopileatus* for 6 weeks, followed by 1 week of high-fat diet only feeding treatment. All of the experimental rats were killed humanely at the end of 49 days. D-2 represents 2 days before the high-fat diet feed began. The normal control is the animals fed with AIN-76 diet only, and negative control is the hyperlipidemic animals fed with high-fat diet only. Data analyses indicated a significant difference at $p < 0.05$ among treatments.

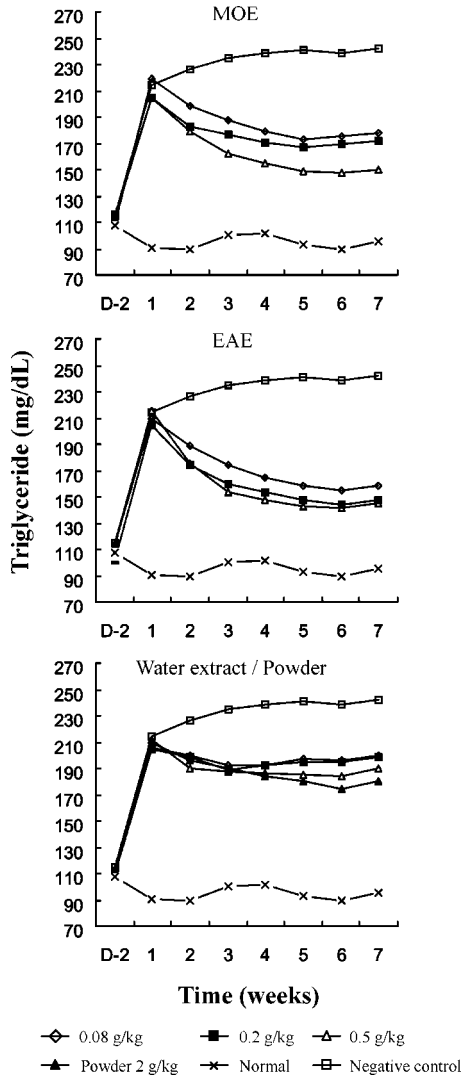

Figure 2. Effect of *P. citrinopileatus* on serum triglyceride in hyperlipidemic rats. The experiment was described in the legend of Figure 1.

but significantly less than the negative control group ($p < 0.05$). Changes in body weight of all experimental and control groups showed no obvious difference in the 7th week, when all additives were stopped ($p > 0.05$).

Changes of Serum Triglyceride Levels. Serum triglyceride levels decreased about 31.1% in the 0.5 g/kg EAE group from the 1st to the 4th week (**Figure 2**). Serum triglyceride levels were decreased most in the highest dosage (0.5 g/kg) EAE group. No differences were found between the two lower dosages (0.2 and 0.08 g/kg) ($p > 0.05$). In the other two EAE groups (0.2 and 0.08 g/kg), levels decreased 24.9% and 21.5%, respectively. The most pronounced decrease was in the 4th week, becoming steady afterward. At the 0.5 g/kg dosage, similar results were found with the MOE group, whereas with the water extract group, serum triglyceride levels were similar at all three feeding dosages ($p > 0.05$). The rate of decrease was lower from the 1st to the 4th week. In the powder group, the rate of decrease was only 16.7% from the 1st to the 6th week.

Changes of Serum Total Cholesterol Levels. The EAE group of 0.5 g/kg had the highest rate of decrease of serum total cholesterol levels in all tested sample groups from the 1st to the 6th week (**Figure 3**). Comparing the 1st week with the 3rd, 4th, and 5th weeks, the rates of decrease in serum total cholesterol were 24.2%, 30.4%, and 34.0%, respectively, becoming steady afterward. Similar results were found in the 0.5 g/kg MOE group. There was no significant difference between the MOE and EAE groups at the 0.5 g/kg dosage ($p > 0.05$). From the 1st to the 6th week, the two other EAE groups (0.2 and 0.08 g/kg) achieved reductions which were lower (67% and 47%, respectively) than those in the 0.5 g/kg EAE group. Lower reductions (12.9%) were found in the powder group, which was 50% of those found in the 0.5 g/kg MOE group. The 0.5 g/kg water extract group showed the lowest rates.

Serum total cholesterol levels increased slightly in all groups in the last week of the experiment when mushroom extract was no longer fed to the rats. Changes in all groups were about 3−8

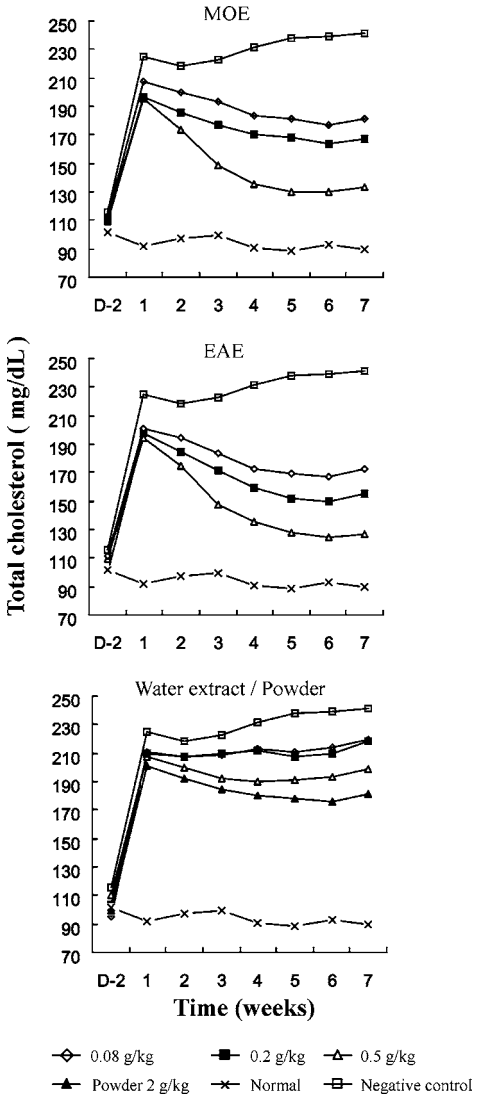

Figure 3. Effect of *P. citrinopileatus* on serum total cholesterol in hyperlipidemic rats. The experiment was described in the legend of Figure 1.

mg/dL. Levels of triglyceride, HDL cholesterol, and LDL cholesterol also showed similar changes.

Neutral steroids in the feces of the MOE and EAE groups at all three different doses were higher than those in the negative control group (**Figure 4**). Fecal steroid amounts in the MOE and EAE groups (both at 0.5 g/kg) were 32.3% and 37.5%, respectively, which were higher than that of the negative control group. The powder group, however, was not significantly different from the negative control group ($p > 0.05$). The water extract group (0.5 g/kg) was 7.4% higher than that of the negative control group.

Fatty acids in the MOE had the highest C20:1 content (2.11%, w/w dry matter, total lipids), followed by C18:1 (1.87%), C16 (1.35%), C18 (0.82%), and C18:3, n6 (0.19%). In the EAE, the highest fatty acid content was C18:1 (3.66%), followed by C20:1 (3.47%), C16 (2.54%), C22 (2.52%), C18 (2.07%), and C18:2, n9 (1.40%).

Changes of Total HDL Cholesterol Levels. Increased rates in serum HDL cholesterol serum levels were the highest (92.9%) with the EAE group at the 0.5 g/kg dosage, as opposed to lower dosages (0.2 g/kg, 88.5%) and (0.08 g/kg, 82.6%) (**Figure 5**). There were no significant differences ($p > 0.05$) between the MOE and EAE groups when compared at the same dosage. In

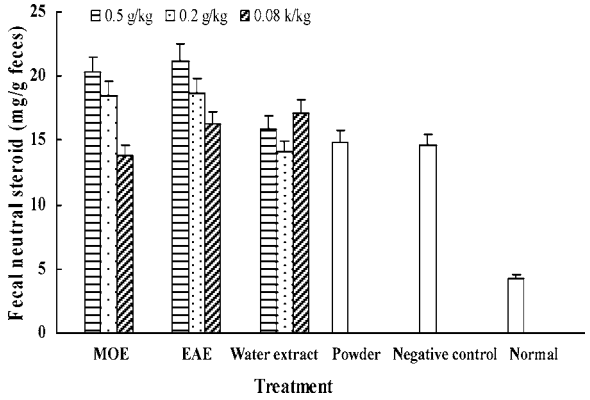

Figure 4. Effect of *P. citrinopileatus* on fecal neutral steroid excretion in hyperlipidemic rats. The experiment was described in the legend of Figure 1. The rats from the powder group were fed with 2 g/kg of body weight daily of powder from the fruiting body. The data were mean ± SD.

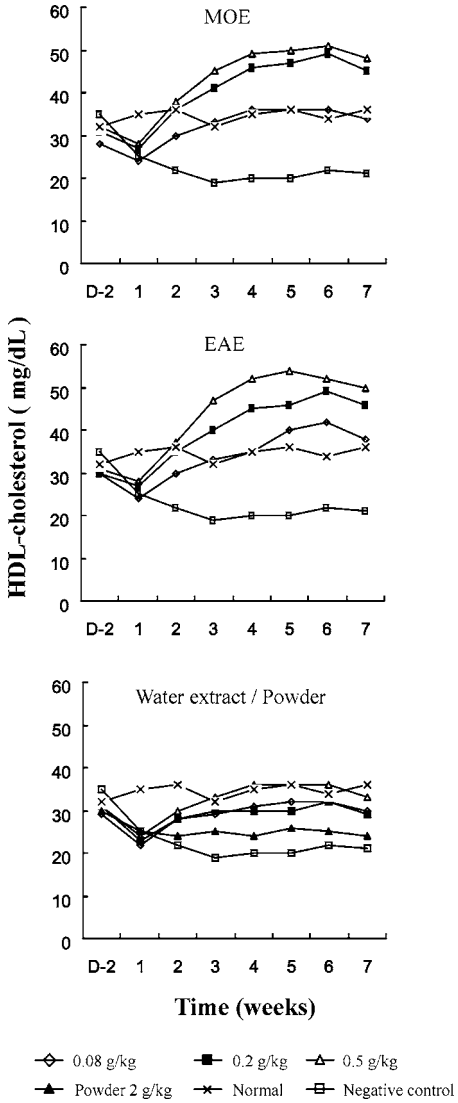

Figure 5. Effect of *P. citrinopileatus* on serum HDL cholesterol in hyperlipidemic rats. The experiment was described in the legend of Figure 1.

the water extract group, at a dose of 0.5 g/kg the results were significantly higher than those at 0.2 g/kg (39.1%) and 0.08 g/kg (40.9%). There was no significant change in the serum HDL cholesterol level in the powder group throughout the experiment.

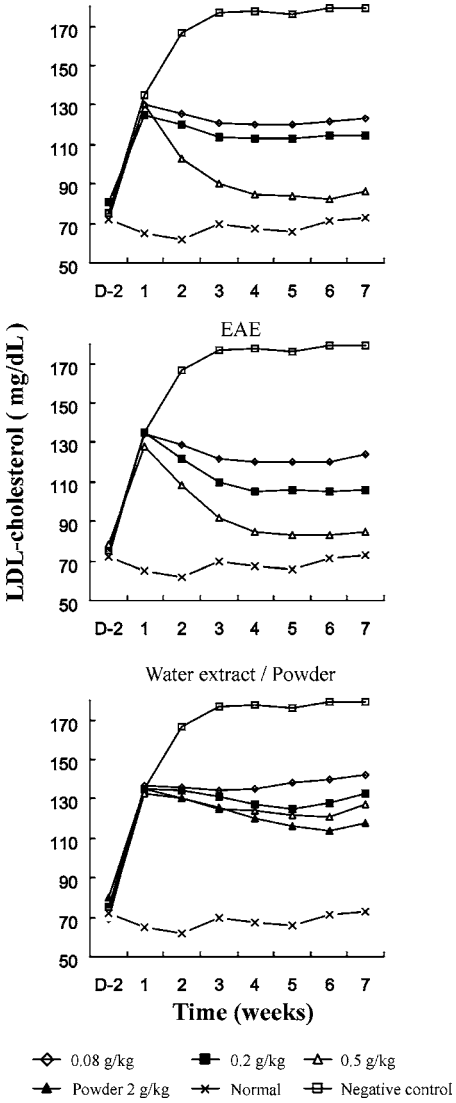

Figure 6. Effect of *P. citrinopileatus* on serum LDL cholesterol in hyperlipidemic rats. The experiment was described in the legend of Figure 1.

Changes of Total LDL Cholesterol Levels. At week 6 there was a 10 mg/dL difference between the 0.5 g/kg MOE group and the normal control group (**Figure 6**). Results were similar at the same dosage with the EAE group. A lower decrease rate was found with both the MOE group (36.9%) and the EAE group (35.2%) at the 0.5 g/kg dosage, which was 12.3% of the powder group by week 6. At doses of 0.2 or 0.08 g/kg there was no significant difference ($p > 0.05$) between the EAE and MOE groups. There was no significant difference in decrease rates of serum LDL cholesterol level between the 0.5 and 0.2 g/kg water extract groups ($p > 0.05$).

Antioxidant Activities in Vivo and in Vitro Tests. The highest glutathion peroxidase activity was found in the EAE group at a dose of 0.5 g/kg daily (**Figure 7**), and the lowest activity was in the powder group, which was lower than the EAE group (0.5 g/kg dosage) by 0.3 in logarithm value. Although at the dietary level of 0.2 g/kg there was no significant difference between the EAE group and the MOE group ($p > 0.05$), at the highest, 0.5 g/kg, level the difference was significant.

The blood superoxide dismutase of the 0.5 g/kg MOE and EAE groups had the highest activities among all the experimental groups; however, the two groups had no significant

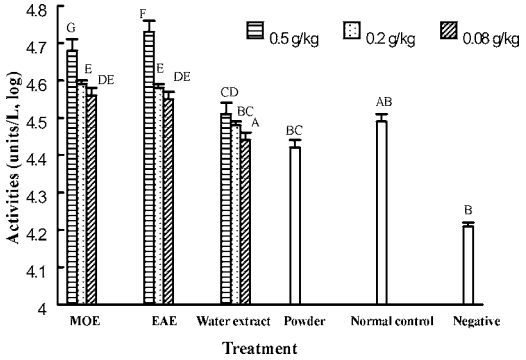

Figure 7. Effect of *P. citrinopileatus* on glutathion peroxidase in hyperlipidemic rats. The experiment was described in the legend of Figure 1. The activities of glutathion peroxidase of the blood from the socket of the eyeball in the 6th week were determined. The activities of the enzyme were 4.46 ± 0.02 unit/L in log in the normal control group, and those of the rest of the experimental groups ranged from 4.28 ± 0.01 to 4.33 ± 0.03 units/L in log before feeding of a high-fat diet. The different capital letters are significantly different at $p < 0.05$.

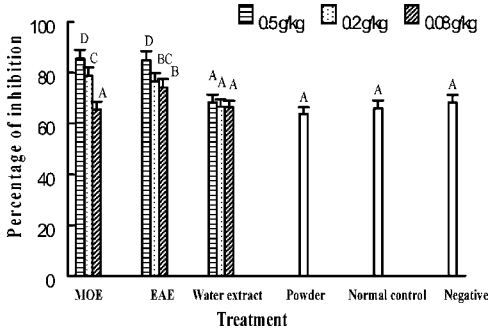

Figure 8. Effect of *P. citrinopileatus* on superoxide dismutase in hyperlipidemic rats. The experiment was described in the legend of Figure 1. The activity of the enzyme of the blood from the socket of the eyeball in the 6th week was determined. The activity of the enzyme is expressed as the percentage inhibition to the superoxide radical, which is generated by the reaction of xanthine and xanthine oxidase. The activities of the enzyme were 66.71 ± 3.05% in the normal control group, and those of the rest of the experimental groups ranged from 65.53 ± 3.35% to 68.22 ± 3.61% before feeding with high-fat diet. The different capital letters are significantly different at $p < 0.05$.

difference ($p > 0.05$) (**Figure 8**). The activities of superoxide dismutase in the water extract groups were the same at all three dosages ($p > 0.05$); as well, there was no significant difference between the powder group and the normal control group ($p > 0.05$).

The highest DPPH free radical scavenging activity was found in the EAE (**Figure 9**), followed by the MOE, then the water extract, and then the powder. Ferric-reducing ability in vitro was highest in the MOE (**Figure 10**). Two percent MOE was similar to 2% α-tocopherol in ferric-reducing ability.

DISCUSSION

In the MOE and EAE groups the rates of decrease in serum total cholesterol levels reached a peak in the 4th week, after which they became steady. MOE and EAE were most effective in reducing serum triglyceride and total cholesterol levels. There was a significant difference in the rate of serum total cholesterol decrease among the three EAE dosages, while with the MOE the difference was only between the 0.5 g/kg group and the lower two dosage groups. Therefore, although dosage details were different in the two test samples, the antihyperlipidemic

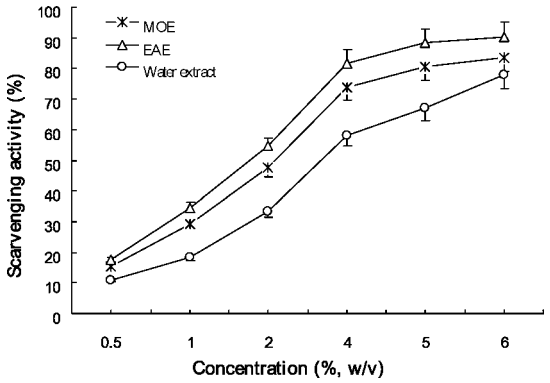

Figure 9. Effect of *P. citrinopileatus* on DPPH free radical scavenging activity tested in vitro. MOE and EAE are described in the legend of Figure 1.

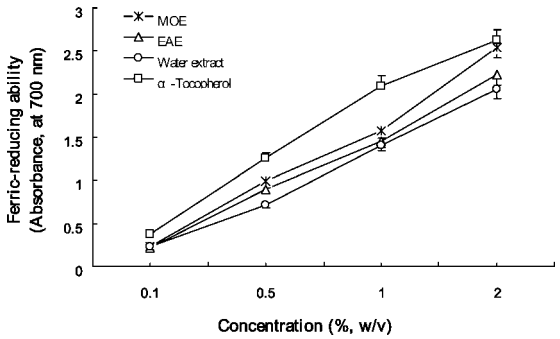

Figure 10. Effect of *P. citrinopileatus* on ferric-reducing ability tested in vitro. MOE and EAE are described in the legend of Figure 1.

effects of both EAE and MOE had demonstrated to be dose-dependent. When Bobek et al. (*17*, *35*, *36*) used *P. ostreatus* with a 0.3% cholesterol diet to feed male Wistar rats, they found the mushroom could reduce the levels of serum total cholesterol levels and suppress cholesterol accumulation in the liver. They found a highly significant negative correlation between the amount of the mushroom in the diet and cholesterol levels in the serum.

Lee et al. (*37*) used cinnamate, a phenolic compound found in cinnamon bark, as an additive to a rat feed at a dose of 1 g of cholesterol/kg, which was administered for 6 weeks. They found the cinnamate could evidently increase plasma HDL cholesterol levels and suppress lipid peroxidation through the enhancement of hepatic antioxidant enzyme activities. Ogawa et al. (*38*) found that a diet containing 0.2% *Angelica keiskei* extract could show an elevation of serum HDL cholesterol levels and a reduction of liver triglyceride levels.

Chen et al. (*39*) used Taiwanese yam to feed Balb/c mice for 21 days. They found that the experimental diet reduced the LDL cholesterol level and prevented hypercholesterolemia. Because blood LDL cholesterol level and its oxidation are related to cardiovascular risk (*40*), the LDL cholesterol level of blood is an index of health (*41*). In our study, there was no difference in serum LDL cholesterol level among dosages of the water extract group during the experimental duration. However, in the MOE and EAE groups at a higher dosage, there were obvious variations from the 1st to the 6th week.

The antihyperlipidemic effect demonstrated in the water extract groups was lower than that of the powder groups. The content of total dietary fiber of the *P. citrinopileatus* powder was 29.7% (dry weight). The antihyperlipidemic effect of the powder groups may be related solely to the dietary fiber content. Fukushima et al. (*19*) found that *Grifola frondosa* and *Flammulina velutipes* fibers were able to decrease the serum total cholesterol level by enhancing fecal cholesterol excretion. Kubo and Nanba (*18*) also found that cholesterol excretion increased after Spraque-Dawley rats on a high cholesterol diet were fed dried *Grifola frondosa* powder. Yamada et al. (*12*) demonstrated an antihyperlipidemic effect from cellulose, guar gum, and pectin in hyperlipidemic Spraque-Dawley rats. Meanwhile, pectin, a soluble dietary fiber, significantly lowered plasma cholesterol (*11*).

Unsaturated fatty acids should help to suppress hyperlipidemia. Monounsaturated fatty acids in particular are considered to be effective in decreasing coronary heart disease rates (*42*), and oleic acids (C18:0) are also known to have an antihyperlipidemic effect (*43*). In our study, the EAE possessed a higher oleic acid content, which might influence the antihyperlipidemic effect (*44*). In MOE and EAE, however, the major components were ergosterol and nicotinic acid, respectively. Yaoita et al. (*45*) previously found two novel steroids in *Pleurotus eryngii*. Steroids have been demonstrated to promote hypolipidemic effects (*46*, *47*). Nicotinic acid is recognized as ideal for treating a wide variety of lipid disorders, including hypertriglyceridemia. At the same time, it also significantly reduces low-density lipoprotein cholesterol levels, triglyceride, while increasing high-density lipoprotein levels (*48*). Therefore, it may be that these components in the MOE and EAE play a key role in the antihyperlipidemic effect, a possibility which could be explored in future research.

Although there was no change in body weight during the final week on the regular AIN-76 diet with no tested samples, serum total cholesterol and triglyceride levels did increase again in the three groups that were previously given the mushroom additives (water extract, MOE, and EAE). In addition, the levels of increase in the water extract group were higher than those in the MOE or EAE groups. Serum total cholesterol levels of all experimental groups remained lower than the negative control group. It appears that the MOE, EAE, and water extract of *P. citrinopileatus* has an antihyperlipidemic effect, although the effect in the water extract was lower. According to present data, reduction of total serum cholesterol in all experimental groups to the level of the normal control group is expected, if the doses were raised or if the feeding period was prolonged. After 5 weeks, however, the rate of decrease in serum total cholesterol tended to stabilize. Although the results might demonstrate that normalization of serum lipids through dietary additives is a slow process, the extracts of *P. citrinopileatus* appeared to have an antihyperlipidemic effect. This was especially apparent with MOE and EAE at a dose of 0.5 g/kg daily. A similar effect was found with serum triglyceride levels. In addition, fecal excretion of neutral steroids in the MOE and EAE groups was significantly higher than that in the negative control group. Chan et al. (*49*) reported similar results in their experiments with rats fed a high-fat diet containing Jasmine green tea.

The antihyperlipidemic effect of water-insoluble extracts was higher than that of water-soluble extracts in all tested samples in our study. Opletal et al. (*23*) also found an antihyperlipidemic effect with ethanol extract of *P. ostreatus*. When Tanaka et al. (*8*) used 0.1% and 1% cholesterol-supplemented diets containing oyster mushroom to feed male rats, they found that both lipid and nonlipid fractions of the diet had a hypolipidemic effect. Certain foodstuff components have been shown to have antihyperlipidemic effects. For example, the cholesterol-lowering effect of green tea has been found to be mainly caused by epigallocatechin gallate, one of the most abundant catechins in

green tea (*9*). The catechin binds with cholesterol in the lumen, thereby reducing the absorptive ability of cholesterol (*50*).

Higher antihyperlipidemic and antioxidant activities, both in vivo and in vitro, were found with EAE and MOE. Hepatic cells were less vacuolized in the MOE and EAE groups than in the other experimental groups (illustrations not shown). There may be an important relationship between the antihyperlipidemic effect and the antioxidant activities of *P. citrinopileatus*. Hunkar et al. (*51*) used cod liver oil to feed diabetic rats for 12 weeks. Hyperglycemia of the rats was partially controlled and accompanied by a significant increase in glutathion peroxidase activity. Antioxidants, which can inhibit LDL cholesterol oxidation, may also reduce early atherogenesis (*41*). Carotenoid antioxidants can modify cholesterol absorption and increase antioxidant status (*7*). Asai and Miyazawa (*52*) indicated that dietary curcuminoids displayed both antioxidant and hypocholesterol activities in their experiment of using dietary curcuminoid mixed with high-fat diet to feed Spraque-Dawley rats.

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