Hypolipidemic Activities of Dietary *Pleurotus ostreatus* in Hypercholesterolemic Rats

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This work was conducted to investigate dietary supplementation of oyster mushroom fruiting bodies on biochemical and histological changes in hyper and normocholesterolemic rats. Six-week old female Sprague-Dawley albino rats were divided into three groups of 10 rats each. Feeding a diet containing a 5% powder of *Pleurotus ostreatus* fruiting bodies to hyper-cholesterolemic rats reduced plasma total cholesterol, triglyceride, low-density lipoprotein (LDL), total lipid, phospholipids, and LDL/high-density lipoprotein ratio by 30.18, 52.75, 59.62, 34.15, 23.89, and 50%, respectively. Feeding oyster mushrooms also significantly reduced body weight in hypercholesterolemic rats. However, it had no adverse effects on plasma albumin, total bilirubin, direct bilirubin, creatinin, blood urea nitrogen, uric acid, glucose, total protein, calcium, sodium, potassium, chloride, inorganic phosphate, magnesium, or enzyme profiles. Feeding mushroom increased total lipid and cholesterol excretion in feces. The plasma lipoprotein fraction, separated by agarose gel electrophoresis, indicated that *P. ostreatus* significantly reduced plasma β and pre- β -lipoprotein but increased α -lipoprotein. A histological study of hepatic cells by conventional hematoxylin-eosin and oil red O staining revealed normal findings for mushroom-fed hypercholesterolemic rats. These results suggest that a 5% *P. ostreatus* diet supplement provided health benefits by acting on the atherogenic lipid profile in hypercholesterolemic rats.

KEYWORDS : Agarose gel electrophoresis, Atherogenic lipid profile, Histopathology, Hypercholesterolemic rats, Hypolipidemic, *Pleurotus ostreatus*

Pleurotus ostreatus, the oyster mushroom, is increasingly being recognized as an important food product with a significant role in human health and nutrition [1]. It is generally accepted that lowering high plasma cholesterol levels plays a significant role in preventing atherosclerosis. Oyster mushrooms are an ideal dietary substance for the prevention and treatment of hypercholesterolemia due to high content of dietary fiber, sterol, proteins, and microelements [2].

The fact that lovastatin is present in high proportions in this mushroom, is an important food supplement for patients suffering from hypercholesterolemia [3]. Besides lovastatin, *P. ostreatus* contains various biologically active phenolic compounds such as gallic acid, protocatechuic acid, chlorogenic acid, naringenin, hesperetin, and biochanin-A [4]. The general idea that controlling blood cholesterol is an important for reducing the risk of developing atherosclerosis [5] has stimulated the investigation and study of natural substances with hypocholesterolemic activity.

Considering the widely accepted concept about the key role of reactive oxygen species in the pathogenesis of atherosclerosis [6], reduced lipid peroxidation in blood is an additional positive effect of oyster mushrooms. Oyster mushrooms and other related mushrooms are used in traditional oriental medicine as components of natural diets with an antisclerotic effect [7].

There is considerable data supporting the hypothesis that the health benefit obtained through lowering blood cholesterol may be derived from the effects of eicosapentaenoic acid and docosahexaenoic acid [8]. In addition to their roles in the development and functioning of the central nervous system, these two fatty acids play an important role in the physiological functions of the cardiovascular system [9].

A hypolipidemic activity study is pertinent because the hypolipidemic activity of *P. ostreatus* is essential for its antiatherosclerotic function. Moreover, *P. ostreatus* has the potential to serve as an effective therapeutic agent for hyperlipidemic diseases, especially cardiovascular disease. Despite the medicinal importance of *P. ostreatus* and its therapeutic potential, no detailed studies on the biochemical and histological function of hypercholesterolemia have been performed, and comprehensive studies on the antihyperlepidemic effects of this mushroom are not available. Therefore we examined the potential hypolipidemic activity of *P. ostreatus* to generate awareness of its health benefit.

Materials and Methods

Mushroom. Fresh fruiting bodies of *P. ostreatus* (cultivar Chun-chu 2) were obtained from Hanultari mushroom farm, Korea. A pure culture was deposited in the

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Culture Collection and DNA Bank of Mushroom, Division of Life Sciences, University of Incheon, Korea with the acquired accession number, IUM-4143. Fresh fruiting bodies were dried with hot air at 40°C for 48 hr and pulverized.

Animals. Thirty female Sprague-Dawley albino rats (101 \pm 4.2 g, 6-week old, purchased from Central Lab. Animal Inc., Seoul, Korea) were used. All animals were acclimated to the animal room for 1-week. The rats were housed in an animal room at $23 \pm 2^{\circ}$ C under a 12 hr darklight cycle (17:00~5:00 hr) and relative humidity of 50~60%. Rats were divided into three feed groups: a basal diet (normocholesterolemic control rats; NC rats), basal diet with 1% cholesterol (hypercholesterolemic rats; HC rats), and a basal diet with 1% cholesterol and 5% *P. ostreatus* powder (oyster mushroom-fed hypercholesterolemic rats; HC + PO rats). The basal diet compositions are presented in Table 1, and the rats were feed for 42 days.

Plasma chemical analysis. At the end of the experimental period, overnight-fasted animals were sacrificed under injectable anesthetic (Zoletil 50; VIRBAC Laboratories, Carros, France). Blood samples were collected with a disposable plastic syringe into heparinized tubes. Plasma was prepared by centrifugation at 2,493 ×g for 10 min. Plasma triglyceride (TG) concentration was measured enzymatically using the glycerophosphate oxidase assay. Plasma total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), very low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C), total lipid (TL), and phospholipid (PL) levels were measured enzymatically by the cholesterol oxidase assay [10] using

Table 1. Basal diet composition

Ingredient	100 g/g
Wheat flour	50.00
Rice power	11.25
Wheat bran	19.00
Casein	8.00
Egg white	10.00
Soybean oil	1.00
Table salt	0.50
Vitamin mixture	0.125
Mineral mixture	0.125

The composition of the vitamin mixture in the diet was as follows (g/ 100~g vitamin mixture): retinyl acetate 9.5×10^{-4} , cholecalciferol 1.2×10^{-3} , α -tocopherol acetate 0.05, thiamine hydrochloride 2.4, nicotinic acid 12, riboflavin 2.4, D-calcium pantothenate 9.6, pyridoxine hydrochloride 1.2, folic acid 9.5×10^{-2} , vitamin K 0.25, cyanocobalamine 9.5×10^{-3} , inositol 47.95 and ascorbic acid 24.0. The composition of the mineral mixture added to diet was as follows (g/100 g of mineral): calcium gluconate 28.5, K_2HPO_4 17.3, $CaCO_3$ 26, $MgSO_4$ 12.6, KCl 12.6, $CuSO_4$ 0.06, $FeSO_4$ 0.3, $MnSO_4$ 0.55, NaF 2.5 $\times10^{-4}$, KI 9×10^{-4} , sodium molybdate 3×10^{-4} , SeO_2 3×10^{-4} , and $CrSO_2$ 1.5×10^{-3} .

commercially available assay kits (Sekisui Medical Co., Ltd., Tokyo, Japan). Plasma albumin, total bilirubin, direct bilirubin, creatinin, blood urea nitrogen, uric acid, glucose, total protein, and electrolyte parameters, including calcium, sodium, potassium, chloride, inorganic phosphate, and magnesium were measured by standard methods using an auto analyzer (Hitachi 7600-210; Hitachi, Tokyo, Japan).

Very low density lipoprotein cholesterol was calculated as follows:

$$VLDL-C = [TC - (HDL-C + LDL-C)]$$

Plasma enzyme analysis. The activity of the plasma transaminases, glutamate pyruvate transaminase (GPT), and glutamate oxaloacetate transaminase (GOT) were determined using the kinetic method [10]. The oxoacids formed in the transaminase reactions were measured indirectly by enzymatic reduction to their corresponding hydroxyacids. The accompanying change in NADH concentration was measured at 340 nm using a spectrophotometer (Optizen POP; Mecasys Co. Ltd., Daejeon, Korea). Plasma alkaline phosphatase (ALP) activity was determined using 4nitrophenyl phosphate. ALP catalyzes the hydrolysis of 4nitrophenyl phosphate, forming phosphate and free 4nitrophenol, which is colorless in dilute acid solutions. But, under alkaline conditions 4-nitrophenol is converted to the 4-nitrophenoxide ion, which is an intense yellow color. The absorbance of this color compound was measured spectrophotometrically at 420 nm to determine plasma ALP activity.

Fecal cholesterol and TL analysis. Feces were collected for 7 days before and at the end of 42 days, lyophilized, and then milled into powder. Total lipids were extracted with chloroform/methanol (2:1 v/v) according to the method of Folch *et al.* [11]. One gram of fecal powder was mixed with 10 mL of chloroform and 5 mL of methanol solution and stirred at 150 rpm for 3 days at room temperature. The suspension was filtered through Whatman No. 2 filter paper (Whatman, Maidstone, UK), the methanol was aspirated, and the chloroform was evaporated. The extracted lipids were then weighed. Two mL of H₂O was added, and a suspension was created using a bath sonicator. This suspension was used to estimate fecal cholesterol content, which was estimated by the enzymatic method using the cholesterol oxidase assay.

Plasma lipoprotein separation by agarose gel electrophoresis. Plasma lipoprotein fractions were determined by agarose gel electrophoresis [12]. Three lipoprotein fractions were detected by electrophoresis, which will henceforth be referred to as β-lipoprotein (LDL), pre-β-lipoprotein (VLDL), and α-lipoprotein (HDL). Sample application (2 μL), electrophoresis (80 V, 30 min), staining (Fat Red 7B), drying, and

densitometric scanning (525 nm) were performed automatically by the Helena TITAN GEL Lipoprotein Electrophoresis System (Helena Laboratories, Beaumont, TX, USA). After electrophoresis, lipoprotein fractions were visualized with enzymatic staining reagents. The visualized gel plate was scanned on a densitometer, and the lipoprotein scanning patterns were identified using analytical software (electrophoresis data bank, K.K. Helena Laboratories, Saitama, Japan). The scanned patterns were divided into lipoprotein fractions using the nadirs of the lipoprotein sequential curve. Lipoprotein levels were estimated from the area percentages and total concentrations.

Histological analysis of liver. Liver tissues were rapidly dissected, fixed in liquid nitrogen and 10% formalin solution, and stored until use at -80°C. A representative part of the frozen tissues was processed with a cryo microtome (Cryotome FSE Cryostat; Thermo Electron Corp., Cambridge, MA, USA) using sections 5-µm thick and stained with oil red-O [13]. A representative part of the formalin fixative liver tissues was processed for 4-µm thick paraffin embedded sections using a microtome (Microtome HM 450; Thermo Electron Corp.) and then stained with hematoxylin and eosin. Both stained tissue samples were then examined and photographed under a light microscope to assess the presence of lipid. Digital images were obtained using an Olympus BX51 microscope equipped with a Camedia C3040ZOOM digital camera (Olympus America Inc., Melville, NY, USA). All images were taken under 40× magnification.

Statistical analysis. Results are expressed as means \pm SDs. Intergroup differences were analyzed by a one-way analysis of variance followed by post-hoc tests. We used the SPSS ver. 11.5 (SPSS Inc., Chicago, IL, USA). A $p \le 0.05$ was considered statistically significant.

Results and Discussion

Effects of feeding oyster mushroom on bodyweight. Feeding *P. ostreatus* reduced body weight significantly in hypercholesterolemic and normocholesterolemic rats by 16.89 and 13.38%, respectively (Table 2). This finding is of special significance because obesity is associated with many diseases including diabetes, atherosclerosis, coronary heart disease, and others [14].

Effects of feeding oyster mushroom on plasma lipid profile. Plasma lipid profile concentrations in NC, HC, and HC + PO rats after *P. ostreatus* feeding for 6-wk are presented in Table 3. Plasma TC, TG, HDL-C, LDL-C, VLDL-C, TL, and PL in HC rats increased by 17.09, 36.68, 12.23, 22.35, 19.01, 19.82, and 16.14%, respectively, compared with levels in NC rats, whereas these parame-

Table 2. Effect of *Pleurotus ostreatus* on the body weight of hypercholesterolemic rats

Rat groups	Initial body weight (g)	Final body weight (g)	Weight gained (g)
NC	101 ± 5.3	243 ± 12.5	$142 \pm 9.1^{\scriptscriptstyle a,b}$
HC	101 ± 4.2	249 ± 11.9	$148\pm13.0^{^a}$
HC + PO	101 ± 3.8	224 ± 9.6	$123 \pm 10.4^{\text{b}}$

The results are mean \pm SDs. Data were analyzed by one-way and then subjected to the LSD post hoc test. Values with different superscripts are significantly different at $p \le 0.05$ in the fourth column. LSD, least significant difference; NC, normocholesterolemic control rats; HC, hypercholesterolemic rats; HC + PO, *Pleurotus ostreatus*-fed hypercholesterolemic rats.

Table 3. Effect of *Pleurotus ostreatus* on plasma lipid profiles in hypercholesterolemic rats

Parameters (mg/dL)	NC	НС	HC + PO
TC	$103.0 \pm 5.3^{\text{b}}$	$120.6 \pm 10.3^{\circ}$	$84.2 \pm 8.6^{\circ}$
TG	$63.8 \pm 11.3^{\text{b}}$	87.2 ± 12.8^{a}	$41.2 \pm 6.8^{\circ}$
HDL-C	$37.6 \pm 2.9^{a,b}$	$42.2\pm2.2^{\text{a}}$	$33.8 \pm 1.8^{\text{b}}$
LDL-C	$17.0 \pm 5.8^{a,b}$	$20.8 \pm 2.3^{\circ}$	$8.4 \pm 2.1^{\text{b}}$
VLDL-C	$48.4 \pm 6.3^{a,b}$	57.6 ± 7.8^{a}	$42.0 \pm 3.6^{\text{b}}$
TL	$328.0 \pm 9.8^{^{a,b}}$	$393.0 \pm 4.8^{\circ}$	$258.8\pm10.4^{\circ}$
PL	$158.6 \pm 9.8^{^{a,b}}$	$184.2 \pm 11.0^{\text{a}}$	$140.2 \pm 5.5^{\circ}$

The results are mean \pm SDs. Values in the same row that do not share a common superscript are significantly different at $p \le 0.05$ (one-way analysis of variance followed by an LSD post-hoc comparison). LSD, least significant difference; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; VLDL-C, very low-density lipoprotein cholesterol; TL, total lipid; PL, phospholipids.

ters decreased significantly by 30.18, 52.75, 19.91, 59.62, 27.08, 34.15, and 23.89%, respectively, in HC + PO rats compared with HC rats. The ratio of plasma LDL and HDL is shown in Fig. 1. In HC rats, this ratio increased by 11%, compared with NC rats, whereas this ratio was reduced significantly by 50% in HC + PO compared with HC rats. The results show that feeding 5% *P. ostreatus* to rats significantly ameliorated the plasma atherogenic lipid profiles in experimentally induced HC rats.

Rats are particularly resistant to the development of hypercholesterolemia and atherosclerosis [15] and have a strong ability to maintain their plasma cholesterol levels [16, 17]. Therefore, to induce hypercholesterolemia or atherosclerosis in rats, cholesterol feeding is used with other additives, including bile acids and propylthiouracil (an anti-thyroid drug), which increase intestinal absorption of cholesterol [18]. However, in the present study, the addition of 1% cholesterol to the basal diet without bile acids and/or anti-thyroid drugs produced hypercholesterolemia in the rats, because cholesterol feeding itself increases bile acid secretion by approximately three to four-fold in rats [19]. The 30.18% increase in plasma cholesterol in the

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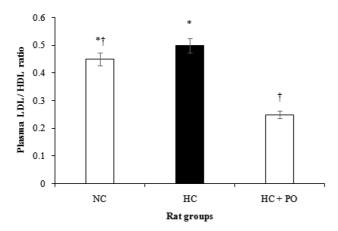


Fig. 1. Effects of *Pleurotus ostreatus* on plasma low density lipoprotein (LDL)/high density lipoprotein (HDL) ratio in hypercholesterolemic rats. Results are mean \pm SDs. Different symbols indicate significant differences at $p \le 0.05$. NC, normocholesterolemic control rats; HC, hypercholesterolemic rats; HC + PO, *P. ostreatus*-fed hypercholesterolemic rats.

HC rats in the present study was comparable with that reported by Bobek *et al.* [20], who feed rats cholesterol (0.3%) diet with added bile acids (0.5%) and showed a 1.7-fold higher cholesterolemia in their cholesterol-feed rats than normal rats. In this experiment, feeding 5% *P. ostreatus* to HC rats significantly repressed the increase in plasma cholesterol. The mechanism by which mushrooms reduce plasma lipoprotein levels in HC rats is not clearly understood. Mushrooms contain the hypocholesterolemic agent mevnolin (monacolin K, lovastatin) [21], which may be involved in decreasing the activity of the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase enzyme [20], the rate-limiting enzyme of cholesterol biosynthesis.

Thus, feeding mushrooms may involve suppression of endogenous cholesterol biosynthesis by inhibiting HMG-CoA reductase activity.

Effects of feeding ovster mushroom on plasma biochemical and electrolyte function. The results of the plasma biochemical and electrolytes concentrations indicated that albumin, uric acid, glucose, total protein, potassium, inorganic phosphate, and magnesium decreased significantly in HC rats by 20.59, 70.83, 27.92, 19.18, 37.33, 40.52, and 36.11%, respectively, compared with levels in oyster mushroom-fed rats. In contrast, no significant difference was found for plasma total bilirubin, direct bilirubin, creatinin, blood urea nitrogen, calcium, sodium, and chloride levels among the normocholesterolemic, hypercholesterolemic, and oyster mushroom-fed HC rats (Table 4). The glucose-lowering effect of propionate is associated with gluconeogenesis and the regulation of serum lipid levels [22]. Reduction in plasma potassium, sodium, and chloride concentrations is one of the mechanisms of action of antihypertensive drugs, particularly diuretics [23]. Diuretics act by diminishing sodium chloride reabsorption at different sites in the nephrons, thereby increasing urinary sodium chloride and water losses and consequently leading to decreased plasma levels of these electrolytes. Antonov et al. [24] reported that plasma electrolyte contents increased significantly in hypertensive rats. Impaired function of Na, K-ATPase and the Na-H antiport, which is typical of arterial hypertension, may promote an increase in plasma electrolytes.

Effects of feeding oyster mushroom on plasma enzyme profile. Lower plasma GOT, GPT, and ALP concentrations were observed in oyster mushroom-fed HC rats than

 Table 4. Effect of Pleurotus ostreatus on biochemical and electrolyte function in hypercholesterolemic rats

Parameters	NC	НС	HC + PO
Albumin (g/dL)	3.3 ± 0.2^{a}	$3.4\pm0.3^{\text{a}}$	$2.7 \pm 0.1^{\text{b}}$
Total bilirubin (mg/dL)	0.1 ± 0.0^{a}	$0.1\pm0.0^{\mathrm{a}}$	$0.1\pm0.0^{\rm a}$
Direct bilirubin (mg/dL)	$0.0\pm0.0^{\rm a}$	$0.0\pm0.1^{\rm a}$	$0.0\pm0.0^{\rm a}$
Creatinin (mg/dL)	$0.6\pm0.0^{\rm a}$	$0.7\pm0.1^{\circ}$	$0.5\pm0.1^{ ext{a}}$
Blood urea nitrogen (mg/dL)	$16.2 \pm 2.3^{\text{a}}$	17.4 ± 3.2^{a}	$16.6\pm1.8^{^{\mathrm{a}}}$
Uric acid (mg/dL)	$2.2 \pm 0.5^{\text{b}}$	4.8 ± 1.4^{a}	$1.4 \pm 0.2^{\circ}$
Glucose (mg/dL)	$106.0 \pm 4.7^{\text{a}}$	118.2 ± 10.7^{a}	$85.2 \pm 6.6^{\text{b}}$
Total protein (g/dL)	$7.2\pm0.2^{^{\mathrm{a}}}$	7.3 ± 0.4^{a}	$5.9 \pm 0.2^{\text{b}}$
Calcium (mg/dL)	$10.5 \pm 0.2^{\text{a}}$	$10.9\pm0.8^{\rm a}$	9.7 ± 0.2^{a}
Sodium (mEg/L)	$142.8\pm0.8^{^a}$	144.8 ± 2.3^{a}	$142.8\pm0.8^{\text{a}}$
Potassium (mEg/L)	$4.8\pm0.3^{^{\mathrm{a}}}$	$7.5 \pm 1.7^{\text{b}}$	$4.7\pm0.4^{\rm a}$
Chloride (mEg/L)	$102.4 \pm 1.5^{\text{a}}$	103.0 ± 1.9^{a}	102.8 ± 0.8^{a}
Inorganic phosphate (mg/dL)	$6.9\pm0.7^{\scriptscriptstyle \rm b}$	11.6 ± 1.6^{a}	$6.9\pm0.4^{\scriptscriptstyle \mathrm{b}}$
Magnesium (mg/dL)	$2.7\pm0.2^{a,b}$	$3.6\pm0.8^{^a}$	$2.3\pm0.2^{\scriptscriptstyle b}$

The results are mean \pm SDs. Values in the same row that do not share a common superscript are significantly different at $p \le 0.05$ (one-way analysis of variance followed by an LSD post-hoc comparison).

LSD, least significant difference; NC, normocholesterolemic control rats; HC, hypercholesterolemic rats; HC + PO, *Pleurotus ostreatus*-fed hypercholesterolemic rats.

Table 5. Effect of *Pleurotus ostreatus* on plasma enzyme profiles related to liver and kidney function in hypercholesterolemic rats

Parameters (U/L)	NC	НС	HC + PO
GOT	63.4 ± 9.1	70.8 ± 8.4	61.2 ± 6.4
GPT	$57.4 \pm 10.9^{a,b}$	$65.6\pm3.0^{\text{a}}$	$58.2 \pm 9.8^{\text{b}}$
ALP	164.8 ± 7.7	177.2 ± 9.4	161.4 ± 8.6

The results are mean \pm SDs. Values in the same row that do not share a common superscript are significantly different at $p \le 0.05$ (one-way analysis of variance followed by an LSD post-hoc comparison). GOT, glutamate oxaloacetate transaminase; GPT, glutamate pyruvate transaminase; ALP, alkaline phosphatase; LSD, least significant difference; NC, normocholesterolemic control rats; HC, hypercholesterolemic rats; HC + PO, *Pleurotus ostreatus*-fed hypercholesterolemic rats.

normocholesterolemic rats (Table 5). No significant difference was observed in the activities of plasma ALP in the NC, HC, or HC + PO rats groups. Plasma GOT and GPT activities were significantly higher in HC rats than in NC rats, whereas 5% HC + PO rats revealed significantly decreased plasma GOT and GPT activities by 13.60 and 11.28%, respectively.

Due to the increasing frequency of antihyperlipidemic drug use and their common side effects, there is a need to identify natural products with few or no side effects. Thus, development continues for highly effective natural ingredients from food, such as mushrooms, which decrease hyperlipidemia [3, 20]. Previous studies have shown that GOT and GPT are typically elevated following cellular damage as a result of enzyme leakage from the cells into blood [25]. Therefore, the increased enzyme activities resulting from oyster mushroom treatment may prevent oxidative damage by detoxifying reactive oxygen species; thus, reducing hyperlipidemia.

Effects of feeding oyster mushroom on fecal TL and cholesterol. The fecal TL and cholesterol of the 5% *P. ostreatus*-fed HC rats significantly increased by 2.7 and 3.2-fold, respectively, compared with NC rats (Table 6).

Table 6. Effects of *Pleurotus ostreatus* on fecal total lipid and cholesterol

Parameters (g/100 g feces)	NC	НС	HC + PO
Total lipid	24.6 ± 3.2^{a}	$55.5 \pm 4.5^{\text{b}}$	$66.3 \pm 5.6^{\circ}$
Cholesterol	$3.8\pm0.6^{\text{a}}$	$13.4\pm0.8^{\circ}$	$12.2 \pm 1.5^{\circ}$

The results are mean \pm SDs. Values in the same row that do not share a common superscript are significantly different at $p \le 0.05$ (one-way analysis of variance followed by an LSD post-hoc comparison). LSD, least significant difference; NC, normocholesterolemic control rats; HC, hypercholesterolemic rats; HC + PO, *Pleurotus ostreatus*-fed hypercholesterolemic rats.

Thus, the decreased plasma cholesterol may have attributed to such a mechanism. The higher level of plasma HDL-C indicates that more cholesterol from peripheral tissues was returning to the liver for catabolism and subsequent excretion. Plasma VLDL-C and TG contents in HC+PO rats were lower compared with hypercholesterolemic control rats. VLDL-C is the major transport vehicle for TG from the liver to extrahepatic tissues, whereas LDL-C is not secreted as such in the liver but seems to be formed from VLDL-C after partial removal of TG by lipoprotein lipase [26]. LDL-C became the prime carrier for cholesterol after feeding cholesterol to the rats, leading to decreased VLDL-C and HDL-C content in HC+PO rats.

Effects of feeding oyster mushroom on the plasma lipoprotein fraction by agarose gel electrophoresis. The α -lipoprotein band was the fast-moving fraction and was located nearest the anode. The β -lipoprotein band was usually the most prominent fraction and was near the origin, migrating only slightly anodic to the point of application. The pre- β lipoprotein band migrated between the α and β -lipoprotein (Fig. 2). The effects of feeding *P. ostreatus* on the plasma lipoprotein fraction are presented in Fig. 3. The results indicated no significant difference in the lipoprotein fractions between NC and HC+PO rats and HC rats. The results revealed that feeding 5% oyster mushrooms significantly reduced plasma β -lipoprotein and pre-

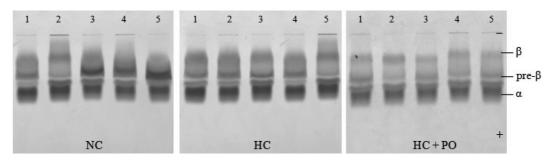


Fig. 2. Separation of plasma lipoproteins by agarose gel electrophoresis. Lanes 1~5 represent the plasma lipoprotein fraction of five different rats from each group. NC, normocholesterolemic control rats; HC, hypercholesterolemic rats; HC + PO, *Pleurotus ostreatus*-fed hypercholesterolemic rats. α, α-lipoprotein; β, β-lipoprotein; pre-β, pre-β-lipoprotein.

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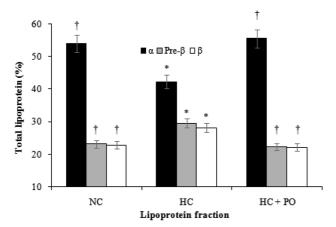


Fig. 3. Effects of *Pleurotus ostreatus* on the plasma lipoprotein fraction following agarose gel electrophoresis. Results are mean \pm SDs. Different symbols indicate significant differences at $p \le 0.05$. NC, normocholesterolemic control rats; HC, hypercholesterolemic rats; HC + PO, *Pleurotus ostreatus*-fed hypercholesterolemic rats; α, α-lipoprotein; β, β-lipoprotein; Pre-β, pre-β-lipoprotein.

β-lipoprotein but increased α-lipoprotein.

The hypocholesterolemic effect of oyster mushrooms is mediated by the interplay of a complex mixture of substances [27]. Water-soluble gel-forming components of the fiber substance (β -1,3-D-glucan with a low degree of polymerization, forming 15~20% of dry matter) interacts with bile acids and affects micelle formation. Such substances might be interfering with the absorption of cholesterol in this manner.

Effects of feeding oyster mushroom on rat liver histo- pathology. The effect of *P. ostreatus* on hepatocyte cells of HC rats is presented in Fig. 4. Liver tissues were

stained with hematoxylin-eosin and oil red O. The hepatic cords were typically arranged and located in liver tissue near the central vein in the NC, HC, and HC + PO groups. Lipid droplets were observed only in the liver tissue of HC rats. This could be attributed to lipid accumulation in the hepatocyte cell cytoplasm. Oxidized LDL induces the expression of scavenger receptors on the macrophage surface. These scavenger receptors promote the accumulation of modified lipoproteins, forming an early atheroma. The histological results indicated that the liver tissues of 5% HC + PO rats were almost similar to NC rats and that the hepatic biosynthesis of cholesterol was suppressed, which might be due to a reduction in the activity of HMG-CoA [28]. Hyperlipidemia is the leading risk factor for atherosclerosis, but the atherosclerotic pathological process could be slowed or reversed by reducing serum LDL, TGs, and PLs and increasing serum HDL. Several studies have demonstrated a protective effect of HDL in atherosclerosis and cardiovascular disease, whereas high levels of LDL constitute a risk factor. Excess LDL in the blood is deposited on the blood vessel walls and becomes a major component of atherosclerotic plaque lesions, whereas HDL facilitates translocation of cholesterol from peripheral tissues, such as arterial walls, to the liver for catabolism [29]. Bobek and Galbavý [7] observed that ovster mushrooms prevented the formation of atheromatous plaques and reduced the incidence and extent of atherosclerotic lesions in the aorta and coronary arteries as well as focal fibrosis in the myocardium of rabbits.

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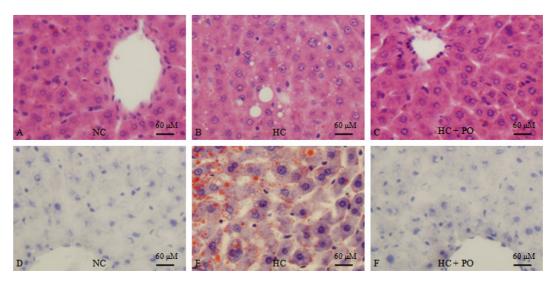


Fig. 4. Effects of feeding *Pleurotus ostreatus* on hepatocyte cells in hypercholesterolemic rats. A~C, hematoxylin-eosin stained photomicrographs (40×); D~F, photomicrographs of Oil red O stain (40×); NC, normocholesterolemic control rats; HC, hypercholesterolemic rats; HC + PO, *Pleurotus ostreatus*-fed hypercholesterolemic rats.

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