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# Antihyperlipidemic Effect of Dietary *Lentinus edodes* on Plasma, Feces and Hepatic Tissues in Hypercholesterolemic Rats

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**We investigated diet supplementation with shiitake mushroom fruiting bodies on biochemical and histological changes in hypercholesterolemic rats. Six-wk old female Sprague-Dawley albino rats were divided into three groups of 10 rats each. A diet containing 5% *Lentinus edodes* fruiting bodies given to hypercholesterolemic rats reduced plasma total cholesterol, triglyceride, low-density lipoprotein (LDL), total lipid, phospholipids, and the LDL/high-density lipoprotein ratio by 34.33, 53.21, 75.00, 34.66, 25.73, and 71.43%, respectively. Feeding mushroom also significantly reduced body weight in hypercholesterolemic rats. However, it had no detrimental effects on plasma albumin, total bilirubin, direct bilirubin, creatinine, 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 *L. edodes* 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 showed normal findings for mushroom-fed hypercholesterolemic rats. These results suggest that shiitake mushrooms could be recommended as a natural cholesterol lowering substance in the diet.**

**KEYWORDS : Agarose gel electrophoresis, Atherogenic lipid profile, Histopathology, Hypercholesterolemic rats, *Lentinus edodes***

*Lentinus edodes*, widely known as the shiitake mushroom, has an established history of use in time-honored oriental therapies. Modern clinical practice in Korea, Japan, China, and other Asian countries continues to rely on mushroom-derived preparations. These practices still form the basis of modern scientific studies of mushroom medicinal activities [1]. Eritadenine is an adenosine analogue alkaloid and lentinacin is a purine alkaloid that reduces cholesterol levels in rats by 25% after 7 days of oral administration at doses as low as 0.005% of feed intake [2]. The main cause of the hypocholesterolemic action of eritadenine seems to be associated with a modification in hepatic phospholipid (PL) metabolism by inducing phosphatylethanolamine N-methyltransferase deficiency [3]. Dietary eritadenine also alters the fatty acid and molecular profile of the liver and plasma by suppressing the metabolic conversion of linoleic acid to arachidonic acid and by decreasing the Δ6-desaturase activity, which can be affected through transcriptional regulation.

The microsomal enzyme 3-hydroxy-3-methylglutarylcoenzymeA (HMG-CoA) reductase is the major rate-limiting enzyme in cholesterol biosynthesis, which converts HMG-CoA to mevalonate. Therefore, inhibiting HMG-CoA reductase decreases intracellular cholesterol biosynthesis [4]. Mevinolin is a known pharmacological HMG-CoA reductase inhibitor. A high quantity of this inhibitor has been found in mushroom fruiting bodies, particularly in the pileus and sporocarps of *Pleurotus ostreatus*. Mushrooms also contain various biologically active compounds such as gallic acid, protocatechuic acid, chlorogenic acid, naringenin, hesperetin, and biochanin-A [5, 6].

The fruiting bodies of *L. edodes* lessen blood platelet effectiveness during coagulation and consequently those who bleed easily or who take anticoagulants should be cautious when chronically consuming *L. edodes* extracts [7]. Nevertheless, the exact mechanisms of action remain to be understood before considering a human treatment to prevent or cure cardiovascular diseases. Various studies have shown that shiitake mushrooms lower both blood pressure and free cholesterol in plasma and accelerate lipid accumulation in the liver by removing them from circulation [8]. The present study was designed to evalu-

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ate the dietary effectiveness of *L. edodes* on plasma, feces, and hepatic tissue function in hypercholesterolemic rats.

## Materials and Methods

**Mushroom.** Fresh fruiting bodies of *L. edodes* were purchased from E-mart at Incheon in Korea. A pure culture was deposited in the Culture Collection and DNA Bank of Mushroom (CCDBM), Division of Life Sciences, University of Incheon, Korea and acquired accession number, IUM-4681. Fresh fruiting bodies were dried with hot air at 40°C for 48 hr and pulverized.

**Animals.** Thirty female Sprague-Dawley albino rats (101 ± 4.2 g, 6 wk old, purchased from Central Lab. Animal Inc., Seoul, Korea) were used. All animals were acclimated to the animal room for 1 wk. The rats were housed in an animal room at 23 ± 2°C under a 12 hr dark-light cycle (17:00~05:00) and a relative humidity of 50~60%. Rats were divided into three feed groups: basal diet (normocholesterolemic control rats; NC), basal diet with 1% cholesterol (hypercholesterolemic rats; HC), and basal diet with 1% cholesterol and 5% *L. edodes* powder (mushroom-fed hypercholesterolemic rats; HC + LE). The basal diet compositions are presented in Table 1, and the rats were fed 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 obtained 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), low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C), total lipid (TL), and PL levels were measured enzymatically by the cholesterol oxidase assay [9] using commercially available assay kits (Sekisui Medical Co., Ltd., Tokyo, Japan). Plasma albumin, total bilirubin, direct bilirubin, creatinine, blood urea nitrogen, uric acid, glucose, total protein, and electrolytes, including calcium, sodium, potassium, chloride, inorganic phosphate, and magnesium were measured by standard methods using an auto analyzer (Hitachi 7600-210; Hitachi, Tokyo, Japan).
VLDL-C was calculated as follows:

$$\text{VLDL-C} = [\text{TC} - (\text{HDL-C} + \text{LDL-C})]$$

**Plasma enzyme analysis.** Plasma transaminases, glutamate pyruvate transaminase (GPT), and glutamate oxaloacetate transaminase (GOT) activities were determined using the kinetic method [9]. Plasma alkaline phosphatase (ALP) activity was determined using 4-nitrophenyl phosphate. ALP catalyzes the hydrolysis of 4-nitrophenyl phosphate, forming phosphate and free 4-nitrophenol, 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 TL and TC analysis.** Feces were collected for 7 days before and at the end of 42 days, lyophilized, and then milled into powder. TLs were extracted with chloroform/methanol (2 : 1, v/v), according to the method of Folch *et al*. [10]. 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 $H_2O$ was added, and a suspension was created using a bath sonicator. This suspension was used to estimate fecal cholesterol content by an enzymatic method using the cholesterol oxidase assay.

**Plasma lipoprotein separation.** Plasma lipoprotein fractions were determined by agarose gel electrophoresis [11]. 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

**Table 1.** Basal diet composition

| Ingredient | g/100 g |
|---|---|
| Wheat flour | 50.00 |
| Rice powder | 11.25 |
| Wheat bran | 19.00 |
| Casein | 08.00 |
| Egg white | 10.00 |
| Soybean oil | 01.00 |
| Table salt | 00.50 |
| 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 $9.5 \times 10^{-4}$, cholecalciferol $1.2 \times 10^{-3}$, 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 $9.5 \times 10^{-2}$, vitamin K 0.25, cyanocobalamine $9.5 \times 10^{-3}$, inositol 47.95, and ascorbic acid 24.0. The composition of the mineral mixture added to the 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 $2.5 \times 10^{-4}$, KI $9 \times 10^{-4}$, sodium molybdate $3 \times 10^{-4}$, $SeO_2$ $3 \times 10^{-4}$, and $CrSO_2$ $1.5 \times 10^{-3}$.

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 5-μm thick section and stained with oil red-O [12]. A representative part of the formalin fixed liver tissues was processed for 4-μm thick paraffin embedded sections using a microtome (Microtome HM 450; Thermo Electron Crop.) 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 ± SDs. Intergroup differences were analyzed by a one-way analysis of variance followed by post-hoc tests. We used SPSS ver. 11.5 (SPSS Inc., Chicago, IL, USA). A $p \leq 0.05$ was considered statistically significant.

## Results and Discussion

**Effects of *L. edodes* on body weight.** Feeding of *L. edodes* reduced body weight significantly in hypercholesterolemic and normocholesterolemic rats by 13.51% and 9.86%, respectively (Table 2), showing that shiitake mushrooms reduced body weight in both hyper and normocholesterolemic rats. This finding is of special significance because obesity is associated with numerous diseases including diabetes, atherosclerosis, coronary heart disease, and others [13].

**Table 2.** Effect of *Lentinus edodes* 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 ± 9.1$^{a,b}$ |
| HC | 101 ± 4.2 | 249 ± 11.9 | 148 ± 13.0$^{a}$ |
| HC + LE | 101 ± 3.8 | 229 ± 13.9 | 128 ± 14.4$^{b}$ |

Results are means ± SD. Data were analyzed by one-way analysis of variance and then subjected to the LSD post hoc test. Values in the fourth column with different superscripts are significantly different at $p \leq 0.05$.
LSD, least significantly different; NC, normocholesterolemic control rats; HC, hypercholesterolemic rats; HC + LE, *L. edodes*-fed hypercholesterolemic rats.

**Effects of *L. edodes* on plasma lipid profiles.** Plasma lipid profile concentrations in NC, HC, and HC + LE rats after feeding *L. edodes* 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,

**Table 3.** Effect of *Lentinus edodes* on plasma lipid profiles in hypercholesterolemic rats

| Parameters (mg/dL) | NC | HC | HC + LE |
|---|---|---|---|
| TC | 103.0 ± 5.3$^{a}$ | 120.6 ± 10.3$^{b}$ | 79.2 ± 9.2$^{c}$ |
| TG | 63.8 ± 11.3$^{a}$ | 87.2 ± 12.8$^{b}$ | 40.8 ± 6.6$^{c}$ |
| HDL-C | 37.6 ± 2.9 | 42.2 ± 2.2 | 37.8 ± 4.8 |
| LDL-C | 17.0 ± 5.8$^{a}$ | 20.8 ± 2.3$^{a}$ | 5.2 ± 0.4$^{b}$ |
| VLDL-C | 48.4 ± 6.3 | 57.6 ± 7.8 | 36.2 ± 8.5 |
| TL | 328.0 ± 9.8$^{a}$ | 393.0 ± 4.8$^{b}$ | 256.8 ± 12.7$^{c}$ |
| PL | 158.6 ± 9.8$^{a}$ | 184.2 ± 11.0$^{b}$ | 136.8 ± 6.3$^{c}$ |

Results are means ± SD. Values in the same row that do not share a common superscript are significantly different at $p \leq 0.05$ (one-way analysis of variance followed by an LSD post-hoc comparison).
LSD, least significantly different; NC, normocholesterolemic control rats; HC, hypercholesterolemic rats; HC + LE, *L. edodes*-fed hypercholesterolemic rats; 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.

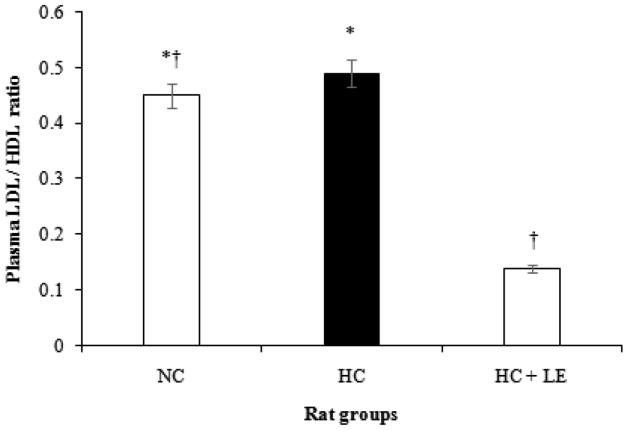


**Fig. 1.** Effects of *Lentinus edodes* on the plasma low density lipoprotein (LDL)/high density lipoprotein (HDL) ratio in hypercholesterolemic rats. Results are means ± SD. Different symbols indicate significant differences at $p \leq 0.05$. NC, normocholesterolemic control rats; HC, hypercholesterolemic rats; HC + LE, *L. edodes*-fed hypercholesterolemic rats.

19.82, and 16.14%, respectively compared with levels in NC rats, whereas these parameters decreased significantly by 34.33, 53.21, 10.43, 75.00, 37.15, 34.66, and 25.73%, respectively in HC + LE rats compared with those in HC rats. The ratio of plasma LDL and HDL is shown in Fig. 1. In HC rats, this ratio increased by 8.89%, compared with that in NC rats, whereas this ratio was reduced significantly by 71.43% in HC + LE compared with that in HC rats. The results show that feeding 5% *L. edodes* to rats significantly ameliorated the plasma atherogenic lipid profiles in experimentally induced hypercholesterolemic rats.

Rats are particularly resistant to developing hypercholesterolemia and atherosclerosis [14] and have a strong ability to maintain their plasma cholesterol levels [15, 16]. Therefore, to induce hypercholesterolemia or atherosclerosis in rats, cholesterol feeding is used with other additives, including bile acids and propylthiouracil (an antithyroid drug), which increase intestinal absorption of cholesterol [17]. However, in the present study, adding 1% cholesterol to the basal diet without bile acids and/or antithyroid drugs produced hypercholesterolemia in the rats, because cholesterol feeding itself increases bile acid secretion by approximately three to four-fold in rats [18]. The 34.33% increase in plasma cholesterol in the hypercholesterolemic rats in the present study was comparable with that reported by Bobek *et al*. [19], who fed rats cholesterol (0.3%) diet with added bile acids (0.5%) and showed a 1.7-fold higher cholesterolemia in cholesterol-fed rats than that in normal rats. In this experiment, feeding 5% *L. edodes* to hypercholesterolemic rats significantly repressed the increase in plasma cholesterol. The mechanism by which mushrooms reduce plasma lipoprotein levels in hypercholesterolemic rats is not clearly understood. Mushrooms contain the hypocholesterolemic agent mevinolin [20], which may be involved in decreasing HMG-CoA activity [19], the rate-limiting enzyme of cholesterol biosynthesis. Thus, feeding mushrooms may involve suppressing endogenous cholesterol biosynthesis by inhibiting HMG-CoA reductase activity.

**Effects of *L. edodes* on plasma biochemical and electrolyte function.** The results of the plasma biochemical and electrolyte concentrations indicated that creatinine, uric acid, glucose, total protein, potassium, inorganic phosphate, and magnesium decreased significantly in hypercholesterolemic rats by 28.57, 58.33, 18.36, 12.33, 30.66, 37.93, and 30.55%, respectively compared with levels in mushroom-fed rats. Conversely, no significant difference was found for plasma albumin, total bilirubin, direct bilirubin, blood urea nitrogen, calcium, sodium, or chloride levels among the normocholesterolemic, hypercholesterolemic, and mushroom-fed hypercholesterolemic rats (Table 4).

The glucose-lowering effect of propionate is associated with gluconeogenesis and the regulation of serum lipid levels [21]. A reduction in plasma potassium, sodium, and chloride concentration is one of the mechanisms of action of antihypertensive drugs, particularly diuretics [22]. Many 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*. [23] reported that plasma electrolyte contents increase significantly in hypertensive rats. Impaired function of Na, K-ATPase, Na-H antiport, which is typical of arterial hypertension, may promote an increase in plasma electrolytes.

**Table 4.** Effect of *Lentinus edodes* on biochemical and electrolyte function in hypercholesterolemic rats

| Parameters | NC | HC | HC + LE |
|---|---|---|---|
| Albumin (g/dL) | 3.3 ± 0.2 | 3.4 ± 0.3 | 2.9 ± 0.2 |
| Total bilirubin (mg/dL) | 0.1 ± 0.0 | 0.1 ± 0.0 | 0.1 ± 0.0 |
| Direct bilirubin (mg/dL) | 0.0 ± 0.0 | 0.0 ± 0.1 | 0.0 ± 0.0 |
| Creatinine (mg/dL) | 0.6 ± 0.0$^{a,b}$ | 0.7 ± 0.1$^{a}$ | 0.5 ± 0.0$^{b}$ |
| Blood urea nitrogen (mg/dL) | 16.2 ± 2.3 | 17.4 ± 3.2 | 15.0 ± 1.9 |
| Uric acid (mg/dL) | 2.2 ± 0.5$^{a}$ | 4.8 ± 1.4$^{b}$ | 2.0 ± 0.6$^{a}$ |
| Glucose (mg/dL) | 106.0 ± 4.7$^{a}$ | 118.2 ± 10.7$^{a}$ | 151.0 ± 5.9$^{b}$ |
| Total protein (g/dL) | 7.2 ± 0.2$^{a}$ | 7.3 ± 0.4$^{a}$ | 6.4 ± 0.3$^{b}$ |
| Calcium (mg/dL) | 10.5 ± 0.2 | 10.9 ± 0.8 | 10.1 ± 0.1 |
| Sodium (mEq/L) | 142.8 ± 0.8 | 144.8 ± 2.3 | 141.6 ± 2.2 |
| Potassium (mEq/L) | 4.8 ± 0.3$^{a}$ | 7.5 ± 1.7$^{b}$ | 5.2 ± 0.5$^{a,b}$ |
| Chloride (mEq/L) | 102.4 ± 1.5 | 103.0 ± 1.9 | 102.0 ± 0.7 |
| Inorganic phosphate (mg/dL) | 6.9 ± 0.7$^{a}$ | 11.6 ± 1.6$^{b}$ | 7.2 ± 0.7$^{a}$ |
| Magnesium (mg/dL) | 2.7 ± 0.2$^{a,b}$ | 3.6 ± 0.8$^{a}$ | 2.5 ± 0.1$^{b}$ |

Results are means ± SD. Values in the same row that do not share a common superscript are significantly different at $p \leq 0.05$ (one-way analysis of variance followed by an LSD post-hoc comparison).

LSD, least significantly different; NC, normocholesterolemic control rats; HC, hypercholesterolemic rats; HC + LE, *L. edodes*-fed hypercholesterolemic rats.

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

| Parameters (U/L) | NC | HC | HC + LE |
|---|---|---|---|
| GOT | 63.4 ± 9.1 | 70.8 ± 8.4 | 66.4 ± 3.5 |
| GPT | 57.4 ± 10.9[a,b] | 65.6 ± 3.0[a] | 51.4 ± 6.5[b] |
| ALP | 164.8 ± 7.7 | 177.2 ± 9.4 | 165.0 ± 7.8 |

Results are means ± SD. Values in the same row that do not share a common superscript are significantly different at $p \leq 0.05$ (one-way analysis of variance followed by an LSD post-hoc comparison).
LSD, least significantly different; NC, normocholesterolemic control rats; HC, hypercholesterolemic rats; HC + LE, *L. edodes*-fed hypercholesterolemic rats; GOT, glutamate oxaloacetate transaminase; GPT, glutamate pyruvate transaminase; ALP, alkaline phosphatase.

**Effects of *L. edodes* on plasma enzyme profiles.** Lower plasma GOT, GPT, and ALP concentrations were observed in shiitake mushroom-fed hypercholesterolemic rats than those in normocholesterolemic rats (Table 5). No significant difference was observed in the activities of plasma GOT and ALP in the NC, HC, or HC + LE rats groups, whereas plasma GPT activity was significantly higher in HC than that in NC rats. Nevertheless, 5% shiitake mushroom-fed hypercholesterolemic rats revealed decreased plasma GOT, GPT, and ALP activity by 6.21, 21.64, and 6.84%, 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, investigation continues to identify highly effective natural ingredients from foods, such as mushrooms, which can decrease hyperlipidemia [19, 24]. 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 mushroom treatment may prevent oxidative damage by detoxifying reactive oxygen species; thus, reducing hyperlipidemia.

**Effects of *L. edodes* on fecal TLs and cholesterol.** Fecal TLs and cholesterol of the 5% *L. edodes*-fed hypercholesterolemic rats increased significantly by 2.5 and 3.8-fold, respectively, compared with those in NC rats (Table 6). Thus, decreased plasma cholesterol may have attributed to such a mechanism.

**Table 6.** Effects of *Lentinus edodes* on fecal total lipids and cholesterol

| Parameters (g/100 g feces) | NC | HC | HC + LE |
|---|---|---|---|
| Total lipid | 24.6 ± 3.2[a] | 55.5 ± 4.5[b] | 60.7 ± 4.2[b,c] |
| Cholesterol | 3.8 ± 0.6[a] | 13.4 ± 0.8[c] | 14.5 ± 1.8[c] |

Results are means ± SD. Values in the same row that do not share a common superscript are significantly different at $p \leq 0.05$ (one-way analysis of variance followed by an LSD post-hoc comparison).
LSD, least significantly different; NC, normocholesterolemic control rats; HC, hypercholesterolemic rats; HC + LE, *L. edodes*-fed hypercholesterolemic rats.

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 + LE rats were lower compared with those in hypercholesterolemic 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 + LE rats.

**Effects of *L. edodes* 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 toward the anode from the point of application. The pre-β lipoprotein band migrated between α- and β-lipoprotein (Fig. 2). The effects of feeding *L. edodes* on the plasma lipoprotein fraction are presented in Fig. 3. The results indicated no significant difference in the lipoprotein fractions between normocholesterolemic and mushroom-fed hypercholesterolemic rats compared to those in hypercholesterolemic rats. The results revealed that feeding 5% shiitake mushrooms significantly reduced plasma β and pre-β lipoprotein, whereas it increased α-lipoprotein.

The hypocholesterolemic effect of 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 and forming 15~20% of dry matter interact with bile acids and affect micelle formation. Such substances might be interfering with the absorption of cholesterol in this manner.

**Effects of *L. edodes* on rat liver histopathology.** The effect of *L. edodes* 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 + LE groups. Lipid droplets were observed only in the liver tissue of HC rats, which was 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

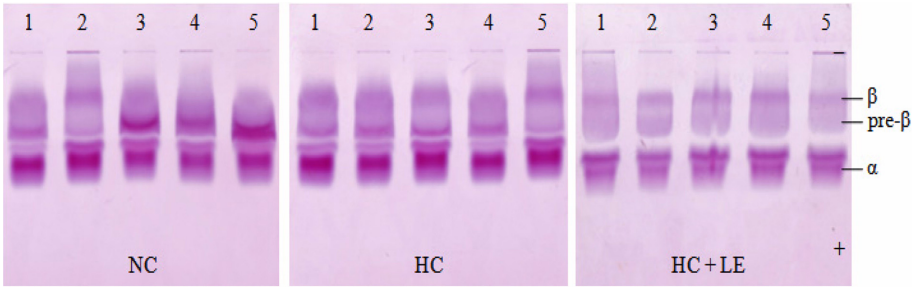


**Fig. 2.** Separation of plasma lipoproteins by agarose gel electrophoresis. Lanes 1~5 represent the plasma lipoprotein fraction of five different rats from each group. NC, normocholesterolemic control rats; HC, hypercholesterolemic rats; HC + LE, *L. edodes*-fed hypercholesterolemic rats; β, β-lipoprotein;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

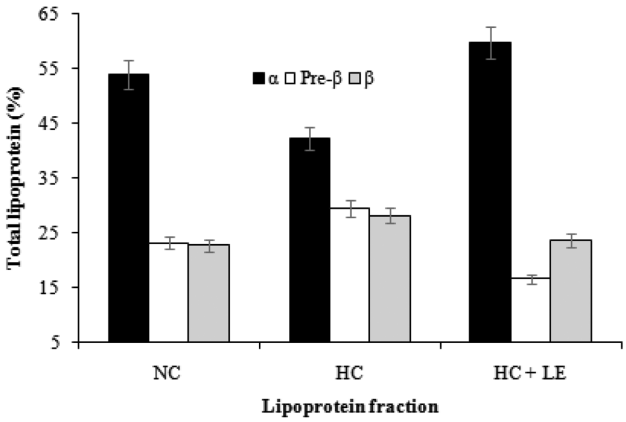


**Fig. 3.** Effects of *Lentinus edodes* on the plasma lipoprotein fraction following agarose gel electrophoresis. Results are means ± SD. NC, normocholesterolemic control rats; HC, hypercholesterolemic rats; HC + LE, *L. edodes*-fed hypercholesterolemic rats; α, α-lipoprotein; pre-β, pre-β-lipoprotein; β, β-lipoprotein.

indicated that the liver tissues of the 5% HC + LE rats were almost similar to those of NC rats and that the hepatic biosynthesis of cholesterol was suppressed, which may have been due to a reduction in HMG-CoA activity [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 [30] observed that oyster mushrooms prevent the formation of atheromatous plaques and reduce the incidence and extent of atherosclerotic

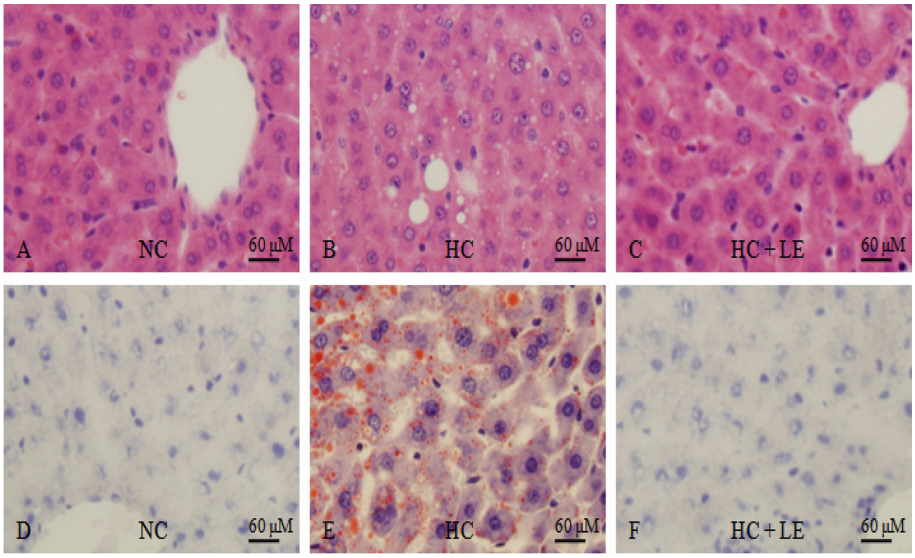


**Fig. 4.** Effects of feeding *Lentinus edodes* on hepatocyte cells in hypercholesterolemic rats. A~C, Hematoxylin-eosin stained photomicrographs (×40); D~F, photomicrographs of oil red-O stain (×40); NC, normocholesterolemic control rats; HC, hypercholesterolemic rats; HC + LE, *L. edodes*-fed hypercholesterolemic rats.

lesions in the aorta and coronary arteries as well as focal fibrosis in the myocardium of rabbits.

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