Appraisal of Antihyperlipidemic Activities of *Lentinus lepideus* in Hypercholesterolemic Rats

Ki Nam Yoon¹, Jae Seong Lee¹, Hye Young Kim¹, Kyung Rim Lee¹, Pyung Gyun Shin², Jong Chun Cheong², Young Bok Yoo¹, Nuhu Alam³, Tai Moon Ha⁴ and Tae Soo Lee¹*¹

¹Division of Life Sciences, University of Incheon, Incheon 406-840, Korea
²Mushroom Division, National Institute of Horticultural and Herbal Science, Rural Development Administration, Suwon 441-707, Korea
³Department of Botany, Jahangirnagar University, Savar, Dhaka 1342, Bangladesh
⁴Mushroom Research Institute, Geonggi Agricultural Research and Extension Service, Gwangju 464-720, Korea

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The wild edible mushroom, *Lentinus lepideus* has recently been cultivated for commercial use in Korea. While the mushroom has been widely used for nutritional and medicinal purposes, the possible anti-hyperlipidemic action is unclear. The effects of dietary *L. lepideus* on plasma and feces biochemical and on the liver histological status were investigated in hypercholesterolemic rats. Six-wk-old female Sprague-Dawley albino rats were divided into three groups of 10 rats each. Biochemical and histological examinations were performed. A diet containing 5% *L. lepideus* fruiting bodies reduced plasma total cholesterol, triglyceride, low-density lipoprotein, total lipid, phospholipids, and the ratio of low-density to high-density lipoprotein. Body weight was reduced. The diet did not adversely affect plasma biochemical and enzyme profiles. *L. lepideus* reduced significantly plasma β- and pre-β-lipoprotein, while α-lipoprotein content was increased. A histological study of hepatic cells by conventional hematoxylin-eosin and oil red O staining revealed normal findings for mushroom-fed hypercholesterolemic rats. The present study suggests that a diet supplemented with *L. lepideus* can provide health benefits by acting on the atherogenic lipid profile in hypercholesterolemic rats.

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

Introduction

*Lentinus lepideus* belongs to the family Tricholomataceae and order Agaricales [1]. It is one of the most popular edible wild mushrooms in China and Japan. Recently, this mushroom was successfully cultivated and is now commercially available in Korea. Traditionally, mushrooms have been used for foods and medicines in Asia. Generally, mushrooms are rich in dietary fiber, minerals, vitamins, but low in fat and calories [2]. Mushrooms also contain various polyphenolic and flavonoid compounds, which are recognized as good antioxidants [3]. Moreover, several important compounds including polysaccharides (β-glucan), ergosterol, vitamins, α-tocopherol, β-carotene, and lovastatin have been isolated from mushrooms [4]. Recent studies have shown that medicinal properties of mushrooms include inhibition of tumors, inhibition of microbial growth, improvement of liver function, improvement of blood pressure, and reduction of harmful cholesterol levels [5, 6].

*Lentinus* spp. are effective for reducing blood pressure and free cholesterol in plasma, as well accelerating the accumulation of lipids in the liver [7, 8]. High dosages of eritadenine may impair the secretion of very low-density lipoprotein cholesterol (VLDL-C) and, in a similar manner to soybean protein, eritadenine lowers cholesterol by decreasing the ratio of phosphatidylcholine to phosphatidylethanolamine in liver microsomes [9].

Despite the clinical importance and therapeutic potential of *L. lepideus*, there is no information concerning anti-hyperlipidemic properties. This study was undertaken to assess the acute dietary effect of *L. lepideus* on the plasma, feces, and hepatic histology in hypercholesterolemic (HC) rats.

Materials and Methods

This study was carried out from February 2010 to January 2011 at the Animal House and Laboratory of Applied Microbiology, Division of Life Sciences. Experimental
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), VLDL-C, total lipid (TL), and phospholipid (PL) levels were measured enzymatically by the cholesterol oxidase assay [10] using commercially available assay kits (Sekisui Medical, Tokyo, Japan). Plasma albumin, total bilirubin, direct bilirubin, creatinin, blood urea nitrogen, uric acid, glucose, and total protein, and electrolyte parameters including calcium, sodium, potassium, chloride, inorganic phosphate, and magnesium were measured by standard methods using a model 7600-210 auto analyzer (Hitachi, Tokyo, Japan).

VLDL-C was calculated as TC – (HDL-C + LDL-C).

Plasma enzyme analyses. The activity of the plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined using the kinetic method [10]. 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 has 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 analyses. 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) as described previously [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 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 using the 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 formalin fixative liver tissues was processed for 4-μm thick paraffin embedded sections using a HM 450 microtome (Thermo Electron) and then stained with hematoxylin and eosin. Both stained tissue samples were then examined and photographed under light microscope magnification to assess the presence of lipid. Digital images were obtained using an Olympus BX51 microscope equipped with a Camedia C3040ZOOM digital camera (Olympus America, Melville, NY, USA). All images were taken at ×40 magnification.

**Statistical analyses.** Data are expressed as mean ± SD. Intergroup differences were analyzed by a one-way analysis of variance followed by Duncan’s new multiple-range test. The SPSS ver. 11.5 (SPSS Inc., Chicago, IL, USA) was used for the analysis. A p ≤ 0.05 was considered statistically significant.

**Results**

**Effect of mushroom feeding on body weight.** There was no significant difference in body weight after 6 wk among the NC, HC, and HC + LL rats (243 ± 12.5, 249 ± 11.9, and 236 ± 12.9, respectively). Feeding of *L. lepideus* reduced body weight in hyper- and normocholesterolemic rats by 8.78% and 4.93%, respectively.

**Effect of mushroom feeding on plasma lipid profile.** Plasma lipid profile concentrations in NC, HC, and HC + LL rats after *L. lepideus* feeding for 6 wk are presented in Table 1. 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 parameters decreased

![Fig. 1. Effects of dietary *Lentinus lepideus* mushroom on plasma low-density lipoprotein/high-density lipoprotein (LDL/HDL) ratio in hypercholesterolemic rats. Values represent mean ± SD (n = 10). Different symbols indicate significant differences at p ≤ 0.05. NC, normocholesterolemic control; HC, hypercholesterolemic; HC + LL, mushroom-fed hypercholesterolemic.](image)

**Table 1.** Effects of dietary *Lentinus lepideus* mushroom on plasma lipid and enzyme profiles and fecal TL and cholesterol in HC rats

| Parameter | NC (n = 10) | HC (n = 10) | HC + LL (n = 10) |
|-----------|------------|------------|-----------------|
| **Plasma parameters** | | | |
| TC (mg/dL) | 103.0 ± 5.3a | 120.6 ± 10.3b | 81.8 ± 6.1a |
| TG (mg/dL) | 63.8 ± 11.3a | 87.2 ± 12.8a | 33.2 ± 5.4a |
| HDL-C (mg/dL) | 37.6 ± 2.9a | 42.2 ± 2.2a | 29.2 ± 2.9a |
| LDL-C (mg/dL) | 17.0 ± 5.8a | 20.8 ± 2.3a | 5.0 ± 0.7a |
| VLDL-C (mg/dL) | 48.4 ± 6.3 | 57.6 ± 7.8 | 47.6 ± 3.6 |
| TL (mg/dL) | 328.0 ± 9.8a | 393.0 ± 4.8b | 248.4 ± 5.7 |
| PL (mg/dL) | 158.6 ± 9.8a | 184.2 ± 11.0b | 132.3 ± 6.5a |
| AST (U/L) | 63.4 ± 9.1 | 70.8 ± 8.4 | 59.4 ± 6.1 |
| ALT (U/L) | 57.4 ± 10.9 | 65.6 ± 3.0 | 57.8 ± 8.4 |
| ALP (U/L) | 164.8 ± 7.7 | 177.2 ± 9.4 | 164.6 ± 5.4 |
| **Fecal parameters** | | | |
| Total lipid (U/L) | 24.6 ± 3.2a | 55.5 ± 4.5b | 62.3 ± 5.8c |
| Cholesterol (U/L) | 3.8 ± 0.6a | 13.4 ± 0.8b | 15.8 ± 1.4c |

Values represent mean ± SD. Values in the same row that do not share a common superscript are significantly different at p ≤ 0.05. TL, total lipid; HC, hypercholesterolemic; NC, normocholesterolemic control; HC + LL, mushroom-fed hypercholesterolemic; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; VLDL-C, very low-density lipoprotein cholesterol; PL, phospholipid; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase.
significantly by 32.17, 61.93, 30.81, 75.96, 17.36, 36.79, and 27.69%, respectively, in HC + LL rats compared with 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 NC rats, whereas this ratio was reduced significantly by 65.31% in HC + LL rats compared with HC rats. The results show that feeding of 5% *L. lepideus* to rats significantly ameliorated the plasma atherogenic lipid profiles in experimentally induced HC rats.

**Effect of mushroom feeding on plasma enzyme profile.** Lower plasma AST, ALT, and ALP concentrations were observed in HC + LL rats than normocholesterolemic rats, and no significant difference was observed in the activities of plasma enzyme profiles in the NC, HC, or HC + LL rats (Table 1). Five percent HC + LL rats displayed decreased plasma AST, ALT, and ALP activity (16.10, 11.89, and 7.11%, respectively).

**Effect of mushroom feeding on fecal TL and cholesterol.** The fecal TL and cholesterol of the 5% *L. lepideus*-fed HC rats significantly increased by 2.5 and 4.2-fold, respectively, compared with NC rats (Table 1).

**Effect of mushroom feeding on plasma biochemical and electrolyte function.** Blood urea nitrogen, uric acid, glucose, total protein, potassium, inorganic phosphate, and magnesium in HC rats were significantly decreased by 24.14, 64.58, 19.46, 13.70, 33.33, 39.66, and 33.33%, respectively, compared with levels in HC + LL rats. In contrast, no significant difference was found in plasma albumin, total bilirubin, direct bilirubin, creatinin, calcium, sodium, and chloride levels among the normo, hyper, and HC + LL rats (Table 2).

**Effect of mushroom feeding on plasma lipoprotein fraction.** 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 α- and β-lipoprotein (Fig. 2). The effects of feeding *L. lepideus* on the plasma lipoprotein fraction are presented in Fig. 3. No significant difference in the

### Table 2. Effects of *Lentinus lepideus* on biochemical and electrolyte function in hypercholesterolemic rats

| Parameter               | NC (n = 10) | HC (n = 10) | HC + LL (n = 10) |
|-------------------------|-------------|-------------|------------------|
| 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 ± 0.1        |
| Direct bilirubin (mg/dL)| 0.0 ± 0.0   | 0.0 ± 0.1   | 0.0 ± 0.0        |
| Creatinin (mg/dL)       | 0.6 ± 0.0   | 0.7 ± 0.1   | 0.5 ± 0.1        |
| Blood urea nitrogen (mg/dL)| 16.2 ± 2.3<sup>abc</sup> | 17.4 ± 3.2<sup>c</sup> | 13.2 ± 0.8<sup>b</sup> |
| Uric acid (mg/dL)       | 2.2 ± 0.5<sup>a</sup> | 4.8 ± 1.4<sup>a</sup> | 1.7 ± 0.2<sup>a</sup> |
| Glucose (mg/dL)         | 106.0 ± 4.7<sup>ab</sup> | 118.2 ± 10.7 | 95.2 ± 8.8<sup>b</sup> |
| Total protein (g/dL)    | 7.2 ± 0.2   | 7.3 ± 0.4<sup>a</sup> | 6.3 ± 0.3<sup>a</sup> |
| Calcium (mg/dL)         | 10.5 ± 0.2  | 10.9 ± 0.8  | 10.0 ± 0.3       |
| Sodium (mEq/L)          | 142.8 ± 0.8 | 144.8 ± 2.3 | 142.8 ± 0.8      |
| Potassium (mEq/L)       | 4.8 ± 0.3<sup>a</sup> | 7.5 ± 1.7   | 5.0 ± 0.3<sup>a</sup> |
| Chloride (mEq/L)        | 102.4 ± 1.5 | 103.0 ± 1.9 | 103.2 ± 1.1      |
| Inorganic phosphate (mg/dL)| 6.9 ± 0.7   | 11.6 ± 1.6<sup>a</sup> | 7.0 ± 0.3<sup>a</sup> |
| Magnesium (mg/dL)       | 2.7 ± 0.2<sup>ab</sup> | 3.6 ± 0.8<sup>b</sup> | 2.4 ± 0.1<sup>b</sup> |

Values represent mean ± SD. Values in the same row that do not share a common superscript are significantly different at *p* ≤ 0.05.

NC, normocholesterolemic control; HC, hypercholesterolemic; HC + LL, mushroom-fed hypercholesterolemic.

![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. α, α-lipoprotein; β, β-lipoprotein; pre-β, pre-β lipoprotein; NC, normocholesterolemic control; HC, hypercholesterolemic; HC + LL, mushroom-fed hypercholesterolemic.](image-url)
Antihyperlipidemic Effects of *Lentinus lepideus*

Lipoprotein fractions was evident between normo and HC + LL rats. The results revealed that feeding of 5% mushroom significantly reduced plasma β- and pre-β lipoprotein but increased α-lipoprotein in HC rats.

**Effect of mushroom feeding on rat liver histopathology.** The effect of *L. lepideus* on hepatocyte cells of HC rats are 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 + LL rats. Lipid droplets were observed only in liver tissue of HC rats. This could be attributed to lipid accumulation in the hepatocyte cell cytoplasm.

**Discussion**

Presently, feeding with a *L. lepideus* supplemented diet reduced body weight in hyper and normocholesterolemic rats. This finding is of special significance because obesity is associated with numerous diseases including diabetes, atherosclerosis, and coronary heart disease [5]. Rats are particularly resistant to the development of 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 anti-thyroid drug), which increases the intestinal absorption of cholesterol [17]. However, in the present study, the addition of 1% cholesterol to the basal diet without bile acids and/or anti-thyroid drugs produced hypercholesterolaemia in the rats, because cholesterol feeding itself increases bile acid secretion by approximately 3-4-fold in rats [18]. The present 32.17% increase in plasma cholesterol in the HC rats is comparable with that reported by Bobek et al. [19], who fed a diet containing 0.3% cholesterol with added bile acids (0.5%) to rats and reported 1.7-fold higher cholesterolemia compared with normal rats. In this experiment, feeding 5% mushroom 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 mevinolin [4], which may be involved in decreasing the activity of the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase [19], which is the rate-limiting activity of cholesterol biosynthesis. Thus, feeding mushrooms may involve suppression of endogenous cholesterol biosynthesis by inhibiting HMG-CoA reductase activity.

Due to the increasing frequency of anti-hyperlipidemic drug use and their common side effects, there is a need to identify natural products with few or no side effects. Thus, identification and of highly-effective natural ingredients from food, such as mushrooms, which decrease hyperlipidemia is an important goal [20]. Previous studies have shown that AST and ALT are typically elevated following cellular damage as a result of enzyme leakage from the cells into the blood [21]. Therefore, the increased enzyme activities resulting from the mushroom treatment may prevent oxidative damage by detoxifying reactive oxygen species, reducing hyperlipidemia.

The decreased plasma cholesterol observed presently might be attributed to such a mechanism. The higher level of plasma HDL-C indicates that more cholesterol from peripheral tissues was returned to the liver for catabolism and subsequent excretion. Plasma VLDL-C and TG contents in HC + LL rats were lower compared with HC 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 [22]. In this scenario, 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 + LL rats.

The glucose-lowering effect of propionate is associated with gluconeogenesis and the regulation of serum lipid levels [23]. Reduction in plasma potassium, sodium, and chloride concentrations is one of the mechanisms of action of antihypertensive drugs, particularly diuretics [24]. Diuretics act by diminishing sodium chloride reabsorption at different sites in the nephrons, thereby increasing urinary sodium chloride and water losses, consequently leading to decreased plasma levels of these electrolytes. Antonov et al. [25] 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.

The hypocholesterolemic effect of mushrooms is mediated by the interplay of a complex mixture of substances [26]. 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.

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 + LL rats were almost similar to NC rats and the hepatic biosynthesis of cholesterol was suppressed, which might be due to a reduction in the activity of HMG-CoA [27]. 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 by 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 [28]. Bobek and Gašparová [29] observed that 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.

The present results demonstrate that dietary L. lepideus significantly reduces body weight and plasma lipid profiles, with no detrimental effects on the liver and kidney in HC rats. On the basis of the results, it is suggested that mushroom intake has significant health benefits through the modulation of physiological functions that consist of various atherogenic lipid profiles in hypercholesterolemia. Consequently, L. lepideus may be a good source of nutrition that may also act as a prophylactic against hypercholesterolemia, hyperlipidemia, and related complications, which are the risk factors of atherosclerosis.

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