

Original Article**Antihyperlipidemic activities of *Pleurotus ferulae* on biochemical and histological function in hypercholesterolemic rats**Nuhu Alam<sup>1</sup>, Ki Nam Yoon<sup>2</sup>, Tae Soo Lee<sup>3</sup>**Abstract**

**BACKGROUND:** *Pleurotus ferulae* is an edible mushroom has been widely used for nutritional and medicinal purposes. Irrespective of the medicinal importance or therapeutic potentials of *P. ferulae*, there have not been studies on anti-hyperlipidemic properties. Therefore, the present study investigates the effects of dietary *P. ferulae* fruiting bodies on plasma and feces biochemical and on the liver histological status in hypercholesterolemic rats.

**METHODS:** Six weeks old female Sprague-Dawley albino rats were divided into three groups of 10 rats each. Then biochemical and histological examinations were performed.

**RESULTS:** Feeding of a diet containing 5% *P. ferulae* fruiting bodies to hypercholesterolemic rat reduced plasma total cholesterol, triglyceride, low-density lipoprotein (LDL), total lipid, phospholipids, and LDL/high-density lipoprotein ratio by 30.02, 49.31, 71.15, 30.23, 21.93, and 65.31%, respectively. Mushroom 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, and enzyme profiles. Feeding mushroom increased total lipid and cholesterol excretion in feces. The plasma lipoprotein fraction, separated by agarose gel electrophoresis, indicated that *P. ferulae* significantly reduced plasma  $\beta$  and pre- $\beta$ -lipoprotein, while increased the  $\alpha$ -lipoprotein. A histological study of hepatic cells by conventional hematoxylin-eosin and oil red O staining showed normal findings for mushroom-fed hypercholesterolemic rats.

**CONCLUSIONS:** The present study suggests that 5% *P. ferulae* diet supplement provides health benefits, at least partially, by acting on the atherogenic lipid profile in hypercholesterolemic rats.

**KEYWORDS:** Agarose Gel Electrophoresis, Atherogenic Lipid Profile, Histopathology, Hypercholesterolemic Rats, *Pleurotus Ferulae*.

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Cardiovascular disease is the most common causes of death in the developed and developing countries of the world.<sup>1</sup> The aetiological risk markers that have been shown to be specially modified by the diet are related to lipid and lipoprotein metabolism, haemostatic function, oxidative damage, homocysteine metabolism and blood pressure changes.<sup>2</sup>

*Pleurotus ferulae* is an edible mushroom, belongs to the family pleurotaceae and order agaricales.<sup>3</sup> This mushroom has been known to

produce various biologically active molecules and novel enzymes.<sup>4</sup> Traditionally, edible mushrooms have been prescribed in oriental medicine due to their hypocholesterolemic effects. In general, intake of edible mushrooms was recommended to reduce the hyperlipidemia,<sup>5</sup> due to the presence of specific substance and other bioactive compounds. The genus *Pleurotus* has several species that produce mevinolin. *P. ferulae* has been shown to produce the highest amount of lovastatin in the fruiting bodies, especially in the lamellae or gills.<sup>6</sup>

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The fatty acid pattern of edible mushrooms seems to contribute to reduce plasma cholesterol.<sup>7</sup> When the fatty acid profile of some edible mushrooms was analyzed, considerable amounts of polyunsaturated fatty acid were found. The presence of trans-isomers of unsaturated fatty acids is associated with the strongest effects on raising the plasma total cholesterol to high-density lipoprotein ratio, increasing cardiovascular diseases risk.<sup>8</sup>

The soluble dietary fiber has shown healthy effects on plasma lipid levels, reducing total cholesterol and LDL-cholesterol amounts.<sup>9</sup> The formation of viscous gels from soluble dietary fiber such as glucans might contribute to inhibit the cholesterol and triglycerol absorption.<sup>10</sup> Their viscous properties are related to an increase on the fecal excretion of bile acids and short-chain fatty acids, which inhibits acetate incorporation to plasma lipids.<sup>11</sup> Despite the clinical importance of *P. ferulae* or the therapeutic potential, there have not been studies on hypercholesterolemia. However, antihyperlipidemic properties of this mushroom are not available. The present investigation was undertaken to generate awareness of the beneficial effects of *P. ferulae* on hypercholesterolemia, which poses serious health problem.

## 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 and the experimental protocols were approved by ethical committee of the University of Incheon, Republic of Korea. All experimental procedures were performed in accordance with the guide for the care and use of experimental animals.

## Mushroom

Fresh fruiting bodies of *P. ferulae* were obtained from Mushmaru mushroom farm at Cheonan 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 acces-

sion number, IUM-4402 was acquired. Fresh fruiting bodies were dried with hot air at 40 °C for 48 hour and pulverized.

## Animals

Thirty female Sprague-Dawley albino rats (101 ± 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 ± 2 °C under a 12 hour dark-light cycle (17:00-5:00 h) 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. ferulae* powder (mushroom-fed hypercholesterolemic rats; HC + PF). The basal diet compositions are presented in Table 1. The rats were fed for 42 days.

## Plasma biochemical analysis

At the end of the experimental period, overnight-fasted rats were sacrificed by injection of 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 2493 × 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 phospholipid (PL) levels were measured enzymatically by the cholesterol oxidase assay<sup>12</sup> using 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).

**Table 1.** Basal diet composition

Ingredient	g/100g
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  $1.2 \times 10^{-3}$ ,  $\alpha$ -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 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  $CrSO_2$   $1.5 \times 10^{-3}$ .

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.<sup>12</sup> 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 total lipid and cholesterol 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.<sup>13</sup> 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, 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.<sup>14</sup> Three lipoprotein fractions were detected by electrophoresis, which will henceforth be referred to as  $\square$ -lipoprotein (LDL), pre- $\square$ -lipoprotein (VLDL), and  $\square$ -lipoprotein (HDL). Sample application (2  $\mu$ l), 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- $\mu$ m thick and stained with oil red-O.<sup>15</sup> A representative part of the formalin fixative liver tissues was processed for 4- $\mu$ m 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 $\times$  magnification.

#### ***Statistical analysis***

Data were expressed as mean  $\pm$  standard deviation (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 P. ferulae on body weight***

Feeding with 5% dietary mushroom, *P. ferulae* reduced body weight significantly in hypercholesterolemic and normocholesterolemic rats by 12.16%, and 8.45%, respectively (Table 2).

#### ***Effects of P. ferulae on plasma lipid profile***

Plasma lipid profile concentrations in NC, HC, and HC + PF rats after *P. ferulae* feeding for 6-

week is 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 parameters decreased significantly by 30.02%, 49.31%, 14.22%, 71.15%, 26.74%, 30.23%, and 21.93%, respectively in HC + PF rats compared with HC rats. The ratio of plasma LDL and HDL is shown in Figure 1. In HC rats, this ratio increased by 8.89%, compared with NC rats, whereas this ratio reduced significantly by 65.31% in HC + PF compared with HC rats.

#### ***Effects of P. ferulae on plasma biochemical and electrolyte function***

The results on plasma biochemical and electrolytes concentrations indicated that, uric acid, glucose, potassium, inorganic phosphate, and magnesium in hypercholesterolemic rats significantly decreased by 64.56%, 23.69%, 37.33%, 37.93%, and 30.56%, respectively compared with levels in mushroom-fed hypercholesterolemic rats. In contrast, no significant difference was found for plasma albumin, total bilirubin, direct bilirubin, creatinin, blood urea nitrogen, total protein, calcium, sodium, and chloride levels among the normocholesterolemic, hypercholesterolemic, and mushroom-fed hypercholesterolemic rats (Table 4).

#### ***Effects of P. ferulae on plasma enzyme profile***

Lower plasma GOT and GPT concentrations were observed in mushroom-fed hypercholesterolemic rats than normocholesterolemic rats (Table 5). No significant difference was in the activities of plasma GOT, GPT, and ALP in the NC, HC, and HC + PF rats groups. Five percent *P. ferulae*-fed hypercholesterolemic rats revealed significantly decreased level of plasma GOT, GPT, and ALP activity by 14.69%, 13.41%, and 5.19%, respectively.

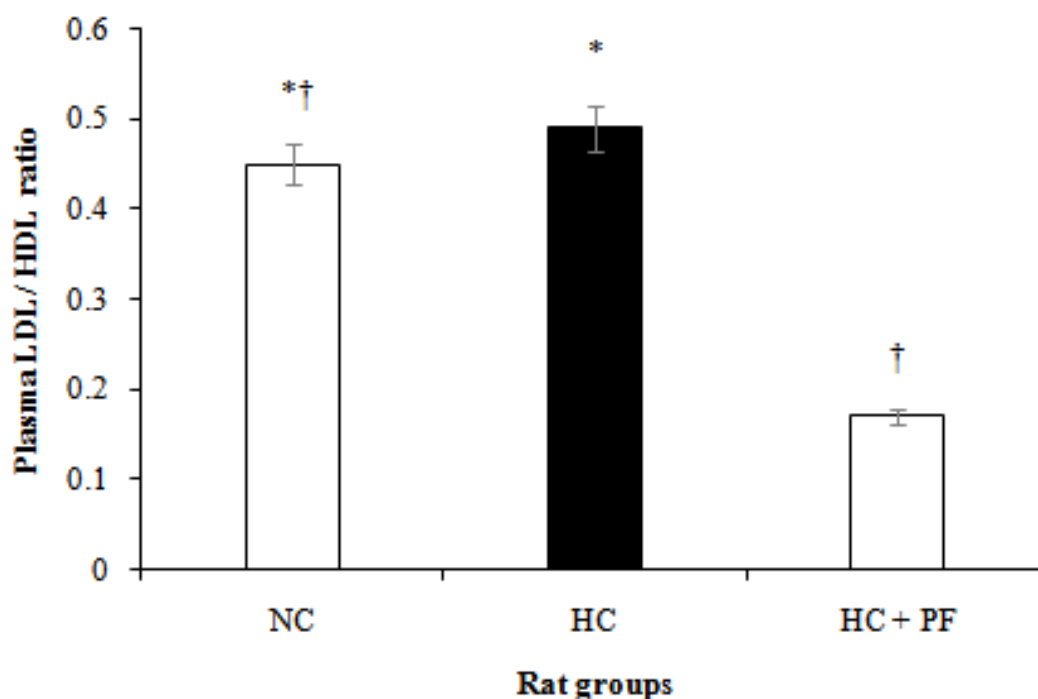
#### ***Effects of P. ferulae on fecal total lipid and cholesterol***

The fecal total lipid and cholesterol of the 5% *P. ferulae*-fed hypercholesterolemic rats signifi-

**Table 2.** Effect of *P. ferulae* on the body weight in hypercholesterolemic rats

Rat Groups	Initial body weight (g) (n=10)	Final body weight (g) (n=10)	Weight gained (g) (n=10)
NC	101 ± 5.3	243 ± 12.5	142 ± 9.1 <sup>a,b</sup>
HC	101 ± 4.2	249 ± 11.9	148 ± 13.0 <sup>a</sup>
HC+PF	101 ± 3.8	231 ± 9.6	130 ± 10.6 <sup>b</sup>

<sup>a,b</sup> Values with different superscripts are significantly different at  $P < 0.05$  in the fourth column.

**Figure 1.** Effects of *P. ferulae* on plasma low density lipoprotein (LDL)/high density lipoprotein (HDL) ratio in hypercholesterolemic rats.

\*,† Different symbols indicate significant differences at  $P < 0.05$ .

**Table 3.** Effect of *P. ferulae* on plasma lipid profiles in hypercholesterolemic rats

Parameters (mg/dl)	NC (n=10)	HC (n=10)	HC+PF (n=10)
TC	103.0 ± 5.3 <sup>a</sup>	120.6 ± 10.3 <sup>b</sup>	84.4 ± 5.7 <sup>c</sup>
TG	63.8 ± 11.3 <sup>a</sup>	87.2 ± 12.8 <sup>b</sup>	44.2 ± 5.9 <sup>c</sup>
HDL-C	37.6 ± 2.9	42.2 ± 2.2	36.2 ± 3.5
LDL-C	17.0 ± 5.8 <sup>a</sup>	20.8 ± 2.3 <sup>a</sup>	6.0 ± 2.0 <sup>b</sup>
VLDL-C	48.4 ± 6.3	57.6 ± 7.8	42.2 ± 3.3
TL	328.0 ± 9.8 <sup>a</sup>	393.0 ± 4.8 <sup>b</sup>	274.2 ± 13.9 <sup>c</sup>
PL	158.6 ± 9.8 <sup>a</sup>	184.2 ± 11.0 <sup>b</sup>	143.8 ± 12.6 <sup>a</sup>

<sup>a,b,c</sup> Values in the same row that do not share a common superscript are significantly different at  $P < 0.05$ .

**Table 4.** Effect of *P. ferulae* on biochemical and electrolyte function in hypercholesterolemic rats

Parameters	NC (n=10)	HC (n=10)	HC+PF (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 ± 0.0
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	0.6 ± 0.1
Blood urea nitrogen (mg/dl)	16.2 ± 2.3	17.4 ± 3.2	15.4 ± 1.9
Uric acid (mg/dl)	2.2 ± 0.5 <sup>a</sup>	4.8 ± 1.4 <sup>b</sup>	1.7 ± 0.5 <sup>a</sup>
Glucose (mg/dl)	106.0 ± 4.7 <sup>a,b</sup>	118.2 ± 10.7 <sup>a</sup>	90.2 ± 5.9 <sup>b</sup>
Total protein (g/dl)	7.2 ± 0.2	7.3 ± 0.4	6.5 ± 0.4
Calcium (mg/dl)	10.5 ± 0.2	10.9 ± 0.8	10.1 ± 0.3
Sodium (mEq/l)	142.8 ± 0.8	144.8 ± 2.3	143.4 ± 0.5
Potassium (mEq/l)	4.8 ± 0.3 <sup>a</sup>	7.5 ± 1.7 <sup>b</sup>	4.7 ± 0.2 <sup>a</sup>
Chloride (mEq/l)	102.4 ± 1.5	103.0 ± 1.9	103.4 ± 2.2
Inorganic Phosphate (mg/dl)	6.9 ± 0.7 <sup>a</sup>	11.6 ± 1.6 <sup>b</sup>	7.2 ± 0.5 <sup>a</sup>
Magnesium (mg/dl)	2.7 ± 0.2 <sup>a,b</sup>	3.6 ± 0.8 <sup>a</sup>	2.5 ± 0.1 <sup>b</sup>

<sup>a,b</sup> Values in the same row that do not share a common superscript are significantly different at  $P < 0.05$ .

cantly increased by 2.6 and 4.03-fold, respectively compared with NC rats (Table 6).

#### Effects of *P. ferulae* on plasma lipoprotein fraction

The  $\beta$ -lipoprotein band was the fast-moving fraction and was located nearest the anode. The  $\beta$ -lipoprotein band was usually the most prominent fraction and was near the origin, migrating only slightly anodic to the point of application. The pre- $\beta$  lipoprotein band migrated between  $\beta$  and  $\beta$ -lipoprotein (Figure 2). The effects of *P. ferulae* on the plasma lipoprotein fraction have been presented in Figure 3. The results indicated that there is no significant difference in the lipoprotein fractions between normocholesterolemic and mushroom-fed hypercholesterolemic rats as compare to hypercholesterolemic rats. Results revealed

that feeding with 5% mushroom significantly reduced plasma  $\beta$ -lipoprotein and pre- $\beta$  lipoprotein but increased  $\beta$ -lipoprotein.

#### Effects of *P. ferulae* on rat liver histopathology

The effect of *P. ferulae* on hepatocyte cells of hypercholesterolemic rats is presented in Figure 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 + PF rats groups. Lipids droplets were observed only in the liver tissue of HC rats. This could be attributed to lipid accumulation in the hepatocyte cell cytoplasm. The histological results indicated that the liver tissues of 5% *P. ferulae*-fed hypercholesterolemic rats were almost similar to normocholesterolemic rats.

**Table 5.** Effect of *P. ferulae* on plasma enzyme profiles in hypercholesterolemic rats

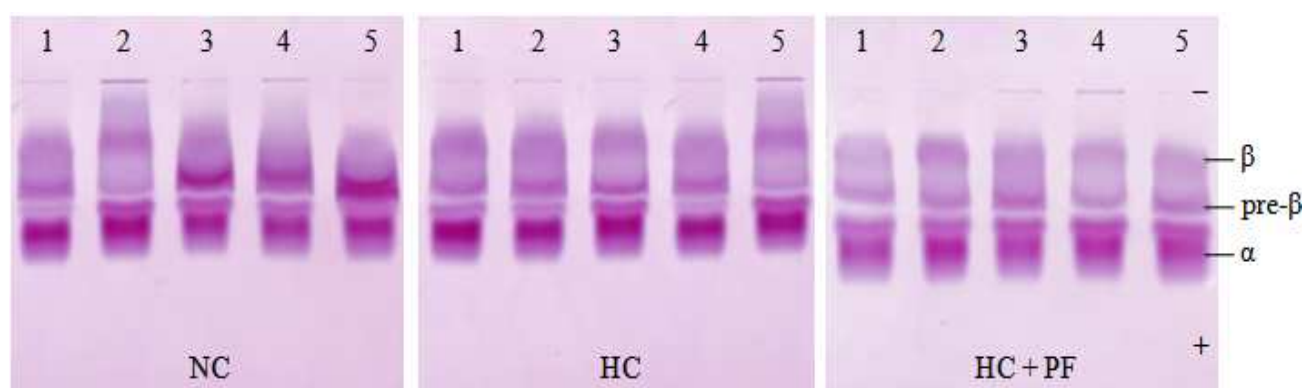
Parameters (U/l)	NC (n=10)	HC (n=10)	HC+PF (n=10)
GOT	63.4 ± 9.1 <sup>a,b</sup>	70.8 ± 8.4 <sup>a</sup>	60.4 ± 5.9 <sup>b</sup>
GPT	57.4 ± 10.9 <sup>a,b</sup>	65.6 ± 3.0 <sup>a</sup>	56.8 ± 8.6 <sup>a,b</sup>
ALP	164.8 ± 7.7 <sup>a,b</sup>	177.2 ± 9.4 <sup>a</sup>	168 ± 10.5 <sup>a,b</sup>

<sup>a,b</sup> Values in the same row that do not share a common superscript are significantly different at  $P < 0.05$ .

**Table 6.** Effects of *P. ferulae* on fecal total lipid and cholesterol

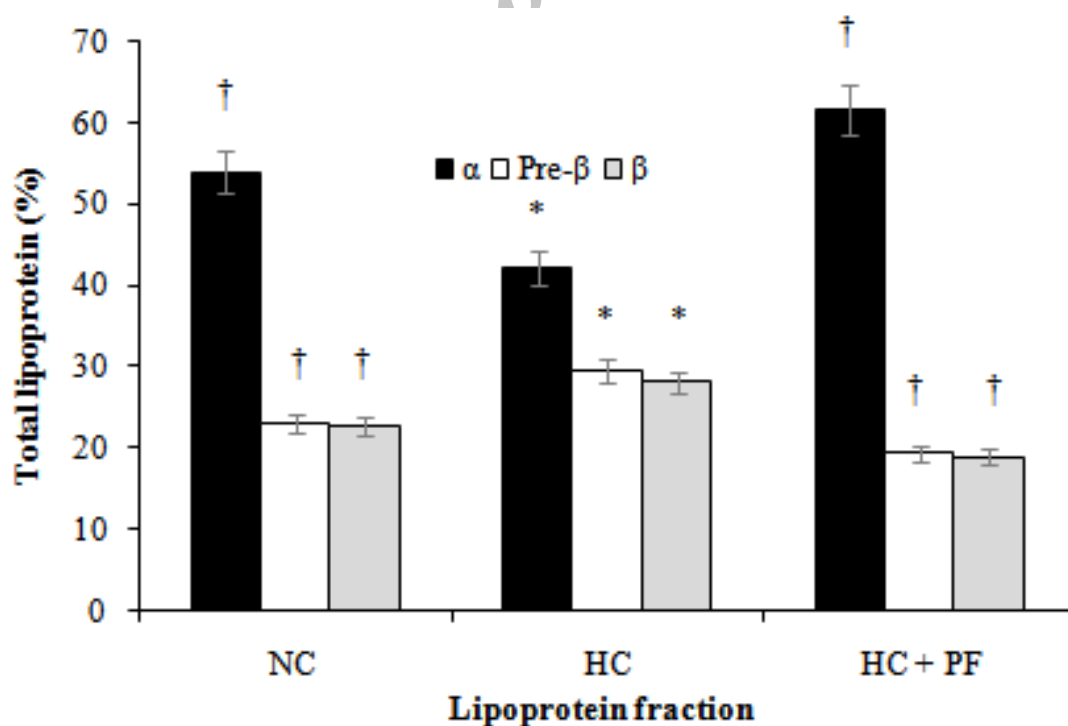
Parameters (g/100g feces)	NC (n=10)	HC (n=10)	HC+PF (n=10)
Total lipid	24.6 ± 3.2 <sup>a</sup>	55.5 ± 4.5 <sup>b</sup>	64.5 ± 7.2 <sup>c</sup>
Cholesterol	3.8 ± 0.6 <sup>a</sup>	13.4 ± 0.8 <sup>c</sup>	15.3 ± 0.9 <sup>c</sup>

<sup>a,b,c</sup> Values in the same row that do not share a common superscript are significantly different at  $P < 0.05$ .



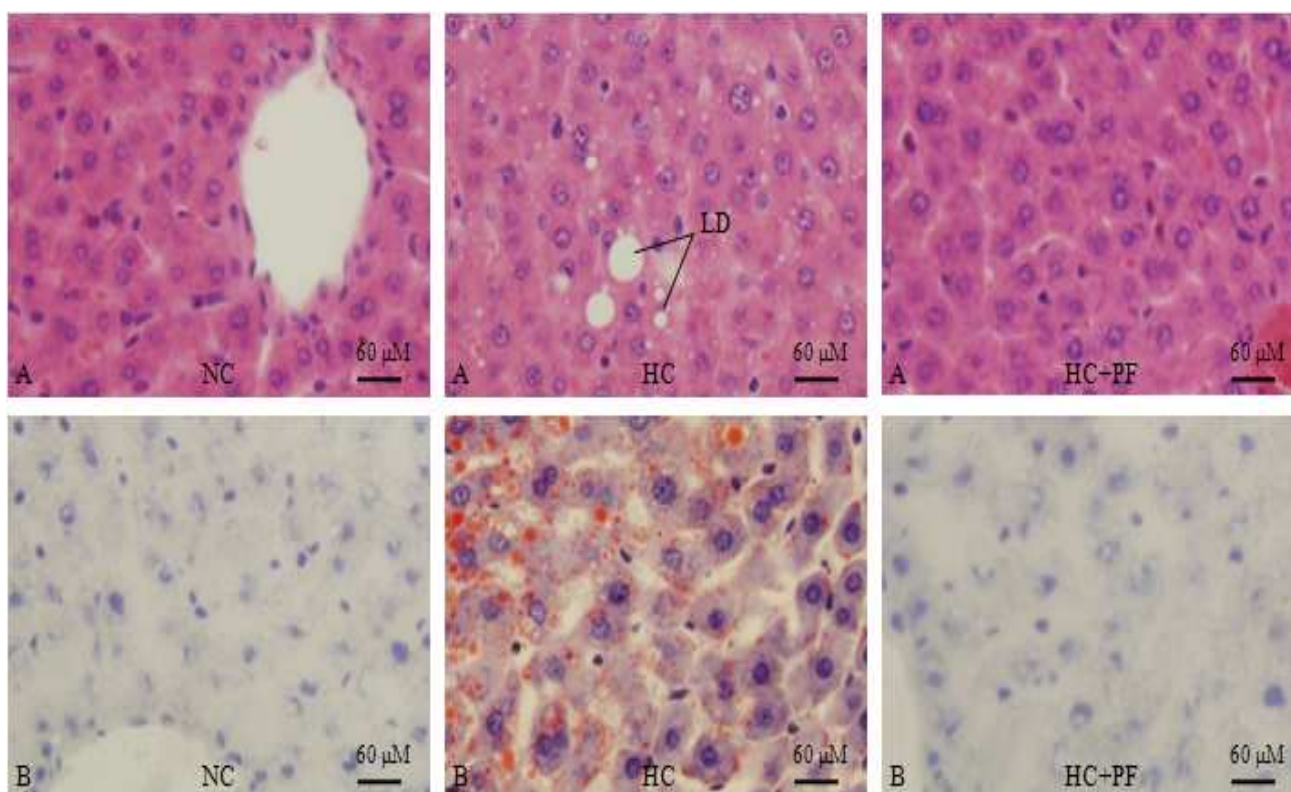
**Figure 2.** Separation of plasma lipoproteins by agarose gel electrophoresis. Lanes 1-5 represent the plasma lipoprotein fraction of five different rats from each group.

β, β-lipoprotein.  
Pre-β, Pre-β-lipoprotein.  
α, α-lipoprotein.



**Figure 3.** Effects of *P. ferulae* on the plasma lipoprotein fraction following agarose gel electrophoresis.

\*† Different symbols indicate significant differences at  $p \leq 0.05$ .



**Figure 4.** Effects of feeding *P. ferulae* on hepatocyte cells in hypercholesterolemic rats.

A-C, hematoxylin-eosin stained photomicrographs at 40 $\times$ .

D-F, photomicrographs of oil red O stain at 40 $\times$ .

LD, Lipid droplets.

## Discussion

Feeding of 5% *P. ferulae* 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.<sup>16</sup>

Rats are particularly resistant to the development of hypercholesterolemia and atherosclerosis<sup>17</sup> and have a strong ability to maintain their plasma cholesterol levels.<sup>18, 19</sup> 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.<sup>20</sup> 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 three to four-folds in rats.<sup>21</sup> The 17.09% increase in plasma cholesterol in the hypercholesterolemic rats in the present study was comparable with that reported by Bobek et al.<sup>22</sup>, who fed rats cholesterol (0.3%) diet with added bile acids (0.5%) and showed a 1.7-fold higher cholesterolemia in their cholesterol-fed rats compared with normal rats. In this experiment, feeding of 5% *P. ferulae* to hypercholesterolemic rats significantly ameliorated the plasma atherogenic lipid profiles. The mechanism by which mushrooms reduce plasma lipoprotein levels in hypercholesterolemic rats is not clearly understood. Mushrooms contain the hypocholesterolaemic agent mevinolin,<sup>6</sup> which may be involved in decreasing the activity of the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase,<sup>22</sup> which is the rate-limiting enzyme of cholesterol biosynthesis. Thus, feeding mushrooms may involve suppression of endoge-



nous cholesterol biosynthesis by inhibiting HMG-CoA reductase activity.

The glucose-lowering effect of propionate is associated with gluconeogenesis and the regulation of serum lipid levels.<sup>23</sup> Reduction in plasma potassium, sodium, and chloride concentrations is one of the mechanisms of action of antihypertensive drugs, particularly diuretics.<sup>24</sup> 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.<sup>25</sup> 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.

Due to the increasing frequency of anti-hyperlipidemic drugs 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.<sup>22, 26</sup> 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.<sup>27</sup> Therefore, the increased enzyme activities resulting from oyster mushroom treatment may prevent oxidative damage by detoxifying reactive oxygen species; thus, reducing hyperlipidemia.

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 mushroom-fed hypercholesterolemic rats were lower compared with 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.<sup>28</sup> LDL-C became the

prime carrier for cholesterol after feeding cholesterol to the rats, leading to decreased VLDL-C and HDL-C content in mushroom-fed hypercholesterolemic rats.

The hypocholesterolemic effect of mushrooms is mediated by the interplay of a complex mixture of substances.<sup>29</sup> Water-soluble gel-forming components of the fiber substance ( $\beta$ -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.

Lipid droplets were observed only in 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% mushroom-fed hypercholesterolemic rats were almost similar to normocholesterolemic control rats and that the hepatic biosynthesis of cholesterol was suppressed, which might be due to a reduction in the activity of HMG-CoA.<sup>30</sup> Hyperlipidemia is the leading risk factor for atherosclerosis, but the atherosclerotic pathological process could be slowed or reversed by reducing serum LDL, TGs, and phospholipids 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.<sup>31</sup> Bobek and Galbavy<sup>32</sup> 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.

## Conclusion

The results indicated that feeding of 5% *P. ferulae* fruiting bodies significantly reduced body weight and atherogenic lipid profiles, and it had no adverse effects on the hepatic tissues in hypercholesterolemic rats. It could be suggested that dietary supplementation of *P. ferulae* might have greater significance in the prevention of hyperlipidemia or cardiovascular disease.

## Limitations of the Study

The effects of dietary *P. ferulae* on normocholesterolemic rats and histology of coro-

nary artery were not evaluated in this experiment. Further studies are needed for additional data.

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## Conflict of Interests

Authors have no conflict of interests.

## Authors' Contributions

NA carried out experiments and prepared the manuscript. KNY contribute histological study and provided assistance for all experiments. TSL carried out the design and supervised the study, and edited the manuscript. All authors have read and approved the content of the manuscript.

## References

1. Cannon CP. Cardiovascular disease and modifiable cardiometabolic risk factors. *Clin Cornerstone* 2007; 8(3): 11-28.
2. Mensink RP, Zock PL, Kester AD, Katan MB. Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. *Am J Clin Nutr* 2003; 77(5): 1146-55.
3. Urbanelli S, Fanelli C, Fabbri AA, Della-Rosa V, Maddau L, Marras F, et al. Molecular genetic analysis of two taxa of the *Pleurotus eryngii* complex: *P. eryngii* (DC.Fr.) Quél. var. *eryngii* and *P. eryngii* (DC.Fr.) Quél. var. *ferulae*. *Biol J Linnean Soc* 2002; 75(1): 125-36.
4. Choi D, Kang SH, Song YH, Kwun KH, Jeong KJ, Cha WS. Exopolysaccharide production in liquid culture of *Pleurotus ferulae*. *J Microbiol Biotechnol* 2005; 146: 209-21.
5. Mori K, Kobayashi C, Tomita T, Inatomi S, Ikeda M. Antiatherosclerotic effect of the edible mushrooms *Pleurotus eryngii* (Eringi), *Grifola frondosa* (Maitake), and *Hypsizygus marmoratus* (Bunashimeji) in apolipoprotein E-deficient mice. *Nutr Res* 2008; 28(5): 335-42.
6. Gunde-Cimerman N, Plemenitas A, Cimerman A. *Pleurotus* fungi produce mevinoles, an inhibitor of HMG CoA reductase. *FEMS Microbiol Lett* 1993; 113(3): 333-7.
7. Hossain S, Hashimoto M, Choudhury EK, Alam N, Hussain S, Hasan M, et al. Dietary mushroom (*Pleurotus ostreatus*) ameliorates atherogenic lipid in hypercholesterolaemic rats. *Clin Exp Pharmacol Physiol* 2003; 30(7): 470-5.
8. Barros L, Ferreira MJ, Queiros B, Ferreira IC, Baptista P. Total phenols, ascorbic acid,  $\beta$ -carotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities. *Food Chem* 2007; 103(2): 413-9.
9. Erkkila AT, Lichtenstein AH. Fiber and cardiovascular disease risk: how strong is the evidence? *J Cardiovasc Nurs* 2006; 21(1): 3-8.
10. Marlett JA, McBurney MI, Slavin JL. Position of the American Dietetic Association: health implications of dietary fiber. *J Am Diet Assoc* 2002; 102(7): 993-1000.
11. Wolever TM, Spadafora PJ, Cunnane SC, Pencharz PB. Propionate inhibits incorporation of colonic [1,2-<sup>13</sup>C]acetate into plasma lipids in humans. *Am J Clin Nutr* 1995; 61(6): 1241-7.
12. Burtis CA, Ashwood ER. *Tietz Fundamentals of Clinical Chemistry*. 6<sup>th</sup> ed. Philadelphia: Saunders; 2006.
13. Folch J, Lees M, Sloanes Stanley GH. A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* 1957; 226(1): 497-509.

14. Kido T, Kurata H, Matsumoto A, Tobiyama R, Musha T, Hayashi K, et al. Lipoprotein analysis using agarose gel electrophoresis and differential staining of lipids. *J Atheroscler Thromb* 2001; 8(1): 7-13.
15. Bayliss-High HO. Lipids. In: Bancroft JD, Stevens A, editors. *Theory and Practice of Histological Techniques*. 3<sup>rd</sup> ed. Edinburgh: Churchill Livingstone; 1990. p. 215-44.
16. Alam N, Amin N, Khan A, Ara I, Shim MJ, Lee MW, et al. Comparative effects of oyster mushrooms on lipid profile, liver and kidney function in hypercholesterolemic rats. *Mycobiology* 2009; 37(1): 37-42.
17. Andrus SB, Fillios LC, Mann GV, Stare FJ. Experimental production of gross atherosclerosis in the rat. *J Exp Med* 1956; 104(4): 539-54.
18. Fujioka T, Nara F, Tsujita Y, Fukushige J, Fukami M, Kuroda M. The mechanism of lack of hypocholesterolemic effects of pravastatin sodium, a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, in rats. *Biochim Biophys Acta* 1995; 1254(1): 7-12.
19. Roach PD, Balasubramaniam S, Hirata F, Abbey M, Szanto A, Simons LA, et al. The low-density lipoprotein receptor and cholesterol synthesis are affected differently by dietary cholesterol in the rat. *Biochim Biophys Acta* 1993; 1170(2): 165-72.
20. Dolphin PJ, Forsyth SJ. Nascent hepatic lipoproteins in hypothyroid rats. *J Lipid Res* 1983; 24(5): 541-51.
21. Uchida K, Satoh T, Chikai T, Takase H, Nomura Y, Nakao H, et al. Influence of cholesterol feeding on bile acid metabolism in young and aged germ-free rats. *Jpn J Pharmacol* 1996; 71(2): 113-8.
22. Bobek P, Hromadova M, Ozdin L. Oyster mushroom (*Pleurotus ostreatus*) reduces the activity of 3-hydroxy-3-methylglutaryl CoA reductase in rat liver microsomes. *Experientia* 1995; 51(6): 589-91.
23. Yang BK, Jung YS, Song CH. Hypoglycemic effects of *Ganoderma applanatum* and *Collybia confluens* exopolymers in streptozotocin-induced diabetic rats. *Phytother Res* 2007; 21(11): 1066-9.
24. Jude IC, atherine IC, rank OC. Effect of aqueous extract of *Tridax procumbens* Linn on plasma electrolytes of salt-loaded rats. *Pak J Nutr* 2010; 9(2): 103-5.
25. Antonov AR, fremov AV, etyagina VV, acharov YV, arkel AL, akobson GS. Plasma and lymph electrolyte and endocrine parameters in rats with genetically-determined arterial hypertension. *Bull Exp Biol Med* 2011; 124(1): 652-4.
26. Alarcon J, Aguila S, Arancibia-Avila P, Fuentes O, Zamorano-Ponce E, Hernandez M. Production and purification of statins from *Pleurotus ostreatus* (Basidiomycetes) strains. *Z Naturforsch C* 2003; 58(1-2): 62-4.
27. Alam N, Yoon KN, Lee TS, Lee UY. Hypolipidemic activities of dietary *Pleurotus ostreatus* in hypercholesterolemic rats. *Mycobiology* 2011; 39(1): 45-51.
28. Mayes PA. Metabolism of lipids. In: Harper HA, Rodwell VW, Mayes PA, editors. *Review of physiological chemistry*. Los Altos: Lange Publications; 1997. p.280-321
29. Bobek P, Ozdin L, Kuniak L. Effect of oyster mushroom (*Pleurotus Ostreatus*) and its ethanolic extract in diet on absorption and turnover of cholesterol in hypercholesterolemic rat. *Nahrung* 1996; 40(4): 222-4.
30. Keim NL, Marlett JA, Amundson CH, Hagemann LD. Comparison of rat hepatic cholesterol biosynthesis during skim milk versus whey permeate ingestion. *J Dairy Sci* 1982; 65(12): 2274-80.
31. Li H, Zhang M, Ma G. Hypolipidemic effect of the polysaccharide from *Pholiota nameko*. *Nutrition* 2010; 26(5): 556-62.
32. Bobek P, Galbavy S. Hypocholesterolemic and antiatherogenic effect of oyster mushroom (*Pleurotus ostreatus*) in rabbits. *Nahrung* 1999; 43(5): 339-42.