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Pleurotus nebrodensis Ameliorates Atherogenic Lipid and Histological Function in Hypercholesterolemic Rats

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Abstract: *Pleurotus nebrodensis* is a popular edible mushroom has been widely used for nutritional and medicinal purposes. The effects of dietary *P. nebrodensis* fruiting bodies on plasma and feces biochemical and on the liver histological status were estimated in hypercholesterolemic rats. Six-week old female Sprague-Dawley albino rats were divided into three groups of 10 rats each. Feeding of a diet containing a 5% *P. nebrodensis* fruiting bodies to hypercholesterolemic rats reduced plasma total cholesterol, triglyceride, low-density lipoprotein, total lipid, phospholipids and LDL/HDL ratio by 31.01, 47.71, 62.50, 31.91, 24.65 and 53.06%, respectively. Feeding mushroom also significantly reduced body weight in hypercholesterolemic rats. Nevertheless, 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. nebrodensis* significantly reduced plasma β and pre-β-lipoprotein, while 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 rat. The present study suggests that a 5% *P. nebrodensis* diet supplement provided health benefits by acting on the atherogenic lipid profile in hypercholesterolemic condition.

Key words: Agarose gel electrophoresis, atherogenic lipid profile, histopathology, white sanctity mushroom

INTRODUCTION

Pleurotus nebrodensis is known as the Bailingu oyster and white sanctity mushroom (Shen *et al*., 2005). It is one of the popular edible mushrooms in China. This mushroom is successfully cultivated and commercially available in Korea. *P. nebrodensis* is abundant in nutrition including sub-oleic acid, non-saturate fatty acids and microelements such as calcium, zinc and manganese (Alam *et al*., 2009a). It is a good source of dietary fiber and other valuable nutrients. This mushroom also contains a number of biologically active compounds with therapeutic activities (Alam *et al*., 2010, 2008; Choi *et al*., 2005; Wang and Ng, 2004).

Investigations in the last decade have revealed that fruiting bodies of oyster mushroom exhibit a hypocholesterolemic effect on rats with normocholesterolemia and hypercholesterolemia induced by intake of a high fat diet or alcohol intake or hereditary cholesterol disorder. A pronounced cholesterol-lowering effect of oyster mushroom consumption has been demonstrated on rats through a decrease in very-low density lipoproteins and also by suppressing the activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase and an enhanced fractional catabolic rate of cholesterol (Alam *et al*., 2009b; Balasubramanian *et al*., 2008). Recently, it has been evidenced by Khatun *et al*. (2007) that *Pleurotus ostreatus* reduced total cholesterol, triglycerides, glucose and blood pressure in hypercholesterolemia and diabetic subjects. The results of this trial suggest that oyster mushroom provides health benefits by acting on the atherogenic lipid profile under hypercholesterolemic and normocholesterolemic conditions. Most animal studies concerning *Pleurotus* spp. were especially focused on oyster mushroom and it is clear that enhanced effectively the effect on lowering cholesterol (Hossain *et al*., 2003). This effect was further supported by the severity of tissue damage as evidenced by histopathological studies on aorta and heart tissues. An increase in bile acid excretion in experimental animals

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could be taken as one of the possible mechanisms explaining in hypocholesterolemic actions of *Pleurotus florida* (Bajaj *et al*., 1997).

Treatment of hyperlipidemia may be with therapeutic medicines or through edible mushrooms which help lower plasma lipid levels. Edible mushrooms have the advantage in that they avoid side effects often associated with medications, while still improving or healing the hyperlipidemia (Rachh *et al*., 2010; Hu *et al*., 2006). Irrespective of the medicinal importance or therapeutic potential of *P. nebrodensis*, there have not been many studies on the antihyperlipidemic properties. Hence, the present study was under taken to create awareness of the beneficial effects of *P. nebrodensis* on hypercholesterolemia which causes serious health problems.

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, University of Incheon, Republic of Korea.

Mushroom: Fresh fruiting bodies of *P. nebrodensis* were obtained from Mushroom Research Institute of Gyeonggi Province 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 with the acquired accession number, IUM-4658. Fresh fruiting bodies were dried with hot air at 40°C for 48 h and pulverized.

Animals: Thirty female Sprague-Dawley albino rats (101±4.2 g, 6 weeks old, purchased from Central Lab. Animal Inc., Korea) were used. All animals were acclimated in the animal room for 1 week. The rats were housed in an animal room at 23±2°C under 12 h dark-light cycle (17:00-5:00 h) and relative humidity 50-60%. Rats were divided into three groups: a basal diet (normocholesterolemic control rats; NC), basal diet with 1% cholesterol (hypercholesterolemic rats; HC) and a basal diet with 1% cholesterol and 5% *P. nebrodensis* powder (mushroom-fed hypercholesterolemic rats; HC+PN). The basal diet compositions are presented in Table 1 and 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 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 (Burtis and Ashwood, 2006) 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).

Very low-density lipoprotein cholesterol was calculated as follows:

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

Plasma enzyme analysis: The activity of the plasma transaminases, Glutamate Pyruvate Transaminase (GPT) and Glutamate Oxaloacetate Transaminase (GOT) were determined using the kinetic method (Burtis and Ashwood, 2006). 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 alkaline phosphatase activity.

Table 1: Basal diet composition

Ingredient	Values (100 g g^{-1})
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 9.5×10^{-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 2.5×10^{-4}, KI 9×10^{-4}, Sodium molybdate 3×10^{-4}, SeO_2 3×10^{-4} and $CrSO_2$ 1.5×10^{-3}

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.* (1957). Two millilire 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 (Kido *et al.*, 2001). 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 (Bayliss-High, 1990). 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±SD. 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 $p \leq 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Effects of feeding *P. nebrodensis* on bodyweight: Feeding *P. nebrodensis* reduced body weight significantly in hypercholesterolemic and normocholesterolemic rats by 20.95 and 17.61%, respectively (Table 2).

It is generally known that high-fat diet is one of the major factors causing obesity. The present study indicates that mushroom 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 (Khairunnuur *et al.*, 2010).

Effects of feeding *P. nebrodensis* on plasma lipid profile: Plasma lipid profile concentrations in NC, HC and HC+PN rats after *P. nebrodensis* feeding for 6 weeks 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 parameters decreased significantly by 31.01, 47.71, 19.43, 62.50, 28.13, 31.91 and 24.65%, respectively, in HC+PN 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 53.06% in HC+PN compared with HC rats.

Feeding 5% *P. nebrodensis* to rats significantly ameliorated the plasma atherogenic lipid profiles in experimentally induced hypercholesterolemic rats. Rats are particularly resistant to the development of hypercholesterolemia and atherosclerosis (Andrus *et al.*, 1956) and have a strong capability to maintain their plasma cholesterol level (Fujioka *et al.*, 1995; Roach *et al.*, 1993). Therefore, to induce hypercholesterolemia or atherosclerosis in rats, cholesterol feeding is used with other additives, including bile acids and propylthiouracil

Table 2: Effect of *Pleurotus nebrodensis* 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+PN	101±3.8	218±10.4	117±14.4^{b}

The results are Mean±SD. Data were analyzed by one-way and then subjected to the LSD post hoc test. Values with different superscripts are significantly different at $p \leq 0.05$ in the fourth column. LSD: Least significant difference; NC: Normocholesterolemic control rats; HC: Hypercholesterolemic rats; HC+PN: *Pleurotus nebrodensis*-fed hypercholesterolemic rats

Table 3: Effect of *Pleurotus nebrodeusis* on plasma lipid profiles in hypercholesterolemic rats

Parameters (mg dL^{-1})	NC	HC	HC+PN
TC	103.0±5.3^{a}	120.6±10.3^{b}	83.2±11.4^{c}
TG	63.8±11.3^{a}	87.2±12.8^{b}	45.6±6.9^{c}
HDL-C	37.6±2.9^{a,b}	42.2±2.2^{a}	34.0±3.9^{b}
LDL-C	17.0±5.8^{a}	20.8±2.3^{a}	7.8±2.0^{b}
VLDL-C	48.4±6.3	57.6±7.8	41.4±5.1
TL	328.0±9.8^{a}	393.0±4.8^{b}	267.6±10.2^{c}
PL	158.6±9.8^{a}	184.2±11.0^{b}	138.8±11.4^{c}

The results are Mean±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 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

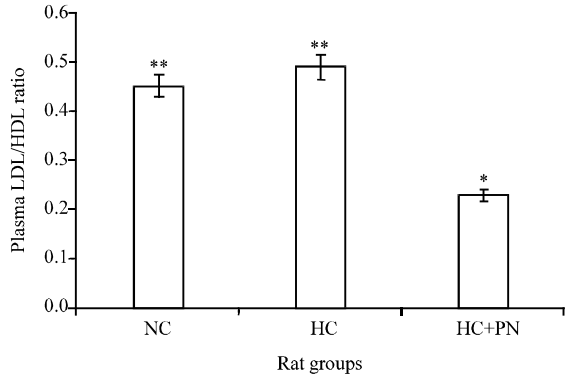

Fig. 1: Effects of *Pleurotus nebrodensis* on plasma Low Density Lipoprotein (LDL)/High Density Lipoprotein (HDL) ratio in hypercholesterolemic rats. Results are Means±SD. *Indicate significant differences at $p \leq 0.05$. NC: normocholesterolemic control rats, HC: hypercholesterolemic rats, HC+PN: *Pleurotus nebrodensis*-fed hypercholesterolemic rats

(an anti-thyroid drug) which increase the intestinal absorption of cholesterol (Dolphin and Forsyth, 1983). However, in the present study, the addition of 1% cholesterol to the basal diet without bile acids and/or anti-thyroid drug produced hypercholesterolemia in the rats, because cholesterol feeding itself increases bile acid secretion by approximately three to four folds in rats (Uchida *et al*., 1996). The 30.01% increase in plasma cholesterol in the hypercholesterolemic rats in the present study was comparable with that reported by Bobek *et al*. (1995) who feed rats cholesterol (0.3%) diet with added bile acids (0.5%) and showed a 1.7-fold higher cholesterolemia in their cholesterol-fed rats than normal rats. In this experiment, feeding 5% *P. nebrodensis* to hypercholesterolemic rats significantly repressed the increment of plasma cholesterol. The mechanism by which

Table 4: Effect of *Pleurotus nebrodeusis* on biochemical and electrolyte function in hypercholesterolemic rats

Parameters	NC	HC	HC+PN
Albumin (g dL^{-1})	3.3±0.2^{a}	3.4±0.3^{a}	2.8±0.2^{b}
Total bilirubin (mg dL^{-1})	0.1±0.0	0.1±0.0	0.1±0.0
Direct bilirubin (mg dL^{-1})	0.0±0.0	0.0±0.1	0.0±0.0
Creatinin (mg dL^{-1})	0.6±0.0	0.7±0.1	0.6±0.1
Blood urea nitrogen (mg dL^{-1})	16.2±2.3	17.4±3.2	16.0±1.2
Uric acid (mg dL^{-1})	2.2±0.5^{a}	4.8±1.4^{b}	1.3±0.1^{c}
Glucose (mg dL^{-1})	106.0±4.7^{a,b}	118.2±10.7^{a}	89.4±6.7^{b}
Total protein (g dL^{-1})	7.2±0.2^{a}	7.3±0.4^{a}	6.1±0.4^{b}
Calcium (mg dL^{-1})	10.5±0.2	10.9±0.8	9.8±0.4
Sodium (mEg L^{-1})	142.8±0.8	144.8±2.3	143.4±1.1
Potassium (mEg L^{-1})	4.8±0.3^{a}	7.5±1.7^{b}	4.3±0.1^{a}
Chloride (mEg L^{-1})	102.4±1.5	103.0±1.9	103.6±0.5
Inorganic Phosphate (mg dL^{-1})	6.9±0.7^{a}	11.6±1.6^{b}	7.2±0.3^{a}
Magnesium (mg dL^{-1})	2.7±0.2^{a}	3.6±0.8^{b}	2.4±0.2^{a}

The results are Mean±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 significant difference, NC: Normocholesterolemic control rats, HC: Hypercholesterolemic rats, HC+PN: *Pleurotus nebrodensis*-fed hypercholesterolemic rats

mushrooms reduce plasma lipoprotein levels in hypercholesterolemic rats is not clearly understood. Mushrooms contain the hypocholesterolemic agent mevnolin (Gunde-Cimerman *et al*., 1993) which may be involved in decreasing the activity of the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase enzyme, the rate-limiting enzyme of cholesterol biosynthesis. Thus, feeding mushroom may involve the suppression of endogenous cholesterol biosynthesis by inhibiting HMG-CoA reductase activity.

Effects of feeding *P. nebrodensis* 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 hypercholesterolemic rats by 17.65, 72.92, 24.37, 16.44, 42.66, 37.93 and 33.33%, respectively compared with levels in 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 mushroom-fed hypercholesterolemic rats (Table 4).

The glucose-lowering effect of propionate is associated with gluconeogenesis and the regulation of serum lipid levels (Yang *et al*., 2007). Reduction in plasma potassium, sodium and chloride concentrations is one of the mechanisms of action of antihypertensive drug, particularly diuretic (Jude *et al*., 2010). Diuretic 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.

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

Parameters (U L^{-1})	NC	HC	HC+PN
GOT	63.4±9.1	70.8±8.4	61.4±6.9
GPT	57.4±10.9^{a,b}	65.6±3.0^{a}	55.6±7.0^{b}
ALP	164.8±7.7	177.2±9.4	165.2±8.1

The results are Mean±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). GOT: Glutamate oxaloacetate transaminase, GPT: Glutamate pyruvate transaminase, ALP: Alkaline phosphatase, LSD: Least significant difference, NC: normocholesterolemic control rats, HC: Hhypercholesterolemic rats, HC+PN: *Pleurotus nebrodensis*-fed hypercholesterolemic rats

Table 6: Effects of *Pleurotns nebrodensis* on fecal total lipid and cholesterol

Parameters (g/100 g feces)	NC	HC	HC+PN
Total lipid	24.6±3.2^{a}	55.5±4.5^{b}	65.3±3.4^{c}
Cholesterol	3.8±0.6^{a}	13.4±0.8^{c}	15.2±1.8^{c}

The results are Mean±SDs. 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 significant difference, NC: normocholesterolemic control rats, HC: hypercholesterolemic rats, HC+PN: *Pleurotns nebrodensis*-fed hypercholesterolemic rats

Antonov *et al*. (1997) reported that plasma electrolyte contents were significantly increased 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.

Effects of feeding *P. nebrodensis* 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 observed in the activities of plasma GOT, GPT and ALP in NC, HC, or HC+PN rats groups. Plasma GOT, GPT and ALP activities were higher in HC rats as compare to NC and HC+PN rats group, while mushroom-fed hypercholesterolemic rats decreased by 13.27, 15.24 and 6.77%, respectively.

Due to the increasing frequency of antihyperlipidemic drug 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 mushroom which decrease hyperlipidemia (Alarcon *et al*., 2003). Previous studies showed that GOT and GPT are typically elevated following cellular damage as a result of enzymes leakage from the cells into blood (Noori *et al*., 2009). Therefore, the decreased enzyme activities resulting from mushroom treatment may prevent oxidative damage by detoxifying reactive oxygen species; thus, reducing hyperlipidemia.

Effects of feeding *P. nebrodensis* on fecal total lipid and cholesterol: The fecal total lipid and cholesterol of the 5% *P. nebrodensis*-fed hypercholesterolemic rats significantly increased by 2.7 and 4.0-folds, respectively compared with NC rats (Table 6). 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 content in mushroom-fed hypercholesterolemic rats were lower compared with the 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 the liver but seems to be formed from VLDL-C after partial removal of TG by lipoprotein lipase (Alam *et al*., 2009b; Mayes, 1997). 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.

Effects of feeding *P. nebrodensis* on plasma lipoprotein fraction by agarose gel electrophoresis: The α-lipoprotein band was the fast moving fraction and was located closest to 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 migrates between α and α-lipoprotein (Fig. 2). The effects of *P. nebrodensis* on plasma lipoprotein fraction have been presented in Fig. 3. The results indicated that no significant difference in the lipoprotein fractions between normocholesterolemic and mushroom-fed hypercholesterolemic rats as compare to hypercholesterolemic rats. Results revealed that mushroom reduced significantly plasma β and pre-β lipoprotein but increased α-lipoprotein.

The hypocholesterolemic effect of mushroom is mediated by interplay of a complex mixture of substances (Bobek *et al*., 1996). Water-soluble gel-forming components of the fibre substances (β-1,3-D-glucan with a low degree of polymerization, forming 15-20% of dry matter) interact with bile acids and affects micelles formation. Such substances might be interfering with the absorption of cholesterol in this manner.

Effects of feeding *P. nebrodensis* on rat liver histopathology: The effect of *P. nebrodensis* on hepatocyte cells of hypercholesterolemic rats is presented in Fig. 4. Liver tissues stained with hematoxylin-eosin and oil red-O. The hepatic cords were typical arranged and located in liver tissue near the central vein in the NC, HC and HC+PN groups. Lipid droplets were observed only in liver tissue of HC rats. This could be attributed to lipid accumulation in hepatocyte cells cytoplasm.

Oxidized LDL induces the expression of scavenger receptors on the macrophages surface. These scavenger receptors promote the accumulation of modified

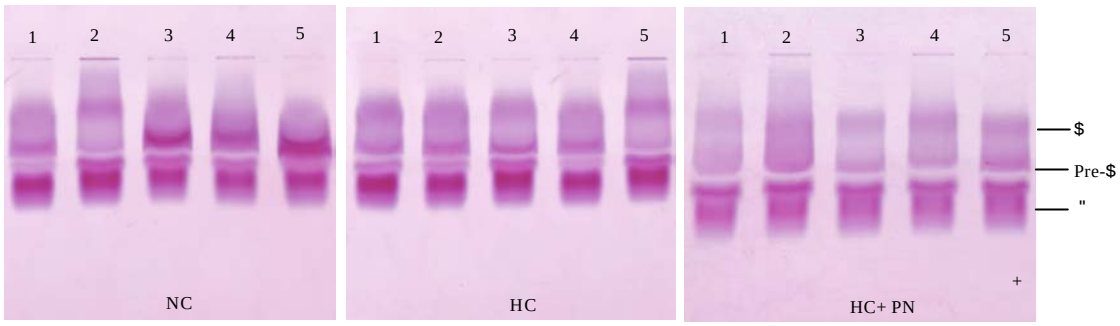

Fig. 2: Separation of plasma lipoproteins by agarose gel electrophoresis. Lanes 1-5 represent the plasma lipoprotein fraction of five different rats from each group. NC: Normocholesterolemic control rats, HC: Hypercholesterolemic rats, HC+PN: *Pleurotus nebrodensis*-fed hypercholesterolemic rats. α: α-lipoprotein; β: β-lipoprotein; Pre-β: Pre-β-lipoprotein

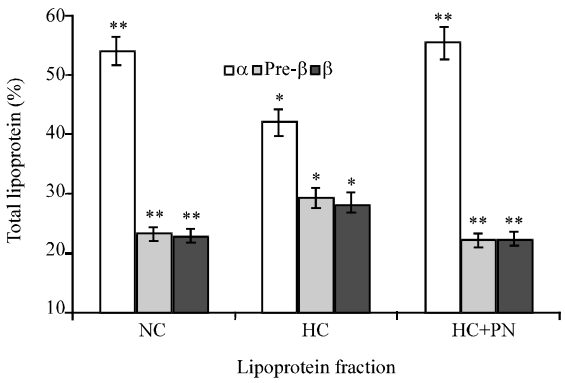

Fig. 3: Effects of *Pleurotus nebrodensis* on the plasma lipoprotein fraction following agarose gel electrophoresis. Results are Means±SD. *Indicate significant differences at $p \leq 0.05$. NC: Normocholesterolemic control rats, HC: Hypercholesterolemic rats, HC+PN: *Pleurotus nebrodensis*-fed hypercholesterolemic rats

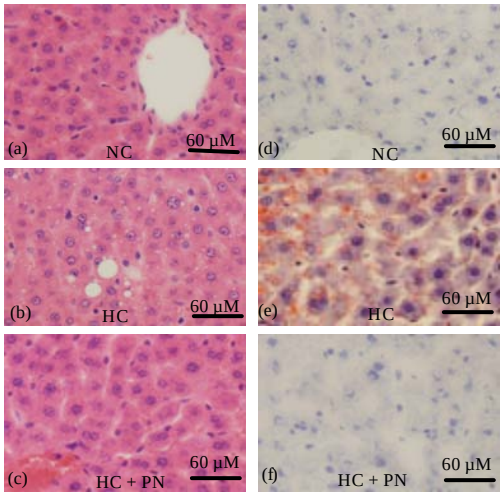

Fig. 4(a-f): Effects of feeding *Pleurotus nebrodensis* on hepatocyte cells in hypercholesterolemic rats. (a-c): Hematoxylin-eosin stained photomicrographs (40x), (d-f): Photomicrographs of oil red O stain (40x), NC: Normocholesterolemic control rats, HC: Hypercholesterolemic rats, HC+PN: *Pleurotus nebrodensis*-fed hypercholesterolemic rats

lipoproteins, forming an early atheroma. The histological results indicated that liver tissues of 5% mushroom-fed hypercholesterolemic rats were almost similar to normocholesterolemic rats. It indicated that the hepatic biosynthesis of cholesterol was suppressed which might be due to a reduction in the activity of the liver enzyme 3-hydroxy-3-methylglutaryl coenzyme-A reductase (Keim *et al*., 1982). Hyperlipidemia is the leading risk factor for atherosclerosis but the atherosclerotic pathological process, could be slowed or reversed by reducing plasma LDL, TGs and phospholipids and increasing plasma 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 (Li *et al*., 2010). Bobek and Galbavy (1999) observed that mushroom prevented the formation of atheromatous plaques and reduced the incidence and extent of atherosclerotic lesions in the aorta and coronary arteries as well as of focal fibrosis of myocardiumin of rabbits.

CONCLUSIONS

Edible mushroom, *P. nebrodensis* is relatively new and commercially available in Korea. The study showed that mushroom significantly reduced body weight and plasma lipid profiles and it had no detrimental effects on the liver and kidney in hypercholesterolemic rats. On the basis of the results, it is suggest that mushroom intake has significant health benefits through the inflection of physiological functions that consist of various atherogenic lipid profiles in hypercholesterolemia. Consequently, *P. nebrodensis* 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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