

Hypolipidemic Effect of Exo-Polymer Produced in Submerged Mycelial Culture of Five Different Mushrooms

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Received: October 1, 2002

Accepted: November 30, 2002

Abstract The hypolipidemic effect of exo-polymer produced in submerged mycelial culture of *Hericium erinaceus* (HE), *Auricularia auricula-judae* (AA), *Flammulina velutipes* (FV), *Pleurotus pini* (PP), and *Grifola frondosa* (GF) was investigated in dietary-induced hyperlipidemic rats. The animals were administered with exo-polymers at the level of 100 mg/kg body weight daily for four weeks. Hypolipidemic effect was achieved in all the experimental groups, however, HE exo-polymer proved to be the most potent one, which significantly reduced the plasma triglyceride (28.9%), total cholesterol (29.7%), low-density lipoprotein (LDL) cholesterol (39.6%), phospholipid (16.0%), and liver total cholesterol (28.9%) level, when compared to the saline administered (control) group. The results of the present investigation strongly demonstrate the potential of HE exo-polymer in combating hyperlipidemia in the experimental animals.

Key words: Exo-polymer, hypolipidemic effect, mushrooms, submerged mycelial culture

Hyperlipidemia is a primary risk factor in the development of cardiovascular disease. It is well established that “Western diets,” known for their high fat, high cholesterol, excess energy, and low fiber contents [5], increase serum cholesterol levels [17]. Recently, lowering of circulating cholesterol (especially LDL cholesterol) levels has been demonstrated to prevent coronary atherosclerosis [31]. Therefore, the search for natural substances capable of lowering blood cholesterol is ongoing in the field of nutrition.

Edible mushrooms are the ideal materials for the dietetic prevention of atherosclerosis, due to their high content of fiber, proteins, microelements, and their low fat content [3, 6, 21]. The hypocholesterolemic effect of some mushrooms has already been studied [18, 32]. Cheung [6, 7] compared

the effects of the fruiting body, mycelia, and exo-polymer produced in submerged fermentation of *Volvariella volvacea*, and suggested that β -glucans in the mushroom were the effective cholesterol-lowering polysaccharides. It is documented that the fiber of *Agaricus bisporus* lowers the serum cholesterol level by increasing the hepatic LDL receptor activity in rats [15]. Sugiyama *et al.* [33] demonstrated that eritadenine, a compound isolated from the fruiting body of *Lentinus edodes*, showed hypocholesterolemic activity by modifying the phospholipid metabolism in rats.

However, the earlier reports on the hypolipidemic effect of mushrooms were mostly restricted to the compounds extracted either from the fruiting bodies or mycelia. Very little effort [6, 35] was made to explore the hypolipidemic potential of the compounds liberated from cultured mycelia into culture media, which, however, were shown to have various bioactive properties [23, 27, 36, 37]. Extraction of these polymeric compounds from the culture media requires relatively simple purification steps [19]. Therefore, it is considered that if the exo-polymer, isolated by relatively simple methods, has the biological activities comparable to that of the endo-polymer, it can be applied to a large scale production process. In view of the above consideration, exo-polymers, of a series of different mushroom species were screened for their hypolipidemic potential. The mushrooms examined in the present study were *H. erinaceus*, *A. auricula-judae*, *F. velutipes*, *P. pini*, and *G. frondosa*.

MATERIALS AND METHODS

Strains and Production of Exo-Polymer

The cultures of *H. erinaceus*, *A. auricula-judae*, *F. velutipes*, *P. pini*, and *G. frondosa* were obtained from the Rural Development Administration in Korea. All organisms were maintained on potato dextrose agar (PDA, Difco) slant at 4°C and subcultured every 3 months. The seed cultures of

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all organisms were grown in 250-ml flasks containing 100 ml of potato dextrose broth (pH 5.0) and incubated on a rotary shaker (120 rpm) at 25°C for approximately 10 days. One hundred ml of the medium with mycelial pellets was then homogenized aseptically in a Sorvall omni-mixer for 3 min in an ice bath [29] and inoculated in the liquid media at the rate of 1% (v/v) for submerged cultivation. The mushroom complete medium (MCM) was used to perform submerged mycelial culture for the production of exo-polymer. The composition of MCM was as follows (g/l): glucose 20, MgSO₄ 0.5, KH₂PO₄ 0.46, K₂HPO₄ 1.0, yeast extract 2, and peptone 2, with the pH was adjusted to 5.0 before sterilization. The submerged mycelial cultures were carried out in 500-ml flasks containing 200 ml of the media on a rotary shaker (120 rpm, pH 5, and 25°C). Culture broth was harvested by centrifugation (10,447 ×g, 20 min) and the supernatant was treated with ethanol. The ethanol precipitate was dissolved in water, dialyzed and lyophilized to obtain an exo-polymer.

Animal Experiments

Sprague-Dawley male rats (60–80 g) obtained from Daehan Biolink Co., Ltd. (Seoul, Korea) were housed individually in stainless steel cages in a room with controlled temperature (22±2°C), humidity (55±5%), and a 12-h cycle of light and dark. The rats were fed with a modified AIN-76 [2] diet for six weeks, which consisted of 55.5% carbohydrates, 14% fats (30% of total energy), 20% protein, 5% fiber, and 0.5% cholesterol by weight. The composition of the diet is shown in Table 1.

The animals were provided with the diets and water *ad libitum*. After two weeks of acclimatization in the growth room, they were brought into the experimental conditions. The exo-polymers of five different mushroom species were screened for their hypolipidemic potential. In this regard, the rats were divided into six experimental groups of eight, based on their body weights. Each group of eight dietary-induced hyperlipidemic rats was orally administered either with saline (control) or the exo-polymers produced from submerged mycelial cultures of *H. erinaceus*, *A. auricula-*

judae, *F. velutipes*, *P. pini*, or *G. frondosa* at a level of 100 g/kg body weight (BW) for four weeks.

The food intake and body weights were recorded every other day and every day, respectively. At the end of the 4-week experiment, the rats fasted for 12 h and then were immediately sacrificed, following an abdominal incision under light ether anesthesia, before blood was collected from the main artery into heparinized tubes. After leaving the samples at room temperature for 2 h, plasma was prepared by centrifugation (1,110 ×g, 10 min). Livers were perfused with cold saline, excised, and kept frozen at -70°C.

Chemical Analysis

The plasma level of triglyceride, total cholesterol, high-density lipoprotein (HDL) cholesterol, and phospholipid in plasma were determined enzymatically using commercial kits (Asan Pharm. Co., Ltd., Chungnam, Korea) based on the methods of glycerol kinase [4], cholesterol oxidase-DAOS [1], phosphotungstic acid-Mg²⁺ precipitation [12], and choline oxidase [34], respectively. LDL cholesterol and atherogenic index (AI) of plasma were calculated by the following equation: LDL cholesterol = total cholesterol - HDL cholesterol - (triglyceride/5) [14], AI = (total cholesterol - HDL cholesterol) / HDL cholesterol. Liver lipid was extracted by treatment with a chloroform/methanol (2:1, v/v) mixture [13]. The liver total cholesterol and triglyceride were assayed using the same method as for the plasma total cholesterol and triglyceride after treatment with Triton X-100 [25].

Statistical Analysis

Each data value is expressed as the mean±SE. The group means were compared using a one-way analysis of variance and Duncan's multiple-range test [10]. The statistical differences were considered significant at *p*<0.05.

RESULTS AND DISCUSSION

Growth Response

The exo-polymers of five different mushrooms were administered to dietary-induced hyperlipidemic rats for four weeks. There were no significant differences in body weight gain, food intake per day, and food efficiency ratio within the experimental groups (Table 2). Moreover, the oral administration of the exo-polymers caused no changes in gross behavior and none of the animals died, which ruled out the possibility of any adverse effect on the experimental animals under the influence of the exo-polymers.

Hypolipidemic Effect

The strongest relationship has been found between the plasma LDL cholesterol and atherosclerosis rather than the total cholesterol. An important recent epidemiological

Table 1. Composition of experimental diet.

Ingredient	Composition (g/kg)
Casein	200
Dextrin	95
Sucrose	460
Lard	90
Soybean oil	50
AIN-mineral mix*	40
AIN-vitamin mix*	10
Cholesterol	5
α-Cellulose	50

*Mineral and vitamin mixture (g/kg mix.) according to AIN-76.

Table 2. Effects of the exo-polymers produced from submerged mycelial cultures of mushrooms on growth parameters of hyperlipidemic rats after four weeks.

Group ¹⁾	Body weight gain (g/day)	Food intake (g/day)	Food efficient ratio ²⁾
Control	7.50±0.10 ^{NS}	24.51±0.25 ^{NS}	0.31
HE	6.66±0.11	22.00±0.18	0.30
AA	6.79±0.07	22.03±0.15	0.31
FV	6.72±0.06	22.78±0.19	0.29
PP	6.62±0.10	22.84±0.19	0.29
GF	6.66±0.14	23.37±0.19	0.28

¹⁾ Described in Materials and Methods.

²⁾ Body weight gain/Food intake.

^{NS} Not significant.

Each value is the mean±SE for eight rats.

finding is that the level of plasma HDL is inversely related to atherosclerosis risk. In other words, an increased HDL level in plasma may play a protective role in it [17].

In the present study, the effect of exo-polymer on plasma total cholesterol, HDL cholesterol, LDL cholesterol, and AI are shown in Table 3. The level in the control group was significantly higher than those in all exo-polymer administered groups. In particular, the plasma total cholesterol level in the HE group (29.7%) was the lowest among the experimental groups. Cheung [6] suggested that the β-glucan was the main factor for the hypocholesterolemic activity of mycelial extracellular polysaccharide produced from submerged culture of *V. volucae*. The hypocholesterolemic effect of many soluble dietary fibers was due to the high viscosity and gel forming properties of dietary fiber, such as pectin and guar gum, thereby interfering with the formation of micelles in the small intestine and increasing the excretion of bile acid and neutral sterol [24, 28]. However, Evans *et al.* [11] suggested that the hypocholesterolemic

Table 3. Effects of the exo-polymers produced from submerged mycelial cultures of mushrooms on plasma total cholesterol, HDL cholesterol, LDL cholesterol, and atherogenic index (AI) in hyperlipidemic rats after four weeks.

Group ¹⁾	Total cholesterol (mg/dl)	HDL cholesterol (mg/dl)	LDL cholesterol ²⁾ (mg/dl)	AI ³⁾
Control	102.27±3.32 ^c	17.41±1.66 ^a	75.24±4.51 ^c	4.86±0.45 ^c
HE	71.73±1.94 ^a	19.61±2.23 ^{bc}	45.43±2.22 ^a	2.66±0.32 ^a
AA	83.87±6.58 ^b	19.24±1.57 ^b	57.47±3.45 ^b	3.36±0.43 ^{ab}
FV	80.07±5.46 ^{ab}	18.10±1.88 ^{ab}	54.45±5.11 ^b	3.42±0.45 ^{ab}
PP	86.42±3.22 ^b	20.37±2.92 ^c	58.75±4.90 ^b	3.24±0.48 ^{ab}
GF	80.88±3.00 ^{ab}	18.98±1.90 ^b	54.54±2.71 ^b	3.26±0.13 ^{ab}

¹⁾ Described in Materials and Methods.

Total cholesterol- HDL cholesterol- (triglyceride/5).

²⁾ (Total cholesterol- HDL cholesterol)/HDL cholesterol.

Each value is mean±SE for eight rats.

³⁾ Values with different superscript letters in the same column indicate significant differences among the groups at *p*<0.05.

effect of galactomannan was not only caused by its viscous effect but also by the composition and the structure itself. Therefore, it is considered that the hypolipidemic effect of exo-polymer may be influenced by the viscous nature and/or chemical composition and structure as well.

In comparison with the control group, all five exo-polymers lowered the level of LDL cholesterol and AI in plasma. The LDL cholesterol level and AI were significantly reduced by the oral administration of the exo-polymers of *H. erinaceus* by 39.6% and 45.3%, respectively. The plasma HDL cholesterol level in the PP group (17.0%) showed the highest increase among the groups.

These results suggest that the decrease of plasma total cholesterol levels in all the treatment groups was due to the lowering of LDL cholesterol levels. Sonoyama *et al.* [30] reported that sugar beet fiber reduces apolipoprotein mRNA in rats. It may be speculated that the effect of all exo-polymers is due to its lowering action on apolipoprotein mRNA or its enhancing action on HDL receptor activity. In general, high dietary cholesterol resulted in elevated plasma cholesterol and this appeared to be related to the suppression of hepatic LDL receptors [22]. Plasma LDL values are affected by modifications of very-low-density lipoprotein (VLDL) metabolism, including the rate of conversion of VLDL to LDL. Basically, nascent VLDL is converted to mature VLDL and to LDL through the loss of triglyceride by the action of lipoprotein lipase. As triglyceride is lost, the concentration of cholesteryl ester also increases, through the action of cholesteryl ester transfer protein. Shen *et al.* [26] suggested that the LDL cholesterol lowering effect of psyllium was due to slow conversion of VLDL to LDL.

In addition, all exo-polymers also reduced the levels of triglyceride and phospholipid in plasma compared with the control group (Table 4). The levels of triglyceride (28.9%) and phospholipid (16.0%) in the HE group were the lowest in all experimental groups as compared to those of the control group. Kinnunen *et al.* [20] demonstrated that the

Table 4. Effects of the exo-polymers produced from submerged mycelial cultures of mushrooms on plasma triglyceride and phospholipid in hyperlipidemic rats after four weeks.

Group ¹⁾	Triglyceride (mg/dl)	Phospholipid (mg/dl)
Control	47.09±3.66 ^c	103.87±8.63 ^c
HE	33.47±2.16 ^a	87.23±1.62 ^a
AA	35.81±1.83 ^{ab}	93.45±5.34 ^{ab}
FV	37.60±4.06 ^b	90.13±6.62 ^{ab}
PP	36.50±2.33 ^{ab}	102.71±5.55 ^c
GF	36.81±2.34 ^{ab}	97.81±4.46 ^b

¹⁾ Described in Materials and Methods.

Each value is mean±SE for eight rats.

^{ab,c} Values with different superscript letters in the same column indicate significant differences among the groups at *p*<0.05.

Table 5. Effects of the exo-polymers produced from submerged mycelial cultures of mushrooms on liver weight, total cholesterol, and triglyceride in hyperlipidemic rats after four weeks.

Groups ¹⁾	Weight (g/100 g BW)	Total cholesterol (mg/g)	Triglyceride (mg/g)
Control	5.04±0.17 ^b	4.98±0.27 ^c	19.25±1.13 ^b
HE	4.68±0.09 ^a	3.54±0.22 ^a	15.87±1.22 ^a
AA	4.51±0.18 ^a	4.53±0.31 ^b	17.09±1.20 ^{ab}
FV	4.68±0.10 ^a	3.85±0.26 ^{ab}	17.94±1.07 ^{ab}
PP	4.91±0.10 ^a	3.83±0.21 ^{ab}	19.18±0.88 ^b
GF	4.68±0.09 ^a	3.75±0.13 ^{ab}	18.99±0.92 ^b

¹⁾Described in Materials and Methods.

Each value is mean±SE for eight rats.

^{a,b,c}Values with different superscript letters in the same column indicate significant differences among the groups at $p < 0.05$.

triglyceride lowering effect in plasma was due to the elevated lipoprotein lipase activity, which catalyzes chylomicron and VLDL on the surface of the endothelium of the capillaries, and Sugiyama *et al.* [33] demonstrated that eritadenine showed hypocholesterolemic activity by modifying phospholipid metabolism in rats.

The effects of the exo-polymers on the weight, total cholesterol, and triglyceride levels in the liver are shown in Table 5. The liver weights were reduced in the HE, AA, FV, PP, and GF groups by 7.1, 10.5, 7.1, 2.6, and 7.1%, respectively, as compared to those of the control group. Moreover, the total cholesterol levels in the liver were lowered in the HE, FV, PP, and GF groups by as much as 28.9, 22.7, 23.1, and 24.7%, respectively. However, the liver triglyceride level in the HE group (17.6%) was the lowest among the experimental groups. It has been demonstrated that the mass of the hepatic cholesterol level is related to the activities of hepatic enzymes of cholesterol synthesis and esterification, and together with the expression of apo B/E receptors, are major determinants of plasma LDL concentrations. Cholesterol accumulation in the liver can result in increased esterification and storage, increased secretion of cholesterol in hepatic lipoproteins, and decreased uptake of plasma cholesterol *via* the LDL receptor [9].

In the present study, the exo-polymers of different fungal groups were screened for their hypolipidemic activity. Among the different fungal exo-polymers, the HE exo-polymer was shown to have the potency to combat hyperlipidemia in alimentary-induced hyperlipidemic rats. Although the exact mechanism of hypolipidemic action of the exo-polymer of HE is not clear at present, the result suggests the fact that different exo-polymers may have different modes of action in the hypolipidemic effect. It also indicates the necessity to perform experiments at a higher dosage. Moreover, further comprehensive chemical and pharmacological investigations are needed to elucidate the exact mechanism of hypolipidemic effects, and to isolate active principles of this mushroom in order for it to

be useful for preventive and therapeutic purposes to alleviate the hyperlipidemic status.

Acknowledgment

This work was supported by a Daegu University research grant, 2002.

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