

## Hypolipidemic Effect of Exo- and Endo-Biopolymers Produced from Submerged Mycelial Culture of *Ganoderma lucidum* in Rats

YANG, BYUNG-KEUN<sup>1</sup>, SANG-CHUL JEONG, AND CHI-HYUN SONG\*

Department of Biotechnology, <sup>1</sup>Research Center for Processing & Application of Agricultural Products, Daegu University, Gyungbuk 712-714, Korea

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**Abstract** The hypolipidemic effect of the exo-biopolymer (EXBP) and endo-biopolymer (ENBP) produced from a submerged mycelial culture of *Ganoderma lucidum* was investigated in dietary-induced hyperlipidemic rats. Hypolipidemic effects were achieved in both the EXBP- and ENBP-treated groups, however, the former proved to be more potent than the latter. The administration of the EXBP (100 mg/kg body weight) substantially reduced the plasma total cholesterol, low-density lipoprotein (LDL) cholesterol, triglyceride, phospholipid levels, and atherogenic index by 31.0, 39.0, 35.4, 28.1, and 53.5%, respectively, when compared to the control group. The EXBP also lowered the liver total cholesterol, triglyceride, and phospholipid levels by 22.4, 23.1, and 12.9%, respectively. Furthermore, the high-density lipoprotein (HDL) cholesterol and ratio of HDL cholesterol to total cholesterol were significantly increased by as much as 24.2% and 47.6%, respectively.

**Key words:** Exo- and endo-biopolymers, hypolipidemic effect, *Ganoderma lucidum*, submerged mycelial culture

Reshi, the fruiting body of *G. lucidum*, has been known since ancient times in Korea, China, Japan, and other countries as an important folk or oriental medicine to treat various human diseases, such as hepatitis, hypertension, hypercholesterolemia, and gastric cancer. Recent studies have also demonstrated that this mushroom has many important biological activities, including antitumor [34], hypotensive [17], hepatoprotective [28], anticomplementary [22], swimming endurance capacity [36], and antiinflammatory [24] effects.

In recent years, considerable attention has been paid to combating hyperlipidemia. It is a well-documented fact that lowering the circulating cholesterol (especially LDL

cholesterol) levels can prevent, arrest, and even reverse coronary atherosclerosis [21, 25, 29]. Several drugs, such as lovastatin and cholesterylamine, are currently used to lower the serum cholesterol, yet they are expensive, and associated adverse side effects limit their use on a broader scale [14]. As such, the search for natural substances to achieve the above goal is ongoing in the field of nutritional research. Various edible mushrooms have already proved themselves as an important natural regimen for controlling hyperlipidemia based on their high fiber, protein, and microelement contents, and low fat content [5, 20, 23]. However, although the hypolipidemic potential of various mushroom species has already been demonstrated [4, 6, 16, 18], most earlier reports are based only on the compounds isolated either from the fruiting bodies or cultured mycelia, while knowledge on the effect of the EXBP produced from a submerged mycelial culture of such mushrooms is rather limited [19, 35]. Chung *et al.* [7] reported on the hypoglycemic activity of a *G. lucidum* fruiting body extract, yet no reports are so far available on the hypolipidemic potential of its submerged culture precipitate.

Accordingly, the present study was undertaken up a detailed investigation to compare the hypolipidemic effect of the EXBP and ENBP of *G. lucidum* extracted from cultured mycelia produced in a submerged culture, by oral administration to dietary-induced hyperlipidemic rats.

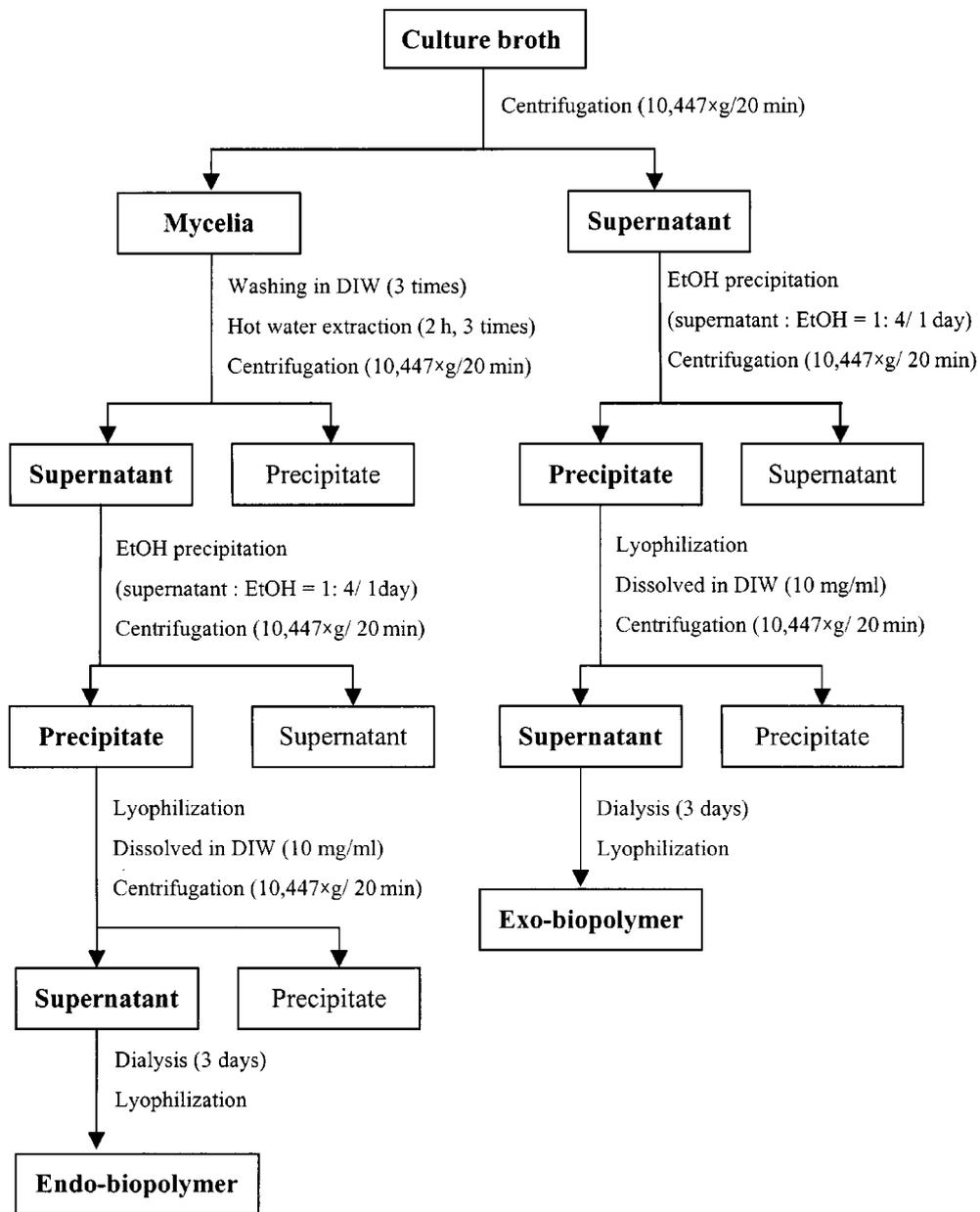
### MATERIALS AND METHODS

#### Strain and Production of EXBP and ENBP

The *G. lucidum* was collected from Kyungbuk province in Korea and then isolated. The culture was grown in a potato/dextrose broth on a rotary shaker (pH 4.5, 120 rpm) at 30°C. After 7 days, 50 ml of the culture broth was homogenized aseptically and inoculated at 1% (v/v) into a culture medium with the following composition (%):

\*Corresponding author

Phone: 82-53-850-6555; Fax: 82-53-850-6559;  
E-mail: chsong@daegu.ac.kr



**Fig. 1.** Schematic diagram depicting recovery process of exo- and endo-biopolymers from submerged mycelial culture of *Ganoderma lucidum*.

galactose 0.1, sucrose 0.9, xylose 0.1, glucose 0.9, yeast extract 0.05, peptone 0.2, potato dextrose broth 0.2,  $\text{NH}_4\text{H}_2\text{PO}_4$  0.05, DL-serine 0.05,  $\text{KH}_2\text{PO}_4$  0.1,  $\text{CaCl}_2$  0.06,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  0.2,  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  0.002,  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  0.002, and  $\text{MnSO}_4 \cdot \text{H}_2\text{O}$  0.002, and the pH was then adjusted to 4.5 before sterilization. The submerged mycelial culture was carried out in 500-ml flasks each containing 200 ml of the medium on a rotary shaker (pH 4.5, 120 rpm) at 30°C for 18 days [28]. The recovery procedure of the EXBP and ENBP from the submerged culture is shown in Fig. 1 [2].

### Animal Experiment

Sprague-Dawley male rats obtained from the Korea Research Institute of Chemical Technology, weighing approximately 100–120 g, were housed individually in a room with controlled temperature (22±2°C), and humidity (55±5%), and a 12-h cycle of light and dark. The rats were fed with a modified AIN-76 diet (Table 1) for 6 weeks [31], which consisted of 55.5% carbohydrate (including 40.5% sucrose), 14.5% fat (30% of total energy), and 20% casein by weight.

The rats were then fed with high fat diets for 2 weeks for the induction of hyperlipidemic state. Thereafter, they

**Table 1.** Composition of experimental diet.

Ingredient	Composition (%)	
	Normal diet	High fat diet
Casein	20.0	20.0
Dextrin	9.5	9.5
Sucrose	55.5	46.5
Lard	-	9.0
Soybean oil	5.0	5.0
AIN-mineral mix*	4.0	4.0
AIN-vitamin mix*	1.0	1.0
Cholesterol	-	4.0
$\alpha$ -Cellulose	5.0	1.0

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

were divided into three treatment groups, based on body weight (BW). Each group was then administered daily for 4 weeks with saline (control), the EXBP, or the ENBP produced from submerged mycelial cultures of *G. lucidum* at a level of 100 mg/kg BW using an oral zonde (Table 2). The Food Intake (FI) and BW were recorded everyday.

At the end of the oral administration of the EXBP and ENBP, the animals were fasted for 14 h, and then sacrificed immediately after an abdominal incision under light ether anesthesia, and blood was collected from the main artery.

### Separation of Plasma and Organs

The blood samples were collected in heparinized tubes and plasma was separated by centrifugation (1,110  $\times$ g for 10 min). Each organ was isolated and their weights were measured after rinsing with 0.9% NaCl. The livers were perfused with cold saline, excised, and kept frozen at  $-70^{\circ}\text{C}$ .

### Chemical Analysis of Plasma and Liver

The total cholesterol, triglyceride, phospholipid, and HDL cholesterol in the plasma were determined using the cholesterol oxidase-DAOS method [1], Bucolo method using glycerol kinase [3], choline oxidase method [10],

and phosphotungstic acid-Mg precipitation method [30], respectively. The LDL cholesterol [11], atherogenic index, and ratio of HDL cholesterol to total cholesterol (HTR) were calculated using the following equations: LDL cholesterol = total cholesterol - HDL cholesterol - (triglyceride/5), Atherogenic index = (total cholesterol - HDL cholesterol)/HDL cholesterol, HTR = HDL cholesterol/total cholesterol.

The liver lipid was extracted by the method of Folch *et al.* [12]. Liver total cholesterol, triglyceride, and phospholipid levels were assayed, using the same method as for the plasma total cholesterol, triglyceride, and phospholipid after treatment with Triton X-100 [26].

### Statistical Analysis

Each data value is expressed as the mean  $\pm$  SE. The group means were compared using a one-way analysis of variance and Duncan's multiple-range test [9]. The statistical differences were considered significant at  $p < 0.05$ .

## RESULTS AND DISCUSSION

### Effect on BW, FI, and Organ Weight

The effect of the EXBP and ENBP produced from a submerged mycelial culture of *G. lucidum* on BW, FI, and food efficiency ratio is presented in Table 3. During the 4-week period, the rats in all the experimental groups remained healthy and showed normal weight gain. No significant differences in either BW or FI were observed under the influence of the EXBP or ENBP of *G. lucidum*. Similarly, the food efficiency ratio did not differ significantly within the experimental groups.

The influence of the EXBP and ENBP on the weight of various organs from the experimental animals is presented in Table 4. The weights of the heart, kidneys, and spleen were not significantly different among the experimental groups. However, the liver weight from the EXBP-fed group was significantly lowered when compared to the control group. The increased liver weight in hyperlipidemic rats is due to accumulation of an excess amount of lipid in the

**Table 2.** Experimental groups for identifying hypolipidemic activity.

Group	Oral administration
Normal <sup>1</sup>	None
Control <sup>2</sup>	0.9% NaCl
EXBP <sup>2</sup>	Exo-biopolymer produced from submerged mycelial culture of <i>Ganoderma lucidum</i>
ENBP <sup>2</sup>	Endo-biopolymer produced from submerged mycelial culture of <i>Ganoderma lucidum</i>

<sup>1</sup>Group of 8 normal rats.

<sup>2</sup>Group of 8 hyperlipidemic rats. The rats in each experimental group were orally administered with either saline (control), the EXBP, or the ENBP at 100 mg/kg body weight daily for 4 weeks.

**Table 3.** Effect of *Ganoderma lucidum* exo- and endo-biopolymers on growth parameters of hyperlipidemic rats after 4 weeks.

Group <sup>1</sup>	Body weight gain (g/day)	Food intake (g/day)	Food efficiency ratio <sup>2</sup>
Normal	7.54 $\pm$ 0.16 <sup>NS</sup>	26.25 $\pm$ 0.61 <sup>NS</sup>	0.30 $\pm$ 0.01 <sup>NS</sup>
Control	7.13 $\pm$ 0.22	23.13 $\pm$ 0.80	0.31 $\pm$ 0.02
EXBP	7.18 $\pm$ 0.12	22.31 $\pm$ 0.46	0.32 $\pm$ 0.01
ENBP	7.26 $\pm$ 0.21	22.51 $\pm$ 0.34	0.32 $\pm$ 0.02

<sup>1</sup>See Table 2.

<sup>2</sup>Body weight gain/Food intake.

<sup>NS</sup>Not significant.

Each value is the mean  $\pm$  SE for 8 rats.

**Table 4.** Effect of *Ganoderma lucidum* exo- and endo-biopolymers on weights of various organs from hyperlipidemic rats after 4 weeks.

Group <sup>1</sup>	Liver (g/100 g BW)	Heart (g/100 g BW)	Kidneys (g/100 g BW)	Spleen (g/100 g BW)
Normal	3.62±0.14 <sup>a</sup>	0.33±0.01 <sup>NS</sup>	0.75±0.06 <sup>NS</sup>	0.18±0.01 <sup>NS</sup>
Control	5.28±0.10 <sup>c</sup>	0.29±0.01	0.66±0.01	0.15±0.00
EXBP	4.64±0.14 <sup>b</sup>	0.30±0.01	0.65±0.02	0.17±0.01
ENBP	4.83±0.12 <sup>bc</sup>	0.30±0.01	0.65±0.02	0.16±0.01

<sup>1</sup>See Table 2.

<sup>NS</sup>Not significant.

Each value is the mean±SE for 8 rats.

<sup>a,b,c</sup>Values with different superscript letters in the same column indicate significant differences among the groups at *p*<0.05.

**Table 5.** Effect of *Ganoderma lucidum* exo- and endo-biopolymers on plasma total cholesterol, LDL cholesterol, triglyceride, and phospholipid in hyperlipidemic rats after 4 weeks.

Group <sup>1</sup>	Total cholesterol (mg/dl)	LDL cholesterol <sup>2</sup> (mg/dl)	Triglyceride (mg/dl)	Phospholipid (mg/dl)
Normal	90.62±3.84 <sup>a</sup>	59.99±3.24 <sup>a</sup>	31.50±3.12 <sup>a</sup>	89.03±6.11 <sup>a</sup>
Control	143.26±7.26 <sup>d</sup>	115.70±4.12 <sup>d</sup>	59.94±4.68 <sup>c</sup>	131.36±6.76 <sup>c</sup>
EXBP	98.86±3.89 <sup>ab</sup>	70.56±3.17 <sup>b</sup>	38.74±3.05 <sup>ab</sup>	94.40±2.49 <sup>ab</sup>
ENBP	120.10±1.27 <sup>c</sup>	93.06±2.89 <sup>c</sup>	43.77±2.93 <sup>b</sup>	103.58±3.57 <sup>b</sup>

<sup>1</sup>See Table 2.

<sup>2</sup>Total cholesterol - HDL cholesterol - (Triglyceride/5).

Each value is the mean±SE for 8 rats.

<sup>a,b,c,d</sup>Values with different superscript letters in the same column indicate significant differences among the groups at *p*<0.05.

liver tissue [15]. Shen *et al.* [27] and Dietschy *et al.* [8] reported that a reduced plasma LDL cholesterol level in dietary-induced hyperlipidemic animals results in an improper lipid metabolism leading to an enhanced level of hepatic acetyl-CoA, which in turn participates in lipogenesis and is accumulated as lipid in the liver tissue. As such, the reduced liver weight found in the present investigation indicates the hypolipidemic potential of the EXBP.

In the current study, the oral administration of the EXBP and ENBP caused no changes in the gross behavior, and none of the animals died. Thus, it would appear that there were no side effects in the rats by the oral administration of the EXBP and ENBP of *G. lucidum*.

**Effect on Levels of Plasma and Liver Lipid**

As seen in Table 5, the plasma total cholesterol, LDL cholesterol, triglyceride, and phospholipid levels were significantly decreased in the rats administered with the EXBP or ENBP, as compared to the control group. However, the EXBP showed a better hypolipidemic potential than the ENBP and lowered the plasma total cholesterol, LDL cholesterol, triglyceride, and phospholipid levels by as much as 31.0, 39.0, 35.4, and 28.1%, respectively, when compared to the control group.

The plasma HDL cholesterol level and HTR were increased, and the plasma atherogenic index was decreased in the rats administered with the EXBP or ENBP, where the EXBP also proved to be more effective (Table 6). The EXBP substantially increased the plasma HDL cholesterol and HTR by 24.2% and 47.6%, respectively, and lowered

the atherogenic index by as much as 53.5%, compared to the control group.

The effects of the *G. lucidum* EXBP and ENBP on the liver lipids are shown in Table 7. The concentrations of the total cholesterol, triglyceride, and phospholipid in the liver of the rats administered with the EXBP were significantly decreased to the extent of 22.4, 23.1, and 12.9%, respectively, and by as much as 15.3, 8.4, and 7.0% in the rats given the ENBP, respectively, as compared to the control group.

In light of the present knowledge, the risk of atherosclerosis is dependent more on the plasma LDL level than on the total cholesterol level in the body system. An important recent epidemiological finding demonstrated that the level of HDL is inversely related to the risk of atherosclerosis. In

**Table 6.** Effect of *Ganoderma lucidum* exo- and endo-biopolymers on plasma HDL cholesterol, atherogenic index, and ratio of HDL cholesterol to total cholesterol (HTR) in hyperlipidemic rats after 4 weeks.

Group <sup>1</sup>	HDL cholesterol (mg/dl)	Atherogenic index <sup>2</sup>	HTR <sup>3</sup>
Normal	24.33±1.38 <sup>c</sup>	2.72±0.03 <sup>a</sup>	0.27±0.01 <sup>c</sup>
Control	15.57±1.21 <sup>a</sup>	8.20±0.32 <sup>d</sup>	0.11±0.00 <sup>a</sup>
EXBP	20.55±1.07 <sup>bc</sup>	3.81±0.13 <sup>b</sup>	0.21±0.01 <sup>b</sup>
ENBP	18.29±1.16 <sup>b</sup>	5.57±0.26 <sup>c</sup>	0.15±0.01 <sup>ab</sup>

<sup>1</sup>See Table 2.

<sup>2</sup>(Total cholesterol- HDL cholesterol)/ HDL cholesterol.

<sup>3</sup>HDL cholesterol/Total cholesterol.

Each value is the mean±SE for 8 rats.

<sup>a,b,c,d</sup>Values with different superscript letters in the same column indicate significant differences among the groups at *p*<0.05.

**Table 7.** Effect of *Ganoderma lucidum* exo- and endo-biopolymers on liver total cholesterol, triglyceride, and phospholipid in hyperlipidemic rats after 4 weeks.

Group <sup>1</sup>	Total cholesterol (mg/g)	Triglyceride (mg/g)	Phospholipid (mg/g)
Normal	1.84±0.04 <sup>a</sup>	7.82±0.78 <sup>a</sup>	7.11±0.18 <sup>a</sup>
Control	5.77±0.22 <sup>c</sup>	19.25±2.05 <sup>bc</sup>	9.32±0.13 <sup>c</sup>
EXBP	4.48±0.24 <sup>b</sup>	14.81±1.47 <sup>b</sup>	8.12±0.19 <sup>ab</sup>
ENBP	4.89±0.13 <sup>bc</sup>	17.64±0.92 <sup>b</sup>	8.67±0.26 <sup>b</sup>

<sup>1</sup>See Table 2.<sup>NS</sup>Not significant.

Each value is the mean±SE for 8 rats.

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

other words, an increased HDL level in plasma could play a protective role [13]. A substantial reduction of LDL and total cholesterol in plasma can be achieved by reduced production of total cholesterol by liver tissue and efficient removal of LDL cholesterol by various tissues without subsequent renewal [32]. Therefore, in the current investigation, the reduced lipid level in the liver tissues under the influence of the EXBP and ENBP was consistent with the above supposition. This was achieved by controlling the lipid metabolism in the liver tissue, being most likely mediated by the influence of the EXBP and ENBP.

Our present preliminary study compared the hypolipidemic potential of the EXBP and ENBP of *G. lucidum*, and the EXBP was found to be more potent. Although the exact mechanism of the EXBP of *G. lucidum* in exhibiting a hypolipidemic effect is still unclear, the possibility of a combined effect of the EXBP in exerting hyperlipidemia can not be ruled out. This includes inhibition of cholesterol absorption and biosynthesis, inhibition of biosynthesis of very-low-density lipoproteins, the precursor of LDL, and acceleration of fractional turnover of LDL [32], and increased excretion of bile acids [33]. Accordingly, further comprehensive chemical and pharmacological investigations are needed to elucidate the exact mechanism of the hypolipidemic effects and isolate the active principles before they are usefully employed for preventive and therapeutic purposes in alleviating a hyperlipidemic status.

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