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Hypoglycemic Effect of Exo- and Endo-biopolymers Produced by Submerged Mycelial Culture of *Ganoderma lucidum* in Streptozotocin-Induced Diabetic Rats

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Abstract The hypoglycemic effect of an exo-biopolymer (EXO) and endo-biopolymer (ENDO) produced from submerged mycelial culture of *Ganoderma lucidum* was investigated in streptozotocin (STZ)-induced diabetic rats. Both the EXO and ENDO showed hypoglycemic potential, however, the former proved to be more potent than the latter. The administration of the EXO at the dose of 100 mg/kg body weight (BW) significantly reduced the plasma glucose level (23.5%) and increased the plasma insulin level (2.2 fold) in the diabetic animals. The EXO also lowered the plasma total cholesterol, triglyceride, low-density lipoprotein (LDL) cholesterol, and athrogenic index by 14.7, 31.4, 24.1, and 45.4%, respectively, and reduced the liver total cholesterol and triglyceride levels by 6.7 and 25.8%, respectively. It increased the plasma high-density lipoprotein (HDL) cholesterol (37.7%), compared to the control group. Furthermore, the alanine transaminase (ALT) and aspartate transaminase (AST) showed lower activities in the EXO administered groups than the other experimental groups. Taken together, these results suggest that the exo-biopolymer may alleviate the blood glucose level by increased insulin secretion.

Key words: Endo-biopolymer, exo-biopolymer, hypoglycemic effect, *Ganoderma lucidum*, submerged mycelial culture

Ganoderma lucidum is a basidiomycete, lamellaless fungus belonging to the family of Polyporaceae. Its fruiting body has long been used in China, Japan, and Korea as a traditional or folk medicine for the treatment of various diseases. Recent studies on this mushroom have demonstrated many interesting biological activities, including antitumor [32], hypotensive [19], cytotoxicity [23], anticomplementary [21], antimicrobial [36], hepatoprotective [37], swimming endurance [35], hypolipidemic [34], and anti-inflammatory [25] effects. Several studies have already been carried out on the hypoglycemic effect of the extracts of *G. lucidum* fruiting body and mycelia [16, 17, 20]. The bioactive biopolymer produced from a submerged mycelial culture of *G. lucidum* has also recently been investigated, because the production process from a culture broth requires only relatively simple purification steps [4]. However, reports on the hypoglycemic effect of the biopolymers secreted by the culture mycelia (i.e. exo-biopolymer) into the culture media are scarce, and there are no reports available so far concerning the hypoglycemic effect of the EXO produced from *G. lucidum*.

Accordingly, the present study was undertaken to compare in detail the hypoglycemic effects of the EXO and ENDO, produced by a submerged mycelial culture of *G. lucidum*, by oral administration to streptozotocin-induced diabetic rats.

Materials and Methods

Strain and Production of EXO and ENDO

The *G. lucidum* was collected in the Gyungbuk Province, Korea. 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 were aseptically homogenized and inoculated at 1% (v/v) in a culture medium with the following composition (g/l): galactose, 1; sucrose, 9; xylose, 1; glucose, 9; yeast extract, 0.5; peptone, 2; potato dextrose, 2; $NH_4H_2PO_4$, 0.5; DL-serine, 0.5; KH_2PO_4, 1; $CaCl_2$, 0.6; $MgSO_4 \cdot 7H_2O$, 2; $FeSO_4 \cdot 7H_2O$, 0.02; $ZnSO_4 \cdot 7H_2O$, 0.02; $MnSO_4 \cdot H_2O$, 0.02; the pH adjusted to 4.5 before sterilization. The submerged mycelial culture was carried out in 500-ml flasks, containing 200 ml of the medium on a rotary shaker

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(pH 4.5, 120 rpm) at 30°C for 18 days. The supernatant and mycelia were harvested from culture broth and were treated with ethanol. The ethanol precipitate was dissolved in water, dialyzed, and lyophilized to obtain an EXO and ENDO, as described previously [33, 34].

Animals and Breeding Condition

Sprague-Dawley male rats (5 weeks of age) obtained from Daehan Biolink Co., Ltd. were housed individually in stainless steel cages in a room with controlled temperature (22±2) and humidity (55±5%), and a 12-h cycle of light and dark. The rats were fed with a commercial pelleted diet (Sam Yang Co., Korea) throughout the experimental period.

Induction of Diabetes and Experimental Design

The rats were adapted for 7 days in the growth room and then fasted for 12 h before an intramuscular injection of STZ [18] (Sigma, 50 mg/kg BW, dissolved in a citrate buffer at pH 4.5). Two days after the STZ treatment, the rats were considered to be diabetic when the non-fasting blood glucose concentrations were higher than 300 mg/dl. The diabetic state was further confirmed by the positive response to glucose in the urine (test strips; Glucotest, Germany). Thereafter, the animals were used as an insulin-dependent diabetes mellitus (IDDM) model. The rats of each group (Table 1) were administered with saline (control), EXO or ENDO at the level of 100 mg/kg BW, using an oral zonde daily for 2 weeks. The food intake and BW were recorded everyday.

At the end of this oral administration, the animals were fasted for 9 h and then immediately sacrificed following an abdominal incision under light ether anesthesia, and blood was then collected from the main artery.

Separation of Plasma and Organ

Blood samples were collected in heparinized tubes, and plasma was separated by centrifugation (1,110 ×*g*/10 min). Each organ was isolated and weighed after washing with 0.9% NaCl. Livers were perfused with cold saline, excised, and kept frozen at -70°C.

Biochemical Assay

The plasma glucose and insulin levels were measured using a glucose oxidase kit (glucose B-test, Wako Chemicals, Japan) [26] and by ^{125}I-radioimmunoassay (Coat-A-Count Insulin kit, DPC Co., LA, U.S.A.), respectively [5]. The plasma total cholesterol, triglyceride, phospholipid, HDL cholesterol, ALT, and AST levels were evaluated by enzymatic test kits (Asan Pharm. Co., Korea). LDL cholesterol [9] and atherogenic index were calculated by the following equations: LDL cholesterol=Total cholesterol-HDL cholesterol-(Triglyceride/5), Atherogenic index=(Total cholesterol-HDL cholesterol)/HDL cholesterol.

Liver lipid was extracted by the method of Folch *et al.* [7]. The liver total cholesterol and triglyceride were assayed by the same method as for the plasma total cholesterol and triglyceride after treatment with Triton X-100 [28].

Statistical Analysis

Each data were expressed as mean±SE. Group means were compared by a one-way analysis of variance and by Duncan's multiple-range test [6]. Statistical differences were considered significant at $p<0.05$.

Results and Discussion

BW Gain, Food Intake, and Organ Weight

The effects of EXO and ENDO produced from the submerged mycelial culture of *G. lucidum* on BW gain, food intake, and food efficiency ratio in diabetic rats are shown in Table 2. The BW and food intake were generally low with overeating in an STZ-induced diabetic rat, however, recovered when the animals were subjected to a hypoglycemic treatment [10]. In the present investigation, ENDO influenced less significantly on the BW gain, food intake, or food efficiency ratio of the experimental animals

Table 1. Experimental protocols in search of hypoglycemic activity.

Groups	Oral administration
Normal[1]	None
Control[2]	0.9% NaCl
EXO[2]	Exo-biopolymer produced from the submerged mycelial culture of *Ganoderma lucidum*
ENDO[2]	Endo-biopolymer produced from the submerged mycelial culture of *Ganoderma lucidum*

[1]Normal rats for 8 rats.
[2]Diabetic rats induced by streptozotocin (50 mg/kg body weight) for 8 rats. Rats of each experimental group were administered orally with either saline (control) or EXO or ENDO at 100 mg/kg body weight daily for 2 weeks.

Table 2. Effect of *Ganoderma lucidum* EXO and ENDO on growth parameters of streptozotocin-induced diabetic rats after 2 weeks.

Group[1]	BW gain (g/day)	Food intake (g/day)	Food efficiency ratio[2]
Normal	8.02±0.28^{b}	25.41±0.92^{a}	0.32±0.02^{c}
Control	6.83±0.16^{a}	34.94±1.40^{b}	0.20±0.01^{a}
EXO	7.44±0.12^{ab}	29.65±0.93^{ab}	0.26±0.01^{b}
ENDO	7.19±0.24^{ab}	32.30±1.66^{b}	0.22±0.01^{ab}

[1]See Table 1.
[2]Body weight gain/Food intake.
Each value is mean±SE for 8 rats.
^{a,b,c}Values with different superscript letters in the same column significantly different among the groups at $p<0.05$.

Table 3. Effect of *Ganoderma lucidum* EXO and ENDO on weights of the various organs in streptozotocin-induced diabetic rats after 2 weeks.

Group[1]	Liver (g/100 g BW)	Kidney (g/100 g BW)	Spleen (g/100 g BW)	Pancreas (g/100 g BW)
Normal	3.60±0.07[a]	0.81±0.03[NS]	0.23±0.01[NS]	0.21±0.01[b]
Control	4.37±0.10[c]	1.03±0.07	0.27±0.01	0.15±0.01[a]
EXO	3.86±0.04[ab]	0.94±0.04	0.24±0.01	0.17±0.01[a]
ENDO	4.11±0.11[bc]	1.01±0.07	0.25±0.01	0.16±0.01[a]

[1]See Table 1.
[NS]Not significant.
Each value is mean±SE for 8 rats.
[a,b,c]Values with different superscript letters in the same column significantly different among the groups at $p<0.05$.

as compared to those of the control group. However, a significant increase in the BW gain and food efficiency ratio was observed in the EXO administered group. In the case of food intake, the EXO group has significantly reduced intake, compared to the control group. In general, the oral administration of EXO and ENDO caused no changes in gross behavior, and none of the animals died, ruling out the possibility of any harmful effect on rats by the oral administration of EXO and ENDO.

The effects of EXO and ENDO on various organs in terms of weight are presented in Table 3. EXO and ENDO did not have much influence on the weight of the kidneys, spleen, and pancreas of the experimental animals. However, the weight of the liver in the EXO fed group was considerably lower than that of the control group. The increase of liver weight in STZ-induced diabetic rats is due to the accumulation of lipid in the liver [24, 29]. Grey *et al.* [14] and Foster & MaGarry [8] reported that a reduced insulin level in the diabetic animals results in improper glucose metabolism, leading to the enhancement of acetyl-CoA level which is used in lipogenesis and accumulates as lipid in the liver tissue.

Effect on Plasma Glucose and Insulin Levels

The effects of EXO and ENDO on plasma glucose retention and insulin level are shown in Table 4. Significant reduction in plasma glucose level and increase in plasma insulin concentration in both the EXO and ENDO groups were observed with different extent. As much as 23.5% reduction in plasma glucose level and 2.2-fold increase in the plasma insulin level were achieved under the influence of EXO, whereas only 14.7% decrease in plasma glucose level and 1.5-fold increase in insulin level were achieved under the influence of ENDO (Table 4).

Table 4. Effect of the *Ganoderma lucidum* EXO and ENDO on plasma glucose and insulin levels in streptozotocin-induced diabetic rats after 2 weeks.

Group[1]	Glucose (mg/dl)	Insulin (μIU/ml)
Normal	112.09±3.04[a]	5.45±0.17[d]
Control	181.86±7.13[c]	1.31±0.13[a]
EXO	139.18±6.07[ab]	2.83±0.09[c]
ENDO	155.16±5.81[bc]	1.94±0.14[ab]

[1]See Table 1.
Each value is mean±SE for 8 rats.
[a,b,c,d]Values with different superscript letters in the same column are significantly different ($p<0.05$).

It is reported that the STZ-treatment inhibits insulin secretion by the pancreas through selective destruction of β-cells of the pancreatic islets [22, 30]. Therefore, it seems that the biopolymers obtained from *G. lucidum* probably repaired the damage of the pancreatic β-cells to some extent and promoted insulin synthesis, consequently influencing glucose metabolism and accumulating glycogen in muscle and liver tissue. The observation made by Gray and Flatt [13] with *Agaricus campestris* aqueous extract might support this postulate. They demonstrated that an aqueous extract of mushroom stimulated 2-deoxyglucose transport, glucose oxidation, and incorporation of glucose into glycogen in abdominal muscle in mice treated with STZ. They also documented stimulation of insulin secretion from the BRIN-BD11 pancreatic β-cell line under the influence of *A. campestris* aqueous extract.

Effect on Plasma and Liver Lipid Levels

Table 5 shows the effect of EXO and ENDO on plasma total cholesterol, triglyceride, and phospholipid in diabetic rats. As compared to the control group, the plasma total cholesterol and triglyceride levels were reduced as much as 14.7% and 31.4% by EXO, and 7.0% and 27.5% by ENDO, respectively. However, no significant change in the plasma phospholipid level was detected among all the experimental groups.

The changes in the levels of plasma HDL cholesterol, LDL cholesterol, and atherogenic index in diabetic rats under the influences of EXO and ENDO are depicted in Table 6. A significant reduction in plasma LDL cholesterol level and atherogenic index was noticed in both the EXO

Table 5. Effect of *Ganoderma lucidum* EXO and ENDO on plasma total cholesterol, triglyceride, and phospholipid in streptozotocin-induced diabetic rats after 2 weeks.

Group[1]	Total cholesterol (mg/dl)	Triglyceride (mg/dl)	Phospholipid (mg/dl)
Normal	78.07±3.84[a]	28.89±1.58[a]	118.54±4.12[NS]
Control	100.57±5.26[d]	47.34±1.69[c]	123.88±1.20
EXO	85.77±3.89[b]	32.46±0.65[ab]	120.32±3.62
ENDO	93.56±4.27[bc]	34.32±2.83[b]	120.91±2.14

[1]See Table 1.
[NS]Not significant.
Each value is mean±SE for 8 rats.
[a,b,c,d]Values with different superscript letters in the same column significantly different among the groups at $p<0.05$.

Table 6. Effect of *Ganoderma lucidum* EXO and ENDO on plasma HDL cholesterol, LDL cholesterol, and atherogenic index in streptozotocin-induced diabetic rats after 2 weeks.

Group[1]	HDL cholesterol (mg/dl)	LDL cholesterol[2] (mg/dl)	Atherogenic index[3]
Normal	25.29±1.71^{c}	47.00±3.24^{a}	2.09±0.02^{a}
Control	16.35±1.34^{a}	74.75±4.12^{b}	5.15±0.15^{d}
EXO	22.52±1.02^{bc}	56.76±3.17^{a}	2.81±0.07^{ab}
ENDO	18.89±1.08^{ab}	69.81±2.89^{a}	4.06±0.09^{c}

[1]See Table 1.
[2]Total cholesterol-HDL cholesterol-(Triglyceride/5).
[3](Total cholesterol-HDL cholesterol)/HDL cholesterol.
Each value is mean±SE for 8 rats.
[a-d]Values with different superscript letters in the same column significantly different among the groups at $p<0.05$.

and ENDO groups. However, the EXO group performed better than the latter, and decreased the LDL cholesterol level as much as 24.1%, and increased the HDL cholesterol level as much as 37.7%, as compared to the control group. A substantial decrease in the atherogenic index (45.4%) was also found under the influence of the EXO.

The effects of EXO and ENDO on the concentrations of liver total cholesterol, triglyceride, and phospholipid are presented in Table 7. The liver total cholesterol and triglyceride levels in the EXO group were significantly lower than the control, whereas the effect of ENDO was less significant. There was no significant change in the phospholipid level in both the EXO and ENDO administered groups. As much as 6.7% and 25.8% reductions in liver total cholesterol and triglyceride levels, respectively, were achieved under the influence of EXO.

The plasma cholesterol and triglyceride levels are related to the diabetic degree in IDDM rats [1, 15]. Abnormalities observed in lipid metabolism in the diabetic state include increased plasma triglyceride and cholesterol levels, and decreased plasma HDL cholesterol [12, 27]. Increased mobilization of free fatty acids and decreased clearance due to improper lipoprotein lipase (LPL) activity result in elevated levels of triglyceride and very-low-density lipoprotein (VLDL) [2] in blood plasma. Insulin regulates both secretion of VLDL into plasma from the liver and its removal at the peripheral tissue through the action of endothelial LPL [2, 11]. The low insulin level in STZ-treated diabetic rats could affect LPL function and result in high triglyceride level, thereby affecting the normal level of total cholesterol, LDL cholesterol, HDL cholesterol, and phospholipid. In the present investigation, a substantial decrease in total cholesterol, triglyceride, phospholipid, LDL cholesterol, and atherogenic index, and increase in HDL cholesterol and HDL-cholesterol/total cholesterol ratio in the diabetic rats were achieved by oral administration of *G. lucidum* EXO, and also ENDOat a lesser extent.

Table 7. Effect of *Ganoderma lucidum* EXO and ENDO on liver total cholesterol, triglyceride, and phospholipid in streptozotocin-induced diabetic rats after 2 weeks.

Group[1]	Total cholesterol (mg/g)	Triglyceride (mg/g)	Phospholipid (mg/g)
Normal	1.78±0.05^{a}	3.16±0.03^{a}	4.56±0.13^{NS}
Control	1.95±0.04^{d}	4.31±0.19^{c}	5.87±0.19
EXO	1.82±0.04^{b}	3.20±0.08^{ab}	5.07±0.16
ENDO	1.90±0.03^{bc}	3.97±0.07^{b}	5.40±0.13

[1]See Table 1.
[NS]Not significant.
Each value is mean±SE for 8 rats.
[a-d]Values with different superscript letters in the same column significantly different among the groups at $p<0.05$.

Effect on ALT and AST Levels

Generally, ALT and AST activities are increased when the liver functions abnormally [3], which can be used as markers to indicate the extent of liver damage. In the present study, the high plasma level of triglyceride and cholesterol of the diabetic animals might be due to abnormal liver function, due to the damage by STZ either directly or indirectly by enhancing the plasma glucose level [31]. The effects of EXO and ENDO on plasma ALT and AST levels are shown in Table 8. Although ENDO slightly reduced the levels of plasma ALT and AST, EXO lowered these levels more effectively (27.8 and 12.9%, respectively) than the control group.

This signifies the fact that *G. lucidum* EXO might play a corrective role in liver function either by reducing the blood glucose level or increasing the plasma insulin level, thereby resulting in reduction of lipid levels in the blood plasma and liver of diabetic animals.

The present preliminary study involved a comparative assessment of the hypoglycemic potential of EXO and ENDO of *G. lucidum*, and showed EXO to be more potent. Although the exact mechanism involved is not clearly understood. The results suggest a combination of mechanisms involved in exhibiting the hypoglycemic effect. Further comprehensive chemical and pharmacological investigations are needed to elucidate the exact mechanism of hypoglycemic

Table 8. Effect of *Ganoderma lucidum* EXO and ENDO on plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in streptozotocin-induced diabetic rats after 2 weeks.

Group[1]	ALT (IU/l)	AST (IU/l)
Normal	27.55±1.12^{a}	109.33±3.36^{a}
Control	44.72±2.22^{c}	137.42±4.57^{c}
EXO	32.27±0.97^{ab}	119.68±2.76^{ab}
ENDO	38.68±2.34^{b}	131.95±3.80^{bc}

[1]See Table 1.
Each value is mean±SE for 8 rats.
[a,b,c]Values with different superscript letters in the same column significantly different among the groups at $p<0.05$.

effects and to study the active principles before their application to preventive and therapeutic purposes to alleviate the hypoglycemic status in diabetes mellitus.

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