Hypoglycemic Effect of a *Lentinus edodes* Exo-polymer Produced from a Submerged Mycelial Culture

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The hypoglycemic effect of an exo-polymer produced from a submerged mycelial culture of *Lentinus edodes* was investigated in streptozotocin-induced diabetic rats. The administration of the exo-polymer (200 mg/kg BW) reduced the plasma glucose level by as much as 21.5%, and increased plasma insulin by 22.1% as compared to the control group. It also lowered the plasma total cholesterol and triglyceride levels by 25.1 and 44.5%, respectively. Gel chromatography of the exo-polymer revealed a single peak which is likely to have been a glycoprotein with a molecular weight of 52 kDa and was found to contain 83.5% carbohydrate and 16.5% protein. The Sugar and amino acid compositions of the exo-polymer were analyzed in detail.

Key words: exo-polymer; hypoglycemic effect; *Lentinus edodes*; submerged mycelial culture

Since the anti-tumoral activity of *Lentinus edodes* was first reported by Chihara et al., it has proved its efficacy for various other diseases and has long been used as an elixir. The hypoglycemic effect of *L. edodes* has been demonstrated by Kim et al. who proved its potential in lowering the blood glucose and TG levels in the serum of rats. Various mushroom species have also been reported for their hypoglycemic effect. However, most of the findings on this hypoglycemic effect were from either the fruiting bodies or mycelia. Bioactive EP produced from a submerged mycelial culture of mushrooms has also recently been investigated, because the production process from a culture broth requires only relatively simple purification. There have been a number of reports on the isolation of various bioactive components from the culture precipitate of *L. edodes*, however, no reports of EP elicted by *L. edodes* showing hypoglycemic activity have been published.

The present study reports the isolation of water-soluble EP from a culture broth of *L. edodes*, a dose-dependent analysis of EP by oral administration to STZ-induced diabetic rats, and an analysis of the sugar and amino acid compositions of EP.

Materials and Methods

**Organism, growth and production of EP.** The culture of *L. edodes* was kindly presented by Dr. K. Y. Cho (Dept. of Microbiology, The University of Sydney). The culture was grown in a potato/dextrose broth on a rotary shaker (120 rpm) at 25°C. After 10 days, 50 ml of the culture broth was aseptically homogenized and inoculated at 1% (v/v) into a culture medium with the following composition (g/l): potato/dextrose broth, 24; malt extract, 10; peptone, 1; the pH value was adjusted to 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 (120 rpm) at 25°C for 24 d. The recovery procedure for EP from the submerged culture is shown in Fig. 1.

**Animals and breeding conditions.** Sprague-Dawley male rats (5 weeks of age) obtained from Korea Research Institute of Chemical Technology were housed individually in stainless steel cages 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 commercial pelleted diet (Sam Yang Co., Korea) throughout the experimental period.

**Induction of diabetes and oral administration of EP.** The rats were adapted for 7-10 d and then fasted for 12 h before an intramuscular injection of STZ

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**Abbreviations:** EP, exo-polymer; STZ, streptozotocin; BW, body weight; TC, total cholesterol; TG, triglyceride; ALT, alanine transaminase; AST, aspartate transaminase; LPL, lipoprotein lipase; VLDL, very-low-density lipoprotein
(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 concentration was higher than 300 mg/dl, and the diabetic state was further confirmed by the positive response to glucose in urine (test strips; Glucotest, Germany). Thereafter, the animals were used as an insulin-dependent diabetes mellitus (IDDM) model. The rats of each group were administered (between 08.00 and 10.00 hr) with saline (control) and EP at the level of 50–200 mg/kg BW, using an oral zonde daily for seven days. At the end of this oral administration, the animals were fasted for 9 h and then immediately sacrificed (between 08.00 and 10.00 hr) following an abdominal incision under light ether anesthesia, before blood was collected from the main artery. The food intake and body weight were respectively recorded every day and weekly.

Biochemical assay. Blood samples were collected in heparinized tubes, and the plasma was separated by centrifugation at 1,100 × g for 10 min. The plasma glucose and insulin levels were respectively measured with a glucose oxidase kit (glucose B-test, Wako Pure Chemicals, Japan) and by a 125I radioimmunoassay (Coat-A-Count Insulin kit, DPC Co., LA, U.S.A.). The plasma TC, TG, ALT and AST levels were evaluated by enzymatic test kits (Asan Pharm. Co., Korea).

Gel filtration. EP obtained from the culture broth was dissolved in 0.2 M NaCl and subjected to gel filtration in a column (2.6 × 99 cm) of Sepharose CL-6B equilibrated with 0.2 M NaCl at a flow rate of 5 ml/tube volume. The molecular weight (MW) markers used were dextran (MW 2,000, 500, 70, 40 and 10 kD) from Sigma Co.

Analysis of component sugars and amino acids in EP. The total protein content of EP was determined by the method of Lowry,21 with bovine serum albumin used as the standard. The amino acid composition was analyzed by a Biochrom 20 amino acid autoanalyzer (Pharmacia Biotech.) with an Na-form column after hydrolysis of the protein.22 The total sugar content of EP was determined by the phenol sulfuric acid method,23 using a mannose and galactose mixture (1:1) as the standard. The sugar composition was analyzed by a GC 3600 gas chromatograph (Varian Co.) based on the hydrolysis and acetylation method described by Jones and Albersheim.24

Statistical analysis. Each data value is expressed as the mean ± SE. Group means were compared by a one-way analysis of variance and by Duncan's multiple-range test.25 Statistical differences were considered significant at p < 0.05.

Results and Discussion

Food intake and body weight gain

The body weight is generally low in an STZ-induced diabetic rat and recovers when the animals is subjected to a hypoglycemic treatment.26 However, the body weights of the animals given different doses of EP were not significantly different from that of the control group, although being slightly higher (Table 1). Likewise, the food intake by the experimental animals did not vary significantly. The oral administration of EP caused no changes in overall behavior and none of the animals died, which rule out any possibility of harmful effects to the rats caused by the oral administration of EP.

Study on the hypoglycemic effect

The effects of L. edodes EP on the plasma glucose retention and insulin synthesis in the diabetic rats are shown in Table 2. The plasma glucose level dropped significantly even at an EP dose as low as 50 mg/kg BW, and dropped further with increasing dose. As
Table 2. Effect of the Lentinus edodes Exo-polymer on the Plasma Glucose and Insulin Levels in STZ-Induced Diabetic Rats for 7 Days

<table>
<thead>
<tr>
<th>Group (exo-polymer, mg/kg/day)</th>
<th>Glucose (mg/dl)</th>
<th>Insulin (μIU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (saline administration)</td>
<td>146 ± 1*</td>
<td>0.87 ± 0.04*</td>
</tr>
<tr>
<td>50</td>
<td>123 ± 2*</td>
<td>1.21 ± 0.03*</td>
</tr>
<tr>
<td>100</td>
<td>120 ± 3*</td>
<td>1.12 ± 0.05*</td>
</tr>
<tr>
<td>150</td>
<td>119 ± 1*</td>
<td>1.21 ± 0.08*</td>
</tr>
<tr>
<td>200</td>
<td>115 ± 1*</td>
<td>1.71 ± 0.06*</td>
</tr>
</tbody>
</table>

Each value is the mean ± SE (n = 8).

Table 3. Effect of the Lentinus edodes Exo-polymer on the Plasma Triglyceride and Total Cholesterol Levels in STZ-Induced Diabetic Rats for 7 Days

<table>
<thead>
<tr>
<th>Group (exo-polymer, mg/kg/day)</th>
<th>Triglyceride (mg/dl)</th>
<th>Total cholesterol (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (saline administration)</td>
<td>105.8 ± 4.0*</td>
<td>100.1 ± 4.6*</td>
</tr>
<tr>
<td>50</td>
<td>91.1 ± 7.1*</td>
<td>80.9 ± 3.9*</td>
</tr>
<tr>
<td>100</td>
<td>74.0 ± 3.8*</td>
<td>86.1 ± 3.2*</td>
</tr>
<tr>
<td>150</td>
<td>73.3 ± 3.5*</td>
<td>82.7 ± 4.8*</td>
</tr>
<tr>
<td>200</td>
<td>58.7 ± 2.9*</td>
<td>75.0 ± 4.7*</td>
</tr>
</tbody>
</table>

Each value is the mean ± SE (n = 8).

Table 4. Effect of the Lentinus edodes Exo-polymer on the Plasma ALT and AST Level in STZ-Induced Diabetic Rats for 7 Days

<table>
<thead>
<tr>
<th>Group (exo-polymer, g/kg/day)</th>
<th>ALT (IU/L)</th>
<th>AST (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (saline administration)</td>
<td>212 ± 4*</td>
<td>269 ± 4*</td>
</tr>
<tr>
<td>50</td>
<td>195 ± 4*</td>
<td>262 ± 2*</td>
</tr>
<tr>
<td>100</td>
<td>188 ± 3*</td>
<td>240 ± 5*</td>
</tr>
<tr>
<td>150</td>
<td>190 ± 3*</td>
<td>252 ± 4*</td>
</tr>
<tr>
<td>200</td>
<td>196 ± 3*</td>
<td>263 ± 2*</td>
</tr>
</tbody>
</table>

Each value is the mean ± SE (n = 8). Values with different superscript letters in the same row are significantly different (p < 0.05).

much as 21% reduction in the plasma glucose level was achieved with a 200 mg/kg BW dose. EP also proved its efficacy to elevate the plasma insulin level by as much as 22%. It has been reported that an STZ treatment inhibited insulin secretion by the pancreas through the selective destruction of β-cells in the pancreatic islets.27,28 It seems that EP of L. edodes could probably repair the damage of the pancreatic β-cells to some extent, promoted insulin synthesis and thus lowered the level of plasma glucose. The observation made by Gray and Flat29 with an Agaricus campestris aqueous extract may support this supposition. They have demonstrated that an aqueous extract of mushroom stimulated 2-deoxyglucose transport, glucose oxidation and the incorporation of glucose into glycogen in the abdominal muscle of mice treated with STZ. They also documented the stimulation of insulin secretion from a BRIN-BD11 pancreatic β-cell line under the influence of an A. campestris aqueous extract. Kiho et al.30 have tested the dose-dependent hypoglycemic effect of extracellular polysaccharide from Pestalotiopsis sp. by oral administration, where a significant glucose-lowering activity was observed until 48 h after the administration at a dose of 100 mg/kg BW.

A substantial decrease in the levels of TG and TC was also found in the present investigation. EP, at a dose of 200 mg/kg BW, lowered the plasma TG and TC levels by as much as 44.5 and 25.1%, respectively, as compared to the control group (Table 3). An EP dose higher than 200 mg/kg/5 ml could not be administered due to the high viscosity of EP in a limited volume of solvent.

The serum TG and cholesterol levels were strongly related to the degree of diabetic control in IDDM rats,30,31 Increased mobilization of free fatty acids and decreased clearance due to reduced LPL activity resulted in elevated levels of TG and VLDL32 in the blood plasma. Insulin regulated both the secretion of VLDL into the plasma from the liver and its removal from the peripheral tissue through the action of endothelial LPL.33 The low insulin level in the STZ-treated diabetic rats might have affected the LPL function and resulted in the high TG level. It can be stated that EP, by promoting insulin production, induced LPL to reduce the plasma TG level; by substantially reducing the plasma TG level, EP could also reduce the plasma TC level.

The AST and ALT levels are generally increased by metabolic changes in the liver such as the administration of a toxin, cirrhosis of the liver, hepatitis and liver cancer.34 These levels can be used as markers to identify the extent of liver damage. In the present investigation, the high plasma levels of TG and cholesterol may have been due to an abnormal liver function caused by the damage done by STZ either directly or indirectly by enhancing the plasma glucose level.35 The plasma AST and ALT values for the different EP groups are depicted in Table 4. As shown in Table 4, the AST and ALT activities were both significantly reduced under the influence of EP. The maximum reduction of their activities was achieved at 100 mg/kg dose, above which their activities tended to increase at higher concentrations. The reason for is obscure to us. However, their overall reduction in activity indicates the corrective role of EP in the liver function, by virtue of its glucose-lowering effect or some other means. This fact might also play a role in reducing the level of TG and TC in the blood plasma of diabetic animals to some extent.

Although the exact mechanism of action by which L. edodes EP exerted its hypoglycemic effect is not properly understood, the results of the study suggest
that a combination of mechanisms might have been involved in this regard.

**Chemical analysis of EP**

EP obtained from the mycelial culture broth yielded only a single peak when subjected to Sepharose CL-6B column chromatography (Fig. 2), and the detected molecular weight was 52 kDa (Fig. 3).

Chemically, this EP seems to have been a glycoprotein and was found to contain 83.5% carbohydrate and 16.5% protein; no acidic sugar was detected. A detailed chemical analysis of EP is summarized in Table 5. Eight different kinds of sugar constituted the carbohydrate moiety, of which the major sugars were found to be mannose (55.1%), galactose (21.8%) and glucose (10.6%). Aspartic acid (14.4%), serine (12.4%), glutamic acid (11.9%) and glycine (10.1%) were found to be the major amino acids of the protein moiety (Table 5). Kim et al., while studying the hypoglycemic potential of *L. edodes* fruiting bodies, have termed the active component as a “protein bound polysaccharide,” but they did not perform detailed chemical analysis. Earlier studies with *L. edodes* EP have demonstrated various other bioactive properties, although the chemical composition of those glycoproteins differed from our present findings. Suzuki et al. and Yamamoto et al. have reported immunoactive and antiviral properties of an *L. edodes* culture precipitate and characterized it as “water-soluble lignin.” Sugano et al. and Tabata et al., who have respectively demonstrated the anticarcinogenic property and mitogenic activities of *L. edodes* EP, suggested the chemical nature as a “xylose-rich proteoglycan.” The difference in the chemical properties of EP of *L. edodes* in different observations may have been due to the different culture conditions used to grow the mycelia. The report on hypoglycemic EP came from the work of Kiho et al. who confirmed that the hypoglycemic exopolysaccharide from *Pestalotiopsis* sp. was a branched galactomannan composed of galactose and mannose in the molar ratio of 1:9. On the other hand, EP obtained in the present investigation consisted of eight different kinds of sugar, with mannose galactose and glucose predominating. Moreover, the hypoglycemic biopolymers obtained from other sources varied chemically from each other. Therefore, it seems that the hypoglycemic activity may not be dependent on the particular chemical composition of the biopolymer; rather, its complex chemical structure is possibly responsible for exhibiting hypoglycemic activity. At the same time, it is also true that not all the biopolymers obtained from the mushroom are hypoglycemic, so there may be some “structural specialty” in showing hypoglycemic activity with varying intensity. Further comprehensive chemical and pharmacological investigations are needed to elucidate the structure-function relationship for the biopolymers with hypoglycemic activity obtained from various sources.

The present investigation has demonstrated the hypoglycemic potential of *L. edodes* EP in rats, which opens up the possibility for its future use in preventive and therapeutic approaches to alleviate the hyperglycemic status in diabetes mellitus.
Table 5. Amino Acid and Sugar Compositions of the Exo-polymer Produced from a Submerged Mycelial Culture of *Lentinus edodes*

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Composition (%)¹</th>
<th>Amino acid</th>
<th>Composition (%)²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhamnose</td>
<td>0.3</td>
<td>Aspartic acid</td>
<td>14.4</td>
</tr>
<tr>
<td>Fucose</td>
<td>5.2</td>
<td>Threonine</td>
<td>9.5</td>
</tr>
<tr>
<td>Ribose</td>
<td>0.1</td>
<td>Serine</td>
<td>12.4</td>
</tr>
<tr>
<td>Arabinose</td>
<td>2.7</td>
<td>Glutamic acid</td>
<td>11.9</td>
</tr>
<tr>
<td>Xylose</td>
<td>4.4</td>
<td>Proline</td>
<td>0.3</td>
</tr>
<tr>
<td>Mannose</td>
<td>55.1</td>
<td>Glycine</td>
<td>10.1</td>
</tr>
<tr>
<td>Galactose</td>
<td>21.8</td>
<td>Alanine</td>
<td>6.6</td>
</tr>
<tr>
<td>Glucose</td>
<td>10.6</td>
<td>Cysteine</td>
<td>1.4</td>
</tr>
</tbody>
</table>

Total sugar content 83.5

Total protein content 16.5

1 Percentages were calculated on the basis of total sugar.

2 Percentages were calculated on the basis of total amino acids.

Acknowledgments

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References


