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Hypoglycemic Effect of *Collybia confluens* Exobiopolymer Produced by Submerged Mycelial Culture on Diabetic Rats

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Abstract The hypoglycemic effect of *Collybia confluens* exobiopolymer was investigated in streptozotocin (STZ)-induced diabetic rats. In a dose-dependent study, the exobiopolymer, at 150 mg/kg body weight (BW) dose substantially lowered the plasma glucose level by 29.3%, as compared to the control group. It also lowered the plasma total cholesterol and triglyceride levels by 23.3 and 30.7%, respectively, and reduced the liver total cholesterol and triglyceride levels by 23.0 and 33.5%, respectively. The activity of alanine transaminase (ALT) and aspartate transaminase (AST) was reduced 34.6% and 23.6% respectively, by the exobiopolymer administration, compared to the control group. The exobiopolymer was found to contain 83.2% carbohydrate and 16.8% protein. The sugar and amino acid of the exobiopolymer were also analyzed in detail.

Key words: *Collybia confluens*, exobiopolymer, hypoglycemic effect, submerged mycelial culture

Mushrooms provide an interesting source for new secondary metabolites with a wide variety of different biological activities [21]. Recent studies on the mushrooms have demonstrated many interesting biological activities [25, 34], including antitumor, hypolipidemic, and hypoglycemic activities, etc. Chihara *et al.* [3] reported antitumor activity of *Lentinus edodes* and proved also its efficacy for various other diseases [12, 31]. The hypolipidemic effects of exobiopolymer produced by submerged mycelial culture of various mushrooms have also been reported [32]. Recently, considerable attention has been paid for natural compounds with hypoglycemic action, and the hypoglycemic effects of *Ganoderma lucidum* has been demonstrated by Hikino *et al.* [8] who proved its potential to lower the blood glucose in rats. The polymers extracted from the fruiting bodies and produced by submerged mycelial culture of various mushroom species have been found to exhibit hypoglycemic activities [16, 20, 31].

Basidiocarps of *C. confluens*, which belongs to the family of Tricholomataceae, are widely used in Japan, China, and Korea as traditional food additives. Various bioactive properties of this mushroom have been explored and attracted considerable attention. The antibiotic activity of *C. confluens* was reported by Simon *et al.* [27], and antitumor [18] and immunological studies [19] have also been published. However, the hypoglycemic activity of *C. confluens* has not yet been reported.

Therefore, a dose-dependent study of exobiopolymer produced by a submerged mycelial culture of *C. confluens* was performed in the present study to assess its hypoglycemic effect in STZ-induced diabetic rats.

Materials and Methods

Strain and Production of Exobiopolymer

The culture of *C. confluens* (KACC 50045) was obtained from the Korean Agricultural Culture Collection. The seed culture was grown in 250-ml flask, containing 100 ml of potato dextrose broth (pH 6.0), and incubated on a rotary shaker (150 rpm) at 25°C for approximately 4 d. One hundred ml of the medium with mycelial pellets were homogenized aseptically in a Sorvall omni-mixer for 3 min in an ice bath and inoculated in the liquid media at the rate of 2% (v/v) for submerged cultivation. The mushroom complete medium (MCM) was used to carry out submerged mycelial culture for the production of exobiopolymer. The composition of MCM was as follows (g/l): glucose 20, yeast extract 2, peptone 2, KH_2PO_4 0.46, K_2HPO_4 1.0, and

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$MgSO_4 \cdot 7H_2O$ 0.5, and the pH was then adjusted to 6.0 before sterilization. The submerged mycelial cultures were carried out in 500-ml flask, containing 200 ml of the medium on a rotary shaker (150 rpm, pH 6) at 25°C for 9 d. Culture broth was harvested by centrifugation (10,447 ×*g*/ 20 min), and the supernatant was treated with ethanol. Ethanol precipitate was dissolved in water, dialyzed, and lyophilized to obtain an exobiopolymer [33, 35].

Animal Experiments

Sprague-Dawley male rats (180-200 g of BW), obtained from Daehan Biolink Co., Ltd., were housed individually in stainless steel cages in a room with controlled temperature (22±2), humidity (55±5%), and a 12-h cycle of light and dark. The rats were fed with a commercial pellet diet (Sam Yang Co., Korea) throughout the experiment. The rats were acclimatized for 7 d in the growth room and then fasted for 12 h before an intramuscular injection of STZ (Sigma, 50 mg/kg BW, dissolved in a citrate buffer at pH 4.5) [11]. Two days after the STZ treatment, the rats were considered to be diabetic when the nonfasting 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 were administered with saline (control) and exobiopolymer at the level of 50-150 mg/kg BW, using an oral zonde daily for 3 weeks. The food intake and body weights were recorded every other day and every day, respectively. At the end of this oral administration, the animals were fasted for 9 h, and sacrificed after an abdominal incision under light ether anesthesia, and blood was then collected from the main artery. Blood samples were collected in heparinized tubes, and plasma was separated by centrifugation (1,110 ×*g*/ 10 min). Livers were perfused with cold saline, excised, weighed after washing with 0.9% NaCl, and kept frozen at -70°C.

Biochemical Assay

The plasma glucose levels were measured using a glucose oxidase kit (glucose B-test, Wako Chemicals, Japan) [26]. The plasma total cholesterol, triglyceride, alanine transaminase (ALT), and aspartate transaminase (AST) levels were evaluated by enzymatic test kits (Asan Pharm. Co., Korea). Liver lipid was extracted by the method of Folch *et al.* [6]. The liver total cholesterol and triglyceride were assayed by the same method as described for plasma total cholesterol and triglyceride after treatment with Triton X-100 [24].

Analysis of Component Sugars and Amino Acids in Exobiopolymer

The total sugar content of exobiopolymer was measured by the phenol-sulfuric acid method [4], using a mixture of mannose and glucose (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 [10]. The total protein content of exobiopolymer was measured by the method of Lowry [22], with bovine serum albumin as the standard. The amino acid composition in the protein hydrolysate was analyzed by Biochrom 20 amino acid auto-analyzer (Pharmacia Biotech.) with a Na^+-column [30].

Statistical Analysis

Each data were expressed as mean±SE. Group means were compared by using a one-way analysis of variance and Duncan's multiple-range test [5]. Statistical differences were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Growth Response

The exobiopolymer produced by a submerged mycelial culture of *C. confluens* was used in the dose-dependent study to confirm its hypoglycemic effect. The effects of *C. confluens* exobiopolymer on BW gain, food intake, and food efficiency ratio in diabetic rats during the 3-week period are shown in Table 1. Generally, the BW and food intake were reduced, and increased, respectively with STZ-induced diabetic rats; however, these anomalies returned to normal when the animals were subjected to a hypoglycemic treatment [7]. In the present study, a significant increase in the BW gain and food efficiency ratio was observed in the exobiopolymer administered group. On the other hand, the exobiopolymer group showed significantly reduced food intake compared to the control group. Furthermore, the oral administration of the exobiopolymer caused no changes in gross behavior, and none of the animals died,

Table 1. Effects of *Collybia confluens* exobiopolymer on the growth parameters in streptozotocin-induced diabetic rats for 3 weeks.

Group (Exobiopolymer, mg/kg/day)	BW gain (g/day)	Food intake (g/day)	Food efficiency ratio[4]
Normal[1]	6.00±0.20^c	24.75±0.11^a	0.24±0.01^c
Control[2,3]	3.50±0.24^a	40.61±0.48^c	0.09±0.02^a
50[2]	4.18±0.37^{ab}	36.53±1.67^b	0.11±0.01^{ab}
100[2]	4.39±0.19^{ab}	34.36±1.47^b	0.13±0.01^{ab}
150[2]	4.24±0.38^{ab}	34.51±1.25^b	0.12±0.02^{ab}

[1]Normal 8 rats.
[2]Diabetic rats induced by streptozotocin (50 mg/kg BW) for 8 rats.
[3]Saline administration.
[4]BW gain/Food intake.
Each value is mean±SE for 8 rats.
^{a,b,c}Values with different superscript letters in the same column indicate significant differences among the groups at $p<0.05$.

Table 2. Effects of *Collybia confluens* exobiopolymer on plasma glucose, total cholesterol, and triglyceride levels in streptozotocin-induced diabetic rats for 3 weeks.

Group (Exobiopolymer, mg/kg/day)	Glucose (mg/dl)	Total cholesterol (mg/dl)	Triglyceride (mg/dl)
Normal[1]	138.91±3.35[a]	68.74±3.26[a]	43.25±1.83[a]
Control[2,3]	375.44±6.95[c]	99.00±7.72[c]	79.25±5.06[d]
50[2]	335.20±5.66[bc]	87.92±6.19[b]	68.20±6.51[c]
100[2]	281.76±9.36[b]	77.00±7.80[ab]	59.00±3.45[bc]
150[2]	265.25±6.25[b]	75.93±4.78[ab]	54.94±7.40[b]

[1]Normal 8 rats.
[2]Diabetic rats induced by streptozotocin (50 mg/kg BW) for 8 rats.
[3]Saline administration.
Each value is mean±SE for 8 rats.
[a,b,c,d]Values with different superscript letters in the same column indicate significant differences among the groups at $p<0.05$.

Table 3. Effects of *Collybia confluens* exobiopolymer on liver total cholesterol and triglyceride levels in streptozotocin-induced diabetic rats for 3 weeks.

Group (Exobiopolymer, mg/kg/day)	Total cholesterol (mg/g)	Triglyceride (mg/g)
Normal[1]	1.19±0.04[a]	1.90±0.04[a]
Control[2,3]	1.78±0.10[c]	3.28±0.12[c]
50[2]	1.53±0.07[b]	2.62±0.18[bc]
100[2]	1.48±0.06[ab]	2.42±0.12[ab]
150[2]	1.37±0.03[ab]	2.18±0.11[ab]

[1]Normal 8 rats.
[2]Diabetic rats induced by streptozotocin (50 mg/kg BW) for 8 rats.
[3]Saline administration.
Each value is mean±SE for 8 rats.
[a,b,c]Values with different superscript letters in the same column indicate significant differences among the groups at $p<0.05$.

thus ruling out any possible harmful effect of the *C. confluens* exobiopolymer in rats.

Hypoglycemic Effects

The effects of *C. confluens* exobiopolymer on plasma glucose, total cholesterol, and triglyceride in the STZ-induced diabetic rats are shown in Table 2. The glucose, total cholesterol, and triglyceride levels in plasma were significantly reduced in all the experimental animals, and further dropped with increase of doses: As much as 29.3% reduction in the plasma glucose level was achieved with a 150 mg/kg BW dose.

This is consistent with increased viscosity with increasing dose of the exobiopolymer. The glucose lowering effect of the exobiopolymer could be explained by the fact that it increases glucose utilization in diabetic animals by promoting insulin secretion and viscosity. An increased viscosity of the intestinal content by the exobiopolymer might result in reduced nutrient movement towards the villi network for efficient absorption, thereby probably lowering the levels of plasma glucose, total cholesterol, and triglyceride. Its activities could be equivalent to a high viscosity dietary fiber, such as guar gum or pectin [9, 23]. Also, since the STZ treatment inhibits insulin secretion by pancreas through selective destruction of the β-cells of Langerhans islets [28], oral administration of *C. confluens* exobiopolymer to STZ-treated animals could repair the damage of the β-cells and promote insulin synthesis, thus lowering the level of plasma glucose. We recently demonstrated the hypoglycemic effect of exopolymer produced in submerged mycelial culture of various mushrooms, in STZ-diabetic rats [17, 31]. Kiho *et al.* [15] also propounded the similar fact, while studying the hypoglycemic effect of *Pestalotiopsis* sp. extracellular polysaccharides. Therefore, the possibility of *C. confluens* exobiopolymer to exhibit hypoglycemic effect appears to be well established.

The levels of total cholesterol and triglyceride in plasma have been shown to be significantly correlated with the degree of diabetic control in IDDM rats [1]. In the present study, maximum 23.3% and 30.7% decreases of total cholesterol and triglyceride, respectively, were achieved at a dose of 150 mg/kg BW (Table 2). The high plasma and liver levels of total cholesterol and triglyceride in diabetic animals may be due to impaired liver function caused by the STZ-induced damage, which enhanced the plasma glucose level either directly or indirectly [29]. The effect of *C. confluens* exobiopolymer on liver total cholesterol and triglyceride levels in the experimental animals is presented in Table 3. The levels of total cholesterol and triglyceride in the liver of the diabetic rats administered with the exobiopolymer were significantly decreased to the extent of 23.0% and 33.5%, respectively, as compared to the control group.

As shown in Table 4, both ALT and AST activities were significantly reduced under the influence of *C. confluens* exobiopolymer. Generally, ALT and AST levels are increased

Table 4. Effects of *Collybia confluens* exobiopolymer on plasma ALT and AST levels in streptozotocin-induced diabetic rats for 3 weeks.

Group (Exobiopolymer, mg/kg/day)	ALT (IU/L)	AST (IU/L)
Normal[1]	6.50±0.86[a]	47.61±1.50[a]
Control[2,3]	23.21±1.23[c]	67.78±3.25[c]
50[2]	18.21±2.20[bc]	53.65±1.66[ab]
100[2]	16.74±1.87[b]	52.12±2.06[ab]
150[2]	15.17±1.89[b]	51.79±1.59[ab]

[1]Normal 8 rats.
[2]Diabetic rats induced by streptozotocin (50 mg/kg BW) for 8 rats.
[3]Saline administration.
Each value is mean±SE for 8 rats.
[a,b,c]Values with different superscript letters in the same column indicate significant differences among the groups at $p<0.05$.

by metabolic changes in the liver, such as administration of toxin, liver cirrhosis, hepatitis, and liver cancer [2]. These levels can be used as markers to identify the extent of liver damage. In the present study, their maximum reduction was observed at 150 mg/kg BW dose, and their activities tended to increase at higher concentrations.

Chemical Analysis of Exobiopolymer

Exobiopolymer produced by the submerged mycelial culture of *C. confluens* seems to be a glycoprotein. A detailed chemical analysis of the exobiopolymer is summarized in Table 5. Total sugar and protein contents of this exobiopolymer were found to be 83.2% and 16.8%, respectively. Fifteenth kinds of amino acids constituted the protein moiety, of which the major amino acids were serine (15.2%), glycine (12.4%), alanine (10.4%), aspartic acid (9.6%), and valine (9.3%). Mannose (13.3%) and glucose (73.0%) were found to be the major carbohydrates of the sugar moiety.

Until now, there exists no report on the hypoglycemic potential of *C. confluens*. However, earlier studies with *C. confluens* polymers have shown various other bioactive properties, although the chemical composition of those glycoproteins was different from our present findings. Simon *et al.* [27] reported antibiotic properties and cytotoxic effects of *C. confluens* fermentation precipitate and characterized it as "water-soluble collybial", and Kim *et al.* [18, 19] demonstrated the immunological and antitumor activities of *C. confluens* polymers, and suggested the chemical nature as a "heteropolysaccharide". The difference in the chemical properties of *C. confluens* polymers by different observers might have been due to different extraction and culture methods. Kim *et al.* [17] reported the hypoglycemic exopolymer produced by submerged mycelial culture of mushrooms, and Kiho and colleagues reported that β-glucan was the main bioactive ingredients with hypoglycemic activity of hot-water extracted polysaccharides of the fruiting bodies of *Tremella aurantia* [13] and hot-water and alkaline extracted polysaccharides of the cultured mycelium of *Cordyceps sinensis* [14]. Furthermore, the hypoglycemic biopolymers obtained from other sources varied chemically from each other [13, 31]. Therefore, it seems likely that the hypoglycemic activity may not solely be dependent on the particular chemical composition of the biopolymer, but rather its complex chemical structure is possibly responsible for exhibiting hypoglycemic activity.

Table 5. Amino acid and sugar compositions of the exobiopolymer produced by submerged mycelial culture of *Collybia confluens*.

Sugar	Composition (%)[1]	Amino acid	Composition (%)[2]
Fucose	3.07	Aspartic acid	9.61
Ribose	0.98	Threonine	9.00
Arabinose	2.29	Serine	15.20
Xylose	2.14	Glutamic acid	6.87
Mannose	13.26	Proline	1.0
Galactose	5.27	Glycine	12.43
Glucose	72.99	Alanine	10.44
		Cysteine	1.35
		Valine	9.28
		Isoleucine	4.32
		Leucine	6.62
		Phenyalanine	3.63
		Histidine	1.22
		Lysine	3.06
		Arginine	5.96
Total sugar content	83.19	Total protein content	16.81

[1]Percentages were calculated on the basis of total sugar.
[2]Percentages were calculated on the basis of total amino acids.

The present study has demonstrated the hypoglycemic potential of *C. confluens* exo-biopolymer, produced by submerged mycelial culture, in STZ-induced diabetic rats. Further pharmacological and chemical studies are needed to elucidate the mechanism and structure-function relationship of the biopolymers obtained from various sources. Such investigations should also be carried out, regarding noninsulin-dependent diabetes mellitus.

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