

## Antidiabetic and Hypolipidemic Effects of *Collybia confluens* Mycelia Produced by Submerged Culture in Streptozotocin-Diabetic Rats

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This investigation was undertaken to study the effects of oral administration (3 weeks) of *Collybia confluens* mycelial powder (CCMP) produced by a submerged culture on plasma glucose and other biochemical parameters in streptozotocin (STZ)-induced diabetic rats. Antidiabetic and hypolipidemic effects were proportionally increased with the increasing concentration of the CCMP for oral administration. The CCMP, at the dose of 400 mg/kg BW, substantially reduced the plasma glucose level by as much as 33.1% as compared to the STZ-induced diabetic rats group. It also lowered the plasma total cholesterol, triglyceride, and low density lipoprotein (LDL) cholesterol by 22.9%, 19.9%, and 37.3%, respectively. The levels of total cholesterol and triglyceride in liver were reduced to the extent of by 13.5% and 18.8%, and the activity of alanine transaminase (ALT) and aspartate transaminase (AST) was decreased by 48.8% and 37.2%, respectively, under the influence of CCMP. The general components of CCMP were found to contain 26.18% carbohydrate, 3.67% crude ash, 4.02% crude fat, 22.55% crude protein, and 43.58% dietary fiber. The amino acid composition of the CCMP was also analyzed in detail.

**Key words:** Antidiabetic effects, *Collybia confluens* mycelia, Hypolipidemic effects, Submerged culture

### INTRODUCTION

For centuries, the mushrooms have been used as a traditional Oriental medicine to prevent and cure of various diseases. Edible mushrooms proved themselves ideal food for diabetic prevention of hyperglycemia due to their high content of fiber, proteins, and low fat content (Yuan *et al.*, 1998), and were thus studied extensively. Although some dietary fiber from mushrooms have been reported to show a hypoglycemic effect on the diabetic rat or mouse (Cho *et al.*, 2002), few edible mushrooms have been investigated in this respect. Many previous studies on the hypoglycemic effect of dietary fiber used STZ-induced animals which is the model for insulin-dependent diabetes mellitus (IDDM) (Rakietyen *et al.*, 1963).

*Collybia confluens*, which belongs to the family of Tricholomataceae, is used as herbal medicine as well as

traditional food additives in Japan, China, and Korea. Recent studies on this mushroom have reported many interesting biological activities, including antibiotic (Simon *et al.*, 1995), antitumor (Kim *et al.*, 1993b), and immunological studies (Kim *et al.*, 1993a). Also, we have previously reported the hypoglycemic effect of the exo-biopolymers produced from submerged culture of *C. confluens* (Yang *et al.*, 2005). However, any biological activity by mycelia of *C. confluens* has not been reported yet.

Therefore, in the present study, detailed investigation was carried out in examining the antidiabetic and hypolipidemic activities of the mycelia (CCMP) produced from the submerged culture of *C. confluens* in STZ-induced diabetic rats.

### MATERIALS AND METHODS

#### Strain and production of mycelia

The culture of *C. confluens* (KACC 50045) was obtained from the Korean Agricultural Culture Collection (Suwon, Korea). The seed culture was grown in 250 mL flask, con-

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taining 100 mL of potato dextrose broth (pH 6.0), and incubated on a rotary shaker (150 rpm) at 25°C for approximately 4 days. One hundred milliliter 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 culture for the production of mycelia (Yang *et al.*, 2002). The composition of MCM is as follows (g/L): glucose 20, yeast extract 2, peptone 2,  $\text{KH}_2\text{PO}_4$  0.46,  $\text{K}_2\text{HPO}_4$  1.0, and  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  0.5, then the pH was adjusted to 6.0 before sterilization. The submerged mycelial cultures were carried out in 500 mL flask, containing 200 mL of the media on a rotary shaker (150 rpm, pH 6) at 25°C for 9 days. Culture broth was harvested by centrifugation ( $10,000 \times g/20$  min) and the precipitate was washed 3 times with DW, lyophilized and homogenized to obtain a CCMP (Fig. 1).

### Animals

Male Sprague-Dawley rats (180-200 g) obtained from the Daehan Biolink Co., Ltd. (Eumsung, Korea), were housed individually in stainless steel cages in a room with controlled temperature ( $22 \pm 2^\circ\text{C}$ ), humidity ( $55 \pm 5\%$ ), and a 12 h cycle of light and dark. The rats were fed with a commercial pelleted diet throughout the experimental period.

### Induction of diabetes and experimental design

The rats were acclimatized for 7 days 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) (Junod *et al.*, 1967). After two days 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 IDDM model. After the induction of STZ diabetes, the rats were divided into six groups as follows: Normal group, normal rats given saline; Control group, STZ diabetic rats given saline; 100 group, diabetic rats given CCMP (100 mg/kg); 200 group, diabetic rats given CCMP (200 mg/kg); 300 group, diabetic rats given CCMP (300 mg/kg); 400 group, diabetic rats given CCMP (400 mg/kg). The rats of each group were administered with saline and mycelia at the level of 100-400 mg/kg, using an oral zonde daily for 3 weeks. The food intake and BW were recorded every other day and every day, respectively.

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 then blood was collected from the main artery. Blood samples were collected in heparinized tubes, and plasma was separated by centrifugation ( $1,110 \times g/10$  min). Livers were perfused with cold saline, excised, weighed after washing with 0.9% NaCl, and kept frozen at  $-70^\circ\text{C}$ .

### Biochemical analysis

The plasma glucose levels were measured, using a glucose oxidase kit (glucose B-test, Wako Chemicals, Japan). The plasma total cholesterol, triglyceride, phospholipid, high-density lipoprotein (HDL) cholesterol, ALT, and AST levels were evaluated by enzymatic test kits (Sigma Co.). LDL cholesterol of plasma was calculated by the following equation:  $\text{LDL cholesterol} = \text{total cholesterol} - \text{HDL cholesterol} - (\text{triglyceride}/5)$  (Fridewald *et al.*, 1979). Liver lipid was extracted by the method of Folch *et al.* (1957). The liver total cholesterol, triglyceride, and phospholipid were assayed by the same method as described for the plasma total cholesterol, triglyceride, and phospholipid after the treatment with Triton X-100 (Sale *et al.*, 1984).

### Statistical analysis

Each data were expressed as mean  $\pm$  SE. Group means were compared by using a one-way analysis of variance and Duncan's multiple-range test (Duncan, 1957). Statistical differences were considered significant at  $p < 0.05$ .

### Analysis of general chemical composition

The general chemical compositions of the CCMP, including crude ash, fat, protein, carbohydrate and fiber, were determined according to the methods of AOAC (1995). The percentage of protein was calculated as  $\text{N} \times 4.38$  (Crisan and Sands, 1978). The amino acid composi-

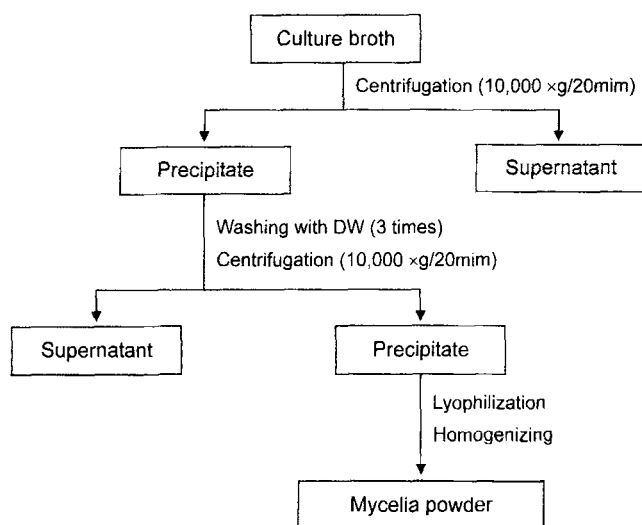


Fig. 1. Schematic diagram depicting recovery process of mycelia from a submerged culture of *Collybia confluens*.

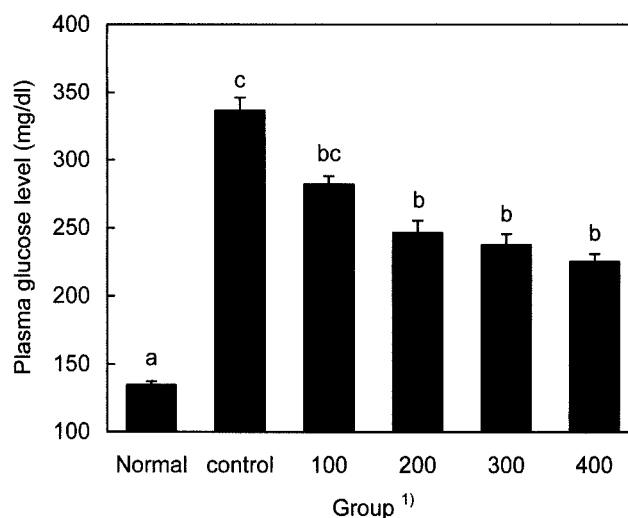
tion in the protein hydrolysate was analyzed by Biochrom 20 amino acid auto-analyzer (Pharmacia Biotech.) with a Na<sup>+</sup>-column (Waters Associates, 1980).

## RESULTS AND DISCUSSION

### Antidiabetic activities

In the present study, a dose-dependent antidiabetic effect of CCMP was investigated in the STZ-treated diabetic rats and relative antidiabetic activities were evaluated with respect to that of saline control group. The changes in BW gain, food intake, and food efficiency ratio on administration of CCMP in all animals groups were presented in Table I. A marked difference of the BW gain and food efficiency ratio was observed in STZ-induced rats compared to the normal group. All CCMP administered group was similarly effective in increasing BW gain and efficiency ratio. Increase in BW gain and food efficiency ratio by the CCMP may prove its antidiabetic potential. Generally, BW gain and food efficiency ratio are reduced in STZ induced diabetic rats and recovered when subjected to antidiabetic treatment (Furuse *et al.*, 1993). The food intake of the CCMP group was also significantly reduced compared to the control group. Furthermore, the oral administration of the CCMP caused no changes in gross behavior and none of the animals died, which rule out any possibility of harmful effect in rats caused by the oral administration of CCMP. This is in agreement with our previously studies (Yang *et al.*, 2002, 2005). Treatments with CCMP decreased the reduction in BW by diabetes, and altered the BW gain and food intake of diabetic animals towards normalcy.

The effects of CCMP on the plasma glucose levels in the STZ-induced diabetic rats are shown in Fig. 2. CCMP treated rats for a period of 3 weeks presented significantly lower plasma glucose levels when compared to untreated diabetic rats, and showed a dose-dependent effect when



**Fig. 2.** Effects of *Collybia confluens* mycelia on plasma glucose levels in streptozotocin-induced diabetic rats for 3 weeks. <sup>1)</sup> Described in Materials and Methods. Each value is mean  $\pm$  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  $p < 0.05$ .

it was administered at dose of 100, 200, 300, and 400 mg/kg. A CCMP dose higher than 400 mg/kg/5 mL could not be administered due to the high concentration of CCMP in a limited volume of solvent. The plasma glucose levels dropped significantly even at a CCMP dose as low as 100 mg/kg BW. Maximum reduction in plasma glucose levels was noticed at 400 mg/kg BW dose, which could reduce by 33.1%. The effect of CCMP due to dietary fiber of high viscosity could be a major factor to cause the antidiabetic action in diabetic animals (Johnson, 1991; Nelson *et al.*, 1991). Antidiabetic state can also be achieved through increased glucose utilization in diabetic animals by promoting insulin secretion (Gray and Flatt, 1998). Previously our observation proved hypoglycemic effects with mycelia and exo-polymer of various mushrooms may explain the above supposition (Yang *et al.*, 2002, 2004). Cho *et al.* (2002) and Kihou *et al.* (1997) also documented reduction of glucose from diabetic rats under the influence of *Lentinus edodes* powders and *Pestalotiopsis* sp. extracellular polysaccharide. Since STZ treatment causes selective destruction of the pancreatic  $\beta$ -cells, the CCMP may produce the antidiabetic effects in diabetic rats by the stimulation of insulin release from the  $\alpha$ -cells and other mechanism.

The effects in ALT and AST values under the influence of the CCMP were shown in Table II. The Activities of AST and ALT were both significantly reduced by the influence of CCMP. Maximum reduction in ALT and AST value was noticed at 400 mg/kg BW mycelial dose, which could reduce the ALT and AST level by 48.8% and 37.2%, respectively. Generally, the ALT and AST levels are increased

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

Group <sup>1)</sup>	Body weight gain (g/day)	Food intake (g/day)	Food efficiency ratio <sup>2)</sup>
Normal	5.72 $\pm$ 0.15 <sup>c</sup>	24.10 $\pm$ 0.43 <sup>a</sup>	0.24 $\pm$ 0.01 <sup>c</sup>
Control	3.02 $\pm$ 0.12 <sup>a</sup>	37.55 $\pm$ 1.54 <sup>b</sup>	0.08 $\pm$ 0.01 <sup>a</sup>
100 mg/kg	3.58 $\pm$ 0.13 <sup>ab</sup>	28.09 $\pm$ 2.15 <sup>ab</sup>	0.13 $\pm$ 0.01 <sup>ab</sup>
200 mg/kg	3.49 $\pm$ 0.24 <sup>ab</sup>	24.84 $\pm$ 1.54 <sup>a</sup>	0.14 $\pm$ 0.02 <sup>ab</sup>
300 mg/kg	3.48 $\pm$ 0.33 <sup>ab</sup>	24.30 $\pm$ 1.21 <sup>a</sup>	0.14 $\pm$ 0.01 <sup>ab</sup>
400 mg/kg	3.65 $\pm$ 0.29 <sup>ab</sup>	24.04 $\pm$ 1.47 <sup>a</sup>	0.15 $\pm$ 0.01 <sup>b</sup>

<sup>1)</sup> Described in Materials and Methods.

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

Each value is mean  $\pm$  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 II.** Effects of *Collybia confluens* mycelia on plasma ALT and AST levels in streptozotocin-induced diabetic rats for 3 weeks

Group <sup>1)</sup>	ALT (IU/L)	AST (IU/L)
Normal	8.04 ± 0.52 <sup>a</sup>	42.14 ± 1.32 <sup>a</sup>
Control	28.84 ± 2.63 <sup>c</sup>	69.91 ± 2.56 <sup>c</sup>
100 mg/kg	16.89 ± 3.84 <sup>b</sup>	49.24 ± 2.58 <sup>ab</sup>
200 mg/kg	15.35 ± 3.69 <sup>b</sup>	47.17 ± 2.44 <sup>ab</sup>
300 mg/kg	15.75 ± 2.03 <sup>b</sup>	45.53 ± 2.44 <sup>ab</sup>
400 mg/kg	14.76 ± 4.60 <sup>b</sup>	43.93 ± 3.22 <sup>ab</sup>

<sup>1)</sup> Described in Materials and Methods.

Each value is 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  $p < 0.05$ .

when functions abnormally of liver such as liver cancer, cirrhosis, and hepatitis (Bursch and Schulte, 1986). These values can be used as a marker to indicate the extent of liver damage. The high plasma level of ALT and AST of the diabetic animals may be due to abnormal liver function caused by STZ treatment or enhancing the plasma glucose level (Van Horn, 1996). The significant reduction of ALT and AST value in the present study seems that CCMP could probably repair the damaged by STZ treatment.

### Hypolipidemic activities

The plasma lipids are usually raised in diabetes, and such an elevation represents a risk factor for coronary heart disease (Betteridge, 1997). In the present study, the continuous treatment with CCMP for a period of 3 weeks caused a significant decrease in the plasma lipid levels of diabetic rats. Table III shows the levels of plasma total cholesterol, triglyceride, phospholipid, LDL cholesterol, and HDL cholesterol in STZ-induced diabetic rats. Oral administration of CCMP significantly reduced the total cholesterol and triglyceride in plasma as compared to

control group, and dropped further with increasing doses. A maximum reduction of total cholesterol and triglyceride levels of 22.9% and 19.9%, respectively, was obtained at a dose of 400 mg/kg BW. However, no significant change in the plasma phospholipid and HDL cholesterol level was evidenced among all the experimental groups. Plasma levels of total cholesterol and triglyceride have a significant correlation with the degree of diabetic control in IDDM rats (Bar-on and Roheim, 1976). Hypercholesterolemia and hypertriglyceridemia have been reported to occur in streptozotocin diabetic rats (Choi *et al.*, 1991; Sharma *et al.*, 1996) and a significant increase observed in our experiment was in accordance to these studies. The hypolipidemic effect was steadily increased with the increasing concentration of the CCMP. The hypolipidemic effect in diabetes rats exerted by the CCMP may result from the rich in dietary fiber of the CCMP. Perhaps for this property it could lower the triglyceride and cholesterol absorption by inhibiting the formation of micelles in the small intestine and by altering the physical characteristics of the intestinal mucosa of rats. This supposition is consistent with that Nelson *et al.* (1991) was observed effects of dietary fiber in diabetes mellitus. We have also observed that in STZ-induced diabetic rats, the level of LDL-cholesterol was significantly lower while concentrations of total cholesterol and triglyceride were decreased. Oral administration of CCMP at 400 mg/kg BW significantly decreased the LDL cholesterol (37.3%) as compared to control group. The strong hypolipidemic activity of CCMP could also be through its control of diabetes, as this is a major determinant of triglyceride, total, and LDL cholesterol levels (Laakso, 1995).

The levels of liver total cholesterol and phospholipid in the CCMP administered group were not significantly different from the control group but their levels tend to be reduced in experimental group (Table IV). And the CCMP,

**Table III.** Effect of the *Collybia confluens* mycelia on plasma total cholesterol, triglyceride, phospholipids, LDL cholesterol, and HDL cholesterol levels in streptozotocin-induced diabetic rats for 3 weeks

Group <sup>1)</sup>	Total cholesterol (mg/dl)	Triglyceride (mg/dl)	Phospholipid (mg/dl)	LDL cholesterol (mL/dl) <sup>2)</sup>	HDL cholesterol (mL/dl)
Normal	72.25 ± 2.07 <sup>a</sup>	44.64 ± 3.32 <sup>a</sup>	119.57 ± 5.07 <sup>NS</sup>	27.20 ± 2.58 <sup>a</sup>	36.12 ± 2.98 <sup>NS</sup>
Control	100.97 ± 5.01 <sup>c</sup>	68.65 ± 6.50 <sup>c</sup>	134.34 ± 6.67	53.31 ± 3.60 <sup>bc</sup>	33.93 ± 3.29
100 mg/kg	84.94 ± 3.87 <sup>b</sup>	63.28 ± 3.44 <sup>bc</sup>	131.80 ± 6.56	39.85 ± 2.43 <sup>b</sup>	32.43 ± 3.36
200 mg/kg	80.90 ± 2.25 <sup>ab</sup>	60.62 ± 3.12 <sup>b</sup>	123.47 ± 4.61	36.25 ± 3.10 <sup>ab</sup>	32.53 ± 2.58
300 mg/kg	81.10 ± 3.81 <sup>ab</sup>	56.56 ± 3.44 <sup>b</sup>	121.66 ± 3.75	38.17 ± 1.94 <sup>ab</sup>	31.62 ± 2.78
400 mg/kg	77.85 ± 3.13 <sup>ab</sup>	55.01 ± 2.93 <sup>b</sup>	123.38 ± 6.15	33.44 ± 2.43 <sup>ab</sup>	33.41 ± 2.16

<sup>1)</sup> Described in Materials and Methods.

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

Each value is mean ± SE for 8 rats.

<sup>NS</sup> Not significant.

<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 IV.** Effects of *Collybia confluens* mycelia on liver total cholesterol, triglyceride, and phospholipid levels in streptozotocin-induced diabetic rats for 3 weeks

Group <sup>1)</sup>	Total cholesterol (mg/g)	Triglyceride (mg/g)	Phospholipid (mg/g)
Normal	2.02 ± 0.01 <sup>NS</sup>	2.77 ± 0.05 <sup>a</sup>	11.19 ± 0.37 <sup>NS</sup>
Control	2.38 ± 0.06	3.93 ± 0.21 <sup>b</sup>	11.95 ± 0.1
100 mg/kg	2.08 ± 0.07	3.52 ± 0.25 <sup>b</sup>	11.39 ± 0.3
200 mg/kg	2.09 ± 0.09	3.45 ± 0.31 <sup>b</sup>	11.11 ± 0.25
300 mg/kg	2.07 ± 0.07	3.41 ± 0.24 <sup>b</sup>	11.10 ± 0.42
400 mg/kg	2.06 ± 0.07	3.19 ± 0.10 <sup>ab</sup>	10.92 ± 0.26

<sup>1)</sup> Described in Materials and Methods.

Each value is mean ± SE for 8 rats.

<sup>NS</sup> Not significant.

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

at the dose of 400 mg/kg BW, reduced the triglyceride levels in liver by 18.8% as compared to that of the control group. A decrease in the triglyceride levels of liver in STZ-induced diabetic rats may indicate an increased mobilization of lipids from the tissues, which may have caused an increase in plasma triglycerides and total cholesterol.

Repeated administration of CCMP thus had a beneficial effect on the hyperlipemia associated with hyperglycemia. The hypolipidaemic effect of CCMP can be explained as a direct reduction in the blood glucose concentration. Although administration of CCMP significantly reduced the plasma glucose and lipid levels, they could not achieve the value as that of normal group.

### General chemical compositions

General chemical compositions of the mycelia produced from the submerged culture of *C. confluens* are shown in Table V. Dietary fiber in general chemical compositions of CCMP was the most abundant component. Seventeen kinds of amino acids were detected by auto-amino acid analyzer and aspartic acid (9.21%), glutamic acid (12.27%), alanine (10.80%), and lysine (9.37%) were detected as the major amino acids. Cho *et al.* (2002) reported that the intake of *Lentinus edodes* was decreased the glucose and lipids levels in diabetic rats, but they did not perform detailed chemical analysis. Frati *et al.* (1991) suggested that the hypoglycemic effect of *Opuntia streptacantha* to the decrease in intestinal glucose absorption provoked by the high content of dietary fiber. Also, we have previously demonstrated the hypoglycemic effect of mycelia produced from submerged culture of *Lentinus edodes* and *Phellinus linteus* in STZ-diabetic rats (Kim *et al.*, 2001; Yang *et al.*, 2002). In the present study, the content of dietary fiber within general chemical compositions of *C. confluens* mycelia occupied almost 43.6%. Meantime, the antidiabetic and

**Table V.** General chemical compositions of the mycelia produced from a submerged mycelial culture of *Collybia confluens*

Component	Composition (%)	Amino acid	Composition (%)
Crude ash	3.67	Aspartic acid	9.21
Crude fat	4.02	Threonine	5.65
Crude protein	22.55	Serine	6.35
Carbohydrate	26.18	Glutamic acid	12.27
Dietary fiber	43.58	Proline	0.47
		Glycine	9.01
		Alanine	10.80
		Cysteine	0.47
		Valine	6.81
		Methionine	0.41
		Isoleucine	4.17
		Leucine	7.53
		Tyrosine	1.99
		Phenylalanine	6.21
		Histidine	2.90
		Lysine	9.37
		Arginine	6.39

hypolipidemic effects of dietary fiber were already proved by many investigators. In particular, Nelson *et al.* (1991) reported effects of dietary fiber supplementation on glycemic control in alloxan-induced diabetes mellitus, and Lee *et al.* (2004) demonstrated effect of hemicellulose extracted soy fiber on glucose and cholesterol concentrations in STZ-induced diabetic rats. Therefore, it is considered that the antidiabetic and hypolipidemic effect of *C. confluens* mycelia was influenced by dietary fiber. This mushroom is rich in dietary fiber, which has a positive effect in the prevention of cardiovascular and diabetic diseases, along with other beneficial effects.

From this study we can state that mycelia produced by submerged culture of *C. confluens* have beneficial effects on plasma glucose level as well as improving hyperlipidemia due to diabetes. Thus, in above-mentioned facts, the present study suggests that CCMP exhibits hypolipidemic as well as antidiabetic activities in the STZ-induced diabetic rats.

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