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A Preliminary Study on the Hypoglycemic Effect of the Exo-Polymers Produced by Five Different Medicinal Mushrooms

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Abstract The hypoglycemic effect of exo-polymers (EPs) produced from submerged mycelial cultures of five types of mushrooms on streptozotocin (STZ)-induced diabetic rats were investigated in this study. The five experimental groups were fed with EPs (50 mg/kg body weight) for 7 days. Significant reduction in plasma glucose, total cholesterol (TC), and triglyceride (TG) levels were observed in rats fed with *Lentinus edodes* and *Cordyceps militaris* EPs. Plasma glucose and TC were also reduced by administration of *Phellinus linteus* EPs, but the TG level was not changed significantly. The EPs of three mushroom species also demonstrated a marked reduction in the level of plasma glutamate-pyruvate transaminase (GPT). The result proves the hypoglycemic activity of EPs of three fungal groups in STZ-induced diabetic rats and indicates their potential in the control of diabetes mellitus.

Key words: Exo-polymers, hypoglycemic effect, submerged mycelial culture

Various researchers have long studied the legendary effect of mushrooms in promoting hypoglycemic activity. For this and other medicinal properties, mushrooms took their place in traditional food additives and Oriental medicine in China, Japan, and Korea over the decade. Edible mushrooms are recognized as the ideal food for dietetic prevention of hyperglycemia because of their high content of fiber, proteins, and a low fat content [27]. Kiho *et al.* [16, 17, 18, 19] investigated the hypoglycemic activity of *Agrocybe cylindracea*, *Tremella fuciformis*, *Cordyceps sinensis*, and *Pestalotiopsis* sp. and reported their outstanding blood glucose lowering effect. Hikino *et al.* [11] stated that hot water and ethanol extracts of fruiting bodies of *Ganoderma lucidum* has an excellent hypoglycemic effect. The hypoglycemic effects of endo-polymer from submerged mycelial cultures of *Lentinus edodes* and *Pleurotus ostreatus* were also documented by Kim *et al.* [20]. Yuan *et al.* [27] investigated the hypoglycemic activity of water-soluble polysaccharide from fruiting bodies of *Auricularia auricula-judae* Quel. However, most of the researches on the hypoglycemic effect were carried out either with the fruiting bodies or the mycelia of mushrooms. In a submerged mycelial culture, some components such as polysaccharides are released into the culture medium, which have been documented to have various biological properties [5, 23]. Recently, these EPs have been investigated extensively because their production process from the culture broth do not require extra steps and they require a relatively simple purification process [1, 5, 14, 25]. Therefore, it was worth to evaluate the hypoglycemic effect of EPs produced by submerged mycelial culture of mushrooms. In the present investigation, the hypoglycemic activity of EPs produced from submerged mycelial culture of five types of mushrooms (*Lentinus edodes*, *Auricularia polytricha*, *Cordyceps militaris*, *Agrocybe cylindracea*, and *Phellinus linteus*) were studied in STZ-induced diabetic rats.

Culture of *Lentinus edodes*, *Auricularia polytricha*, *Cordyceps militaris*, *Agrocybe cylindracea*, and *Phellinus linteus* were obtained from the Rural Development Administration in Korea. All organisms were maintained on the potato dextrose agar (Difco) slant at 4°C and subcultured every 3 months. The composition of culture medium for the production of EPs was as follows (g/l): potato dextrose broth 24, malt extract 10, peptone 1, and the pH was adjusted to 5.0 before sterilization. The seed culture of five strains were grown in potato dextrose broth on a rotary shaker (120 rpm) at 25°C. After a 10-days incubation period, 50 ml of the culture broth with mycelial pellets were homogenized aseptically in a Sorvall omni-

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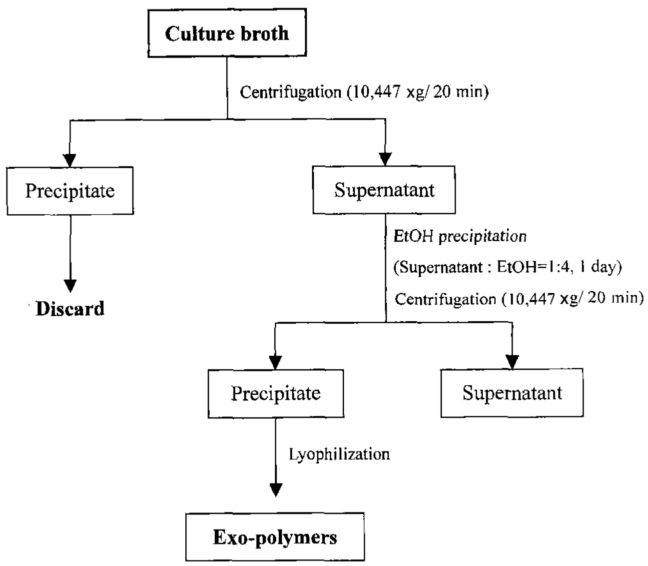

Fig. 1. A schematic diagram depicting the recovery process of exo-polymers from submerged mycelial culture of mushrooms.

mixer for 3 min in an ice bath [23]. Two-percent of the mycelial suspensions (v/v) were used as inoculum. The submerged mycelial culture was carried out in a 5-l jar fermenter (working volume: 3-l; agitation speed: 100 rpm; pH 5; temperature: 25°C; aeration rate: 1 vvm) for 25 days. Culture broth was harvested by centrifugation (10,447 ×g for 20 min) and the supernatant was treated with four times of ethanol (v/v) to collect the EPs. The recovery process of crude EPs from culture broth is shown in Fig. 1.

Sprague-Dawley male rats were obtained from the Korea Research Institute of Chemical Technology (KRICT) and housed individually in stainless steel cages in a room with controlled temperature (22±2°C), humidity (55±5%), and a 12 h cycle of light and dark. Rats were fed with a commercial pellet diet (Sam Yang Co., Korea). They were adapted for 7-10 days and fasted for 12 h before the intramuscular injection of STZ (Sigma, 50 mg/kg body weight, dissolved in citrate buffer, pH adjusted 4.5). Five days after STZ treatment, the diabetic state was confirmed by the positive response of glucose in urine (using strips; Glucotest, Germany). They were then used as the insulin dependent diabetes mellitus (IDDM) model. Rats of each group were administered saline (control) and EPs at the level of 50 mg/kg body weight using an oral zonde daily for seven days. At the end of the oral administration, the animals were fasted for nine hours and sacrificed by ether anesthesia. Food intake and body weight were recorded every day. Blood samples were collected in heparinized tubes and plasma was separated by centrifugation at 1,100 ×g for 10 min. Plasma glucose, TC, TG, and GPT were measured using enzymatic kits (Asan Pharm. Co., Ltd.).

Data were expressed as mean±SE. Group means were compared by one-way analysis of variance and Duncan's multiple-range tests. The statistical differences were considered significant at $p<0.05$.

Table 1. Effects on food intake and body weight of STZ-induced diabetic rats after the administration of exo-polymers produced from submerged mycelial culture of mushrooms.

Experimental groups	Food intake (g/day)	Body weight gain (g/week)
Control (Saline)	23.20±4.59^{NS}	18.99±6.76^{NS}
Lentinus edodes	21.95±7.32	21.45±7.76
Agrocybe cylindracea	21.49±5.77	23.54±5.78
Auricularia polytricha	22.89±5.61	19.76±6.54
Cordyceps militaris	20.93±4.35	22.49±7.44
Phellinus linteus	21.28±7.25	21.87±6.25

Values are mean±SE (n=8).
^{NS}Not significant.

Effects of Food Intake and Body Weight

The final body weights of the animals fed on five experimental diets were not significantly different from that of the control diet, though slightly increased (Table 1).

The levels of food intake for the five EPs-administered groups were also not substantially different from that of the control group. Generally, body weights were reduced in STZ-induced diabetic rats [7] and recovered when subjected to the hypoglycemic treatment. The body weight of all groups increased slightly and oral administration of EPs caused no change in gross behavior, and none of the animals died. Thus, it could be stated that there were no harmful effects in rats following the oral administration of EPs.

Hypoglycemic Activity

The effects of EPs of five different fungal cultures on plasma glucose, TC, and TG retention in diabetic rats were

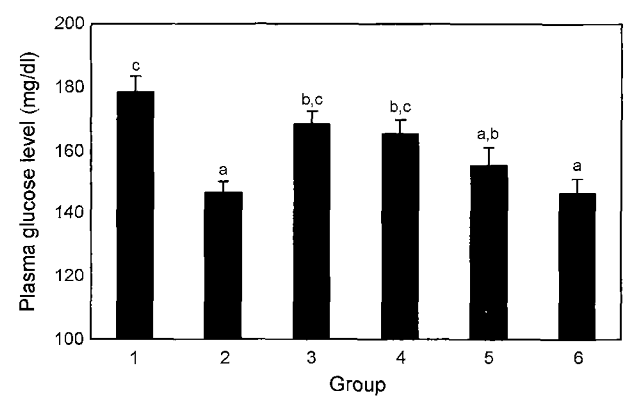

Fig. 2. Hypoglycemic effects of exo-polymers, produced from submerged mycelial culture of mushrooms, in rats by oral administration (50 mg/kg body weight).

1. Control (saline administration), 2. *Lentinus edodes*, 3. *Agrocybe cylindracea*, 4. *Auricularia polytricha*, 5. *Cordyceps militaris*, 6. *Phellinus linteus*. Values are mean±SE (n=8). Values with different letters (a~c) were significantly (p<0.05) different among the groups by Duncan's multiple range test.

evaluated with respect to that of the saline-administered control group. The EPs of different fungal culture behaved differently in a plasma-glucose retention pattern in diabetic rats (Fig. 2).

The plasma glucose level was significantly reduced by 18% after the oral administration of EPs produced by *L. edodes* and *P. linteus*, and about 14% by *C. militaris*. The extent of this decrease in plasma glucose in two other diet groups was less significant. However, the endo-polymers of *C. sinensis* and extracts of fruiting bodies of *A. cylindracea* significantly lowered the plasma glucose level in diabetic rats [15, 18]. This may be due to structural and compositional differences between extracts from the fruiting body and EPs produced from the submerged mycelial culture. The hypoglycemic effect exerted by the EPs of three mushroom species could be explained by the viscous nature. An increased viscosity of the intestinal content imposed by the EPs might have resulted in reduced nutrient movement towards the villi network for efficient absorption, which probably played a role to lower the levels of plasma glucose, TG, and TC. Its effects can be compared to a high viscosity water-soluble dietary fiber such as guar gum or pectin [12, 13, 22]. The difference in viscosity of the EPs of different mushroom species could bring the difference in their hypoglycemic effect. However, this fact cannot exclude the other mechanisms involved in exhibiting the hypoglycemic effect by EPs. The corrective role of EPs in pancreatic β-cells function cannot be ruled out. The STZ treatment inhibits insulin secretion by the pancreas through selective destruction of β-cells of the pancreatic islets [21, 24]. Perhaps the EPs of *L. edodes* could repair the damage of the pancreatic β-cells and promote insulin synthesis, thus lowering the level of plasma glucose. In fact, the findings of Gray and Flatt [9] with a water-soluble extract of *Agaricus campestris* may possibly support this supposition. They demonstrated that an aqueous extract of mushroom stimulated 2-deoxyglucose transport, glucose oxidation, and incorporation of glucose into glycogen in the abdominal muscle in mice treated with STZ. They also documented the stimulation of insulin secretion from the BRIN-BD11 pancreatic β-cells line under the influence of the *A. campestris* aqueous extract.

Plasma TG and TC levels are strongly related to the degree of diabetic control in IDDM rats [2, 10]. In the present investigation, the TG level in plasma was significantly reduced by oral administration of EPs produced by *C. militaris* (14.6%) and *L. edodes* (10.1%), as shown in Table 2.

However, the effect of other EP diets on the plasma TG level was less significant. The increased TG level in the diabetic rats is associated with the low insulin level in the plasma. Increased mobilization of free fatty acids and decreased clearance due to reduced lipoprotein lipase (LPL) activity result in elevated levels of TG and a very low density lipoprotein (VLDL) [3] in the blood plasma. Insulin regulates both the secretion of VLDL into the plasma from the liver and its removal at the peripheral tissue through the action of the endothelial LPL [3, 8]. The low insulin level in STZ-treated diabetic rat might have affected LPL function and resulted in the high TG level. The significant TG lowering effect of *C. militaris* and *L. edodes* EPs can be explained by their probable insulin-inducing activity, which promotes LPL activity to reduce the plasma TG level.

Although *P. linteus* EPs exhibited little effect on the TG level, it substantially reduced the level of plasma TC. Effect of different EPs on the plasma TC level was depicted in Table 2. Maximum depletion (15.8%) of plasma TC was achieved under the influence of *C. militaris* EPs, which was followed by *P. linteus* (13.7%) and *L. edodes* (13.3%). The substantial reduction of TC in plasma may be a result of a reduced production of TC by the liver tissue (by suppressing the activity of the hepatic enzyme HMG Co-A reductase) and/or inhibition of reabsorption of cholesterol and bile from the small intestine by increasing the viscosity of the intestinal content [6]. In addition, they may increase the excretion of bile acids through the excreta [26].

Generally, GPT activity is increased by metabolic changes in the liver such as administration of toxin, cirrhosis of the liver, hepatitis, and liver cancer [4]. In fact, it can be used as a marker to indicate the extent of liver damage. Increased TG and TC levels in the diabetic rats may be due to an

Table 2. Effects of exo-polymers produced from submerged mycelial culture of mushrooms on plasma triglyceride, total cholesterol, and GPT levels in STZ-induced diabetic rats.

Experimental groups	Triglyceride (mg/dl)	Total cholesterol (mg/dl)	GPT (Karmen unit: IU/l)
Control (Saline)	70.58±3.70^{b}	89.44±2.01^{ab}	53.54±1.73^{a}
Lentinus edodes	63.43±4.72^{bc}	77.52±2.62^{def}	52.45±2.19^{a}
Agrocybe cylindracea	66.66±2.03^{bc}	81.62±1.59^{cbe}	51.14±3.80^{ab}
Auricularia polytricha	74.96±3.42^{ab}	89.78±1.91^{a}	48.58±2.70^{ab}
Cordyceps militaris	58.84±3.86^{c}	75.27±1.64^{f}	51.51±3.17^{a}
Phellinus linteus	66.75±4.03^{bc}	77.21±2.27^{ef}	44.45±1.52^{b}

Values are mean±SE (n=8).
Values with different superscript letters (a~f) in the same row significantly (p<0.05) different among the groups by Duncan's multiple range test.

abnormal liver function. Its activity could be correlated with plasma TG and TC levels of the experimental animals. The effect in GPT value under the influence of different EPs was depicted in Table 2. In the present investigation, only *P. linteus* EPs significantly lowered GPT level in serum that was raised by injection of STZ. Perhaps the EPs from *P. linteus* could enable the liver tissue to rectify the damage induced by STZ administration to some extent. However, the *C. militaris* and *L. edodes* EPs having significant hypolipidemic effect could not reduce GPT activity at all. The reason behind this is still unclear.

This preliminary study involved screening EPs of different fungal groups for their hypoglycemic activity. Among the different fungal EPs, the EPs of *L. edodes*, *C. militaris*, and *P. linteus* proved their potency to combat against hyperglycemia in STZ-induced diabetic rats. Although the exact mechanism of hypoglycemic action of EPs of these three mushrooms is not clear as of this time, our result signifies the fact that different EPs may have different modes of action in exhibiting their hypoglycemic effect. It also indicates the necessity to perform the experiment at a higher dosage. Moreover, further comprehensive chemical and pharmacological investigations are needed to elucidate the exact mechanism of hypoglycemic effects and to isolate the active principles of these mushrooms before they can be useful for preventive and therapeutic purposes to alleviate the hyperglycemic status in diabetes mellitus.

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