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# Antihyperglycemic and antioxidant activities of polysaccharide produced from *Pleurotus ferulae* in streptozotocin-induced diabetic rats

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**Abstract**−We investigated the antihyperglycemic and antioxidant activities of polysaccharides produced from *Pleurotus ferulae* mutant in streptozotocin-induced diabetic rats. Blood glucose level in the STZ-induced diabetic rat administered the extract polysaccharides, 250 mg/kg b·w/d (EPSG) was 196.97 mg/dL, approximately 54.12% less compared to that of the STZ-induced diabetic rats administered 0.9% NaCl solution (NCG). The insulin level in the EPSG was approximately 1.64-fold higher than that in the NCG. HDL and LDL cholesterol levels in the EPSG were 29.15 mg/L and 20.35 mg/dL, respectively, representing an approximate increase of 69.18% and decrease of 38.52%, respectively. The activities of aspartate aminotransferase (AST) and alanine transferase (ALT) in the EPSG decreased approximately by 49.27 and 50.43%, respectively, while the alkaline phosphatase (ALP) activity decreased by 34.25%, relative to the NCG. Antioxidant activities in the NCG decreased relative to the normal group. In contrast, for EPSG, these values increased relative to the NCG. The malondialdehyde level in the EPSG was 12.95 mmol/mg protein, which was approximately 70.64% of that in the NCG. These results suggest that the polysaccharides of *Pleurotus ferulae* mutant could be developed as potential antidiabetic agents or functional foods for people with a high risk of diabetes mellitus.

Keywords: *Pleurotus ferulae*, Antihyperglycemic Activity, Antioxidant Activity, Polysaccharide, STZ-induced Diabetic Rat

## INTRODUCTION

Diabetes mellitus, one of the leading causes of morbidity and mortality worldwide, refers to a group of endocrine metabolic disorders with different etiologies. It is a chronic disease involving alterations in carbohydrate, fat, and protein metabolisms with serious complications including heart disease, blindness, and kidney failure. Furthermore, diabetic patients in Korea develop various diabetic complications, and the diabetes-related mortality has rapidly increased over the last decades. The World Health Organization estimates that more than 220 million people worldwide have diabetes, a number likely to more than double by 2030 [1]. Diabetes mellitus is a serious and growing health problem that is characterized by chronic hyperglycemia and the development of diabetes-specific microvascular pathology in the retina, renal glomerulus, and peripheral nerve [2]. Oxidative stress is regarded as the major factor in the pathogenesis of diabetes mellitus and its associated health disorders. Studies have shown that increased generation of free radicals and reduced antioxidant defense mechanisms could lead to increased lipid peroxidation and development of insulin resistance [3]. To date, many oral hypoglycemic drugs with different mechanisms of action have been developed against diabetes. However, insulin and sulfonylureas, especially, potent and long-acting agents such as glibenclamide, cause hypoglycemia, while metformin carried the risk of lactic acidosis, vomiting, and diarrhea. In addition, these drugs have been associated with high rates of secondary failures [4,5]. Hence, there is a need for an alternative approach, including the use of an effective and safe natural drug with strong antioxidative effect and hypoglycemic action.

*Pleurotus ferulae* is a hymenomycetes fungus belonging to the order Agaricales and family Pleurotaceae. *P. ferulae* has been reported to inhibit acetylcholinesterase and protect brain cells during Alzheimer disease induction and have antimicrobial and fibrinolytic activity [6]. Hong et al. [7] reported that nutritional components such as amino acid, mineral, vitamin, sugar, and fatty acid contents of artificially cultivated of *P. ferulae* were higher than those of *P. ostreatus* and *P. eryngii*. Rho and Park reported that the vitamin $D_2$ contents of *P. ferulae* irradiated by ultraviolet (UV)-B showed a

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significant increase when compared to the vitamin $D_2$ content before UV-B irradiation [8]. Recently, we researched the optimal media and growth conditions for *P. ferulae* liquid cultures for effective production of exopolysaccharide and mycelial growth [9] and determined the viability of human cancer cell lines for use in screening the antitumor substances contained in the *P. ferulae* extract [10]. We also investigated the *in vitro* antioxidant effects and the nitrite scavenging activities of the extracts from *P. ferulae* fruiting body grown on a solid-state culture using corncob and activated bleaching earth and its mycelium grown in the liquid state [11]. Despite the clinical importance of *P. ferulae*, there have not been studies on antihyperglycemic and antioxidant effects of polysaccharides produced from *P. ferulae*.

In this study, to investigate the antihyperglycemic and antioxidant effects of polysaccharides produced from *P. ferulae* mutant in streptozotocin-induced diabetic rats, glucose, insulin, glycogen, fructosamine, lipid levels, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, lactate dehydrogenase, and organ weights were determined. In addition, the antioxidant activities were evaluated.

## MATERIALS AND METHODS

### 1. Cultivation

The composition of the culture media used for the mycelium and polysaccharide production from *P. ferulae* mutant was as follows: glucose 50.0 g/L, CSL 4.0 g/L, yeast extract 4.00 g/L, polypeptone 10.0 g/L, $KH_2PO_4$ 1.0 g/L, and $MgSO_4$ 0.5 g/L. All media were sterilized at 121 °C for 40 min. The culture medium was inoculated with 5% of the mycelial homogenate and then cultivated at 25 °C in a 50 L air-lift bioreactor with 25 L of working volume under 2.0 vvm for 10 days.

### 2. Induction of *Pleurotus ferulae* Mutant

One hundred microliters of the protoplast suspension was spread out onto Petri dishes (90 mm in diameter) containing 15 mL semi-synthetic medium (casamino acid 2 g, glucose 20 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, $KH_2PO_4$ 1.0 g, $CaCl_2 \cdot 2H_2O$ 0.1 g, $FeSO_4 \cdot 7H_2O$ 1 mg, $ZnSO_4 \cdot 7H_2O$ 0.9 mg, $MnSO_4 \cdot 4H_2O$ 0.8 mg, $H_3BO_3$ 1 mg, $CuSO_4 \cdot 5H_2O$ 0.15 mg, $Co(NO_3)_2$ 0.1 mg, $(NH_4)_6Mo7O_{24} \cdot 6H_2O$ 0.2 mg, thiamine hydrochloride 0.1 mg, adenine hydrochloride 5 mg, and agar 15 g per liter of distilled water) containing 0.5 M sucrose as osmotic stabilizer. Ultraviolet (UV) light was used as a mutagen. The plates were irradiated with a Toshiba 10-W germicidal lamp 10 cm apart for 17-22 s in the darkroom. The survival rate of protoplasts after this treatment was 1%-3%. The plates were then incubated at 25 °C in the dark for 10 days.

### 3. Preparation of Polysaccharides Extract

The powdered mycelium was defatted with petroleum ether and extracted with double distilled water at 80 °C for 8-10 hr in several batches. The extracts were combined, filtered, and concentrated to about one-third of the original volume and chilled ethanol about five times the original volume was added and kept at 4 °C for 24 hr. The precipitate was collected after centrifugation, redissolved in distilled water, treated with Sevag's reagent several times to remove protein, and then dialyzed against deionized water for 24 hr at 4 °C. The polysaccharides were again precipitated with ethanol and the precipitate thus obtained was lyophilized. The lyophilized polysaccharide was dissolved in water, reprecipitated with equal volume of cetyltrimethylammonium hydroxide and kept overnight. The supernatant obtained was precipitated with chilled ethanol. After centrifugation, the polysaccharides extract (0.2 g) was dissolved in distilled water, dialyzed against distilled water, and applied to a DEAE-cellulose ion-exchange column (5×50 cm). Stepwise elution was performed with distilled water and a linear gradient of NaCl solution (0-2 M) in Tris-HCl buffer (pH 8.5). The polysaccharides were collected, lyophilized and stored at 15 °C for further analyses.

### 4. Monosaccharide Composition Analysis

The monosaccharide was determined by GC-MS. Briefly, 5.0 mg of polysaccharide was dissolved in 4 mL of trifluoroacetic acid (TFA, 2 M) solution and hydrolyzed at 120 °C for 4 hr in a sealed glass. The hydrolyzate after removing TFA was reduced with $NaBH_4$ and acetylated with acetic anhydride. The acetylated products were dissolved in 4 mL of chloroform and dried with anhydrous $Na_2SO_4$. The dried products were analyzed by GC-MS using an Agilent 7890 GC/5975 MS system fitted with a fused silica HP-5 capillary column (0.25 mm×0.25 mm×30 m).

### 5. Animals and Experimental Design

White male Sprague-Dawley rats weighing approximately 200-250 g were raised. Before starting the experiments, all the animals were acclimatized to the laboratory conditions for three weeks. They were housed at an ambient temperature of 25±2 °C, 12/12 hr light-dark cycle, and 50-60% humidity conditions with pellet diet (Sam Yang, Seoul, Korea) and water. All animals used in this study were cared for according to the Care and Use of Laboratory Animals Guidelines. All procedures were approved by the Animal Ethics Committee of Chosun University Laboratory Animal Research Center. All the animals were randomly divided into four groups with eight animals in each group: 1) normal group (NG), normal rats administered 0.9% NaCl solution; 2) negative control group (NCG), streptozotocin (STZ)-induced diabetic rats administered 0.9% NaCl solution; 3) positive control group (PCG), STZ-induced diabetic rats administered metformin (250 mg/kg/day); and 4) extract polysaccharide group (EPSG), STZ-induced diabetic rats administered the extract polysaccharides (250 mg/kg/day). Polysaccharides and metformin were administered by mixing with pellet diet. The animals were injected intraperitoneally with a single dose of 60 mg/kg body weight of STZ (N-(Methylnitrosocarbamoyl)-$\alpha$-D-glucosamine) dissolved freshly in cold 20 mM citrate buffer adjusted to pH 4.5.

### 6. Oral Glucose Tolerance Test

After being administered polysaccharide for 21 days to STZ-induced diabetic rats, the rats were used for oral glucose tolerance test (OGTT). Rats fasted for 12-15 hr before the OGTT. On the test day, purified water (control) or polysaccharide was orally administered to each group. Thirty minutes later, 50% glucose solution was administered at a dose of 2 g/kg (i.p.). Blood samples were taken from the tail vein at 0 (just before glucose administration), 30, 60, 90, and 180 min for the measurement of blood glucose.

### 7. Collection of Blood and Tissue Samples

At the end of the experimental period, animals fasted for 12 hours prior to sacrifice. Rats were anesthetized with ether and

blood samples were collected via the abdominal aorta. Plasma was separated by centrifugation (3,000 rpm) at 4 °C for 15 min. Liver and kidney were excised and weighed, and the weights of organs were calculated as the organ weight per 100 g of body weight. After measurement, organs were immediately frozen and stored at –70 °C until further analysis.

**8. Measurements of Body Weight and Intakes of Food and Water**

Body weight, feed, and water intakes were measured every day at the same hour during the experiment period. The FER was calculated as daily weight gain (g)/daily dietary intake (g).

**9. Analysis of Biochemical Parameters**

Blood glucose was determined using glucose assay kits. The blood was collected from the rats after 10-hr fasting and the insulin content was assessed using insulin ELISA kit (R&D Systems, USA). The insulin sensitivity index was presented as the ratio of insulin content to fasting serum glucose content. To determine the glycogen concentration, the liver and gastrocnemius (80-100 mg) were homogenized with lye and heated in boiling water for 30 min. After cooling in an ice bath, the homogenized sample was centrifuged at 8,500 rpm for 30 min. The precipitate was then suspended in 1.5 mL distilled water and the glycogen concentration was measured with a glycogen test kit. The levels of triglyceride, HDL and LDL cholesterol and the activities of aspartate aminotransferase (AST), alanine transferase (ALT), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) from blood were determined using an ADVIA Model 1650 chemistry analyzer and Hitachi Model 7180 chemistry analyzer, respectively.

**10. Activities of Catalase, Superoxide Dismutase, and Glutathione Peroxidase**

To determine the catalase (CAT) activity, a 0.1 ml aliquot of surfactant was added to 50 mM potassium phosphate buffer at pH 7.0 and 10.5 mM $H_2O_2$. The reaction took place for 30 sec at 25 °C. The amount of enzyme activity required to decompose 1 μmole $H_2O_2$/sec via this reaction was defined as one unit of activity. Superoxide dismutase (SOD) activity was determined by recording the inhibition of ferricytochrome C reduction with EDTA. In each sample, the amount of enzyme sufficient to inhibit the rate of cytochrome C reduction by 50% was determined. Malondialdehyde (MDA) level was measured by using 2-thiobarbituric acid (TBA) method and expressed as nM/mg protein in the liver or nM/mL in serum. To analyze the glutathione peroxidase (GSH-Px) activity, 500 μL mixture of potassium phosphate buffer (0.1 M, pH 7.0) containing $1\times10^{-3}$ M sodium azide, 1 mM EDTA, 10 μL of enzyme solution, 100 μL of glutathione reductase (2.768 U/mL), and 100 μL of glutathione ($1\times10^{-2}$ M) was mixed and precultured for 10 min at 37 °C. Next, an aliquot of $NaHCO_3$ (0.1%) containing 100 μL of NADPH ($1.5\times10^{-3}$ M) and 100 μL of $H_2O_2$ ($1.5\times10^{-3}$ M) was added to the reaction mixture. The absorbance was measured for 1 min at 340 nm.

**11. Statistical Analysis**

All experiments were performed in triplicate and the results were expressed as mean±SEM. One-way analysis of variance (ANOVA) followed by Duncan's multiple comparison test was performed. Statistical Package for the Social Science (SPSS, Version 19.0, SPSS Inc., Chicago, IL, USA) was used. The criterion for statistical significance was a *p*-value less than 0.05.

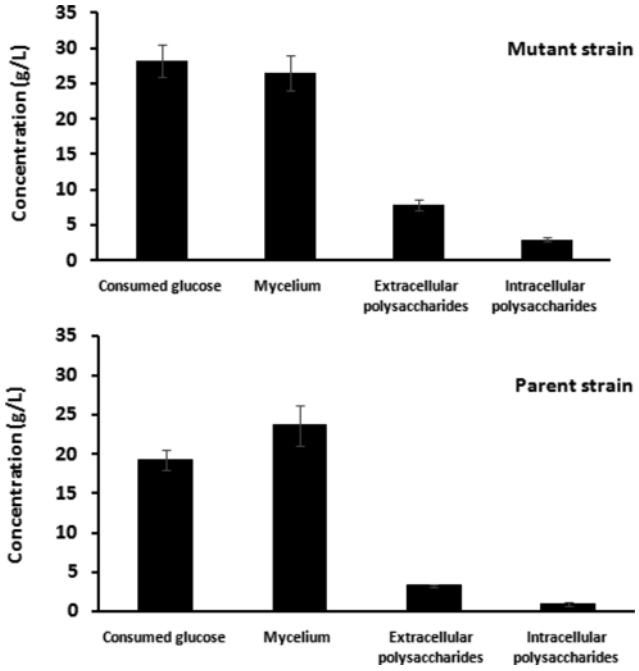


**Fig. 1. Polysaccharides production and mycelium growth from *P. ferulae*. All experiments were performed in triplicate. The results are expressed as mean±SEM.**

## RESULTS

**1. Polysaccharides and Mycelium Production from *P. ferulae***

For efficient polysaccharides production and mycelium growth from submerged culture of *P. ferulae* mutant and parent in an air-lift bioreactor, various environmental factors were investigated. The optimal temperature and pH were 25 °C, and 7.0, respectively (data not shown). Culture medium composed of glucose 50.0 g/L, CSL 4.0 g/L, yeast extract 4.00 g/L, polypeptone 10.0 g/L, $KH_2PO_4$ 1.0 g/L, and $MgSO_4$ 0.50 g/L was the most effective energy source for mycelium growth and exopolysaccharides production. Using the optimal culture condition and medium composition, batch cultures were carried out in a 50 L air-lift bioreactor with 25 L working volume for 10 days. The mycelium growth, exopolysaccharide production, and the residual glucose concentration observed are shown in Fig. 1. When the parent strain (*P. ferulae* PF-23) was used, the glucose consumption increased up to eight days, but consumption did not increase after ten days of culture. The glucose consumed was 19.26 g/L and mycelium production was 23.67 g/L while the extracellular and intracellular polysaccharide productions were 3.26 and 0.92 g/L, respectively after ten days. The production yield for extracellular and intracellular polysaccharides was 0.17 and 0.05 g/g of consumed glucose, respectively. On the other hand, when *P. ferulae* mutant (PFM-68) was used, the glucose consumption increased with culture time up to eight days of culture but did not increase after ten days of culture. After ten days, the glucose consumed was 28.12 g/L while the maximum mycelium production was 25.63 g/L, which was similar to that of *P. ferulae* PF-23 (parent strain). Extracellular and intracellular polysaccharide productions were 7.83 and 2.96 g/L, respectively, which was approximately 2.4-

**Table 1. Chemical composition of polysaccharides**

| Neutral sugar (%) | Uronic acid (%) | Protein (%) | Neutral sugar (molar ratio) | | | | |
|---|---|---|---|---|---|---|---|
| | | | Glucose | Mannose | Galactose | Arabinose | Xylose |
| 71.91±3.32 | 0.93±0.21 | 11.22±1.1 | 6.94±0.45 | 9.14±1.22 | 4.26±0.23 | 2.12±0.01 | 0.13±0.001 |

All experiments were performed in triplicate. The results are expressed as mean±SEM

and 3.0-fold higher than those of parent strain (*P. ferulae* PF-23). The production yields of extracellular and intracellular polysaccharides were 0.28 and 0.11 g/g of consumed glucose, respectively. The developed model for an air-lift bioreactor showed good agreement with experimental data and simulated results for mycelium production and exopolysaccharide production in a culture of *P. ferulae* mutant (data not shown). These results indicate that the polysaccharide production increased in parallel with the growth of mycelium, and was associated with the mycelial growth in an air-lift bioreactor using *P. ferulae* mutant. These results also suggest that an air-lift bioreactor has the potential for use in polysaccharide and mycelium production from *P. ferulae* mutant.

**2. Composition of Polysaccharides**

Table 1 gives the chemical composition of polysaccharides produced from *P. ferulae* mutant. Neutral sugar, uronic acid, and protein concentrations of extracted polysaccharides were 71.91, 0.93, and 11.22%, respectively. Among neutral sugars, mannose and glucose were the major ones of the sugar moiety with molar ratio of 9.14 : 6.94, which shows that chemical properties may have a great influence on antioxidant and antihyperglycemic activities. In the case of galactose, arabinose, and xylose, the molar ratio was 4.26 : 2.12 : 0.13. Lo et al. [12] found a direct relationship between monosaccharide composition and conformation of side chains with scavenging ability of polysaccharides from culture broth filtrates of *L. edodes*. They confirmed that the glucose 1→6 linkage and arabinose 1→4 linkages of the side-chain were significantly related to the scavenging on DPPH radicals. The radical scavenging ability of amylopectin and starch for the observed concentration range was 30.3, 41.8, 30.2, and 26.6%, respectively.

**3. Effect of Polysaccharides on Body Weight, Feed and Water Intake, and Feed Efficiency**

The effect of polysaccharides administration on body weight, feed and water intake, and feed efficiency in STZ-induced diabetic rats is shown in Fig. 2. While the body weight of STZ-induced diabetic rats decreased, the oral administration of polysaccharides and metformin caused no change in gross behavior and none of the animals died. The body weight gain in the NCG was 2.02 g/day. However, the weight gain in STZ-induced diabetic rats provided with oral administration of polysaccharides (250 mg/kg body weight) and metformin (250 mg/kg body weight), was 3.78 and 3.86 g/day, respectively, which was about 72.15 and 70.65% compared to NG. Both feed and water intake increased in all STZ-induced diabetic rats. When STZ-induced diabetic rats were orally administered with metformin and polysaccharides, the feed intake was 28.63 and 28.72 g/day, respectively. In the case of the NCG, the feed intake was about 13.45% more than that of EPSG. Water intake in STZ-induced diabetic rats orally administered with metformin and polysaccharides was 134.16 and 144.54 mL/day, respectively. In the case of the NCG, it increased by about 23.40% as

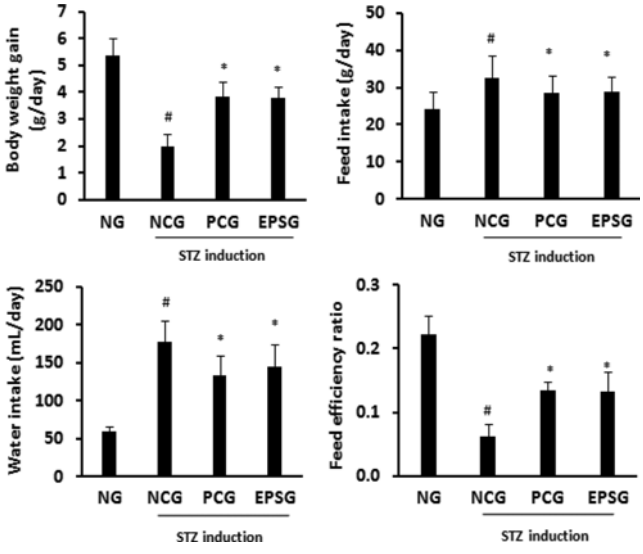


**Fig. 2. Effect of polysaccharides on body weight, feed intake, water intake, and feed efficiency in rats. NG, Normal rats administered 0.9% NaCl solution; NCG, STZ-induced diabetic rats administered 0.9% NaCl solution; PCG, STZ-induced diabetic rats administered metformin (250 mg/kg b·w/d); EPSG, STZ-induced diabetic rat administered the extract polysaccharides (250 mg/kg b·w/d). The results are expressed as mean ±SEM (n=8). $^{\#}p<0.05$ compared with NG, $^{*}p<0.05$ compared with NCG (ANOVA followed by Duncan's multiple comparison test).**

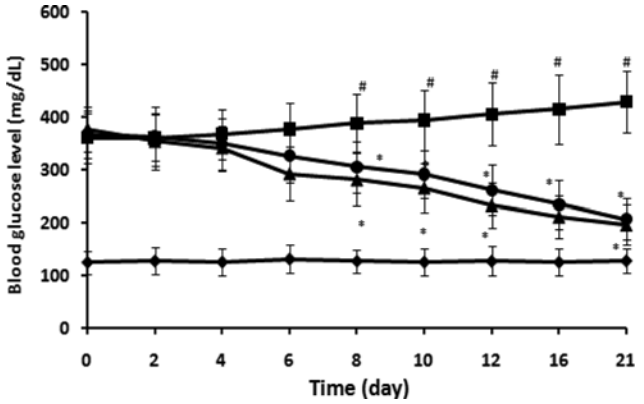


**Fig. 3. Effect of polysaccharides on the blood glucose levels in rats. Symbol: ◆, NG; ■, NCG; ▲, PCG; ●, EPSG. The results are expressed as mean±SEM (n=8). $^{\#}p<0.05$ compared with NG, $^{*}p<0.05$ compared with NCG (ANOVA followed by Duncan's multiple comparison test).**

compared to the EPSG. Feed efficiency ratio was greatly decreased in STZ-induced diabetic rats. Especially, in STZ-induced diabetic rats orally administered with polysaccharide, it was only 0.13. In addition, it further decreased by about 53.84% in the NCG as compared to EPSG.

**4. Effect of Polysaccharides on Glucose Levels**

Changes in fasting blood glucose levels for 21 days are shown in Fig. 3. The blood glucose levels were almost similar until four days in STZ-induced diabetic rats. However, blood glucose levels from the fifth day increased with the increase of feeding period in the NCG. The blood glucose levels in the NCG increased to 429.41 mg/dL after 21 days, which was about 18.80% increase as compared to their initial glucose levels. This could be due to the side effects of peritoneal injection of STZ, which include pancreatic beta-cell destruction and inhibition of insulin secretion. On the other hand, when STZ-induced diabetic rats were orally administered with polysaccharides and metformin, the blood glucose level decreased to 196.94 and 208.36 mg/dL after 21 days, respectively, which was about 43.43 and 47.92% reduction compared to the initial values. In addition, when compared to the NCG, the blood glucose level in the EPSG was reduced by 54.16% after 21 days. The results showed that STZ-induced diabetic rats fed with polysaccharides produced from *P. ferulae* mutant might help alleviate the elevation of fasting blood glucose levels. These results indicate that polysaccharides of *P. ferulae* mutant exhibited considerable hypoglycemic effect in STZ-induced diabetic rats and may prove to be useful for the management of diabetes mellitus.

**5. Effect of Polysaccharides on Insulin, Glycogen, and Fructosamine Levels**

Fig. 4 shows the effect of polysaccharides on insulin, glycogen, and fructosamine levels in rats. The insulin levels decreased in STZ-induced diabetic rats, when compared to NG. Especially, the insulin level was lowered by 35.63% in the NCG after 21 days. On the other hand, the insulin level was increased by 22.33% in the EPSG when compared to the NCG. These results showed that the polysaccharide treatment improved glucose tolerance and decreased blood glucose level by increasing the insulin sensitivity. The glycogen level in liver also decreased in STZ-induced diabetic rats, when compared to NG. It was 2.06 mg/kg liver in the NCG, which was about 35.63% less compared to NG. On the other hand, in the case of the EPSG, the glycogen level was 2.56 mg/kg liver, which was about 22.38% more than that of NCG. Fructosamine is a risk factor for the development of hyperinsulinemic insulin resistance and type 2 diabetes mellitus. STZ-treatment inhibited insulin secretion from the pancreas through the selective destruction of beta cells in the pancreatic islets. Inhibited insulin secretion due to the beta cells destruction in the pancreatic islets in STZ-diabetic rats has been closely correlated with elevated blood level. Blood fructosamine level was increased significantly in the STZ-induced diabetic rats compared with the normal rats. On the other hand, when STZ-induced diabetic rats were orally administered with metformin and polysaccharides, it was 168.81 and 168.10 mg/dL, respectively, which was about 39.41% decrease compared to the NCG. These results established the correlation between fructosamine and blood

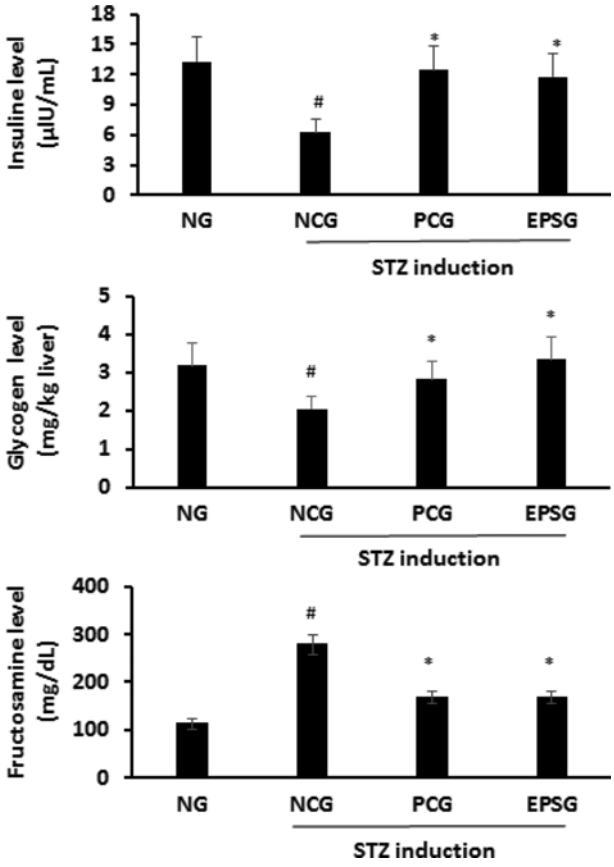


**Fig. 4. Effect of polysaccharides on insulin, glycogen, and fructosamine levels. The results are expressed as mean±SEM (n=8). $^{\#}p<0.05$ compared with NG, $^{*}p<0.05$ compared with NCG (ANOVA followed by Duncan's multiple comparison test).**

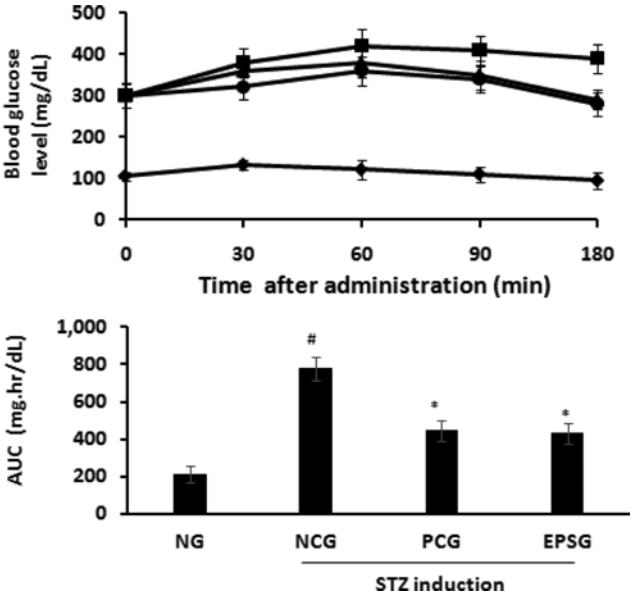


**Fig. 5. Effect of polysaccharides on the oral glucose tolerance test. Symbol, ◆, NG; ■, NCG; ▲, PCG; ●, EPSG. The results are expressed as mean±SEM (n=8). $^{\#}p<0.05$ compared with NG, $^{*}p<0.05$ compared with NCG (ANOVA followed by Duncan's multiple comparison test).**

glucose levels observed in STZ-induced diabetic rats.

**6. Effect of Polysaccharides on Oral Glucose Tolerance**

An oral glucose tolerance test (OGTT) was conducted using the OGTT methodology to investigate the effects of polysaccharides on the glucose level in normal and diabetic rats after administration of a large amount of glucose. The physiological influence of the extract including liver and kidney toxicity during the 180 min following the administration of glucose was also analyzed. The level of blood glucose in different groups during OGTT is shown in Fig. 5. In the NG, the blood glucose level increased up to 132.65 mg/dL at 30 min after glucose administration and decreased thereafter. On the other hand, the glucose level, in the STZ-induced diabetic group increased only at 60 min of glucose administration. However, the glucose levels significantly increased to 420.56 mg/dL in the NCG and did not recover to baseline even after 120 min. In the EPSG, the glucose level was 360.28 mg/dL at 60 min after glucose administration, and returned to the initial glucose level after 180 min. These results indicate that the EPSG showed improvement in overall glucose response compared to the NCG. The area under the curve (AUC) in the NCG was 779.26 mg·hr/dL, which was 3.67-fold higher than that of the NG. These results established the anti-postprandial hyperglycemic effect of polysaccharides in STZ-induced diabetic and normal rats after consumption of glucose.

**7. Effect of Polysaccharides on Lipid Levels**

Fig. 6 shows the effects of polysaccharides on the level of triglyceride, total cholesterol, HDL cholesterol, and LDL cholesterol in rats. Triglyceride, total cholesterol, and LDL cholesterol levels were significantly increased while the HDL cholesterol level was decreased in the NCG compared to the NG. On the other hand, when polysaccharides were administered in STZ-induced diabetic rats, the triglyceride level in the blood decreased to 80.36 mg/dL, which was about 29.32% reduction compared to that of NCG. The total cholesterol was also decreased to 61.87 mg/dL, which was about 46.73% reduction compared to that of the NCG. Meanwhile, the HDL and LDL cholesterol levels were 29.15 mg/L and 20.15 mg/dL, respectively, representing an approximate increase of 69.13% and decrease of 38.54%, respectively, compared to the NCG. The calculated atherogenic index significantly increased to 5.75 in NCG relative to NG. However, when polysaccharides were administered to STZ-induced diabetic rats, the atherogenic index decreased to 1.12, which was about 80.46% less compared to that of the NCG. These results indicate that polysaccharides effectively improved hypertriglyceridemia and hypercholesterolemia in STZ-induced diabetic rats.

**8. Effect of Polysaccharides on Aspartate Aminotransferase, Alanine Transferase, Alkaline Phosphatase, and Lactate Dehydrogenase Activities**

Liver disease is one of the leading causes of death in persons with diabetes mellitus. Diabetic patients can present abnormal liver chemistries, from benign non-alcoholic fatty acid liver disease to severe cirrhosis of the liver. Fig. 7 shows activities of aspartate aminotransferase (AST), alanine transferase (ALT), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) in rats. The activities of AST and ALT in the NCG were 230.24 and 111.25 U/L, respectively, which was 2.87 and 2.16-fold higher than those of NG. On the other hand, they were 131.64 and 56.35 U/L, respectively in the EPSG. In the case of the PCG, the values were similar to those of EPSG. Thus, it can be postulated that the polysaccharides enabled the liver tissue to recover from the liver damage induced by STZ administration. ALP activity in the NCG and EPSG was 468.36 U/L and 309.34 U/L, respectively. The PCG showed similar ALP activity as that of the EPSG. LDH activity in the NCG was 3714.61 U/L. On the other hand, it was 2998.63 U/L in the EPSG. These results are indicative of the hepatoprottctive effect of polysaccharides against hepatic injury as a complication in STZ-induced diabetic rats.

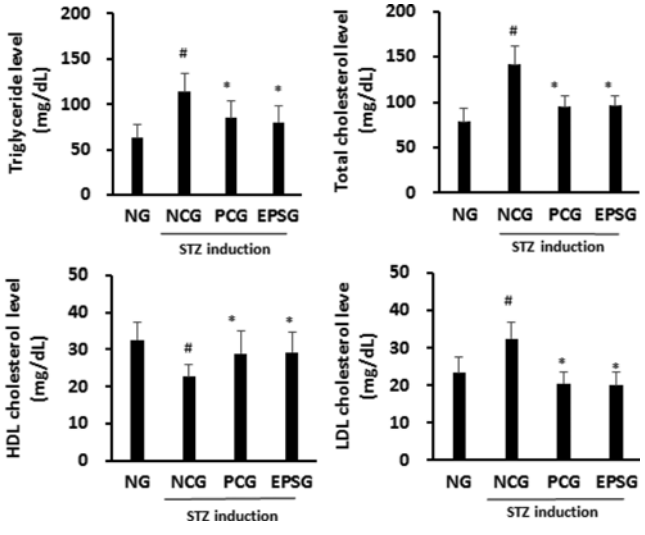


**Fig. 6. Effect of polysaccharides on triglyceride, total cholesterol, HDL cholesterol, and LDL cholesterol levels. The results are expressed as mean±SEM (n=8). $^{\#}p<0.05$ compared with NG, $^{*}p<0.05$ compared with NCG (ANOVA followed by Duncan's multiple comparison test).**

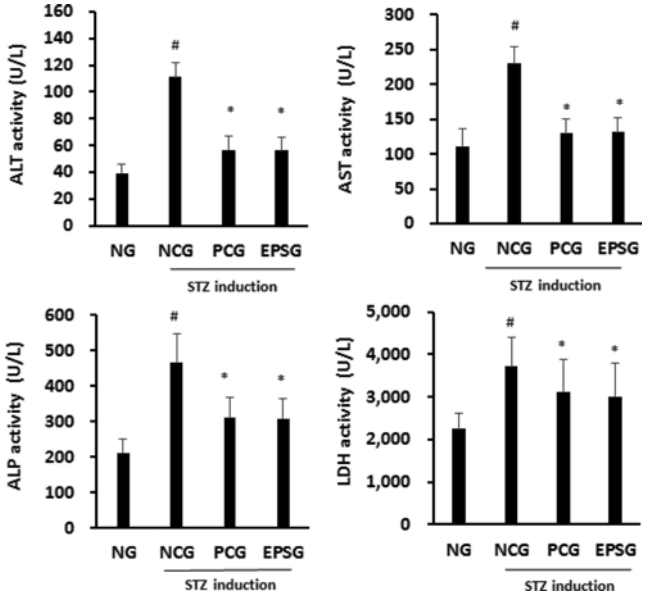


**Fig. 7. Effect of polysaccharides on the AST, ALT, ALP, and LDH activities. The results are expressed as mean±SEM (n=8). $^{\#}p<0.05$ compared with NG, $^{*}p<0.05$ compared with NCG (ANOVA followed by Duncan's multiple comparison test).**

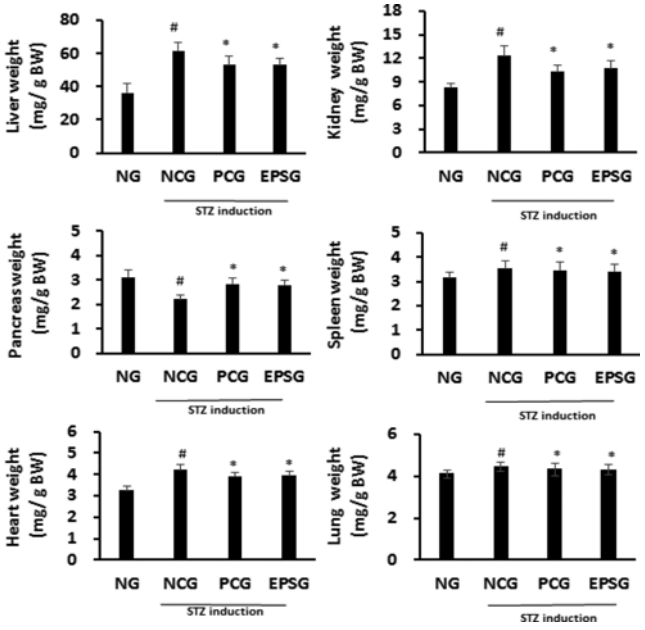


**Fig. 8. Effect of polysaccharides on organ weights. The results are expressed as mean±SEM (n=8). $^{\#}p<0.05$ compared with NG, $^{*}p<0.05$ compared with NCG (ANOVA followed by Duncan's multiple comparison test).**

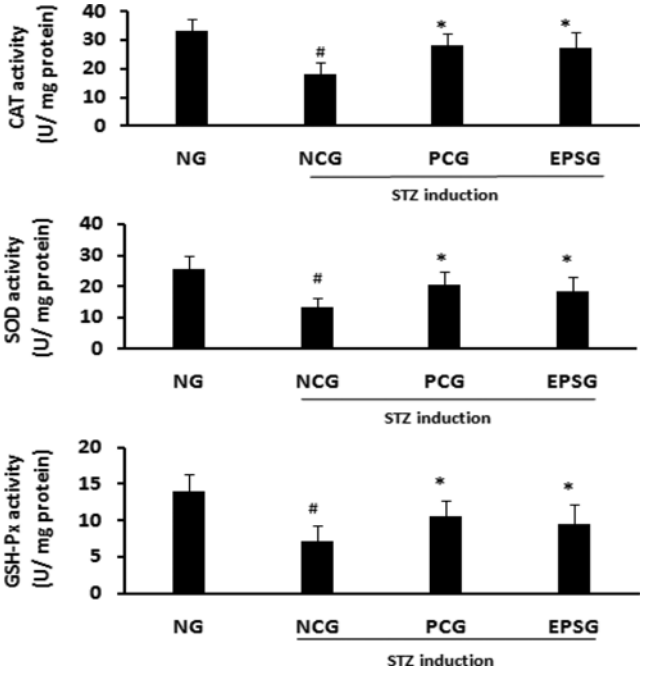


**Fig. 9. Effect of polysaccharides on CAT, SOD, and GSH-Px activities. The results are expressed as mean±SEM (n=8). $^{\#}p<0.05$ compared with the NG, $^{*}p<0.05$ compared with NCG (ANOVA followed by Duncan's multiple comparison test).**

### 9. Effect of Polysaccharides on Organ weights

Fig. 8 shows the weights of liver, kidney, pancreas, spleen, heart, and lung (as percent per body weight) after feeding for 21 days in normal rats and STZ-induced diabetic rats. The weights of liver, kidney, and heart in STZ-induced diabetic rats significantly increased. Specifically, weights of the liver and kidney in the NCG were 1.72 and 1.53-fold higher than that of NG. The liver and kidney weights in the EPSG were 14.24 and 13.41% less, respectively, compared to NCG. In the case of the PCG, they were similar to the EPSG. Spleen and lung weights in all experimental groups were similar. On the other hand, pancreas weight in the NCG significantly decreased compared to NG. It decreased to 2.21 mg/g body weight, which was about 29.25% less compared to the NG. This could be due to the damage to the pancreas by STZ induction. However, in the EPSG, it was increased to 2.79 mg/g body weight, which was similar to that in the PCG. Generally, liver, kidney, and testis in rats with diabetes induced by STZ are enlarged because of abnormal glucose metabolism and the accumulation of lipids caused by reduced insulin formation and insulin resistance [14]. These results suggest that the polysaccharide administration may improve the organ damage resulting from hyperglycemia as typically seen in the case of the liver.

### 10. Effect of Polysaccharides on Catalase, Superoxide Dismutase, and Glutathione Peroxidase Activities

Antioxidants have been shown to prevent the destruction of beta cells [14] by inhibiting the peroxidation chain reaction and thus may provide protection against the development of diabetes [1]. Low levels of plasma antioxidants are implicated as a risk factor for the development of the diabetes, while throughout the progression of disease, high levels of circulating radical scavengers have been recorded [15]. To investigate the effects of polysaccharides on

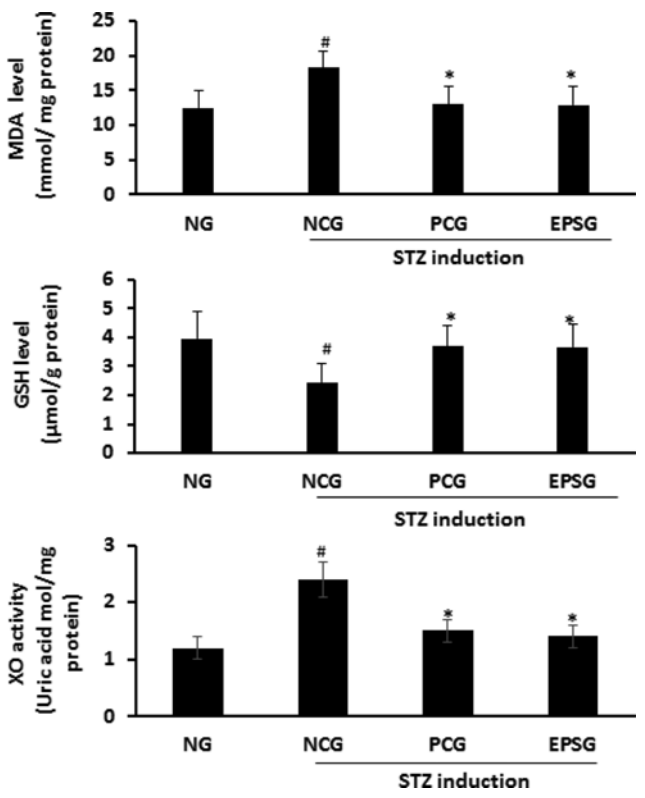


**Fig. 10. Effect of polysaccharides on malondialdehyde and glutathione level and xanthine oxidase activity. The results are expressed as mean±SEM (n=8). $^{\#}p<0.05$ compared with NG, $^{*}p<0.05$ compared with NCG (ANOVA followed by Duncan's multiple comparison test).**

the antioxidant activity in STZ-induced diabetic rats, SOD, CAT, and GSH-Px activities were measured. The results are shown in Fig. 9. SOD, CAT, and GSH-Px activities in the NCG were 13.24, 18.26, and 7.16 U/mg protein, respectively. On the other hand, they were 18.56, 27.14, and 9.52 U/mg protein in the EPSG, respectively, which was 72.36, 81.94, and 68.73% more than that in NG. The values for PCG were similar to that of the EPSG.

**11. Effect of Polysaccharides on Malondialdehyde and Glutathione Level and Xanthine Oxidase Activity**

Fig. 10 shows effect of polysaccharides on malondialdehyde and glutathione level and xanthine oxidase activity. Malondialdehyde (MDA) reflected the degree of lipid peroxidation, and the increased malondialdehyde production played an important role in the progression of diabetic pancreas damage [16]. The MDA level in the NCG significantly increased compared to the NG (12.44 mmol/mg protein). On the other hand, the MDA level in the EPSG was 12.91 mmol/mg protein, which was approximately 70.64% of the NCG. In addition, the MDA level in the PCG was similar to that of the EPSG, GSH is used as a substrate for the antioxidant defense system against oxidative stress [17]. The GSH production in response to polysaccharides was approximately 1.53-fold higher than the NCG. When compared to the PCG, the GSH production of polysaccharide was similar. In the case of the NG, it was 3.96 μmol/g protein. Xanthine oxidase (XO) is a non-specific enzyme involved in the metabolism of purine, pyrimidine, aldehydes and heterocyclic compounds; *in vivo*, it oxidizes primarily hypoxanthine via xanthine into uric acid. It has been reported that XO activity of diabetic rats is increased in liver and plasma. Generally, with low XO activity there is a decrease in the generation of ROS such as superoxide, hydroxyl radical, and hydrogen peroxide [18]. The XO activity in the STZ-induced diabetic group was markedly higher than that of the NG. Specifically, XO increased to 2.43 uric acid mmol/mg protein in the NCG, which was about 2.01-fold higher than that of the NG. On the other hand, it decreased to 1.44 uric acid mmol/mg protein when polysaccharides were administered. In the case of the PCG, similar results as that of the EPSG were obtained. These results indicate that polysaccharides could decrease the blood glucose and increase the antioxidant activity in a similar manner, which suggests that the antioxidant activity of polysaccharides is likely to be one of mechanisms of hypoglycemic activity.

## DISCUSSION

Many investigators have endeavored to study the hypoglycemic effect of various polysaccharides produced from fermentation broth and fruiting body of mushrooms. For example, the antidiabetic activity of crude exopolysaccharides produced from fermentation broth of *Phellinus baumii* [19], *Laetiporus sulphureus* [20] in STZ-induced diabetic rats was investigated. Xiao et al. [21] reported the hypoglycemic effects of *Ganoderma lucidum* polysaccharides administered in type II diabetic mice. The protein-bound polysaccharides produced from *Pleurotus ostreats* and *Lentinus edodes* [22] and acid polysaccharides isolated from the fruiting bodies of *A. auricular-judge*, *Tremella aurantia*, and *T. fuciformis* have also been studied for their antidiabetic effects [23]. Although there have been many reports on the antihyperglycemic properties of different mushrooms, much less is known about the antihyperglycemic and antioxidant activities of polysaccharides produced from the mycelium cultured on *P. ferulae* mutant.

In the present study, we investigated the antihyperglycemic and antioxidant activities of polysaccharides produced from *P. ferulae* mutant in STZ-induced diabetic rats. The body weight gain was significantly lower in the NCG compared to the NG and the hypoglycemic treatment group recovered (70.65-72.15%). STZ administration causes beta cell destruction within the pancreas, leading to type 1 diabetes mellitus, and consequently insulin production deficiency and a decline in insulin action. Insufficiency in energy production from glucose metabolism affects growth and development. Insulin is involved in the protein metabolism and stimulates the influx of amino acids into skeletal muscles, resulting in an increase of protein synthesis. In animals with induced diabetes, the decline in such actions of insulin leads to the decline in cellular glucose utilization and starvation [24]. This is why all the diabetic groups had greater weight reductions than the NG. Feed and water intake increased in STZ-induced diabetic rats compared to the NG. Specifically, when STZ-induced diabetic rats were orally administered with polysaccharide and metformin, the feed and water intake was about 1.2% more compared to normal rats. Feed efficiency was greatly decreased in STZ-induced diabetic rats, but was slightly restored by polysaccharide and metformin administration. Diabetes induced by STZ leads to enormous loss of body weight because of reduced tissue protein and muscle exhaustion. It is already reported that protein synthesis is reduced in all tissues as a result of decrease in ATP production and insulin deficiency [25].

Blood glucose level after 21 days in the NCG increased about 3.32-fold compared to NG. Although no difference could be observed between the NCG, PCG, and the EPSG before sample administration, the PCG and EPSG showed significant reduction in blood glucose levels from day four of sample administration. Blood glucose levels further decreased significantly with increase in duration of the feeding period after ten days of sample administration in the EPSG. In particular, after 21 days of polysaccharide administration, the glucose concentration was 196.97 mg/dL, which was 54.1% less compared to the NCG. A similar effect was observed in the PCG. Studies have demonstrated that STZ-induced rats administered *Ganoderma applanatum* (white rot fungus) exopolymer showed decreased blood glucose levels [26], and hypoglycemic effects of exopolymers of mushrooms may explain the above result. Previous results have also shown that the fasting blood glucose levels in genetically diabetic rats and STZ-induced diabetic rats, fed a diet containing water-soluble polysaccharide from *Auricularia auricular* and *Tremella aurantia*, significantly decreased. The beneficial component containing exopolymers of mushrooms repairs the damage to pancreatic beta cells and ameliorates insulin synthesis, decreasing the level of plasma glucose in rats treated with STZ [27].

The insulin as well as the glycogen level decreased in STZ-induced diabetic rats. The insulin level in the EPSG was 1.88-fold higher than that of the NCG. In the case of glycogen in the liver, it was 1.24-fold higher than that of the NCG, showing polysaccharides produced from *P. ferulae* mutant facilitated the anabolism of glycogen in the liver. Recently, there has been increasing evidence that

an aqueous extract of mushroom stimulated the insulin secretion from BRIN-BDII pancreatic beta cell line and from isolated islet cells of rats fed with mycelial *Lentinus edodes* [28]. Hypoglycemic effects were also reported in ob/ob mice fed a diet containing exopolysaccharides from *Tremella fuciformis* and *Phellinus baumii* [29]. The possible mechanisms by which polysaccharides exert their hypoglycemic action in diabetic mice could involve a potentiating effect of insulin in plasma or as enhancing either the pancreatic secretion of insulin from the existing beta cells or its release from the bound form. Generally, insulin resistance leads to hyperinsulinemia, resulting in high blood glucose levels and type II diabetes. However, it was reported that polysaccharides from fruiting bodies of *Garnoderma lucudum* reduced serum glucose and increased serum insulin levels for ten days in alloxan-induced diabetic rats in a dose-dependent manner [30]. It has also been reported that polysaccharides from fruiting bodies of *Garnoderma lucudum* produced the hypoglycemic effect potentially by increasing the plasma insulin level in normal rats [31]. Cha et al. [32] reported that the insulin level in STZ-induced diabetic rats, fed with a diet supplemented with fermentation mushroom milk product containing polysaccharides, was strongly related to the degree of diabetic control. Thus, these results suggested that the hypoglycemic effect of polysaccharides produced from *P. ferulae* mutant in the STZ-induced diabetic rats may be at least in part due to the enhancement of insulin secretion from pancreas or insulin-like action. Consequently, the increase in the blood insulin level caused by polysaccharides produced from *P. ferulae* mutant may be an important factor in improving the hyperglycemia of STZ-induced diabetic rats.

The polysaccharides administration group had a lower (about 44.62%) AUC of the glucose response curve than the control group of diabetic rats. Controlling not only fasting but also postprandial hyperglycemia is important in achieving tight control of blood glucose levels, which is the major target of diabetic therapy [33]. Furthermore, postprandial hyperglycemia has been shown to increase the production of free radicals, which induce vasoconstriction and stimulate prothrombotic pathways leading to an increased risk of cardiovascular disease, the major cause of premature death among type 2 diabetic patients [34]. Type 2 diabetic patients have both postprandial hyperglycemia and atherogenic dyslipidemia. Postprandial hyperglycemia is also involved in a variety of metabolic disorders and other diseases, including virus-based diseases and cancer [36]. The increase in postprandial glucose levels was suppressed significantly in both STZ-induced diabetic and normal rats when treated with polysaccharide. These results demonstrated that polysaccharides produced from *P. ferulae* mutant may delay the absorption of dietary carbohydrates, resulting in the suppression of an increase in postprandial blood glucose concentration. Inoue et al. [35] reported that the medication, which flattens peak of postprandial blood glucose, reduces the AUC of the blood glucose response curve. In this study, polysaccharides were shown to reduce both the blood glucose concentration at the peak time point and the AUC.

Hypercholesterolemia is regarded as a major risk factor for cardiovascular diseases such as atherosclerosis, myocardial infarction, heart attack, and cerebrovascular disease, which are the leading causes of death in advanced countries. Reducing the circulating cholesterol levels can reduce the risk of these diseases. Hypercholesterolemia is related to increased levels of oxidative stress and lipid metabolism, with LDL generation being identified as a major contributor to the vascular damage induced by high cholesterol levels [37]. Generally, LDL cholesterol is the main carrier of blood cholesterol and is linearly related to the levels of serum cholesterol. Circulating HDL cholesterol is regarded as 'good cholesterol', which carries cholesterol from peripheral cells to the liver for metabolic conversion into bile acids. This pathway is crucial for maintaining cholesterol homeostasis between blood and peripheral tissues. HDL cholesterol protects against coronary heart disease [38]. When polysaccharides were administered to STZ-induced diabetic rats, HDL cholesterol levels increased by 69.13% while the LDL cholesterol levels decreased approximately 38.54% as compared to the NCG. However, when compared to NG, the values were approximately 10.62 and 14.47% less, respectively. *Grifola frondosa*, *Hypsizygus marmoreus*, and *P. eryngii* had hypolipidemic effects *in vivo*. *Grifola frondosa* and *Hypsizygus marmoreus* decreased plasma lipids in apolipoprotein E-deficient mice, and *P. eryngii* improved dyslipidemia in apolipoprotein E-deficient and db/db mice [39]. These findings agree with previous reports demonstrating the beneficial effects of *Cordyceps militaris* and *C. sinensis* on blood lipid profiles. The fruiting body of *Cordyceps militaris* (3% of the diet) reduced triglyceride levels in high-fat diet-fed rats [40], and *C. sinensis* reduced triglyceride and cholesterol levels and increased HDL-cholesterol levels in STZ-injected rats [41]. Cordycepin has shown similar effects in hamsters and rats fed a high-fat diet [42]. The polysaccharides from *C. sinensis* mycelia mediated the hypolipidemic effect in mice [43]. Thus, the anti-dyslipidemic activity of *Cordyceps militaris* could have been partly mediated by polysaccharides and cordycepin in this study. Improved insulin sensitivity may be the underlying mechanism of the polysaccharide anti-dyslipidemic effect. Insulin resistance plays an important role in the development of hypertriglyceridemia in type 2 diabetes. Insulin resistance increases free fatty acid flux, leading to increased production of triglycerides and very-low-density lipoprotein (VLDL) in the liver [44]. The conversion of VLDL to triglyceride-rich remnants by lipoprotein lipase interferes with chylomicron remnant clearance [45], resulting in hypertriglyceridemia and elevated lipoproteins enriched with triglycerides, which are susceptible to hepatic lipase and cause a decrease in HDL cholesterol [46]. Control of diabetic dyslipidemia, elevated triglyceride, and lowered HDL cholesterol levels is important for reducing the risk of cardiovascular complications. Reducing blood cholesterol levels decreases the risk for cardiovascular disease in patients with diabetes [47]. Therefore, polysaccharides of *P. ferulae* mutant may be useful in preventing or improving cardiovascular complications in diabetes. Kim et al. [48] also reported that plasma levels of total triglyceride and cholesterol were decreased in cinnamon extract-treated *ob/ob* mice. The mechanism was explained by the adenosine monophosphate-activated protein kinase (AMPK)-enhanced triacylglycerol lipase activity that increases glycogen synthesis in the liver and enhances glucose uptake in skeletal muscle and adipocytes. These results suggest that dietary polysaccharides improved the composition of blood lipid profiles in STZ-induced diabetic rats and therefore might possess another potential as an anti-obesity ingredient in the appli-

cation of oriental medicine compounds.

It is well known that ALT and AST are elevated in some diseases such as diabetes mellitus and infectious hepatitis. The activities of AST and ALT generally increase with metabolic changes in the liver due to the administration of toxins such as diabetic-inducing STZ or alloxan. Thus, the AST and ALT activities can be used as biomarkers for monitoring the extent of hepatic injury in diabetic mellitus [49]. When polysaccharides were administered to STZ-induced diabetic rats, the activities of AST and ALT were reduced by about 49.2 and 50.4%, respectively, compared to the NCG. ALP activity in EPSG reduced by about 34.27% compared to the NCG, whereas the LDH activity in the EPSG reduced by about 20.14 %. It has been reported previously that the ALT and AST activities significantly decreased in type I STZ-induced diabetic rats and type II Zucker diabetic rats fed with diet supplemented with fermented mushroom milk containing mushroom polysaccharides [50]. They also reported that the marked increase in the serum ALT and AST activities in D-galactosamine-induced hepatic injury rats significantly decreased with each of the following mushrooms: *Fulammulia velutipes* by 73% and 75.1%, *Lentinus edodes* by 55.5% and 65.2% and *Pleueotus ostreatus* by 28.2% and 51.2%, respectively. Yang et al. reported that ALT and AST activities were significantly reduced under the influence of *Lentinus edodes* exopolymers in STZ-induced diabetic rats [28].

It is widely accepted that oxidative stress plays a key mediatory role in the development and progression of diabetes and its complications due to increased production of free radicals and impaired antioxidant defenses. Oxidative stress combined with mitochondrial dysfunction leads to the activation of inflammatory signaling pathways, which may damage insulin-producing cells and further aggravate diabetes complications [51]. In this study, SOD, CAT, and GSH-Px activities decreased in the NCG compared to NG. On the other hand, they were found to increase in the case of the EPSG as compared to NCG. Enzyme antioxidants and MDA play a major role in scavenging toxic free radicals *in vivo*. Evidence suggests that the diabetogenic capacity of alloxan may depend on its ability to damage beta cells and induce oxidative stress. Lowered activities of enzymatic antioxidants, such as SOD, CAT, and GSH-Px, have been well documented in alloxan-induced rats [52,53] SOD, CAT, and GSH-Px activities significantly decreased in the alloxan-induced diabetic group compared to normal group. However, a significant increase of SOD, CAT, and GSH-Px activities in the *Inonotus obliquus* polysaccharide-treated diabetic group was observed when compared with the alloxan-induced diabetic group [35]. MDA level significantly increased in the alloxan-induced diabetic group. However, the polysaccharide from dry matter of culture broth of *Inonotus obliquus* and glibenclamide treatment significantly decreased MDA level as compared to the alloxan-induced diabetic group [35]. GSH, which is widely distributed in most living cells, is a principal antioxidant and low-molecular weight nonprotein thio compound, and plays an important role in maintaining the intracellular thiol redox state and protecting cells against oxidative damage, xenobiotic organic chemicals, and heavy metals. The GSH production decreased in the NCG but in response to polysaccharide administration, it was found to be approximately 1.5-fold higher than the NCG. This result indicated that the antidiabetic effect of polysaccharides could be related to the antioxidative stress effect by polysaccharides. Diabetes, an important chronic disorder, is related to diverse factors and hence, there is a need to assess the hypoglycemic/anti-hyperglycemic effect and therapeutic potential of mushroom extracts. XO activity decreased in STZ-induced group; however, when polysaccharides were administered in STZ-induced diabetic rats, there was 39.84% reduction in XO activity compared to the NCG. Therefore, these results suggest that polysaccharides may inhibit the XO activity, an ROS generator, and increase the activities of the ROS scavengers, SOD, CAT and GSH-Px. Thus, the main mechanisms that can be attributed to these polysaccharides include their action on the glucose level, reduction in the MDA level, and their influence on the activities of SOD, GSH-Px, and CAT. STZ is a chemical that can cause oxidative damage and, therefore, the activity of antioxidants decreased after the injection of STZ. These results also suggest that the polysaccharides produced from *P. ferulae* mutant are able to reduce the oxidative stress in diabetic rats.

Taken together, these results establish that polysaccharides could decrease the blood glucose and increase the antioxidant activity in a similar manner, which suggests that antioxidant activity of polysaccharides is likely to be one of mechanisms for their hypoglycemic activity. However, further studies are necessary to elucidate the relationship between glucose and lipid in blood and the pharmacological activity of the polysaccharides produced from *P. ferulae* mutant.

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