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Antidiabetic Activities of Ethanol Extract of Dry Matters of Culture Broth of *Coriolus versiolor* in Submerged Culture

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ABSTRACT

The study aimed to investigate the antihyperglycemic and antilipidperoxidative effects of ethanol extract of the dry matter from culture broth of Coriolus versicolor (ECBC) in streptozotocin-induced diabetes mice. Blood glucose level, insulin level, total cholesterol (TC), triglyceride (TG), low density lipoprotein-cholesterol(LDL-C), high density lipoprotein -cholesterol (HDL-C) in serum and reduced glutathione level (GSH), lipid peroxidation, glycogen, antioxidant enzymes in liver were evaluated. Moreover, histopathological observation was conducted. Streptozotocin treatment (150 mg/kg body weight) induced the decrease of GSH level, antioxidant enzymes activities, glycogen content in liver, HDL-C content and insulin level in serum, accompanied by the elevation of the lipid peroxidation in liver, serum blood glucose level, contents of TC, TG and LDL-C. Treatment with ECBC restored the changes in the above parameters up to the basal level. The protective effects were further supported by the attenuation of the degree of pancreas damage in ECBC treated mice.

Key words: *Coriolus versiolor;* Antihyperglycemic; Antilipidperoxidative; Diabetes

INTRODUCTION

Diabetes mellitus, the most common endocrine disease, is not a single disease but a group of disorders of varying etiology and pathogenesis. The management of diabetes is considered a global problem. Modern drugs, including insulin and other hyperglycemia agents such as biguanides, sulphonylureas, etc. control the blood glucose level only when they are regularly administered, but these treatments are tedious and have several disadvantages (such as hypoglycemia, obesity, etc.) (Bhatnagar, 1998; May et al., 2002; Galende et al., 2009). The management of hyperglycemia or hyperlipidemia with minimal side effects in clinical experience and relatively low costs is still a challenge to the medical system.

Coriolus versicolor, known as Yunzhi in China, is a mushroom belonging to the Basidiomycetes class of fungi. Its medicinal value was recorded in the Compendium of Chinese Materia Medica and has been gaining acceptance among the patients worldwide (Kidd, 2000; Wasser and Weis, 1999). Because the growth of this mushroom need several months and the difficulty in cultivation, so it is generally recognized that growing its mycelia in a

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defined medium by submerged fermentation could be a rapid and alternative method to obtain the fungal biomass of consistent quality (Lu et al., 2007; Ramadas et al., 2009; Scheidt et al., 2009). Although several studies have focused on the therapeutic effects of the fruit body of the *C.versicolor*, little information is available about its antihyperglycemic and antilipidperoxidative effects in submerged culture. Accordingly, the objective of this study was to evaluate the antihyperglycemic, lipids modulating and antioxidative effects of ethanol extract of the dry matter of culture broth of *C. versicolor* (ECBC) in submerged culture using murine diabetic model induced by streptozotocin, and to investigate if the culture broth of this mushroom was helpful to repair the damaged pancreas of diabetic in mice.

MATERIALS AND METHODS

Preparation of the ECBC

The voucher specimen of *Coriolus versicolor* (No17002) is deposited in the Lab of Pharmaceutical Engineering of Mushroom, Institute of Horticulture, Zhejiang Academy of Agricultural Science, Hangzhou, China. The ECBC was fermented in the lab. The mycelia of *C. versicolor* was inoculated into a culture medium composed of 3% glucose and 0.75% soybean milk in distilled water and adjusted to the initial pH of 5.0. The culture was grown in 500 mL Erlenmeyer flask containing 200mL of medium by incubating it at 26 ^{o}C for a week by shaking at 150 rpm. Thereafter, the fermentation product was then harvested and the culture broth (mycelia and mycelial extracellular medium) was concentrated under vacuum and freeze-dried to powder form (1.2 and 0.9 % in field). The dried powder was then extracted with 75% EtOH at room temperature three times for 3h each time. The solution was concentrated under reduced pressure to get a dark-brown extract (8.6%).

Reagents

The blood glucose measurement kits were obtained from Shanghai Kaiyang Biotech Institute (Shanghai, China); the kits of insulin were purchased from Shanghai Xitang Biological Engineering Company (Shanghai, China), the kits of total cholesterol (TC), triglyceride (TG), high density lipoprotein-cholesterol (HDL-C) and low density lipoprotein-cholesterol (LDL-C), catalase (CAT), malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPx) and the content of reduced glutathione (GSH) were purchased from the Nanjing Jiancheng Bioengineering Institute (Jiangsu, China). Streptozotocin was obtained from the Sigma-Aldrich Chemical Co., USA. The other chemicals used were reagent grade from commercial source.

Animal modeling, group and treatment

Male ICR mice (20±2 g) were purchased from the Animal Experiment Center of Medical College, Zhejiang University, China. All the procedures were conducted in accordance with the P.R. China legislation under NO.8910MO047 on the use and care of laboratory animals and with the guidelines established by the Institute for Experimental Animals of Zhejiang University. Efforts were made to minimize animal suffering and to reduce the number of animals used. Animals were housed in a climate- and light-controlled room with a 12-h light/dark cycle. Twelve hours before the experiments, food was withheld, but animals had free access to drinking water. At the end of the experiment, animals were euthanized in a CO_2 chamber. No side effects were observed in any of the studied animal groups.

All the animals were randomly divided into the five groups with eight animals in each group. Group 1 served as normal controls and received vehicle only. Group 2 served as diabetic control and received vehicle. Group 3 received the standard drug glibenclamide (25 mg/kg body weight). Groups 4 and 5 received the ECBC (100 and 200 mg/kg body weight, respectively). The vehicle of test drugs were administered orally (1% CMC-Na in distilled water). Blood samples were collected by tail nipping.

Assessment of ECBC on streptozotocin-induced diabetic animals

The procedure was same as described by Vats et al. (2004). Mice were made diabetic by a single intraperitoneal injection of sreptozotocin (150 mg/kg body weight) after overnight fasting for 12 h. Sreptozotocin was dissolved in a freshly prepared 0.01 M citrate buffer (pH 4.5) and given to the mice according to the weight. Seventy two hours after streptozotocin injection, mice with blood glucose levels above 11.1 mmol/l were included in the study, and then treated with drugs 48h later. Blood samples were collected by tail nipping at weekly intervals till the end of study

(five weeks) and centrifuged (3000 g×15 min at 4 °C) for separating the serum. After that, the serum was frozen at -70 °C for the following, assessment for blood glucose. Finally, blood was collected from the eyes (venous pool) under aether anesthesia, then the animals were sacrificed by cervical dislocation, and the liver and pancreas were removed immediately. The serum was stored at -70 °C after separation for the biochemical analysis. The tissues were also stored at -70 °C until required.

Measurement of blood glucose, lipids and lipoprotein in serum

Blood glucose was estimated by commercially available glucose kit based on glucose oxidase method (Trinder, 1969). TC, TG, LDL-C and HDL-C levels were measured following the commercial kit's instructions.

Measurement of CAT, SOD, GPx activities and MDA and GSH levels in liver homogenates

Livers were thawed, weighed and homogenized with Tris-HCl (5 mmmol/l containing 2 mmol/l EDTA, pH 7.4). Homogenates were centrifuged (3000 g ×15 min, 4 °C) and the supernatant was used immediately for the assays of CAT, SOD, GPx activities and MDA, GSH levels following the commercial kits instructions.

Measurement of hepatic glycogen content in liver

Hepatic glycogen content was measured according to the anthrone-H_2SO_4 methods with glucose as the standard (Minzhu, 1994). Briefly, liver tissue (<100 mg) was homogenized in five volumes of an ice-cold 30% (w/v) KOH solution and dissolved in a boiling water-bath (100 °C) for 20 min. Then the glycogen was resolubilized in distilled water. The glycogen concentration was determined by treatment with an anthrone reagent (2 g anthrone/1 L of 95%, v/v H_2SO_4), and the absorbance was measured at 620 nm.

Measurement of insulin level in serum

Serum insulin was measured by insulin RIA kit according to the instruction.

Histopathological examination

The pancreas was removed immediately from the animals after sacrificing and rinsed in ice-cold saline. The tissue samples were fixed with 10% formaldehyde, dehydrated in a graded series of ethanol, and embedded in paraffin wax before sectioning. Then the paraffin sections were cut into sections (about 5 μm thickness) then dewaxed and rehydrated. The sections were then stained with H&E dye and studied using light microscope for histopathological changes.

Statistical analysis

The data are expressed as means±standard error for all the groups. Statistical comparisons were compared by one-way analysis of variance (ANOVA). The level of significance was taken as $p<0.05$.

RESULTS

Effect of ECBC on streptozotocin-induced diabetic mice

The antihyperglycemic effect of ECBC on the fasting blood glucose levels of diabetic mice is shown in Table 1. Intraperitoneal injection of streptozotocin (150 mg/kg body weight) led to over 3-fold elevation of blood glucose level, which was maintained over a period of five weeks. Daily treatment with 100 and 200 mg/kg body weight of ECBC for five weeks led to fall in blood glucose level by 13.7 and 30.7% at 21 days, 30.1 and 37.3% at 35 days, respectively. The percentage reduction of blood glucose levels in glibenclamide-treated mice was 18.7 and 32.8% at 21 and 35 days, respectively.

Table 1 - Effects of the ethanol extract of the dry matter from culture broth of of *Coriolus versicolor* (ECBC) on blood glucose levels in streptozotocin-induced diabetes mice.

Group	Dose (mg/kg)	Serum glucose (mmol/L)		
		0 week	3 week	5 week
Normal control	-	7.64 ± 0.89**	7.82 ± 0.76**	8.21 ± 0.65**
Diabetic control	-	23.23 ± 1.47	26.66 ± 2.21	28.92 ± 1.57
Glibenclamide	25	24.82 ± 1.79	21.61 ± 1.53*	19.4 ± 2.01*
ECBC	100	23.91 ± 1.22	22.94 ± 3.01	20.27 ± 1.36*
ECBC	200	20.31 ± 2.15	18.41 ± 1.39*	18.09 ± 0.98*

Data were represented as mean±s.e.m. (N=8). *P<0.05 compared with untreated diabetic mice. **P<0.01 compared with untreated diabetic mice.

Effect of ECBC on lipids and lipoprotein in serum

Table 2 shows the levels of lipids and lipoprotein in serum of normal and experimental animals in each group. The TG, TC, and LDL-C levels were significantly increased, while HDL-C level was decreased in streptozotocin-induced diabetic mice as compared to normal mice. When diabetic mice were treated for five weeks with the ECBC (100 and 200 mg/kg body weight) and glibenciamide (25 mg/kg body weight), there was significant decrease in the levels of TG, TC and LDL-C and simultaneously increase in the HDL-C level.

Effect of ECBC on MDA level, GSH content and activities of antioxidant enzyme

Table 2 also shows the MDA level, antioxidant enzyme (SOD, CAT and GPx) activities and the content of GSH in liver homogenates of normal and experimental animals. MDA was significantly increased in diabetic mice whereas the activities of antioxidant enzyme and the content of GSH were significantly decreased in streptozotocin-induced diabetic compared to normal mice. The ECBC (100 and 200 mg/kg body weight) and glibenclamide (25 mg/kg body weight) treatment significantly decreased in MDA and were associated with a marked increase CAT, SOD and GPx activities and the GSH content in the liver compared to streptozotocin-induced diabetic mice.

Effect of ECBC on hepatic glycogen content in liver

As shown in Table 2, the level of hepatic glycogen was decreased significantly in diabetic mice. The treatment with ECBC at the dose of 100 mg/kg body weight slightly enhanced it, which was further enhanced in mice treated with ECBC at the dose of 200 mg/kg body weight. Similar increase in the hepatic glycogen level was observed in mice treated with glibenclamide (25 mg/kg body weight).

Effect of the ECBC on insulin level in serum

As shown in Table 2, the level of insulin was decreased significantly in diabetic mice. The treatment with ECBC (100 and 200 mg/kg body weight) and glibenclamide (25 mg/kg body weight) obviously increased the insulin level in serum.

Table 2 - Effects of ethanol extract of the dry matter from culture broth of of *Coriolus versicolor* (ECBC) on biochemical parameters in streptozotocin-induced diabetes mice.

Group	Normal control	Diabetic control	Glibenclamide (25mg/kg)	ECBC (100mg/kg)	ECBC (200mg/kg)
Serum					
Insulin level(μIU/mL)	3.85±0.42*	2.04±0.31	2.99±0.51*	2.47±0.28	2.87±0.18*
LDL-C (mmol/L)	1.21±0.09*	2.59±0.24	1.33±0.17*	2.01±0.18*	1.67±0.31*
HDL-C (mmol/L)	3.31±0.24*	2.01±0.14	3.37±0.41*	2.44±0.31	2.89±0.20*
TC (mmol/L)	1.28±0.18*	3.54±0.41	1.96±0.51*	2.59±0.33*	1.94±0.25*
TG (mmol/L)	0.49±0.06*	1.31±0.28	0.85±0.11*	1.21±0.09	0.66±0.18*
Liver tissue					
SOD (Unites)	138.4±18.5*	58.1±12.4	102.5±13.5*	79.5±10.9	110.3±18.8*
CAT (Unites)	288.5±38.3**	149.1±16.5	212.3±19.5*	188.0±21.3	201.7±15.6*
GPx (mU/mg protein)	88.4±11.2*	26.5±8.3	66.5±13.2*	48.7±11.7*	57.3±10.3*
GSH (μg/mg protein)	8.87±0.86*	3.86±0.69	7.31±1.84*	4.40±0.27	6.91±3.87*
Glycogen content (mg/kg liver tissue)	13.85±0.47*	8.21±0.39	10.33±0.34*	8.92±0.57	10.57±0.28*

Data were represented as mean±s.e.m. (N=8). *P<0.05 compared with untreated diabetic mice. **P<0.01 compared with untreated diabetic mice.

Histopathological studies

Histopathological changes of pancreas are given in Fig. 1. Streptozotocin administration elicited severe injury in the pancreas, such as decreasing the β-cells' numbers and diminishing the diameter of pancreatic island. The treatment with the ECBC (100 and 200 mg/kg body weight) exhibited modedrate expansion of islets and significantly reduced the injuries of pancreas.

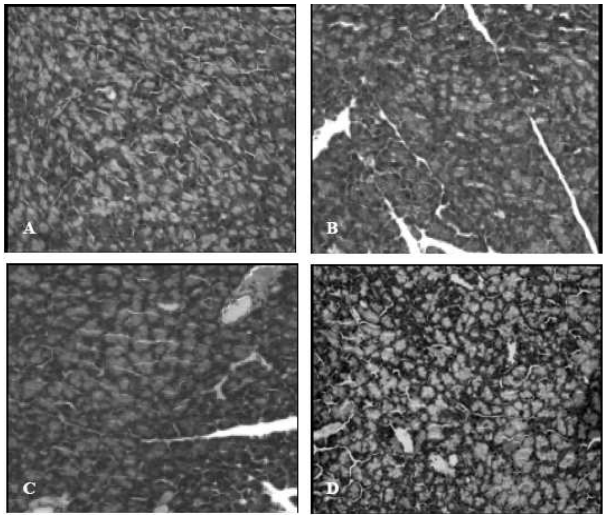

Figure 1 - Effect of ethanol extract of the dry matter from culture broth of of *Coriolus versicolor* (ECBC) on the histological morphology of mice pancreas (H&E100×). A: Normal control mice showed normal pancreas. B: Streptozotocin treatment elicited severe injury of pancreas and the number of β-cells were greatly reduced. C and D: the ECBC at the doses of 100 and 200mg/kg body weight significantly weakened the damage of pancreas.

DISCUSSION

Streptozotocin-induced diabetes in rodents appears to be the most suitable animal model because it reflects the symptoms of diabetes in human. These animals show low production of insulin and high level of blood circulationg glucose (Schechter, 1990; Bolaffi et al., 1987). Changes in blood glucose and insulin levels reflect abnormalities in β-cells function of structure. Streptozotocin impairs glucose oxidation and decreases insulin biosynthesis and secretion. Streptozotocin generated reactive oxygen species, which contributed to DNA fragmentation and evoke other deleterious changes in the β-cells (Nukatsuka et al., 1990; Takasu et al., 1991).

In this study, the effects of ECBC in normal and streptozotocin-induced diabetic mice were evaluated. The ECBC at different doses (100 and 200mg/kg body weight) showed marked hypoglycemic, hypolipidemic and antioxidant effects *in vivo*. Histological examination also showed that the ECBC evidently reduced the injuries of pancreas induced by streptozotocin.

As the most predominant characteristic of diabetes mellitus, hyperglycemia is in itself very dangerous for diabetic patients. The elevated blood glucose levels in diabetes are thought to lead to cell death through oxidative stress induction that occurs as a common sequel of diabets-induced modification of sugar moieties on proteins and lipids (Donnini et al., 1996). Hyperglycemia increases oxidative stress through the overproduction of reactive oxygen species, which results in an imbalance between free radicals and the antioxidant defense systems of the cells. In the present study, the ECBC showed a mild hypoglycemic effect in streptozotocin loaded mice which could be attributed to the potentiation of the insulin effect of plasma by increasing the pancreatic secretion of insulin from existing β-cells or their radical scavenging activities.

Hypertriglyceridemia is a common finding in the patients with diabetes mellitus and is responsible for vascular complications. It has been reported that the treatment of diabetes with insulin served to lower plasma triglyceride level by regulation lipoprotein lipase and hydrolyzing triglycerides (Shirwaikar et al., 2004). The administration of ECBC significantly decreased serum triglycerides and cholesterol in diabetic mice. These results are comparable with those of previous studies (Ravi et al., 2005). The present results also showed that the ECBC increased the serum levels of insulin in the treated group of mice as compared to diabetic mice. Thus, cholesterol- and triglyceride-lowering properties of ECBC could be attributed to hypocholesteromic compounds that might act as

inhibitors or activators for some enzymes with participate in cholesterol metabolism and also its potentiality to release insulin (Babu et al., 2007). The levels of serum lipids is usually elevated in diabetes mellitus and such an elevation represents the risk factor for coronary heart diseases (Davidson, 1981). The market hyperlipemia that characterizes the diabetic states may be regarded as consequence of the uninhibited actions of lipolytic hormones on the fat depots (Goodman and Gilman, 1985). So lower of serum lipids concentration through dietary or drug therapy seems to be associated with the decrease in the risk of vascular disease (Rhoads et al., 1976).

Glycogen is the primary intracellular storable form of glucose and its levels in various tissues, especially in liver, are a direct reflection of insulin activity which regulates glycogen deposition by stimulating glycogen synthase and inhibiting glycogen phosphorylase. Since streptozotocin causes selective destruction of β-cells of islets, resulting in marked decrease in insulin levels, it could be predicted that glycogen levels in liver decrease as the influx of glucose in the liver is inhibited in the absence of insulin (Golden et al., 1979). However, this alteration in hepatic glycogen content is normalized by insulin treatment (Vats et al., 2004). The results of this study showed although the levels of glycogen in ECBC-treated diabetic were lower than the control mice, they could significantly improve hepatic glycogen contents. This was supported by the results of Sharma et al. (2008). This indicated one of the possible ways of ECBC might act by improving the process of glycogenesis in the liver.

Increased lipid peroxidation under diabetic conditions can be due to increased oxidative stress in the cell as a result of depletion of antioxidant scavenger systems. Associated with the changes in lipid peroxidation the diabetic tissues showed decreased activities of key antioxidants SOD, CAT, GPx which play an important role in scavenging the toxic intermediate of incomplete oxidation. SOD and CAT are the two major scavenging enzymes that remove the toxic free radical *in vivo*. Previous studies have reported that the activity of SOD is low in diabetes mellitus (Feillet-coudray et al., 1999). A decrease in the activity of these antioxidants can lead to an excess availability of superoxide anion and hydrogen peroxide in biological systems, which in turn generate hydroxyl radicals, resulting in initiation and propagation of lipid peroxidation (Kumuhekar and Katyane, 1992). The present results indicated that the activities of SOD and CAT were significantly decreased on diabetic-mice but the effect was not significant on ECBC cotreatment. The result of increased activities of SOD and CAT suggested that ECBC contained free radical scavenging activity, which could exert a beneficial effect against the pathological alterations caused by the presence of O^{2-} and OH·. The increased activity of SOD accelerates dismutation of O^{2-} to hydrogen peroxide, which is removed by CAT (Aebi, 1984). The above results indicated the protective effect of ECBC on liver damage.

GSH is an important inhibitor of free radical mediated lipid peroxidation (Meister, 1987). The decrease levels of GSH in diabetes may be due to increased utilization in trapping the oxyradical. Several studies have also reported decreased levels of erythrocytes GSH in experimental diabetic rats (Anuradha and Selvam, 1993). GSH is the first line of defense against the proxidant status (Ahmed et al., 2000) and GSH system may have the ability to manage the oxidative stress with adaptional changes in enzymes regulating GSH metabolism. In the present study, the restoration of GSH level in the groups treatment with the ECBC might account for the protective efficacy of them.

Histopathological investigation is a very important approach to study the pharmacological activity of some active compounds or extracts. In the present study, histopathological assessment showed prominent reduction in the number of β-cell and diminishing of the diameter of pancreatic island in streptozotocin-induced diabetic mice, which was again normalized in diabetic mice treated with the ECBC. This showed that ECBC could be useful in repairing β-cell in pancreatic islet injury and the protective effect would be closely related with its improving function.

CONCLUSION

In conclusion, the present investigation showed that ECBC possessed several healthful properties, including the control of hyperglycemia, hyperlipemia, antioxidant effects and pancreatic β-cell protection that together contributed to its protective effect in streptozotocin-diabetic animals.

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