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Hypoglycemic Properties of Polysaccharides Extracted from *Ganoderma lucidum* in Alloxan-Induced Diabetic Rats

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Abstract

A recent randomized and double-blind placebo-controlled clinical study has indicated that *Ganoderma lucidum* polysaccharides (GLP) decrease blood glucose in patients with type II diabetes. The aim of this study was to investigate the effect of the GLP extract in alloxan-induced diabetic rats. Oral administration of GLP at 0.25, 0.5 and 1.0 g/kg for 4 weeks resulted in a reduction of blood glucose levels by 12.5, 18.7 and 33.7% respectively, while glibenclamide treatment brought the hyperglycemic value down to normal. The hypoglycemic effect was supported by a significant decrease in glycosylated haemoglobin and increased plasma insulin levels ($p<0.01$) in a dose- and time-dependent manner. This study showed that GLP has similar hypoglycemic effects as glibenclamide in alloxan-induced diabetic rats.

Key words: *Ganoderma lucidum*, polysaccharide, diabetes, rat

INTRODUCTION

Diabetes is characterized by disrupted insulin production and sensitivity, leading to high blood glucose and a series of complications such as renal dysfunction, neuropathy and cardiopathy (1-3). Lowering blood glucose has been shown to reduce the risk of serious complications from diabetes (1,3,4). Many oral hypoglycemic drugs with different mechanisms of action have been developed to treat diabetes. However, the sulphonylureas, especially potent and long acting agents such as glibenclamide, may cause hypoglycemia, while metformin carried the risk of lactic acidosis (5-7). Thus, safe and effective therapeutic approaches for type II diabetes are needed.

A recent randomized and double-blind placebo-controlled clinical study indicated that water-extracted *Ganoderma lucidum* polysaccharides (GLP) decrease blood glucose and increase plasma insulin level in patients with type II diabetes (8). *G. lucidum* is a medicinal mushroom which has been extensively used to treat various chronic diseases, such as chronic hepatopathy, hypertension, diabetes and cancer (9-12). We undertook this study to investigate the efficacy of GLP for the treatment of diabetes in alloxan-induced diabetic rats.

MATERIALS AND METHODS

Preparation of polysaccharides from *Ganoderma lucidum*

The fruiting bodies of *G. lucidum* were collected from southern China and the polysaccharide fractions were extracted twice with hot water at 70°C for 3 hr as described previously (13). All extracts were pooled, and the polysaccharide enriched fractions were precipitated by the addition of 75% (v/v) ethanol. The polysaccharide-enriched fraction was further purified by high performance anion-exchange and gel filtration chromatography using a 1.6×100 cm column packed with Sephadex G-25 (Pharmacia, Uppsala, Sweden). The extracted polysaccharides had an average molecular size of 4.85×10^5, as determined by gel filtration chromatography and the phenol-sulfuric acid method (14,15). They consisted of glucose (61.2%), xylose (15.5%), fructose (14.4%), galactose (4.8%) and rhamnose (4.1%) linked together by β-glycosidic linkages. The protein concentration was 0.35 % w/w as determined by the bicinchoninic acid method (16,17). Triterpenes were not detected in the final extracts by silica gel thin layer chromatography or visualized by UV light shadowing. There was no detectable level of endotoxin (lipopolysaccharide) in the extracted polysac-

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charide fractions as determined by the chromogenic limulus amebocyte lysate assay (18,19).

Animals

Experiments were performed on healthy male Wistar Kyoto rats (185~220 g). Rats were housed under constant temperature (22±1°C), lighting (12-h cycle) and humidity (55%) according to institutional guidelines. Sterile food and water was available *ad libitum*. Animals described as fasting were deprived of food for at least 18 h but were allowed free access to drinking water. The Institutional Animals Ethics Committee approved all animal procedures. Blood was collected by tail nipping.

Experimental induction of diabetes

Rats were made diabetic by a single intravenous injection of 45 mg/kg body weight alloxan monohydrate (Sigma-Aldrich Chemical Co.) given in the tail vein. Alloxan was first weighed individually in Eppendorf's tube for each animal according to the body weight and then dissolved in 0.2 mL sterile normal saline just prior to injection. Two days after alloxan injection, rats with plasma glucose levels >175 mg/dL were included in the study. Treatment with test extract was started 48 h after alloxan injection.

Drug treatment of animals

Rats were randomly divided into the following 7 groups with six animals in each group. Rats in Group I and II were normal (i.e., no alloxan induction), whereas those in Group III-VII were alloxan-induced diabetic. Group I rats received no GLP (used as the baseline control). Group II rats received 1.0 g/kg GLP intragastrically for 28 days. Group III rats received vehicle only (used as the diabetic control). Group IV was given intragastric glibenclamide, 0.5 mg/kg (used as the standard positive control). Group V- VII rats were treated with GLP at 0.25, 0.5 and 1.0 g/kg intragastrically for 28 days, respectively. After the treatment period, all rats were sacrificed by injection of sodium pentobarbital at a dose of 65 mg/kg. Blood was collected in a tube containing potassium oxalate and sodium fluoride solution for the estimation of blood glucose and insulin at the end of study.

Determination of blood glucose, haemoglobin, glycosylated haemoglobin and insulin

Fasting blood glucose was measured using the *o*-toluidine-boric acid method (20). Briefly, 0.1 mL of rat plasma sample was mixed with 0.5 mL of *o*-toluidine-boric acid reagent and boiled for 10 min to react with the terminal aldehyde group of glucose to produce a blue-green color. The tubes were cooled down by placing in tap water for 3 min and absorbance was read at 630 nm by a Tecan spectrometer (Maennedorf, Switzerland). Haemoglobin was estimated using the cyanmethaemoglobin method (21), and glycosylated haemoglobin using the modified phenol sulphuric acid method (22). Briefly, an aliquot (20 μL) of rat blood was added in 5 mL alkaline Drabkin's solution containing potassium ferricyanide, potassium cyanide, sodium bicarbonate and a surfactant and protected from light. The mixture was incubated for 20 min and absorbance measured at 540 nm. For the measurement of glycosylated haemoglobin, hemolyzates (0.10 mL) from red blood cells were hydrolyzed with 4 M HCl (0.10 mL) for 4 h at 100°C, and the protein was precipitated with 50 μL 40% trichloroacetic acid. After centrifugation at 2000×g for 10 min at 4°C, an aliquot (0.1 mL) of the supernatant containing the free sugars was treated with 1 mL 2.5% (v/v) phenol and 2.5 mL concentrated sulphuric acid to form the color and absorbance read at 490 nm. Plasma insulin was determined using the commercially available radioimmunoassay kits.

Statistical analysis

Data were expressed as means±SD. Statistical analyses of multiple groups or changes from a basal measurement were performed using one-way or repeated measure analysis of variance (ANOVA) and post hoc Dunnet's *t*-test. P values of less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

As shown in Table 1, administration of alloxan (45 mg/kg) led to 2.9-and 2.7-fold increases in blood glucose and glycosylated haemoglobin levels ($p<0.001$), and a 2-fold decrease in plasma insulin concentration ($p<0.01$), which was maintained over a period of 4 weeks. Four weeks of daily treatment with 0.25, 0.5 and 1.0 g/kg GLP resulted in a reduction of blood sugar levels by 12.5, 18.7 and 33.7% respectively, while glibenclamide treatment brought the hyperglycemic value down to normal. Furthermore, there was a significant decrease in glycosylated haemoglobin and increase in plasma insulin levels ($p<0.01$) after 4 weeks treatment of GLP in a dose- and time-dependent manner; The highest dosage, GLP 1.0 g/kg for 4 weeks, was required for the maximal response in reducing blood glucose and increasing in plasma insulin concentrations ($p<0.05$).

This study showed that GLP treatment had significant hypoglycemic effects in alloxan-induced diabetic rats. Our results agree with those reported by Zhang et al. (23). There is increasing interest in the uses of complementary and alternative medicines to treat patients with type II diabetes (24), and a number of herbal medicines have been shown to have hypoglycemic effects in animal studies (25,26). For example, extracts from *Enicostemma*

Table 1. The effects of GLP treatment in alloxan-induced diabetic rats

Group	Fasting blood glucose (mg/dL)			Glycosylated hemoglobin (mg/g Hb)			Plasma insulin (U/mL)		
	Basal	14 days	28 days	Basal	14 days	28 days	Basal	14 days	28 days
Normal	78.3±8.67*	80.2±6.51*	81.3±7.33*	0.21±0.03*	0.22±0.04*	0.22±0.04*	24.6±8.82*	25.8±9.90*	25.5±9.4*
Diabetic control	228.1±12.3[a,c]	234.3±10.1[a]	222.6±13.1[a]	0.57±0.07[a,c]	0.57±0.06[a]	0.56±0.05[a]	12.2±1.16[a,c]	12.7±1.22[a]	13.6±1.45[a]
Diabetic+GLP (0.25 g/kg)	231.4±11.1[c]	215.6±14.5	202.5±10.8[b]	0.56±0.06[c]	0.53±0.06	0.51±0.05	11.8±0.87[c]	12.3±0.73	13.2±0.89
Diabetic+GLP (0.5 g/kg)	226.8±9.77[c]	206.8±11.7[b]	184.3±8.62[b,d]	0.55±0.06[c]	0.49±0.05	0.45±0.04[b]	12.3±1.17[c]	13.5±0.89	14.1±1.15[b]
Diabetic+GLP (1.0 g/kg)	228.3±11.9[c]	187.7±9.42[b,d]	151.3±9.91[b,d]	0.57±0.06[c]	0.46±0.05[b]	0.42±0.03[b,d]	12.7±1.36[c]	14.8±1.28[b]	16.2±1.42[b,d]
Diabetic+Glibenclamide (0.5 mg/kg)	229.3±14.2[c]	94.3±6.52[b,d]	83.2±5.93[b,d]	0.56±0.05[c]	0.25±0.03[b,c]	0.20±0.02[b,d]	11.9±1.04[c]	15.6±1.05[b,d]	20.3±1.27[b,d]
Normal+GLP (1.0 g/kg)	76.5±6.21[c]	70.6±4.21	65.6±5.21[d]	0.20±0.02[c]	0.20±0.03	0.19±0.03	25.3±6.75[c]	25.8±7.56	27.6±5.23[d]

Data are the mean±SD (n=6).

*Values with normal rats are significantly different compared to diabetic control rats by Student *t*-test ($p<0.05$).

[a,b]Values with diabetic control rats are significantly different compared to diabetic rats treated with GLP by ANOVA with Duncan's multiple range test at $p<0.05$.

[c,d]Values for rats by days 14 and 28 are significantly different compared to basal level (day 0) by ANOVA with Duncan's multiple range test at $p<0.05$.

littorale, *Taraxaci radix*, *Lentinus edodes*, as well as Diamed (the aqueous extracts of *Azardirachta indica*, *Cassia auriculata* and *Momordica charantia*) and Quei Fu Di Huang Wan have demonstrated antihyperglycemic effects in mouse or rat models (27-29).

Alloxan has been widely used to produce experimental diabetes. This compound causes severe necrosis of pancreatic β-cells (30), due mainly to the production of H_2O_2 and of some free radicals such as $O_2^{\cdot}$ and $^{\cdot}OH$ (31). The mechanisms of the antidiabetic activity of GLP are not fully defined. *In vivo* and *in vitro* studies in the mouse indicate that GLP had strong scavenging ability to protect the pancreatic islets from free radicals damage induced by alloxan (23). However, other mechanisms may be involved. For example, polysaccharides or triterpenes from *G. lucidum* may bind to β-cells or other functional cells in the pancreas to stimulate the secretion of insulin and inhibit glucagons production. There is evidence that the β-D-glucans purified from medicinal mushrooms induce biological response by binding to membrane complement receptor type three (CR3, $\alpha_M\beta_2$ integrin, or CD11b/CD18) on immune effector cells such as macrophage (32,33). GLP may increase glycogenesis, and decrease intestinal absorption of glucose and glycogenolysis, which have been associated with the hypoglycaemic effect of *Coccinia indica* (34). Therefore, more studies are required to identify the mechanisms for the hypoglycemic activity of GLP.

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