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Evaluation of in vivo antioxidant activities of *Ganoderma lucidum* polysaccharides in STZ-diabetic rats

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ABSTRACT

Effect of *Ganoderma lucidum* polysaccharides treatment on blood glucose, serum insulin level, lipid peroxidation, nonenzymic and enzymic antioxidants in the plasma and liver of streptozotocin (STZ)-induced diabetic rats was studied. Adult male rats of Wistar strain, weighing 195 to 250 g, were randomized into control and experimental groups. Experiment group rats were induced diabetes by administration of STZ (45 mg/kg b.wt.) intraperitoneally. The diabetic rats were treated with *G. lucidum* polysaccharides (60, 120, 180 mg/kg b.wt.) dissolved in 15% dimethyl sulphoxide (DMSO) for 30 days. The normal control rats were treated with 15% DMSO for 30 days. Streptozotocin treatment elevated the levels of lipid peroxidation markers (thiobarbituric acid reactive substances, lipid hydroperoxides and conjugated dienes), and reduced nonenzymic antioxidants (vitamin C and reduced glutathione, vitamin E) levels, and enzymic antioxidants (superoxide dismutase, catalase and glutathione peroxidase) activities in the plasma and liver of untreated diabetic control rats. Decreased level of serum insulin and increased level of blood glucose (BG) were observed in the plasma of untreated diabetic control rats. *G. lucidum* polysaccharides treatment significantly and dose-dependently increased nonenzymic and enzymic antioxidants, serum insulin level and reduced lipid peroxidation, blood glucose levels in STZ-diabetic rats. From the present study, it can be concluded that *G. lucidum* polysaccharides can be considered as a potent antioxidant.

1. Introduction

Cellular oxidative damage is a well-established general mechanism for cell and tissue injury and primarily caused by reactive oxygen species (ROS). These ROS can bind with most normal cellular components; they react with unsaturated bonds of membrane lipids, denature proteins, and attack nucleic acids (Adachi, Fujiwara, & Ishii, 1998; Agarwal & Sohal, 1993; Aksenova, Aksenov, Carney, & Butterfield, 1998). A disturbance of the balance between formation of active oxygen metabolites and the rate at which they are scavenged by enzymic and nonenzymic antioxidants is referred to as oxidative stress (Papas, 1996). It has been suggested that oxidative stress plays an important role in some physiological conditions and in many diseases, including diabetes mellitus (DM), myocardial infarction and carcinogenesis. Cells and biological fluids have an array of protective antioxidant mechanisms such as glucose-6-phosphate dehydrogenase, superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST) and reduced glutathione, for both preventing the production of free radicals and repairing oxidative damage (Chandra et al., 1994).

An earlier study has shown that treatment with antioxidant reduces diabetic complications (Wohaieb & Godin, 1987). Efforts to discover antioxidants as useful drug candidates to combat diabetic complications are going on relentlessly. *Ganoderma lucidum* (Fr.) Krast (Polyporaceae) is a fungus usually used in traditional Chinese medicine. Its fruiting body, called "Lingzhi", contains a variety of chemical substances. *Ganoderma lucidum* (Lingzhi) is a member of the fungus family (lamellae basidiomycete of the family Polyporaceae) that naturally grows on fallen trees and logs of other broad leaf trees. The use of *G. lucidum* as a longevity- and vigor-promoting "magic herb" dates back more than 2000 years in China. Scientific investigations have repeatedly confirmed beneficial effect of *G. lucidum* on health in general; it is now frequently promoted as an effective agent against cancers in the Pacific Rim areas, such as China, Japan, Korea, and other Asian countries. Recent studies on *G. lucidum* have shown many interesting biological activities, including anti-tumour and anti-inflammatory effects and cytotoxicity to hepatoma cells. The polysaccharides of *G. lucidum* are the major source of its biological activity and therapeutic uses. Polysaccharide extracts from many species of fungi exhibit immunostimulating and/or anti-tumour activities (Lin, 1991, 2001; Shao, Dai, Xu, Lin, & Gao, 2004). Recent study showes that polysaccharide of *G. lucidum* promises to be a new type of carcinostatic agent,

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which might be useful in immunotherapy (Borchers, Stern, Hackman, Keen, & Gershwin, 1999; Wasser, 2002). The polysaccharides which demonstrate this activity are all glucans that are closely related in their structure to scleroglucan but vary in their water solubility and in the degree and nature of their side-chains. Because of its perceived health benefits, polysaccharides of *G. lucidum* have gained wide popularity as a health food, in both Japan and China (Smith & Sivasithamparam, 2003; Xu, 2001). The importance of the antioxidant constituents of plant materials in the maintenance of health and protection against heart diseases and cancer is also raising interest among scientists, food manufacturers and consumers as the trend for the future is toward functional food with specific health effects (Loliger, 1991). Recently, it has been reported that *G. lucidum* polysaccharides has the ability to scavenge the free radicals (Gui, Wang, & Yang, 1996; Kim & Kim, 1999; Lee, Kwon, Jeong, & Lee, 1999; Lin, Lin, Chen, Ujiie, & Takada, 1995; Shi, Anthony, Iris, & John, 2002; You & Lin, 2002).

No detailed study has been carried out on the effect of *G. lucidum* polysaccharides on lipid peroxidation and antioxidants in STZ-diabetic rats. Hence, the present study was planned to evaluate the effect of *G. Lucidum* polysaccharides on lipid peroxidation, blood glucose and serum insulin levels, nonenzymic and enzymic antioxidants activities in plasma and liver of STZ-diabetic rats.

2. Materials and methods

2.1. Materials

Streptozotocin was purchased from BeiJing CCRO International Medical Consulting Co. Ltd. (Beijing, China). All other chemicals were of analytical grade obtained from the Nanjing jiancheng Biochemistry Co. Ltd (Nanjing, China). *Ganoderma lucidum* was purchased from local herbs market.

2.2. Preparation of Ganoderma lucidum polysaccharides

The polysaccharides were prepared from the fruiting bodies of *G. lucidum* by boiling water extraction. All extracts were finally pooled, and the polysaccharide extracts were precipitated by the addition of 75% (v/v) ethanol and further purified by high-performance anion-exchange and gel filtration chromatography. The molecular size and chemical components of polysaccharide was determined by gel filtration chromatography and the phenol–sulphuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956; Gibbs, Lightfoot, Edwin, & Root, 1992), gas chromatography and mass spectrogram (Grové, Rohwer, Laurens, & Vorster, 2006), and the concentrations of uronic acids and proteins were also determined (Immerzeel, Schols, Voragen, & de Vries, 2004).

2.3. Animal modeling, group and treatment

A total of 30 male Wistar rats weighing from 195 to 250 g were provided by the experimental Animal Breeding Centre associated to Chinese Academy of Sciences, and were maintained in an air conditioned room (25 ± 1 °C) with a 12 h light:12 h dark cycle. They were fed with standard laboratory diet and given tap water. All experimental animals were overseen and approved by the Animal Care and Use Committee of our Institute before and during experiments.

Twenty-four fasted rats were intraperitoneally injected with STZ (45 mg/kg b.wt) in freshly prepared citrate buffer (0.1 M, pH 4.5). The development of hyperglycemia in rats was confirmed by plasma glucose estimation 90 h post STZ injection. The rats with fasting plasma glucose level of above 11.1 mmol/L were considered diabetic and only uniformly diabetic rats were included in the study (Liang, 2004).

The diabetic rats were randomly divided into four groups consisting of six rats each. Six healthy rats were served as normal control. The polysaccharides were administered using vehicle solution (15% DMSO).

Group I: Normal control received 15% DMSO only.

Group II: Diabetic control (15% DMSO).

Group III: Diabetic + polysaccharides (60 mg/kg/b.wt. in 15% DMSO).

Group IV: Diabetic + polysaccharides (120 mg/kg/b.wt. in 15% DMSO).

Group V: Diabetic + polysaccharides (180 mg/kg/b.wt. in 15% DMSO).

At the end of 30 days, the animals were deprived of food overnight and sacrificed by decapitation. Blood was collected in tubes containing potassium oxalate and sodium fluoride mixture for the estimation of blood glucose, lipid peroxidation and antioxidants. Plasma was separated for the estimation of insulin. The liver was removed promptly, and weighed. The tissues were stored at −70 °C until required. A 20% homogenate was prepared in 50 mM phosphate buffer, pH 7.4 and were centrifuged and the supernatant was used immediately for the assays of vitamin C (vC), vitamin E (vE), reduced glutathione (GSH), glutathione peroxidase (GPx), catalase (CAT), Glutathione reductase (GR) and superoxide dismutase (SOD).

2.4. Biochemical analysis

The concentrations of thiobarbituric acid reactive substances (TBARS), lipid hydroperoxides (LP) and conjugated dienes (CD) were estimated by the methods of Danova et al. (2005), Shang, Qin, Cheng, and Miao (2006) and Klein (1979), respectively. The levels of blood glucose, vC and E, GSH and serum insulin were estimated by the methods of Heyliger, Tahiliani, and McNeill (1985), Lin et al. (2003), Chow and Omaye (1983), Beutler (1975) and Di Marco, Ghisalberti, Martim, and Oliver (2008), respectively. The activities of SOD, CAT, GR and GPx were measured by the methods of Reveillaud, Niedzwiecki, Bensch, and Fleming (1991), Beutler (1975), Carlberg and Mannervik (1975), and Flohé and Gunzler (1984), respectively.

2.5. Statistical analysis

The results were analyzed by two-way ANOVA followed by Duncan's Multiple Range Test (DMRT) using SPSS-12.0 and expressed as the mean values ± S.D for six rats in each group.

3. Results

3.1. Ganoderma lucidum polysaccharides

Results indicated that *G. lucidum* polysaccharides (GLP) was a glycopeptide with a molecular weight of 585,100 Da and the ratio of polysaccharides to peptides was 90.23:6.71%. The polysaccharides were found to contain D-rhamnose, D-xylose, D-fructose, D-galactose, D-mannose and D-glucose groups in mole ratios of 0.752:0.979:2.921:0.188:0.403:7.74 and linked together by β-glycosidic linkages. The peptides consist of 14 kinds of amino acid. These are basically in agreement with result of Zhu and Lin (2006) and You and Lin (2002).

3.2. Effect of polysaccharides on lipid metabolic parameters

The TBARS, LP, CD levels in liver and plasma of untreated diabetic control rats were significantly higher than those of the untreated normal control rats. When diabetic rats were treated for 30 days, polysaccharides significantly decrease the levels of TBARS, LP, CD in a dose-dependent manner (Table 1).