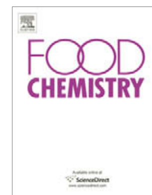
Food Chemistry 115 (2009) 32–36

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem



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

Jie Jia [a], Xi Zhang [b], Yong-Shan Hu [a,*], Yi Wu [a], Qing-Zhi Wang [b], Na-Na Li [b], Qing-Chuan Guo [c], Xin-Cun Dong [c]

[a] Department of Rehabilitation, Huashan Hospital, Fudan University, Shanghai 200040, China
[b] Department of Anatomy, Xinxiang Medical College, Henan, China
[c] Department of Rehabilitation, Third Hospital, Xinxiang Medical College, Henan, China

ARTICLE INFO

Article history:
Received 3 November 2006
Received in revised form 6 November 2008
Accepted 13 November 2008

Keywords:
Ganoderma lucidum polysaccharides
Streptozotocin-induced diabetes
Thiobarbituric acid reactive substances
Antioxidant
Oxidative stress

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.

© 2008 Elsevier Ltd. All rights reserved.

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,

* Corresponding author. Tel./fax: +86 21 52887820.
E-mail address: drjiajie@163.com (Y.-S. Hu).

0308-8146/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2008.11.043

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).

Table 1
Concentration of thiobarbituric acid reactive substances (μmol MDA equivalents/ml serum, μmol MDA equivalents/mg protein), lipid hydroperoxides and conjugated dienes (□ absorbance at 233 nm) in the plasma and liver of diabetic rats.

	I	II	III	IV	V
Plasma					
TBARS	0.22 ± 0.06	0.37 ± 0.02[a]	0.32 ± 0.02[c]	0.26 ± 0.03[c]	0.22 ± 0.02[c]
LP ($\times 10^{-5}$ mM)	9.58 ± 0.37	25.69 ± 2.45[a]	21.47 ± 2.55[b]	16.58 ± 2.60[c]	12.58 ± 1.10[c]
CD	0.76 ± 0.06	1.17 ± 0.13[a]	0.88 ± 0.06[c]	0.75 ± 0.04[c]	0.67 ± 0.05[c]
Liver					
TBARS	1.12 ± 0.12	4.07 ± 0.12[a]	3.00 ± 0.16[c]	2.24 ± 0.15[c]	1.21 ± 0.10[c]
LP(mM/100 g tissue)	76.38 ± 7.54	153.23 ± 9.99[a]	123.63 ± 7.51[c]	100.47 ± 6.89[c]	87.71 ± 5.57[c]
CD	83.84 ± 5.25	112.65 ± 8.53[a]	110.62 ± 7.73	92.56 ± 6.98[c]	80.51 ± 7.12[c]

In each group $n = 6$ rats. Values are expressed as means ± SD of 6 parallel measurements.
[a] $P < 0.01$ compared with normal control group (I).
[b] $P < 0.05$.
[c] $P < 0.01$ compared with diabetic control group (II).

3.3. Effects of polysaccharides on fasting blood glucose and serum insulin levels

Fasting blood glucose and serum insulin levels were measured after rats were fasted for 12 h on day 30 (last day of treatment) (Table 2). In the diabetic control group, the fasting blood glucose increased significantly, while serum insulin was significantly decreased compared to the untreated normal control rats. Administration of polysaccharides dose-dependently and significantly decreased fasting blood glucose, elevated serum insulin levels of diabetic rats (Table 2).

3.4. Effect of polysaccharides on nonenzymic antioxidant levels and antioxidant enzymes activities in the plasma and liver of diabetic rats

Nonenzymic antioxidants, measured as vC, vE, GSH, and antioxidant enzymes, measured as SOD, CAT, GR and GPx, were significantly decreased in both the plasma and liver of untreated diabetic control rats compared to the untreated normal control rats. Polysaccharides treatment dose-dependently and significantly restored the decreased nonenzymic antioxidant levels and antioxidant enzymes activities near normal levels in diabetic rats (Table 3).

4. Discussion

According to World Health Organization, around 171 million people worldwide were suffering from diabetes in 2000. This figure is predicted to double by 2030 (Wild, Roglic, & Green, 2004). Diabetic retinopathy, nephropathy and cardiovascular disease are among the most common complications of diabetes. Around 85% of all diabetics eventually develop diabetic retinopathy, which is the commonest cause of blindness in the fourth and seventh decades of life (Tewari & Venkatesh, 2004). Diabetes is also one of the leading causes of kidney failure, whereas heart disease accounts for the majority of deaths among people with diabetes in developed countries.

Table 2
Concentration of blood glucose and serum insulin in diabetic rats.

	I	II	III	IV	V
BG (mmol/l)	5.71 ± 0.7	22.14 ± 1.91[a]	17.32 ± 0.98[b]	14.38 ± 1.23[b]	8.43 ± 0.72[b]
Insulin (Ut/ml)	29.16 ± 1.85	13.89 ± 1.44[a]	16.56 ± 1.31[b]	19.96 ± 2.65[b]	25.71 ± 2.04[b]

In each group $n = 6$ rats. Values are expressed as means ± SD of 6 parallel measurements.
[a] $P < 0.01$ compared with normal control group (I).
[b] $P < 0.01$ compared with diabetic control group (II).

Table 3
Concentration of nonenzymetic antioxidant and activities of antioxidant enzymes in the plasma and liver of diabetic rats.

	I	II	III	IV	V
Plasma					
SOD (U/ml)	6.87 ± 0.38	3.14 ± 0.87[b]	4.04 ± 1.05	5.83 ± 0.95[d]	7.04 ± 0.84[d]
GPx (U/ml)	18.97 ± 1.37	8.35 ± 1.86[b]	10.77 ± 1.25[d]	13.88 ± 1.63[d]	19.45 ± 1.74[d]
CAT (U/ml)	38.6 ± 4.11	19.29 ± 2.23[b]	22.56 ± 2.53[c]	28.83 ± 1.27[d]	36.72 ± 2.71[d]
GR (U/ml)	15.43 ± 1.22	5.73 ± 0.93[b]	8.04 ± 0.8[d]	11.65 ± 1.34[d]	17.93 ± 2.14[d]
vC (μg/ml)	2.16 ± 0.08	1.04 ± 0.06[b]	1.37 ± 0.06[d]	1.69 ± 0.05[d]	2.07 ± 0.07[d]
vE (μg/ml)	8.24 ± 0.35	3.75 ± 0.59[b]	6.78 ± 0.68[d]	8.04 ± 0.31[d]	12.97 ± 0.27[d]
GSH (μg/ml)	41.79 ± 2.58	22.48 ± 1.93[b]	26.50 ± 1.40[d]	32.32 ± 2.87[d]	35.02 ± 3.44[d]
Liver					
SOD (U/mg)	9.61 ± 0.49	4.53 ± 0.07[b]	5.07 ± 0.12[d]	6.82 ± 0.54[d]	8.67 ± 0.42[d]
GPx (U/mg)	11.15 ± 0.95	5.76 ± 0.43[b]	7.39 ± 0.29[d]	8.47 ± 1.02[d]	9.26 ± 0.28[d]
CAT (U/mg)	96.38 ± 6.29	53.86 ± 3.21[b]	70.65 ± 9.06[d]	78.86 ± 6.02[d]	88.12 ± 6.73[d]
GR (U/mg)	22.46 ± 1.94	10.28 ± 1.61[b]	15.87 ± 1.25[d]	18.47 ± 1.52[d]	21.95 ± 2.28[d]
vC (μg/mg)	0.96 ± 0.10	0.64 ± 0.07[b]	0.74 ± 0.03[d]	0.85 ± 0.04[d]	1.00 ± 0.09[d]
vE (μg/mg)	1.02 ± 0.05	0.41 ± 0.08[b]	0.74 ± 0.03[d]	1.26 ± 0.04[d]	1.32 ± 0.05[d]
GSH (μg/mg)	145.05 ± 5.89	52.62 ± 5.05[b]	73.60 ± 4.76[d]	87.34 ± 5.06[d]	105.23 ± 6.83 [d]

In each group $n = 6$ rats. Values are expressed as means ± SD of 6 parallel measurements.
[b] $P < 0.01$ compared with normal control group (I).
[c] $P < 0.05$.
[d] $P < 0.01$ compared with diabetic control group (II).

Streptozotocin-induced diabetes is a well-documented model of experimental diabetes. Streptozotocin-diabetes provides a relevant example of endogenous chronic oxidative stress due to the resulting hyperglycemia (Like & Rossini, 1976). The roles of oxidative stress and antioxidants in organs and tissues damage have been studied extensively in experimental diabetes and diabetic patients (West, 2000). In the present study, streptozotocin treatment significantly increased lipid peroxides and decreased nonenzymetic antioxidant levels and antioxidant enzymes activities in the plasma and livers of rats studied. These results confirm the previous reports that streptozotocin-induced diabetes is accompanied by an increased generation of reactive species (Winiarska, Fraczyk, Malinska, Drozak, & Bryla, 2006). One reason for the elevated lipid peroxidation in streptozotocin-induced diabetes is the reduction in the levels of glutathione, a potent endogenous antioxidant. In agreement with the previous findings herein significantly increased blood glucose and decreased serum insulin levels were observed in untreated diabetic rats. This study has revealed that the increased blood glucose and decreased serum insulin levels is closely associated with the elevated lipid peroxidation. The ROS scavenging capacity by antioxidants is decreased in diabetes such that constant oxidative stress develops and oxidation of lipids, proteins and other macromolecules such as DNA is increased. Augmentation of plasma antioxidative capacity would also attenuate lipid peroxidation through this mechanism (Ohkawa, ohishi, & Yagi, 1979).

Ganoderma lucidum is an herb commonly used to treat diabetes in traditional Chinese medicine. An early study reported that *G. lucidum* could prevent alloxan-induced activation of processes of lipid peroxidation in the pancreas and demonstrated definite insulinogenic properties (Zhang, He, Yuan, & Lin, 2003), whereas a double-blind placebo-controlled trial found that 200 mg of *G. lucidum* per day could improve blood sugar levels in non-insulin-dependent diabetic patients (Zhang et al., 2003; Zhao, Li, & Hu, 2006). In this study, we used polysaccharides of *G. lucidum* as a sample to investigate its antioxidant activity and mechanism in streptozotocin-induced diabetic rats. We focused on the endogenous antioxidant augment activity with GLP treatment. A significant antihyperglycemia action on the fasting blood glucose and serum insulin was observed after a 30 day treatment of diabetic rats with different doses of GLP (60–180 mg/kg b.wt.). This finding provided a valuable evidence for past reports on the antidiabetic action of GLP (Chen, Huang, Luo, Luo, & Yang, 2005). Although it is generally believed that streptozotocin-induced hyperglycemia is mainly due to its ability to induce oxygen free radicals, there were also other mechanisms (Levy, 2006). Our study revealed that one mechanism of antihyperglycemia action of GLP may be through its scavenging ability to protect the pancreatic islets from free radicals-damage induced by streptozotocin in vivo and in vitro.

Antioxidative enzymes form the first line of defense against ROS in the organism. This defense includes the enzymes SOD, CAT and GPx (Ames, Shigenenga, & Hagen, 1993). The diabetogenic action of streptozotocin can be prevented by the superoxide dismutase, catalse and other hydroxyl radical scavengers, such as ethanol and dimethyl urea; hence there is evidence to suggest that the incidence of diabetes involves superoxide anion and hydroxyl radicals (Ames et al., 1993). The deleterious effects of superoxide anion and hydroxyl radicals can be counteracted by antioxidant enzymes, such as SOD, CAT and GPx. In addition to these enzymes, GR and GST provide glutathione and help to nutralize toxic electrophiles, respectively. There is clear cut evidence to show the role of free radicals in diabetes and studies indicate that tissue injury in diabetes may be due to free radicals (Grankvist, Marklund, & Tajedal, 1981).

Earlier workers had reported a decrease in the activities of these antioxidant enzymes (SOD, CAT, GPx and GST) in the plasma and liver of diabetic rats (Anuradha & Selvam, 1993). We have also observed the decrease in GSH, vC, vE levels and SOD, CAT, GPx, GR activities in liver and plasma in diabetic rats. GPx, an enzyme with selenium and GR, catalyses the reduction of hydrogen peroxide to non-toxic compounds (Carlberg & Mannervik, 1975). Administration of GLP increased the activities of GPx and GR in diabetic conditions. SOD and CAT are two major scavenging enzymes that remove the toxic-free radical in vivo. The enzyme SOD scavenges superoxide radicals (O_2^-) by catalysing the conversion of two of these radicals into hydrogen peroxide and molecular oxygen. The hydrogen peroxide formed by SOD and other processes is scavenged by CAT, a ubiquitous heme protein that catalyses the dismutation of hydrogen peroxide into water and molecular oxygen. Reduced activities of SOD in erythrocytes and CAT in liver and kidney have been observed during diabetes and this may result in a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide (Santhakumari, Prakasam, & Pugalendi, 2003). We also found that the activities of SOD and CAT in untreated diabetic control animals were significantly ($p < 0.01$) lower than the normal control animals. This could be attributed to higher levels of superoxide radicals and hydrogen peroxide as indicated by increased ROS levels in these rats, which reduced the antioxidant enzymes activities. GLP-treated rats showed decreased lipid peroxides that are associated with increased activity of SOD and CAT in a dose-dependent manner. The result of increased activities of SOD and CAT suggest that GLP contains a free radical scavenging activity, which could exert a beneficial effect against pathological alterations caused by the presence of O_2^- and OH^-. Administration of GLP has been reported to prevent or attenuate decrease in tissue antioxidant enzymes in number of animal models of oxidative stress to provide cellular protection against reactive oxygen species (Lakshmi, Ajith, Jose, & Janardhanan, 2006; Zhang et al., 2003). However, compounds possessing antioxidant activity are shown to protect hepatic and nephritic tissues by attenuating the increased antioxidant enzymes due to their compensatory elevation of antioxidant defense mechanism (White, 2006). In the present investigation GLP decreased the levels of antioxidant enzymes in plasma and liver tissues of diabetic rats by reducing the oxidative stress due to its potential antioxidant activity and these results agree with the previous studies on antioxidants (Anuradha & Selvam, 1993). Antioxidant treatment was reported to alleviate the oxidative injury by enhancing MnSOD and GSH-Px activities and increasing the mRNA expression of the two antioxidant enzymes (Pang, Chen, & Zhou, 1999; Pang, Chen, & Zhou, 2000). It is possible that the effect of GLP on SOD and GSH-Px was associated with its induction on the expression of genes of the antioxidant enzymes.

The results presented here demonstrate that the orally administered *G. lucidum* possesses significant antihyperglycemia action and could effectively normalise the impaired oxidative stress in the plasma and liver of the diabetic rats. The promising antioxidant and antihyperglycemia efficacies of *G. lucidum* demonstrated in this study may open new avenues in the treatment of diabetes and its complications.

Acknowledgment

Authors would like to express their thank for financial support from the foundation of science technology department of Henan province of China (# 0324410028).

References

Adachi, H., Fujiwara, Y., & Ishii, N. (1998). Effects of oxygen on protein carbonyl and aging in *Caenorhabditis elegans* mutants with long (age-1) and short (mev-1) life spans. *Journal of Gerontology, 53*, B240–B244.

Agarwal, S., & Sohal, R. S. (1993). Relationship between aging and susceptibility to protein oxidative damage. *Biochemical and Biophysical Research Communications, 194*, 1203–1206.

Aksenova, M. V., Aksenov, M. Y., Carney, J. M., & Butterfield, D. A. (1998). Protein oxidation and enzyme activity decline in old brown Norway rats are reduced by dietary restriction. *Mechanisms of Ageing and Development, 100*, 157–168.

Ames, B. N., Shigenenga, M. T., & Hagen, M. (1993). Oxidants, antioxidants and the degenerative diseases of aging. *Proceedings of the National Academy of Sciences of the USA, 90*, 7915–7922.

Anuradha, C. V., & Selvam, R. (1993). Effect of oral methionine on tissue lipid peroxidation and antioxidants in alloxan induced diabetic rats. *Journal of Nutritional Biochemistry, 4*, 212–217.

Beutler, E. (1975). *Red cell metabolism: A manual of biochemical methods* (2nd ed.). New York: Grune and Stratton.

Borchers, A. T., Stern, J. S., Hackman, R. M., Keen, C. L., & Gershwin, M. E. (1999). Mushrooms, tumors, and immunity. *Proceedings of the Society for Experimental Biology and Medicine, 221*, 281–293.

Carlberg, I., & Mannervik, B. (1975). Purification and characterisation of the flavoenzyme glutathione reductase from rat liver. *Journal of Biological Chemistry, 250*, 5475–5480.

Chandra, M., Chandra, N., Agrawal, R., Kumar, A., Ghatak, A., & Pandey, V. C. (1994). The free radical system in ischemic heart disease. *International Journal of Cardiology, 43*, 121–125.

Chen, W. Q., Huang, J. W., Luo, L. Q., Luo, S. H., & Yang, H. (2005). Study of *Ganoderma lucidum* polysaccharides on blood glucose and blood lipid levels in diabetic rats. *Chinese Journal of Gerontology, 8*, 91–92 (in Chinese).

Chow, F., & Omaye, S. (1983). Use of antioxidants in the analysis of vitamins A and E in mammalian plasma by high-performance liquid chromatography. *Lipids, 18*, 837–841.

Danova, K., Dobisova, A., Fischer, V., Halcak, L., Minarova, H., Olejarova, I., et al. (2005). Production of reactive oxygen species and antioxidant defense systems in patients after coronary artery bypass grafting: One-week follow-up study. *Journal of Clinical and Basic Cardiology, 8*, 33–36.

Di Marco, P. N., Ghisalberti, A. V., Martim, C. E., & Oliver, I. T. (2008). Perinatal changes in liver corticosterone, serum insulain and plasma glucagon and corticosterone in the rat. *European Journal of Biochemistry, 87*, 243–247.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Calorimetric method for determination of sugars and related substances. *Analytical Chemistry, 28*, 350–356.

Flohé, L., & Gunzler, W. A. (1984). Assays of glutathione peroxidase. *Methods in Enzymology, 105*, 114–121.

Gibbs, S. J., Lightfoot, J., Edwin, N., & Root, T. W. (1992). Protein diffusion in porous gel filtration chromatography media studied by pulsed field gradient NMR spectroscopy. *Journal of Physical Chemistry, 96*, 1458–1462.

Grankvist, K., Marklund, S., & Tajedal, I. B. (1981). Superoxide dismutase is a prophylactic against alloxan diabetes. *Nature, 294*, 158–160 (London).

Grové, A. A., Rohwer, R. E., Laurens, B. J., & Vorster, C. B. (2006). The analysis of illicit methaqualone containing preparations by gas chromatography–mass spectrometry for forensic purposes. *Journal of Forensic Sciences, 51*, 376–380.

Gui, X. F., Wang, L., & Yang, Y. H. (1996). The study of protecting kidney cortical from radical with the use of *Ganoderma lucidum* injection. *Chinese Journal of Biochemistry and Pharmacology, 17*, 188–190 (in Chinese).

Heyliger, C. E., Tahiliani, A. G., & McNeill, J. H. (1985). Effect of vanadate on elevated blood glucose and depressed cardiac performance of diabetic rats. *Science, 227*, 1474–1477.

Immerzeel, P., Schols, A. H., Voragen, J. G. A., & de Vries, C. S. (2004). Different arabinogalactan proteins are present in carrot (*Daucus carota*) cell culture medium and in seeds. *Physiologia Plantarum, 122*, 181–189.

Kim, K. C., & Kim, I. G. (1999). *Ganoderma lucidum* extract protects DNA from strand breakage caused by hydroxyl radical and UV irradiation. *International Journal of Molecular Medicine, 4*, 273–277.

Klein, R. A. (1979). The detection of oxidation in liposome preparation. *Biochimica et Biophysica Acta, 210*, 486–489.

Lakshmi, B., Ajith, T. A., Jose, N., & Janardhanan, K. K. (2006). Antimutagenic activity of methanolic extract of *Ganoderma lucidum* and its effect on hepatic damage caused by benzo[a]pyrene. *Journal of Ethnopharmacology, 107*, 297–303.

Lee, J. M., Kwon, H., Jeong, H., & Lee, J. W. (1999). Inhibition of lipid peroxidation and oxidative DNA damage by *Ganoderma lucidum*. *Phytotherapy Research., 13*, 529–531.

Levy, A. P. (2006). Application of pharmacogenomics in the prevention of diabetic cardiovascular disease: Mechanistic basis and clinical evidence for utilisation of the haptoglobin genotype in determining benefit from antioxidant therapy. *Pharmacology and Therapeutics, 112*, 501–512.

Liang, X. C. (2004). Study on the pathogenesis mechanism of diabetic neuropathy and intervention on it by Chinese and western medicine. *Chinese Journal of Integrated Traditional and Western Medicine, 6*, 45–47.

Like, A. A., & Rossini, A. A. (1976). Streptozotocin-induced pancreatic insulitis: New model of diabetes mellitus. *Science, 193*, 415–417.

Lin, Z. B. (1991). *Recent advances in Chinese herbal drugs/actions and uses* (pp. 133–140). Beijing: Scientific Publishing House.

Lin, Z. B. (2001). *Modern research of Ganoderma lucidum* (2nd ed.). Beijing: Beijing Medical University Press.

Lin, H. Y., Licza, P., Kazutaka, Y., Tohru, M., Masayuki, K., & Hideo, Y. (2003). Determination of plasma vitamin C concentration in fattening cattle. *Animal Science Journal, 74*, 7–10.

Lin, J. M., Lin, C. C., Chen, M. F., Ujiie, T., & Takada, A. (1995). Radical scavenger and antihepatotoxic activity of *Ganoderma formosanum*, *Ganoderma lucidum* and *Ganoderma neo-japonicum*. *Journal of Ethnopharmacology, 47*, 33–41.

Loliger, J. (1991). The use of antioxidants in food. In O. I. Aruoma & B. Halliwell (Eds.), *Free radicals and food additives* (pp. 129–150). London: Taylor and Francis.

Ohkawa, H., ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry, 95*, 351–358.

Pang, Z., Chen, Y., & Zhou, M. (1999). The effect of polysaccharide krestin in GPx gene expression in macrophages. *Acta Biochemica et Biophysica Sinica, 31*, 284–288.

Pang, Z. J., Chen, Y., & Zhou, M. (2000). Polysaccharide krestin enhances manganese superoxide dismutase activity and mRNA expression in mouse peritoneal macrophages. *American Journal of Chinese Medicine, 28*, 331–341.

Papas, A. M. (1996). Determinants of antioxidant status in humans. *Lipids, 31*(Suppl.), 77–82.

Reveillaud, I., Niedzwiecki, A., Bensch, K. G., & Fleming, J. E. (1991). Expression of bovine superoxide dismutase in *Drosophila melanogaster* augments resistance to oxidative stress. *Molecular and Cellular Biology, 11*, 632–640.

Santhakumari, P., Prakasam, A., & Pugalendi, K. V. (2003). Modulation of oxidative stress parameters by treatment with piper betle leaf in streptozotocin induced diabetic rats. *Indian Journal of Pharmacology, 35*, 373–378.

Shang, Y. Z., Qin, B. W., Cheng, J. J., & Miao, H. (2006). Prevention of oxidative injury by flavonoids from stems and leaves of *Scutellaria Baicalensis* georgi in PC12 cells. *Phytotherapy Research, 20*, 53–57.

Shao, B. M., Dai, H., Xu, W., Lin, Z. B., & Gao, X. M. (2004). Immune receptors for polysaccharides from *Ganoderma lucidum*. *Biochemical and Biophysical Research Communications, 323*, 133–141.

Shi, Y. L., Anthony, E., Iris, F. F., & John, A. (2002). Mushroom-derived preparations in the prevention of HO-induced oxidative damage to cellular DNA. *Teratogensis, Carcinogensis and Mutagensis, 22*, 103–111.

Smith, B. J., & Sivasithamparam, K. (2003). Morphological studies of *Ganoderma* (Ganodermataceae) from the Australasian and Pacific regions. *Australian Systematic Botany, 16*, 487–503.

Tewari, H. K., & Venkatesh, P. (2004). Diabetic retinopathy for general practitioners. *The Journal of the Indian Medical association, 102*, 720–723.

Wasser, S. P. (2002). Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. *Applied Microbiology and Biotechnology, 60*, 258–274.

West, I. C. (2000). Radicals and oxidative stress in diabetes. *Diabetic Medicine, 17*, 171–180.

White, C. W. (2006). Commentary on "Hypoxia, hypoxic signaling, tissue damage, and detection of reactive oxygen species (ROS)". *Free Radical Biology and Medicine, 40*, 923–927.

Wild, S., Roglic, G., & Green, A. (2004). Global prevalence of diabetes: Estimates for the year 2000 and projections for 2030. *Diabetes Care, 27*, 1047–1053.

Winiarska, K., Fraczyk, T., Malinska, D., Drozak, J., & Bryla, J. (2006). Melatonin attenuates diabetes-induced oxidative stress in rabbits. *Journal of Pineal Research, 40*, 168–176.

Wohaieb, S. A., & Godin, D. V. (1987). Alterations in free radical tissue-defence mechanisms in streptozotocin-induced diabetes in rat. Effect of insulin treatment. *Diabetes, 36*, 1014–1018.

Xu, Y. (2001). Perspectives on the 21st century development of functional foods: Bridging Chinese medicated diet and functional foods. *International Journal of Food Science and Technology, 36*, 229–242 (in Chinese).

You, Y. H., & Lin, Z. B. (2002). Protective effects of *Ganoderma lucidum* polysaccharides peptide on injury of macrophages induced by reactive oxygen species. *Acta Pharmacologica Sinica, 23*, 787–791 (in Chinese).

Zhang, H. N., He, J. H., Yuan, L., & Lin, Z. B. (2003). In vitro and in vivo protective effect of *Ganoderma lucidum* polysaccharides on alloxan-induced pancreatic islets damage. *Life Sciences, 73*, 2307–2319.

Zhao, J., Li, P., & Hu, Y. (2006). Effect of *Ganoderma lucidum* polysaccharides on reducing blood sugar in mice with diabetes. *Journal of North China Coal Medical College, 3*, 13–14 (in Chinese).

Zhu, X. L., & Lin, Z. B. (2006). Modulation of cytokines production, granzyme B and perforin in murine CIK cells by *Ganoderma lucidum* polysaccharides. *Carbohydrate Polymers, 63*, 188–197.