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Attenuating effects of *Ganoderma lucidum* polysaccharides on myocardial collagen cross-linking relates to advanced glycation end product and antioxidant enzymes in high-fat-diet and streptozotocin-induced diabetic rats

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ABSTRACT

In the present study, polysaccharides were isolated from *Ganoderma lucidum* and their effects on myocardial collagen cross-linking were discussed in high-fat-diet/streptozotocin diabetic rats to investigate whether collagen-linked advanced glycation end products (AGE) and antioxidant enzymes were involved in the progress. Rats were grouped into the normal control, diabetic control and polysaccharides-treated groups. Blood glucose, insulin, blood fat, antioxidant enzymes activities, myocardial collagen and AGE were measured. After polysaccharides treatment, blood glucose and fat decreased, while insulin increased. It was also found that *G. lucidum* polysaccharides attenuated myocardial collagen cross-linking in diabetic rats, which was related to the decreased level of AGE and augmented activities of antioxidant enzymes. The results provided a possible use of *G. lucidum* polysaccharides in the treatment of myocardial fibrosis of diabetes.

1. Introduction

Diabetes mellitus (DM) is a chronic dysbolism disease caused by the interaction of heredity and environment. According to World Health Organization, around 171 million people suffered from diabetes in 2000. This number was predicted to double by 2030 (Wild, Roglic, Green, Sicree, & King, 2004). Suffering from DM for a long time may cause myocardial fibrosis, gradually leading to the development of myocardial stiffness, which is a risk factor for cardiovascular disease (Asbun & Villarreal, 2006).

Accumulating evidence suggests that advanced glycation end products (AGE) and cross-linked collagen play an important role in the process of this cardiac complication in DM (Paul & Bailey, 1996; Sajithlal, Chithra, & Chandrakasan, 1998). AGE, which can be increased by hyperglycemia, usually modifies proteins by forming covalent bonds with amino groups on other proteins. As a result, it can alter physical and structural properties of extracellular matrix, making flexible proteins rigid, decreasing the susceptibility of collagen to proteolytic and chemical degradation, and encourage collagen deposition and myocardial stiffness (Brownlee, Cerami, & Vlassara, 1988). AGE is also an indication of the levels of oxidative stress (Gopal & Indira, 2010). Breakers of AGE can reduce collagen crosslinking and improve cardiac function in aging diabetic heart (Candido et al., 2003; Liu et al., 2003). These data suggest that addition of an AGE breaker may provide a potential new therapeutic approach for the treatment of cardiovascular fibrotic disease in diabetes. Therefore, a number of synthetic and natural compounds have been studied to test whether they can prevent AGE formation, attenuate collagen cross-linking and reduce the degree of myocardial fibrosis (Asif et al., 2000; Vasan, Foiles, & Founds, 2003).

Scientific investigation has repeatedly confirmed beneficial effects of *Ganoderma lucidum* on the prevention and treatment of human disease. There are several active components in *G. lucidum*. And *G. lucidum* polysaccharides (GLP), which exhibit immunostimulating, anti-tumour, antioxidant, anti-angiogenic activities, are major sources of its biological activities and therapeutic uses (Boh et al., 2003; Song et al., 2004). GLP also have the ability to reduce lipid peroxidation and blood glucose levels in diabetic rats (Chen et al., 2009; Jia et al., 2009). Recently, a phase I/II study found that polysaccharide fractions extracted from *G. lucidum* could improve blood glucose levels in non-insulin-dependent diabetic patients (Gao, Jin, Dai, Ye, & Zhou, 2004). But no information about the effect of GLP on diabetic cardiac complications was available. Whether GLP can be used for an AGE breaker to attenuate myocardial fibrosis in diabetes is also unknown.

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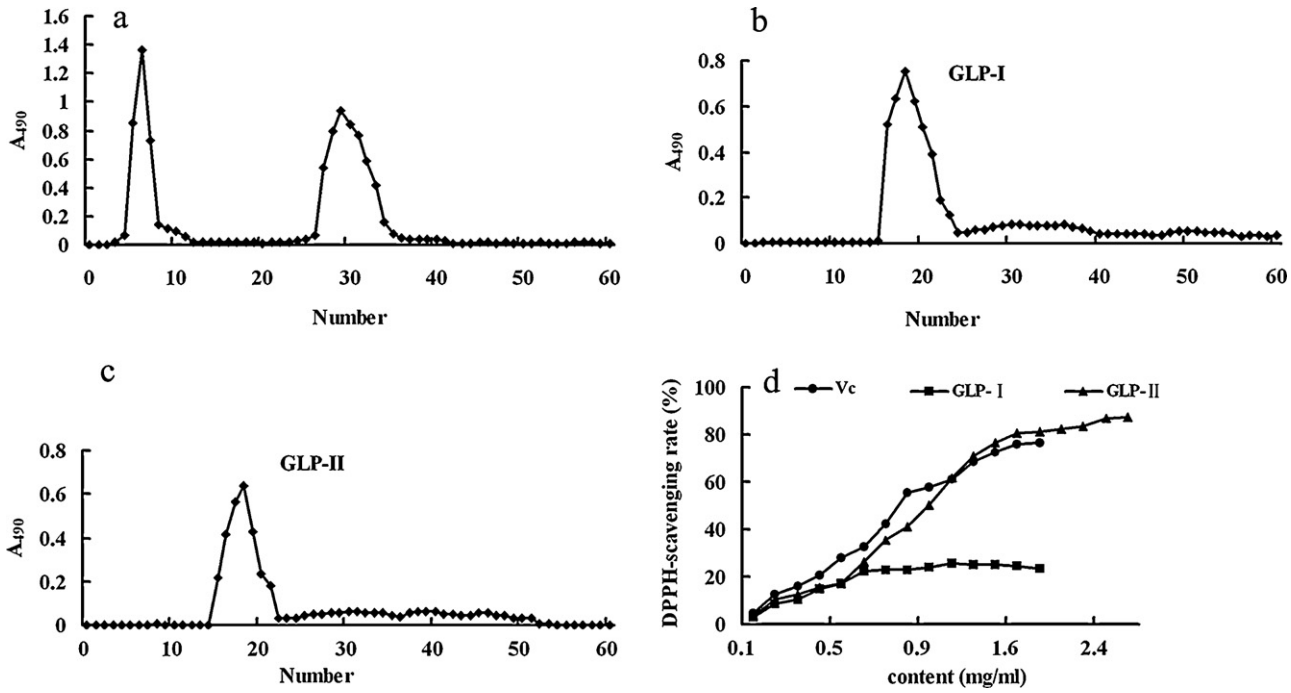

Fig. 1. Isolation, purification of *Ganoderma lucidum* polysaccharides. (a) Isolation and purification of *G. lucidum* polysaccharides by DEAE-cellulose anion-exchange chromatography. (b and c) Purification of *G. lucidum* polysaccharides by Sephadex G-100 gel column chromatography. (d) DPPH radical-scavenging activities of *G. lucidum* polysaccharides (Vc refers to vitamin C).

Therefore, our study was conducted to determine whether *G. lucidum* polysaccharides could ameliorate the modification of myocardial collagen and attenuate myocardial fibrosis in diabetic rats. Our study also investigated the possible mechanisms, such as whether collagen linked AGE and oxidative stress were involved in the progress.

2. Materials and methods

2.1. Extraction of G. lucidum polysaccharides

The fruiting bodies of *G. lucidum* were provided by Jiangsu Alphay Biological Technology Co. Ltd. (Nantong, Jiangsu, China). Their sporocarps were cut into small pieces, dried and powdered, followed by ultrasonic wave extraction with hot water. Polysaccharides were isolated by the method of previous report with slight modification (Chen et al., 2009). The working parameters of ultrasonic wave extraction were as follows: extraction time of 17 min, ultrasound power of 680 W, and water to material ratio of 28:1. The yield and purity of polysaccharides after extraction were (20.79 ± 0.11) mg/g and 48.09%, respectively. The water extract was further subjected to ultra-filtration with different filter membranes to yield a condensed solution of polysaccharides with more than 50 kDa molecular weight. After ultra-filtration the purity of polysaccharides was increased to 61.32%.

2.2. Isolation and purification of G. lucidum polysaccharides

The polysaccharides extract was purified by DEAE-cellulose anion-exchange chromatography, and phenol-sulfuric acid assay was conducted to obtain an elution curve, a plot of absorbance against effluent volume. Two fractions, corresponding to the two peaks in the elution curve (Fig. 1a), were isolated and named GLP-I and GLP-II, respectively, which were then subjected to Sephadex G-100 gel column chromatography. This analysis indicated that both GLP-I and GLP-II were homogeneous with concentrated molecular weight distribution in terms of their single, symmetric chromatography peaks (Fig. 1b and c). Confirmation of the polysaccharides was done by Anthrone (Yemn & Wills, 1954) and phenol sulphuric acid tests (Dubois, Gilles, & Hamilton, 1956) as previously described. Then the purity was available. The purity of polysaccharides in two fractions was 74.03% and 75.61%, respectively. The main impurity might be some free proteins, polypeptides, residual extraction media, etc. Each of them had less ability or no ability at all.

2.3. Measurement of DPPH free radical-scavenging activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging activities of the above polysaccharides were measured as previously described (Duan, Zhang, Li, & Wang, 2006). Both GLP-I and GLP-II exhibited a dose-dependent DPPH radical-scavenging activity. At the same concentration, the anti-oxidative activity of GLP-II was significantly higher than that of GLP-I and so GLP-II was chosen for further study in our research.

2.4. Animal experiments

2.4.1. Animals modeling, grouping and treatment

The animal experiments were conducted according to NIH Guidelines for the Care and Use of Laboratory Animals. Sprague-Dawley rats weighing from 160 to 180 g were provided by Experimental Animal Central of Nantong University. Rats were housed individually in the specific room at constant temperature (20–22 °C) and humidity (45–55%) with a 12-h light–dark cycle. Ten animals as normal controls were fed with standard laboratory diet and given physiological saline. The others were fed with high fat diet (fat: 20%, milk: 5%, power of egg yolk: 5%, standard laboratory diet: 70%) for 4 weeks. Then streptozotocin (STZ, 30 mg/kg) in freshly prepared citrate buffer solution (0.1 M, pH 4.5) was intraperitoneally injected. Control rats were injected with citrate buffer alone. Three days later, the development of hyperglycemia in rats was confirmed by fasting blood glucose (FBG) estimation from tail vein with a One-Touch blood glucose meter (Johnson, USA). The

Table 1
Effects of *Ganoderma lucidum* polysaccharides on blood glucose, HbA_{1c}, insulin, TC and TG in experimental animals.

Group	Blood glucose (mmol/L)	HbA_{1c} (%)	Insulin (mIU/L)	TC (mmol/L)	TG (mmol/L)
NC	3.61 ± 0.66	4.54 ± 0.61	38.57 ± 3.38	1.25 ± 0.08	0.43 ± 0.05
DC	19.26 ± 2.94[b]	9.64 ± 0.73[b]	18.73 ± 1.62[b]	4.71 ± 0.36[b]	1.42 ± 0.19[b]
GLP-L	16.17 ± 3.11[b]	9.05 ± 0.86[b]	19.86 ± 3.10[b]	4.66 ± 0.32[b]	1.43 ± 0.14[b]
GLP-M	13.31 ± 2.65[bd]	8.20 ± 1.03[bc]	27.38 ± 1.89[bd]	3.60 ± 0.30[bd]	0.90 ± 0.04[bd]
GLP-H	7.08 ± 3.28[d]	6.49 ± 0.93[bd]	33.98 ± 3.09[bd]	3.27 ± 0.29[bd]	0.77 ± 0.11[bd]

Each value represents mean ± SD; $n = 10$. (b) $P < 0.01$, compared with normal control; (c) $P < 0.05$, (d) $P < 0.01$, compared with diabetic control.

rats with FBG above 11.1 mmol/L were considered to be diabetic (Jia et al., 2009) and only uniformly diabetic rats were included in the study.

Forty diabetic rats were randomly divided into four groups ($n = 10$ per group): the diabetic control (DC group), and three drug groups receiving different doses of *G. lucidum* polysaccharides (GLP-L: 200 mg/kg, GLP-M: 400 mg/kg and GLP-H: 800 mg/kg). The dose of 400 mg/kg is in line with Chinese traditional phytotherapy. For three drug groups, administration by gastric gavage was conducted once daily over a 16-week period. Ten normal rats served as normal controls (NC group), receiving the same volume of physiological saline instead of drug once a day over the same period. All animals continued on their original diets for the duration of the study.

2.4.2. Biochemical analysis

After 16 weeks' treatment, the rats were deprived of food overnight. The level of FBG was tested again. Then blood was collected from the carotid artery into tubes for various analyses. Glycated hemoglobin (HbA_{1c}) values and antioxidant enzymes activities, including superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT), were assessed by using commercially available kits (Nanjing Jianchen Biochemistry Co. Ltd., China). Insulin was estimated by radioimmunity. Blood-fat was tested with automatic biochemistry analyser (Olympus AU1000, Japan).

2.4.3. Myocardial collagen measurement

Myocardial hydroxyproline concentration was determined as previously described (Stegemann & Stalder, 1967). Myocardial collagen was extracted and digested with cyanogen bromide (CNBr), according to the procedure described previously (Mukherjee & Sen, 1990). The remaining portion of the CNBr-digested collagen sample was subjected to acid hydrolysis and hydroxyproline determination. This was non-cross-linked (soluble) collagen. Then, the amount of cross-linked (insoluble) collagen in the myocardium was determined based on the total myocardial collagen amount. The ratio between insoluble and soluble collagen was used as an index of the degree of collagen cross-linking (Meng et al., 2009).

2.4.4. AGE assessment

Myocardial AGE formation was determined based on the fluorescence assay methods as described earlier (Babu, Sabitha, Srinivasan, & Shyamaladevi, 2007; Monnier et al., 1986). Serum AGE levels were analyzed by the method of Münch et al. (1997). Fluorescence was measured at 440 nm upon excitation at 370 nm with a spectrofluorimeter (Shimadzu, Japan). The degree of myocardial AGE was determined by fluorescence expression demonstrated as arbitrary units of fluorescence (AUF) per gram hydroxyproline, while serum AGE-associated fluorescence level was determined as AUF per gram protein.

2.4.5. Statistical analysis

The statistical analysis was performed by using Stata software. Data were presented as mean ± standard deviation (SD). Multiple comparisons were performed by one-way analysis of variance followed by a Student–Newman–Keuls test. Differences were considered statistically significant when P was less than 0.05.

3. Results and discussion

3.1. Effects of GLP on blood glucose, HbA_{1c}, insulin and blood fat

At the beginning of treatment, the level of fasting blood glucose in all five groups was similar to each other (data not shown). Three days after STZ was injected, induction of diabetes mellitus was confirmed by fasting blood glucose values above 11.1 mmol/L, which was higher than the level of normal rats (data not shown). In our study, animals were developed into a model of type 2 diabetes mellitus in a non-obese, outbred rat strain that replicated the natural history and metabolic characteristics of the human syndrome and was suitable for pharmaceutical research (Bi et al., 2007; Reed et al., 2000). It was found that diabetic rats in our study showed distinctive performances of type 2 diabetes mellitus, such as elevated blood glucose, HbA_{1c}, blood fat and decreased insulin.

After 16 weeks' treatment, blood glucose and HbA_{1c} were increased significantly in the DC group, while insulin was significantly decreased compared to the untreated normal control rats. Administration of middle or high doses of GLP significantly decreased blood glucose and HbA_{1c}, but elevated insulin levels in rats, yet, there was no significant difference between the DC group and the GLP-L group (Table 1). Previous studies have confirmed that GLP can reduce blood glucose levels in diabetic animals (Jia et al., 2009; Mohammed, Adelaiye, Abubakar, & Abdurahman, 2007; Zhang, He, Yuan, & Lin, 2003). Our study also provided a valuable evidence for past reports on the anti-hyperglycemic effects.

GLP have shown to have hypoglycemic potentials in normal and glucose loaded animals by increasing the insulin levels, improving pancreatic cell function or elevating glucose uptake (Hikino, Ishiyama, Suzuki, & Konno, 1989; Hikino, Konno, Mirin, & Hayashi, 1985; Jung et al., 2006; Ni et al., 2007). In our study, insulin was increased accompanied by decreased blood glucose after GLP treatment, which is in line with the previous work. Another general hypothesis is that glycated hemoglobin, as an average level in blood for a long term, is in proportional to the blood glucose levels (Koenig et al., 1976). In our study, GLP also reduced blood HbA_{1c} coupled with a decrease in blood glucose, which might be interesting and appealing in further studies.

Meanwhile, it was noticed that GLP could decrease total cholesterol (TC) and triglyceride (TG) in high-fat-fed/STZ diabetic rats. In the DC group, TC and TG were increased compared with the NC group. After treatment, TC and TG in the GLP-M or GLP-H group were reversed to the value that was significantly lower than that in DC group (Table 1). The improvements in blood cholesterol and triglyceride levels could be due to the reduced blood glucose levels in diabetic rats (Jain, Rains, & Croad, 2007; Yang, Wilson, Cho, & Song, 2004). Previous studies discovered that *G. lucidum* consumption caused a marked suppression of the elevated total cholesterol levels, which might be responsible for, or be associated with the inhibition of the hepatic phosphoenolpyru-

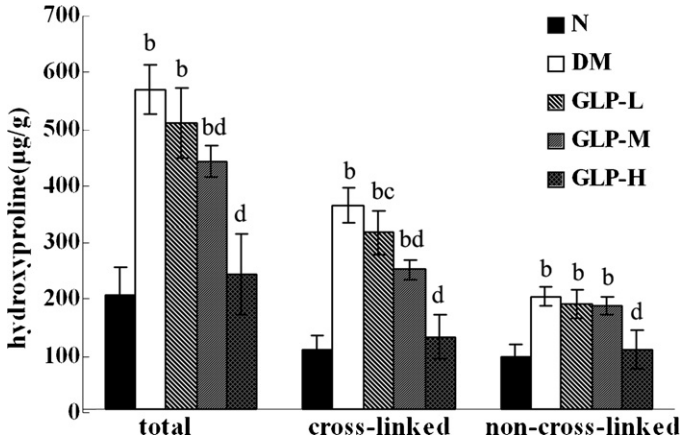

Fig. 2. Effects of *G. lucidum* polysaccharides on myocardial total collagen and cross-linked characteristics in experimental animals. (b) $P < 0.01$, compared with normal control; (c) $P < 0.05$, (d) $P < 0.01$, compared with diabetic control.

vate carboxykinase gene expression (Seto et al., 2009). HMG CoA reductase activities, lipoprotein metabolism might also contribute to hypolipemia effects after GLP treatment similar to other plant medicines (Lemhadri, Hajji, Michel, & Eddouks, 2006; Sharma, Nasir, Prabhu, Murthy, & Dev, 2003). The detailed mechanism for the observed cholesterol and triglyceride lowering activities of GLP might be elucidated in further studies.

3.2. Effects of GLP on myocardium collagen

Moreover, epidemiological, clinical, and laboratory studies have confirmed the existence of cardiomyopathy (including left ventricular hypertrophy, myocardial fibrosis, and even congestive heart failure) associated with diabetes (Asbun & Villarreal, 2006). Myocardial fibrosis, probably one of the major threatening cardiac complications in diabetes (Maya and Villarreal, 2010), was characterized by excess collagen accumulation or altered collagen composition in myocardium. The content of the total hydroxyproline, as an index of total collagen quantity, was determined in left ventricular myocardium of rats. It was found that the DC group tended to have a higher hydroxyproline concentration than the NC group, which suggested that there had been more collagen accumulation and obvious myocardial fibrosis at the stage of our experiment. After treatment with GLP, it was discovered that the total hydroxyproline concentration showed a significant dose dependent decrease. And there was the lowest concentration in the GLP-H group with a significant difference compared with the GLP-L or GLP-M group (Fig. 2).

There does not seem to be a simple inverse correlation between total collagen content and heart function (Kim et al., 2000). A previous report has showed that loss of myocardial interstitial collagen is coincident with a marked deterioration of systolic or diastolic function, and attenuation of cardiac fibrosis decreases heart systolic performance in rats with myocardial fibrosis (Cingolani et al., 2004). In a word, it seems that collagen deposition might play an important role in maintenance of myocardial structure and contractility, although too much collagen may decrease the systolic function (Baicu et al., 2003). In other words, total left ventricular collagen reduction essentially does not necessarily benefit the cardiac function. Examination only from the aspect of reducing the total collagen amount is not adequate for a comprehensive evaluation on the inhibition of myocardial fibrosis in diabetic rats with GLP. In the present investigation, our study indicated that *G. lucidum* polysaccharides influenced not only myocardial hydroxyproline but also the concentration of both soluble (to CNBr digestion) and insoluble (cross-linked) myocardial collagen. In the NC group, insoluble (cross-linked) and soluble (non-cross-linked) collagen was relatively minor. The ratio between them was 1.12 ± 0.05. However, the DC group produced an increase in both of cross-linked and non-cross-linked collagen. The ratio went up to 1.78 ± 0.11, and was higher than that in the NC group ($P < 0.01$), which suggested that the elevated collagen was mainly the cross-linked collagen in diabetic rats. After 16-week treatment, both cross-linked and non-cross-linked collagen of each treated group produced a certain degree of decreasing. But the ratio in the GLP-L group was 1.66 ± 0.07 and there was no significant difference compared with DC group. The ratios in the GLP-M and GLP-H groups were 1.34 ± 0.10 and 1.23 ± 0.07, which were significantly less than that of the DC group ($P < 0.01$). Moreover, the ratio in the GLP-H group was of normal value as in the NC group, implying that a moderate dose of GLP might effectively attenuate the cross-linked degree of myocardial collagen.

Table 2
Effects of *G. lucidum* polysaccharides on serum and myocardial AGE in experimental animals.

Group	AGE	
	Serum (AUF/g protein)	Myocardium (AUF/g hyproxyproline)
NC	66.33 ± 14.47	630.5 ± 71.4
DC	139.28 ± 19.23[b]	934.7 ± 70.0[b]
GLP-L	119.22 ± 12.56[b]	845.3 ± 105.8[b]
GLP-M	108.32 ± 15.66[bd]	806.5 ± 60.4[bc]
GLP-H	86.95 ± 14.17[d]	730.3 ± 75.2[d]

Each value represents mean ± SD; $n = 10$. (b) $P < 0.01$, compared with normal control; (c) $P < 0.05$, (d) $P < 0.01$, compared with diabetic control.

3.3. Effects of GLP on myocardial and serum AGE

Meanwhile, there was a significant increase in myocardial and serum AGE in the DC group. The accumulation of AGE and AGE-related collagen was proposed to be responsible for the decrease of cardiac compliance in diabetes mellitus. After treatment, there was no significant improvement in the GLP-L group. But in the GLP-M and GLP-H groups, the content of AGE was significantly reduced, which implied that treatment with moderate doses of GLP efficiently attenuated the enhanced AGE appearing in diabetic rats (Table 2). As is well known, the form of AGE is a slow process. First, glucose interacts with collagen to generate a Schiff base, which can then rearrange to produce a reversible earlier period of glycosylation products: an Amadori product. Then the Amadori product can undergo further complicated chemical modification to yield irreversible AGE (Ulrich & Cerami, 2001). This advanced glycation of proteins is accelerated in the hyperglycemic environment of diabetes (Aronson, 2003). Previous studies indicate that persistent hyperglycaemia may result in non-enzyme-glycosylation of many structural, functional and nucleate proteins in vivo, and finally forming the irreversible AGE (Lorenzi, 1992; Pamplona, Bellmunt, Portero, & Prat, 1993). AGE can form covalent bond with other proteins at the end of collagen protein amino terminal and lead to the cross-linked collagen, deposited fiber and enhanced stiffness. Treatment with AGE breaker in diabetic rats significantly reduced the cardiac accumulation of AGE and increased collagen solubility to the normal level (Pamplona et al., 1993). Our experiment was consistent with the viewpoint. Our study discovered that, in the high-fat-fed/STZ diabetic rats, there was serious myocardial fibrosis following with high concentration of AGE, which was enhanced in hyperglycemic rats. This indicated that AGE was involved in the increasing of myocardial collagen cross-linkages. After the treatment with GLP, not only blood glucose but also AGE and cross-linked collagen decreased significantly. It revealed that preventing AGE production was beneficial to protect myocardial collagen from

Table 3
Effects of *G. lucidum* polysaccharides on antioxidant enzymes activities in myocardium of experimental animals.

Group	SOD (U/mg protein)	GSH-Px (U/mg protein)	CAT (U/mg protein)
NC	246.59 ± 16.18	23.72 ± 4.76	25.88 ± 6.62
DC	132.05 ± 9.10[b]	13.52 ± 2.55[b]	12.98 ± 1.95[b]
GLP-L	132.91 ± 11.33[b]	13.70 ± 4.41[b]	14.37 ± 2.79[b]
GLP-M	176.16 ± 5.41[bd]	19.42 ± 3.62[c]	19.79 ± 3.75[c]
GLP-H	189.68 ± 6.55[bd]	23.16 ± 2.41[d]	21.47 ± 3.33[d]

Each value represents mean ± SD; $n = 10$. (b) $P < 0.01$, compared with normal control; (c) $P < 0.05$, (d) $P < 0.01$, compared with diabetic control.

cross-linking. Because of the attenuated fibrosis, cardiac functions were also augmented after treatment (data not shown).

3.4. Effects of GLP on myocardial antioxidant enzymes activities

Because DPPH was useful to determinate antioxidant properties of natural compounds, the DPPH radical-scavenging assay system was successfully used for the evaluation on the antioxidant activities of GLP before the animal experiment. In our study, antioxidant activities of two polysaccharides were different, which might be related to molecular weight and glycosidic linkages (Liu, Wang, Pang, Yao, & Gao, 2010). It was found that GLP at high concentration (>1.2 mg/ml) had similar DPPH radical-scavenging activities to vitamin C, which was a well-known antioxidant (Fig. 1d). This discovery suggested that *G. lucidum* polysaccharide was a fairly good scavenger for DPPH radicals.

Moreover, recent studies have found that an obvious oxidative stress existed in diabetes. And cellular oxidative damage is primarily caused by reactive oxygen species (ROS) in the condition of oxidative stress (Jia et al., 2009). Brownlee M presumed that ROS was a common up-stream event in the pathway of AGE, PKC, polyalcohol pathobiology during diabetic complications (Brownlee, 2005). Antioxidant enzymes, including SOD, GSH-Px and CAT, are vital defenses against ROS. They are important in inhibiting oxyradical formation and usually used as biomarkers to indicate ROS production. Our study found that antioxidant enzymes activities (SOD, GSH-Px and CAT) were significantly decreased in the serum of untreated diabetic rats (DC group) compared to normal controls (NC group). Thus there was a low antioxidant defense in diabetes. And excess free radical production exceeded the scavenging activity and even resulted in chemical alterations of bio-molecules, structural modifications and functional deficiencies (Cuzzocrea & Reiter, 2001). And administration of middle or high doses of GLP significantly enhanced the SOD, GSH-Px and CAT activities (Table 3), which indicated that GLP might enhance myocardial antioxidant capacity in diabetic rats. One mechanism of the anti-hyperglycemic actions of GLP may be through its scavenging ability to protect pancreatic cells from oxyradical damage. So the section of insulin was able to increase to reduce the high level of blood glucose (Jia et al., 2009).

Once there is excessive oxidation in diabetes, it can enhance the process of auto-oxidized glycosylation, contribute to the oxidation of glycosylated proteins and further promote the formation of AGE (Gopal & Indira, 2010). ROS can also increase the presence of AGE via up-regulation of receptors for AGE (RAGE) depending on NF-kappa B pathway (Yamamoto & Gaynor, 2001). And accelerated formation of AGE during hyperglycemia has also been implicated in the development of diabetic complications (Thorpe & Baynes, 1996). AGE, as a potential surrogate biomarker for oxidative stress, not only results in serious myocardium collagen cross-linking directly but also combines with its specific receptor and increases the production of ROS which lead to impairing cells and increasing incidences of cardiomyopathy by attacking membrane proteins, intracellular enzyme systems as well as nucleic acids (Gopal & Indira, 2010; Price, Rhett, Thorpe, & Baynes, 2001). So AGE and reactive oxygen species can result in a vicious circle. They interact with each other, elevate formations or effects, and finally augment degrees of collagen cross-linking. In our study, there was enhanced oxidation stress because of decreased activities of SOD, GSH-Px and CAT in diabetic rats. It was also confirmed that the level of AGE and cross-linked collagen increased in high-fat-fed/STZ diabetic rats. These findings were consistent with the previous reports. Moreover, moderate doses of GLP attenuated myocardium collagen cross-linking in treated diabetic rats, which was related to decreased formation of AGE and increased activities of SOD, CAT and GSH-Px. Therefore, GLP, as an effective antioxidant, contains a free radical oxygen scavenging activity, which can exert a protective effect against AGE related cross-linked collagen accumulation.

4. Conclusion

In summary, it has been found that adequate doses of GLP used in this study could attenuate myocardial collagen cross-linking in diabetic rats. This action was related to the decreased level of AGE and augmented activities of antioxidant enzymes. Moreover, our data might provide a possibility that GLP may be used for the treatment of myocardial fibrosis, which was one of the common cardiovascular complications in diabetes. However, further studies are needed to explain the detailed mechanism of the protective effect.

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