

# Hypoglycemic effect of *Gynostemma pentaphyllum* saponins by enhancing the Nrf2 signaling pathway in STZ-inducing diabetic rats

Dawei Gao · Min Zhao · Ximing Qi ·  
Yanping Liu · Nan Li · Zhiwei Liu ·  
Yanhong Bian

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**Abstract** *Gynostemma pentaphyllum* (GP) is a natural plant resources for diabetes therapy, however, there is little research on the mechanisms of GP. The present study was undertaken to characterize if *G. pentaphyllum* saponins (GPs) is the principal active compound of GP responsible for anti-diabetes, and to examine the relativity between blood glucose modulate and antioxidation. The GPs-treated streptozotocin diabetic rats had a more effective hypoglycemic status than those of diabetic control rats, which also ameliorate dyslipidemia. GPs has increased SOD and GSH-px activities, and the spleen and thymus indexes in diabetic rats. The insulin levels in the GPs-treated groups were significantly higher than diabetic control group. Our finding provides a new insight into the application of GPs for the treatment of oxidative stress related diseases.

**Keywords** *Gynostemma pentaphyllum* saponins · Hypoglycemia · Nuclear factor erythroid 2-related factor 2 (Nrf2) · Insulin · Reactive oxygen species

## Introduction

The prevalence of diabetes mellitus is rising at an alarming rate, embracing the major population of the world. At present, diabetes incidence rate is 6.7 % in China, which higher

than the world average level (6.4 %). People suffering from diabetes are not able to produce or properly use insulin in the body, so they have a high content of blood glucose. As a very common chronic disease, diabetes is becoming the third “killer” of the health of mankind along with cancer, cardiovascular and cerebrovascular diseases due to its high prevalence, morbidity and mortality. The development and progression of nephropathy and cardiomyopathy in the patients with diabetes remains unpreventable.

At present, research showed that reactive oxygen species (ROS) has been implicated in more than 100 diseases (Hwang and Kim 2007), which also has a close relationship with diabetes incidence (Newsholme et al. 2007). ROS is continuously produced by the body’s normal use of oxygen such as respiration and some cell-mediated immune functions. Excess ROS can react with many biomolecules such as DNA, lipids and proteins, initiating the peroxidation of membrane lipids, leading to the accumulation of lipid peroxides and the damage of DNA and proteins, and finally resulting in disease conditions. Extra generation of ROS, induced by hyperglycemia, is considered as the main reason for the development of these diabetic complications (Tuba and Ilhami 2008).

As transcription factor, NFE2-related factor 2 (Nrf2) is a master regulator of cellular detoxification response and redox status, and also provides a protective action from various oxidative stresses and damages (Inoguchi et al. 2000). Under physiological conditions Nrf2 locates in the cytoplasm and combines with its inhibitor kelch-like ECH-associated protein 1 (KEAP1). KEAP1 could mediate a rapid ubiquitination and subsequent degradation of Nrf2 by the proteasome (McMahon et al. 2003). Upon exposure of cells to oxidative stress or electrophilic compounds, Nrf2 is free from KEAP1 and translocates into the nucleus to bind to antioxidant-responsive elements (AREs) in the genes

D. Gao (✉) · Y. Liu · N. Li · Z. Liu · Y. Bian  
Applied Chemical Key Lab of Hebei Province, Department of  
Biological Engineering, Yanshan University, No. 438 Hebei  
Street, Qinhuangdao 066004, China  
e-mail: dwgao@ysu.edu.cn

M. Zhao · X. Qi  
Department of Pathology, The First Hospital of Qinhuangdao,  
No. 258 Cultural Road, Qinhuangdao 066004, China

encoding antioxidant enzymes such as NADPH quinone oxidoreductase (NQO1), glutathione S-transferase, heme oxygenase-1 (HO1), and  $\gamma$ -glutamylcysteine synthetase, increasing their expression to play a role of detoxification, antioxidation, and anti-inflammation (McMahon et al. 2003; Haan 2011). Recently, several studies have indicated preventive effect of Nrf2 on high glucose induced oxidative damage in the cultured cells and potentially on the diabetic complications in animal models (Xue et al. 2008).

Therefore, antioxidant prevention or therapy of diabetic complications is attractive, but to date, there were few antioxidants that were found to be efficiently applied in clinics (Nishikawa et al. 2000; Rask-Madsen and King 2007). Herbal remedies have become a key component for human health care because they may be perceived to have fewer side effects and multiple effects when compared with modern western medicine (Matschinsk 2009). There are many kinds of plant drug used for diabetes therapy, including *Ligustrum Lucidum* Ait, *Cortex Lycii Radicis*, *Berberine*, etc. (Gao et al. 2009, 2012; Zhang et al. 2008). *Gynostemma pentaphyllum* is a dioecious, herbaceous climbing vine of the genus *Gynostemma* in family Cucurbitaceae, which also named Jiaogulan (Chinese name), *pentapanax leschenaultii*, sweet tea vine etc. Main components of *G. pentaphyllum* extracts are as much as 82 different saponins, several amino acids and vitamins (Deng et al. 1994). The structures of 4 kinds of *G. pentaphyllum* saponins are same with those of ginsenoside Rb1, Rb3, Rd and F2, and the ginsenoside content of GP is eight times of ginseng's (Zhang et al. 2007). Abroad spectrum of beneficial effects had been reported including regulation of blood pressure (Tanner et al. 1999), antiallergic activity (Huang et al. 2007), anti-hyperlipidemic and anti-hyperglycemic activities (Megalli et al. 2005), and anticancer effect (Chen et al. 2009). It is a popular Chinese medicinal and edible plant resource. Whether *G. pentaphyllum* saponins produce protective effects mainly through antioxidant mechanisms is unknown. If this hypothesis is the truth, whether *G. pentaphyllum* saponins play as an antioxidant through quenching ROS, inhibiting lipid peroxidation or indirectly stimulating cellular antioxidant defenses is to be clarified.

The study was carried out to develop a preparative column chromatographic method for isolation of saponins from *G. pentaphyllum*, determine their composition by high performance liquid chromatography (HPLC). To our knowledge, the mechanism by which *G. pentaphyllum* saponins (GPs) affect of blood glucose metabolism is not completely understood, in particular, their effect on treatment of diabetes in streptozotocin (STZ)-induced diabetic rats. The study was undertaken to evaluate hypoglycemic and hypolipidemic effects of *G. pentaphyllum* saponins, at

the same time, determine the relationship between hypoglycemic mechanisms and Nrf2 signaling pathway.

## Materials and methods

### Reagents and herb sample

Glucose analyzer and strips were purchased from Roche Diagnostic Co. (USA). Streptozotocin (STZ) was bought from Sigma Co., (USA). Seven-leaf *G. pentaphyllum* was grown in Shanxi Province and purchased from Tong ren tang Traditional Chinese Medicine Ltd. (China), and its identity was verified by the botanist. Ginseng saponins standard was purchased from Chengdu Manst Biological Technology Co., Ltd. Other reagents were all analytical grade.

### Preparation of standard curve

Ginsenoside Rb<sub>1</sub> standard 10 mg was added in methanol to total volume 10 mL, and the different volume Rb1 solution (20, 40, 60, 80, 100 and 120  $\mu$ L) were added to a new tubes, then soak in 60 °C water bath to remove the solvent. 0.2 mL 5 % vanillin glacial acetic acid solution and 0.8 mL perchloric acid were added the tubes, mixed, heated in 60 °C water bath for 15 min, then added cooled ice acetic acid 5 mL. The corresponding reagent as blank, light absorption values of the solutions were measured at 550 nm. The absorbance value (x) and the content of ginsenoside Rb1 (y) were regressed to obtain the standard curve.

### Preparation and characterization of *G. pentaphyllum* saponins

Briefly, dried *G. pentaphyllum* powder was mixed with 10 volume of 70 % ethanol for 1 h, and then extracted with ultrasound for 30 min, repeated the process two times, and combined the extract solution. The supernatant was concentrated by a rotary evaporator and vacuum dried to obtain GP extract powder. The powdered extract was resuspended in dH<sub>2</sub>O, and separated by column chromatography using adsorbent resins (HP20) columne. dH<sub>2</sub>O was used for elution repeatedly until the detective result showing sugar free by the molish reaction. The solution was eluted with 20 % ethanol and 70 % ethanol respectively. Finally, the 70 % ethanol phase was collected, freeze-dried to obtain GP extract powder. The content of GPs was determined using vanillin perchloric acid colorimetric method, which was same with the preparation of standard curve.

### Measurement of superoxide anion radical scavenging activity

Free radicals are mainly produced inside organelles, such as the mitochondria, and also released into the cytosol. Numerous biological reactions generate the superoxide radical which is a highly toxic species. Although they cannot directly initiate lipid oxidation, superoxide radicals are potential precursors of highly reactive species. In this study, DPPH free radical scavenging activity was estimated at 25 °C using the spectrophotometric monitoring as our previous method, the ascorbic acid served as positive control (Gao et al. 2013). 0.1 mM DPPH solution in methanol was prepared and 2 mL of this solution was added to 0.1 mL of GPs and ascorbic acid solutions, respectively. The decrease in absorbance at 517 nm was measured at 0, 5 min and then every 15 min until the reaction reached a plateau. The percentage of DPPH remaining at the steady-state was calculated as a function of the molar ratio of antioxidant to DPPH. The vehicle without the sample was employed as a control. All tests were conducted in triplicate. The radical scavenging activity of different concentrations of GPs sample and ascorbic acid solutions were calculated according to the following formula:

$$\text{DPPH radical scavenging activity (\%)} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100\%$$

### Animals experiment

Male Wistar rats, weighing 200–220 g, were provided by the Zoology Department of the Beijing Institute of Traditional Medical and Pharmaceutical Sciences. The rats were individually housed in stainless cages at a controlled temperature (20–22 °C) and 60–65 % relative humidity with normal 12 h light and dark cycle. Following 1 week of acclimation, experimental diabetes was induced by a single intraperitoneal injection of streptozotocin at a dose of 60 mg/kg body mass (using a freshly prepared solution in 0.1 M citrate buffer, pH 4.5) to animals fasted overnight; and control rats were injected with citrate buffer alone. Blood samples were obtained from the tail vein after an overnight fast at 72 h post-injection. Rats with fasting blood glucose levels in the range of 15.0–25 mM were defined as diabetic model rats. 24 diabetic rats were chosen and randomly divided into three groups: DM control group (DM), DM + GPs low dose group (DM + GPs LD), and DM + GPs high dose group (DM + GPs HD). The GPs treated diabetic rats each was given GPs powder, which was dissolved in dH<sub>2</sub>O, 200 mg/kg b.w. (for DM + GPs LD group) and 400 mg/kg b.w. (for DM + GPs HD group), respectively, daily by gavage for 40 days. In contrast, the control rats (NC & DM groups) were given the

same volume of dH<sub>2</sub>O only. On days 0, 10, 20, 30 and 40, following overnight fasting, blood samples were collected from tail veins and measured.

### In vivo biochemical assays

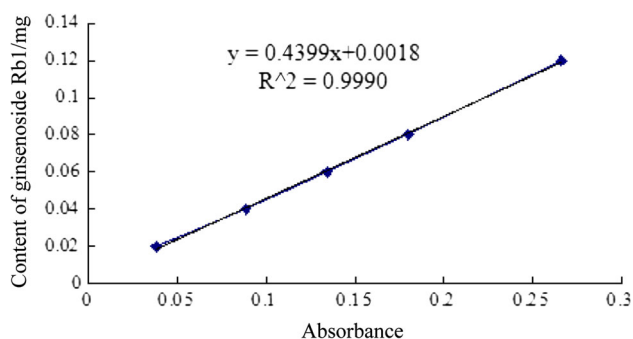
On day 40, blood samples were collected from tail vein of fasted experimental rats, and placed at room temperature for 2 h, and then centrifuged at 1,500×g for 15 min at 4 °C. The supernatant was immediately separated from the pellet for serum preparation. The serum was used for measuring the levels of total triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c) and high-density lipoprotein cholesterol (HDL-c) following the instructions of the manufacturer (TC, TG, LDL-c and HDL-c assay kits, Nanjing Jiancheng Bioengineering Co., China). The serum insulin levels were then determined by insulin-Elisa kit (San Diego, CA, USA) according to the manufacturer's instruction.

### Effect of GPs treatment on organs weights of test rats

On day 40, the rats were sacrificed by cervical dislocation under aether anesthesia. Liver, thymus, pancreas, spleen and kidney were rapidly dissected and weighed and rinsed twice with a cold saline solution. The weight of various organs was recorded, homogenized with Tris-HCl (pH 7.4), and centrifuged (1,500×g, 10 min, 4 °C). The supernatants were used immediately to assays for MDA, GSH-px and SOD using commercial kits according to the manufacturer's instructions (Nanjing Jiangcheng Bioengineering Co., China).

### Analysis of Nrf2 expression in liver tissues

The single hepatocyte suspension of the rats was prepared in ice-cold 0.1 M PBS, and the antibodies were diluted in 0.1 M PBS with 0.1 % NaN<sub>3</sub>. 10<sup>6</sup> cells were incubated with primary anti-Nrf2 antibody for 30 min on ice, then added 4 % paraformaldehyde 200 μL for 30 min at 4 °C. The cells were washed two times in 0.1 M PBS with 0.1 % NaN<sub>3</sub>, and discarded the supernate by centrifugation. 3 % BSA, 0.1 % NaN<sub>3</sub> and 10 % Saponin (Sigma Co., USA) were added and mixed for 15 min, then added 500 μL PBS (pH 7.4). The supernate was removed and 0.5 μg of secondary FITC-conjugated rabbit anti-mouse antibody were added and incubated on ice for 60 min. Finally, the cells were resuspended in 1 mL of 0.1 M PBS. The hepatocytes were scanned using a FACSCalibur (Becton-Dickinson, USA). Nonspecific binding of secondary antibody was excluded by incubating the cells only with the FITC-labelled secondary antibody. For reproducibility, the experiment was repeated three times. The software used



**Fig. 1** Standard curve of ginsenoside Rb1

was BD CellQuest Pro (Becton–Dickinson Biosciences, USA) and the data were expressed as fluorescence intensity formula ( $I = \text{Log}(x\text{-mode}) \times 340$ ).

### Statistical analysis

Statistical analyses were performed using the SPSS statistical software package. The effects of GPs from *G. pentaphyllum* on the blood glucose were determined using analysis of variance (ANOVA) for repeated measurements. To analyze the specific differences, the levels of blood lipids, and organ weights were analyzed by one-way ANOVA followed by Scheffe test. Results were considered significantly different at the level of  $p < 0.05$ .

## Results

### Characterization of GPs from *G. pentaphyllum*

#### Standard curve

Absorbances of standard solution and blank solution were determined using wavelength 550 nm. The absorption of ginsenoside Rb1 acts as abscissa and the content of ginsenoside Rb1 regard as the vertical to draw the standard curve. As shown in Fig. 1, curvilinear regression equation was  $y = 0.4399x + 0.0018$ , which the coefficient of correlation was  $R^2 = 0.9990$ , and the linear range was 0.02–0.12 mg.

#### Quality of *G. pentaphyllum* extract

*Gynostemma pentaphyllum* dry powder 200 g was extracted using water to obtained total saponins of *G. pentaphyllum* extract 50.252 g. After HP20 macroporous resin column chromatography purification, the content of total saponins of *G. pentaphyllum* was 1.135 g. The content of GPs was calculated by standard curve and vanillin

perchloric acid colorimetric method, the value was 8.7554 % before HP20 chromatography, the result was 76.3956 % after HP20 chromatography purification.

### Effect of GPs on DPPH free radical clearance

DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical method is an antioxidant assay based on electron-transfer that produces a violet solution in ethanol (Garcia et al. 2012). This free radical, stable at room temperature, is reduced in the presence of an antioxidant molecule, giving rise to colorless ethanol solution. The use of the DPPH assay provides an easy and rapid way to evaluate antioxidants by spectrophotometry. DPPH is a stable free radical with red color (absorbed at 517 nm). If free radicals have been scavenged, DPPH will generate its color to yellow. This assay uses this character to show GPs free radical scavenging activity (Fig. 2). In the DPPH assay, the GPs solution was able to reduce the radical DPPH to the yellow-colored diphenyl-picrylhydrazine, and the DPPH free radical scavenging activity for GPs was 38.2 % (100  $\mu\text{g/mL}$ ), and its activity reached 64.3  $\mu\text{g/mL}$  at 500  $\mu\text{g/mL}$  GPs concentration. The ascorbic acid, as a control agent, exhibits a high scavenging effect upon DPPH free radical of 62.1 % (100  $\mu\text{g/mL}$ ) and 95.1 % (500  $\mu\text{g/mL}$ ). The results indicated that the increase of the superoxide anion dismutase by GPs might lead to the elevated DPPH free radical scavenging efficiency.

### Effect of GPs treatment on blood glucose level

Changes of plasma glucose level as a result of GPs treatment are shown in Table 1. Fasting blood glucose levels were measured on days 0, 10, 20, 30 and 40. They were consistently staying at similar levels within the time course from day 0 to day 40 in normal control and DM control groups. However, in DM + GPs treated groups significantly lower blood glucose levels were observed as compared to DM control group at the same time points ( $p < 0.05, 0.01$ ). The decreasing rates of the plasma glucose levels at day 40 were 47.95 % (in DM + GPs LD group) and 60.84 % (in DM + GPs HD group), respectively. It indicated that DM + GPs HD group showed favorable hypoglycemic status compared with DM + GPs LD group, despite there was no statistical difference.

### Effect of GPs treatment on blood lipid levels

The effect of GPs treatment on serum lipids of tested groups is given in Table 2. The levels of TG, TC, LDL-c in diabetic control rats were  $1.19 \pm 0.09$ ,  $2.02 \pm 0.1$  and  $1.23 \pm 0.12$  mM, respectively, and were significantly higher ( $p < 0.01$ ) than those of normal rats. While their

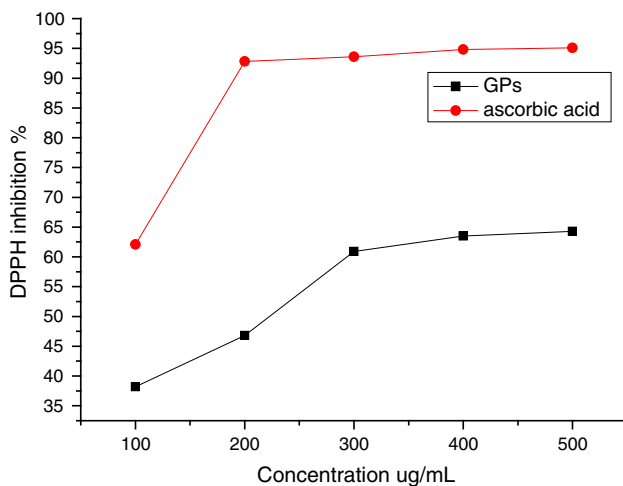
**Table 1** Effect of GPs treatments on blood glucose of STZ-induced diabetic rats

Groups	Blood glucose level (mmol/L)				
	Day 0	Day 10	Day 20	Day 30	Day 40
NC	4.79 ± 0.57	4.89 ± 0.49	4.65 ± 0.43	4.64 ± 0.35	4.78 ± 0.43
DM	17.3 ± 2.02	17.93 ± 1.95	18.38 ± 1.98	19.41 ± 2.02	18.93 ± 2.1
DM + GPs LD	17.54 ± 1.77	15.49 ± 2.01 <sup>#</sup>	13.69 ± 1.91*	11.5 ± 2.04*	9.13 ± 1.47*
DM + GPs HD	18.13 ± 0.27	15.33 ± 0.45 <sup>#</sup>	12.01 ± 0.40*	8.89 ± 0.28*	7.10 ± 0.28*

The values represent the mean ± SD of eight rats per group

<sup>#</sup>  $p < 0.05$  versus diabetes control group (DM)

\*  $p < 0.01$  versus diabetes control group (DM)



**Fig. 2** This assay uses this character to show GPs free radical scavenging activity

level of HDL-c was significantly lower ( $p < 0.01$ ) than the normal control rats'. When diabetic rats were treated with GPs for 40 days, TG, TC, LDL-c serum levels were reduced ( $p < 0.05, 0.01$ ) compared to diabetic control rats. The GPs-treated groups also had a significantly higher HDL-c level than diabetic control rats ( $p < 0.05, 0.01$ ), which had HDL-c levels close to that of normal rats.

#### Insulin level of GPs-treatment diabetic rats

The serum insulin levels of GPs-treatment diabetic rats were determined, and the results were summarized in Fig. 3. The serum insulin level of NC group was higher than that of DM group, which indicated that STZ damaged the pancreas islet cells. The fasting serum insulin level in DM rats was found to be 43.17 % lower when compared with NC rats ( $p < 0.01$ ). The serum insulin levels in GPs-treated groups were significantly higher than that of DM group ( $p < 0.05, 0.01$ ), which implied that GPs treatment stimulated the insulin secretion on diabetic rats. In the GPs treated high dose group, the insulin level was higher than that of the low dose group. But there were no significant differences between two groups. The results implied that GPs improved insulin secretion on the STZ induced diabetic rats.

#### Effect of GPs treatment on organ weights of the rats

The weight of various organs is presented in Table 3. There were significant differences in the weight of kidney, pancreas, spleen and thymus between normal and diabetic control rats ( $p < 0.05, 0.01$ ), which means STZ can induce the above organs damage. The weight of the thymus and spleen was significantly higher in the GPs-treated groups

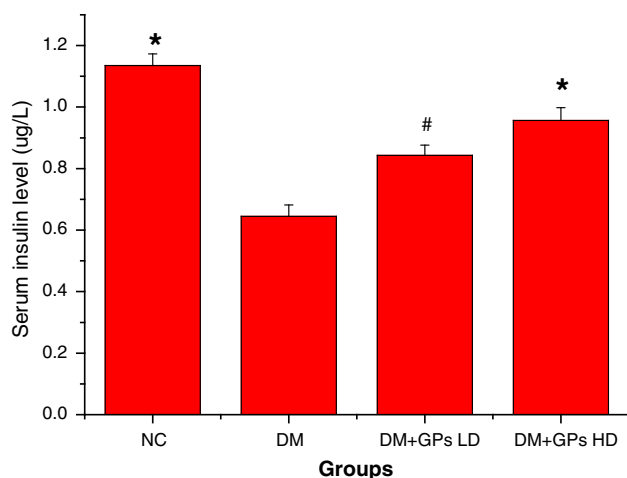
**Table 2** Effect of GPs treatments on blood lipids of STZ-induced diabetic rats

Groups	TG (mmol/L)	TC (mmol/L)	LDL-c (mmol/L)	HDL-c (mmol/L)
NC	0.94 ± 0.09*	1.81 ± 0.07*	0.98 ± 0.08*	0.97 ± 0.1*
DM	1.19 ± 0.09	2.02 ± 0.1	1.23 ± 0.12	0.73 ± 0.09
DM + GPs LD	1.10 ± 0.07	1.86 ± 0.09 <sup>#</sup>	1.04 ± 0.07*	0.85 ± 0.09 <sup>#</sup>
DM + GPs HD	0.94 ± 0.09*	1.83 ± 0.1*	1.01 ± 0.07*	0.90 ± 0.08*

The values are mean ± SE of eight rats per group

<sup>#</sup>  $p < 0.05$  versus diabetic control group (DM)

\*  $p < 0.01$  versus DM



**Fig. 3** Effects of GPs on the levels of fasting serum insulin in diabetic rats. The values represent the mean  $\pm$  SE of eight rats per group; #  $p < 0.05$  versus diabetic control group (DM); \*  $p < 0.01$  versus DM

comparing with those of the DM and normal control groups ( $p < 0.05$ ), while there was no marked difference in high and low dose GPs-treated groups. The results indicated that GPs treatment may alleviate the organs injury induced by STZ.

**Table 3** Effect of GPs treatments on organ weights of STZ-induced diabetic rats

Groups	Liver (g)	Kidney (g)	Pancreas (g)	Spleen (g)	Thymus (g)
NC	7.87 $\pm$ 0.52	2.06 $\pm$ 0.15*	1.35 $\pm$ 0.12#	1.01 $\pm$ 0.06*	0.66 $\pm$ 0.06*
DM	8.25 $\pm$ 0.41	2.32 $\pm$ 0.14	1.13 $\pm$ 0.08	0.80 $\pm$ 0.09	0.45 $\pm$ 0.09
DM + GPs LD	8.20 $\pm$ 0.32	2.21 $\pm$ 0.13	1.20 $\pm$ 0.12	0.91 $\pm$ 0.07#	0.57 $\pm$ 0.07#
DM + GPs HD	8.16 $\pm$ 0.34	2.20 $\pm$ 0.14	1.19 $\pm$ 0.10	0.91 $\pm$ 0.08#	0.58 $\pm$ 0.11#

The values represent the mean  $\pm$  SD of eight rats per group

#  $p < 0.05$  versus diabetic control group

\*  $p < 0.01$  versus diabetic control group

**Table 4** Effect of GPs on tissue MDA, SOD and GSH-px activities of diabetic rats

Group	Tissue	Evaluated parameter		
		MDA (nmol/mg pro)	SOD (U/mg pro)	GSH-px (U/mg pro)
NC	Liver	8.25 $\pm$ 0.89*	49.78 $\pm$ 6.59*	26.57 $\pm$ 4.19*
	Kidney	9.80 $\pm$ 1.05*	52.81 $\pm$ 5.89*	33.54 $\pm$ 3.69*
DC	Liver	11.36 $\pm$ 1.42	36.22 $\pm$ 6.18	16.77 $\pm$ 2.87
	Kidney	12.33 $\pm$ 1.21	40.15 $\pm$ 4.58	16.84 $\pm$ 3.67
DM + GPS LD	Liver	9.62 $\pm$ 1.24#	46.35 $\pm$ 4.59#	21.27 $\pm$ 2.81#
	Kidney	11.12 $\pm$ 1.26	47.67 $\pm$ 4.06#	22.87 $\pm$ 4.12#
DM + GPS HD	Liver	9.58 $\pm$ 1.07#	46.57 $\pm$ 4.60#	21.89 $\pm$ 3.72#
	Kidney	10.56 $\pm$ 1.06#	48.16 $\pm$ 4.37#	23.95 $\pm$ 4.54#

The values are mean  $\pm$  SD for eight rats per group

#  $p < 0.05$  versus diabetic control group

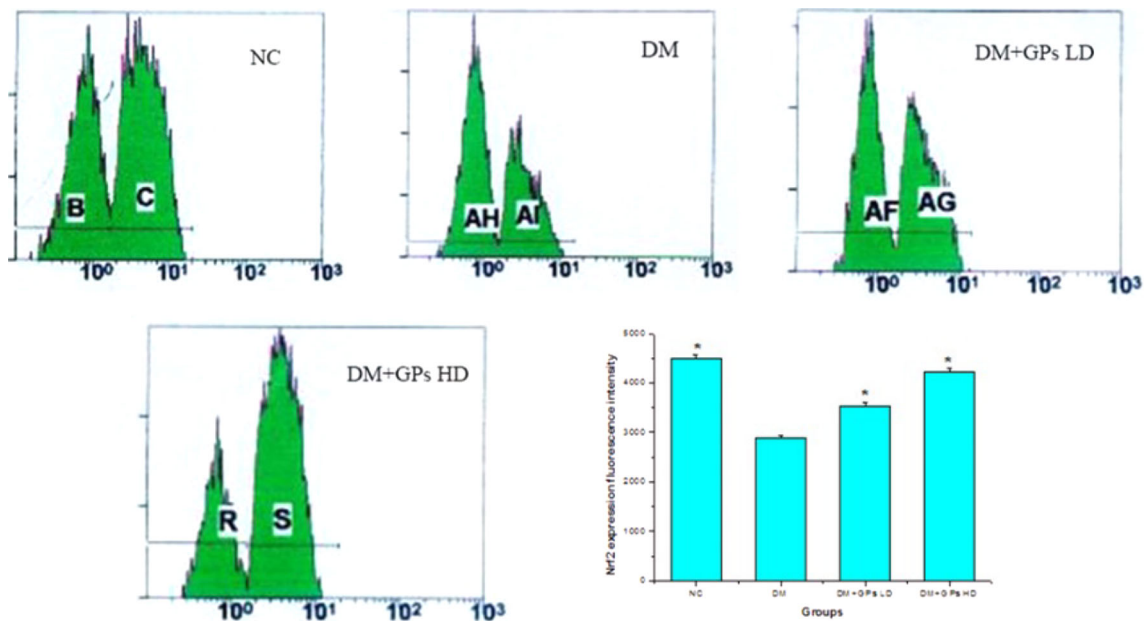
\*  $p < 0.01$  versus diabetic control group

### Changes on MDA content and SOD and GSH-px activities

The levels of SOD, GSH-px and MDA in normal, diabetic and GPs-treated rats are shown in Table 4. In STZ-induced diabetic control rats, the SOD and GSH-px levels decreased significantly by 27.2 and 36.8 % in the liver of diabetic rats, and by 23.9 and 49.7 % in the kidney, respectively. MDA level was increased in the liver and kidney tissues of the diabetic rats ( $p < 0.01$ ). GPs treatment have significant raise on SOD and GSH-px activities in the liver and kidney of diabetic rats ( $p < 0.05$ ). MDA levels in the liver and kidney of GPs-treated groups decreased significantly. GPs treatment therefore significantly blocked the increase of MDA and was associated with a partial elevation in liver and kidney total antioxidant ability, including SOD and GSH-px activities.

### Effects of GPs on Nrf2 expression

Nrf2 plays key role on cellular defense system against oxidative stress. In order to evaluate the effects of GPs on Nrf2 expression for diabetic rats, the Nrf2 levels were



**Fig. 4** Flow cytometric analysis of Nrf2 expression in the hepatocyte of GPs-treated diabetic rats and control rats. Single-colour histograms represent hepatocyte staining with anti-Nrf2 antibodies; x-axis,

fluorescence intensity; y-axis, frequency of cells displaying certain fluorescence intensity. The values represent the mean  $\pm$  SE of eight rats per group; \* $p < 0.01$  versus diabetic control group

measured in the hepatocyte nuclear extracts for GPs-treated rats by flow cytometry, and the results showed GPs significantly increased translocated nuclear expression of Nrf2 (vs. normal control rats;  $p < 0.01$ ), in accordance with a decrease in the DM group ( $p < 0.01$ , Fig. 4). The data also indicated that GPs treatment promoted further translocation of Nrf2 into the nucleus, and activated expression of Nrf2 in the hepatic nucleus of STZ induced diabetic rats, as evidenced by a significant elevation of Nrf2 in the nuclear fractions ( $p < 0.05$ ).

## Discussion

*Gynostemma pentaphyllum* mainly distributes in Asia, which grows wild in Southern China, Japan, India, Korea, and so on. As a Chinese medicinal tea, this is being promoted and sold in Europe for advantage to one's health and beauty. It has been used in traditional medicine to treat bronchitis and other diseases. The earliest record of its use as a drug comes from herbalist Li Shi-Zhen's book Compendium of Materia Medica published in 1578 for treating various ailments such as hematuria, edema in the pharynx and neck, tumors, and trauma. Phytochemical studies of *G. pentaphyllum* have identified about 82 dammarane-type glycosides closely related to the ginseng saponins. Because of this similarity to the expensive ginseng root, *G. pentaphyllum* has attracted much interest as a potential new plant drug. Pharmacological studies of *G. pentaphyllum* and/or

the isolated saponins have shown a variety of interesting activities, such as antitumor, cholesterol-lowering, immunopotentiating (Razmovski Naumovski et al. 2005), antioxidant and others (Hou et al. 1991; Li et al. 1993). We purchased *G. pentaphyllum* from Shanxi province, and optimized the ultrasound extraction and HP20 adsorbent resins column purification technology to obtain GPs from *G. pentaphyllum*. Its authenticity to GPs was confirmed by vanillin perchloric acid colorimetric method analysis.

It is well known that in uncontrolled diabetes mellitus, there is an increase in TC, LDL-c and TG with decrease in HDL-c levels which associates with the risk of the development of atherosclerosis in diabetes mellitus (Assmann et al. 2006; Whitney et al. 2005). 40-day treatment with GPs brought down the levels of TC, LDL-c and TG, and elevated the level of HDL-c in diabetic animals. From this point of view, it is encouraging that GPs may be beneficial to diabetic individuals with atherosclerosis and hyperlipidemia.

Several studies have documented the influence of free radicals on blood glucose, lipid peroxidation and low-density lipoprotein in the progress of diabetes (Cohen et al. 2013). Peroxidation of the membrane increases its fluidity and permeability, resulting in loss of membrane integrity (Hunt et al. 1990). The amount of free radicals generated in diabetes is incremental due to an increase in the protein glycation, glucose autooxidation and excess accumulation of sorbitol in cells (Gacche and Dhole 2011). Oxygen free radicals exert their cytotoxic effects on membrane

phospholipids resulting in the formation of MDA. As a product of lipid peroxidation, levels of MDA reflect the degree of oxidation in the body. Masad reported that amylin is capable of forming  $H_2O_2$  from  $O_2$  and can also generate  $\cdot OH$  via Fenton Chemistry, which will damage the pancreatic islets of patients with type 2 diabetes mellitus (Masad et al. 2011). Free radicals can diffuse intracellularly and result in mitochondrial enzyme damage and DNA breaks, all of which impair cellular function and contribute to the pathophysiology of diabetes (Bonnefont-Rousselot et al. 2000). As components of the free radical scavenging system, SOD and GSH-px exist in all oxygen-metabolizing cells, prevent damage to cells by free radicals and provide a repair mechanism for oxidized membrane components. In the present study, the blood glucose was significantly improved in rats received GPs. It is likely that lipid peroxidation and free radical generation can induce diabetes by different means. Increases in antioxidant enzymes provides effective protection from oxidative damage, while we observed a significant increase in SOD and GSH-px activities in GPs-treated diabetic rats compared with the control rats. The results indicated that GPs protected liver cells against oxidative injury induced by STZ through increasing SOD and GSH-px activities and decreasing MDA content, and it was able to regulate the balance of oxygenation effectively in the diabetic rats. Therefore, GPs possesses significant free radical scavenging activity.

Nrf2 serves as master regulator of a cellular defensive system against oxidative stress (Nguyen et al. 2009). Under basal conditions, Nrf2 is sequestered in the cytoplasm by Keap1, and upon exposure of cells to inducers such as oxidative stress and certain chemo-preventive agents, Nrf2 dissociates from Keap1, translocates to the nucleus, binds to antioxidant response elements (ARE), and transactivates phase II detoxifying and antioxidant genes (Jeong et al. 2006). As enhancer elements, ARE initiate the transcription of a battery of genes encoding phase II enzymes (Johnson et al. 2008). Nrf2–ARE signaling is also mainly responsible for stimulating of SOD and GSH-px gene expression and hence constitutes a crucial cellular response to environmental stress (Surh et al. 2009). In this study, GPs treatment also promoted expression of Nrf2 in the liver tissue of the rats, it is further capable of activation of Nrf2 and preventing high blood glucose induced inhibition of antioxidant enzymes. The flow cytometry results clearly show that high blood glucose induced oxidative stress is associated with activation of Nrf2, as evidenced by a significant elevation of Nrf2 in the nuclear fractions ( $p < 0.05$ ). GPs treatment markedly promoted further translocation of Nrf2 into the nucleus. Thus, GPs could inhibit oxidative stress through the Nrf2–Keap1 signaling pathway. As expected, GPs stimulates anti-oxidative enzyme gene expression in diabetic rats via Nrf2-

dependent transcriptional activation of ARE sites. Nrf2 is known to be a key factor of regulating a battery of genes that protect cells against deleterious environmental insults (Zhang 2006).

Yoh found that female Nrf2<sup>-/-</sup> mice have significantly fewer CD4<sup>+</sup> CD8<sup>-</sup> T cells than female Nrf2<sup>+/+</sup> mice, and they also suffer oxidative stress. That oxidative stress is responsible, at least in part, for the acceleration of autoimmune abnormalities (Yoh et al. 2001). Intervention through the Nrf2 pathway provides a rational mechanism to improve cellular immune function on hyperglycemic rats. It is possible that a decrease in Nrf2 activity could lead to oxidative stress-mediated proinflammatory responses in cells from the innate immune system. GPs treatment showed that the weights of thymus and spleen were significantly increased in diabetic rats administered with GPs. Thymus plays an important role in the development of the immune system, and its cells form a part of the body's normal immune system (Pertsov 2006). Spleen also is an important lymphoid organ, also is an important site of producing immunological effect molecular. We speculated that the hypoglycemic mechanisms of GPs maybe regulate the Nrf2 pathway to improve cellular immune function and scavenge the free radicals on hyperglycemic rats.

## Conclusion

The present study provides further evidence in support of *G. pentaphyllum* saponins application on the regulation of hypoglycemia, hypolipidemia and immunocompetence. The antioxidant effect of GPs was demonstrated by stimulation of the Nrf2 antioxidant pathway to elevate the enzymes activities of SOD and GSH-px, decrease of MDA content, and stimulate secretion of insulin, then achieving the effect of eliminating ROS. Therefore, GPs could be used as an effective anti-oxidative compound and offer potential new therapeutic approaches for diabetes and other diseases.

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