Carbohydrate Polymers 94 (2013) 378–385

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

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

Protective effect of the polysaccharide from *Ophiopogon japonicus* on streptozotocin-induced diabetic rats

Xiaoming Chen[a,*,1], Jia Tang[a,1,2], Wenyuan Xie[b,1], Junjun Wang[a,3], Jing Jin[a], Jun Ren[a], Liqin Jin[a], Jianxin Lu[a,*]

[a] *Institute of Glycobiological Engineering/School of Laboratory Medicine & Life Science, Wenzhou Medical College, Wenzhou 325035, China*
[b] *Department of Medicine, The People's Hospital of Yongjia, Wenzhou 325035, China*

ARTICLE INFO

Article history:
Received 25 September 2012
Received in revised form
12 December 2012
Accepted 4 January 2013
Available online 24 January 2013

Keywords:
Ophiopogon japonica polysaccharides
Diabetes
Oxidative stress
Diabetic complication

ABSTRACT

The present study was designed to investigate the effect of OJP1, a polysaccharide isolated from the roots of *Ophiopogon japonica*, on blood lipid metabolism, antioxidant activity, as well as its protective effect on the liver and kidneys in diabetic rats. Results showed that OJP1 significantly reduced the MDA concentration and increased the activity of both GPx and SOD in the serum, liver and kidneys of diabetic rats. Moreover, the values of TG, TC, LDL-C and HDL-C in diabetic rats were significantly reversed by OJP1 treatment. Biochemical and histopathological analyses also showed that OJP1 can alleviate liver and kidneys injury in diabetic rats. The mRNA expression of transforming growth factor-β1 (TGF-β1) and connective tissue growth factor (CTGF) in diabetic rats decreased significantly after administration of OJP1. Altogether, these results suggest that OJP1 possess potent antioxidant activity and can protect the liver and kidneys from the injurious effects of diabetes.

1. Introduction

Diabetes mellitus (DM) is a complex metabolic disorder characterized by high blood glucose level due to the inability of the body's cells to utilize glucose properly (Ugochukwu & Babady, 2002), often accompanied by hyperphagia (obesity), a selective loss of pancreatic islet β-cell mass, and microvascular complications (Adikwu, Yoshikawa, & Takada, 2004; Yamac et al., 2008). It is the most significant chronic disease and cause of death in modern society. With the number of people with diabetes mellitus rising exponentially, the disease represents one of the greatest medical and socioeconomic challenges worldwide (Spinetti, Kraenke, Emanueli, & Madeddu, 2008). While exogenous insulin and other medications can control many aspects of diabetes, numerous complications affecting the vascular system, kidney, retina, lens, peripheral nerves, and skin are common and are extremely costly in terms of longevity and quality of life (Maritim, Sanders, & Watkins, 2003). Therefore, there is a strong need for safe and effective oral antihyperglycaemic agents that provide an alternative option for preventing, treating and managing diabetes and its complications.

Many herbal extracts or derivatives have been documented in traditional Chinese medicine (TCM) as having clinical effectiveness in treating diabetes mellitus (Jia, Gao, & Tang, 2003; Zhang, Zheng, Zhang, & Hai, 2012). *Ophiopogon japonicus* (Thunb.) Ker-Gawl, widely distributed in South-east Asia, is a well known traditional Chinese medicine that has been used to treat cardiovascular and chronic inflammatory diseases for thousands of years, and has also been confirmed in various experiments as having anti-inflammatory, anti-arrhythmia, and microcirculation-improving properties. (Zhou et al., 2003). In recent years, the polysaccharides isolated from the roots of *O. japonicus* have drawn the attention of researchers and consumers due to their nutritional and health protective value as an immunostimulant, as anti-ischaemetic and hypoglycemic agent, and for their ability to inhibit platelet aggregation. (Fan & Zhang, 2006; Zheng, Feng, Xu, Lin, & Chen, 2009). In our previous research, a polysaccharide isolated from *O. japonicus* named as OJP1 had an anti-diabetic effect on STZ-induced diabetic rats (Chen et al., 2011b), however, no detailed study has been carried out on the protective effect of the polysaccharide on lipid peroxidation or on antioxidants in STZ-induced diabetic rats, or to what extent OJP1 can prevent or alleviate diabetic complications.

Therefore, the present study was conducted to evaluate the effect of OJP1 on blood lipid metabolism and antioxidant activity, and to evaluate its protective effect on the liver and kidneys in diabetic rats in order to better understand the possible hypoglycemic mechanisms and the health benefits of the polysaccharide.

* Corresponding authors. Tel.: +86 577 86699652; fax: +86 577 86689717.
E-mail address: xmchen01@163.com (X. Chen).
[1] These authors contributed equally to this work.
[2] Present address: The Traditional Chinese Medicine Hospital of Wuxi, China.
[3] Present address: The People's Hospital of Pingyang, China.

0144-8617/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2013.01.037

2. Materials and methods

2.1. Materials and chemicals

The roots of *O. japonicus* were collected in Zhejiang province (China). Sephadex G-100, DEAE-cellulose, and streptozotocin (STZ) were purchased from Sigma. RPMI-1640 medium was purchased from Gibco. TRIZOL Reagent, DNA ladder marker and cDNA reverse transcription kits were purchased from TaKaRa Biotechnology Co., LTD, Dalian, China. Blood Glucose Meters were purchased from Lifescan (Milpitas, CA, USA). The assay kits for total cholesterol (TC), triglyceride (TG), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), glutathione peroxidase (GPx), superoxide dismutase (SOD), malondialdehyde (MDA), aspartate transaminase (AST), alanine transaminase (ALT), serum creatinine (SCr), uric acid (UA) and urea were purchased from Jiancheng Bioengineering Institute, Nanjing, Jiangsu Province, China. All other chemicals used were of analytical grade.

2.2. Animals

Sprague-Dawley rats (body weight 250 ± 20 g) used for experiments were purchased from Experimental Center, Wenzhou Medical College, Wenzhou, Zhejiang, China. The rats were acclimatized for at least one week before starting the experiment. Before and during the experiment the rats were housed under controlled environmental conditions of temperature (22 ± 2 °C) in a 12 h light and dark cycle, and were maintained on (unless otherwise stated) standard food pellets and tap water *ad libitum.*

All animal handling procedures were performed in strict accordance with the P. R. China legislation on the use and care of laboratory animals, adherence to the guidelines established by the Institute for Experimental Animals of Wenzhou Medical College, and were approved by the college's committee for animal experimention.

2.3. Establishment of the diabetic rat model

The diabetic rats were induced by the intraperitoneally (i.p.) injection of freshly prepared STZ at a dose of 60 mg/kg body weight. Three days after STZ injection, the glucose level of blood from the tail vein was determined, and hyperglycemic rats (blood glucose level > 16.7 mmol/L) were used as the diabetic rats for further experiments.

2.4. Experimental design

The STZ-induced diabetic rats (mentioned above) were randomly divided into four groups (8 rats per group), and normal rats were used as the control group.

Group I ($n = 8$): normal control (NC), normal rats were allowed free access to a normal diet and treated with saline solution for 28 days.

Group II ($n = 8$): diabetic control (DC), the diabetic rats were allowed free access to a normal diet and treated with saline solution for 28 days.

Group III ($n = 8$): OJP1-150, diabetic rats were put on a normal diet and treated with 150 mg/kg/d of OJP1 for 28 days.

Group IV ($n = 8$): OJP1-300, diabetic rats were put on a normal diet and treated with 300 mg/kg/d of OJP1 for 28 days.

Group V ($n = 8$): Metformin, diabetic rats were put on a normal diet and treated with 200 mg/kg/d of metformin for 28 days.

Weekly body weights were also recorded.

On the last day of experimentation, the animals were deprived of food overnight and sacrificed by cervical dislocation. Blood was collected in polystyrene tubes without tan anticoagulant, and the serum was immediately separated by centrifugation at 3000 rpm at room temperature for 10 min. Samples were stored at -70 °C until assayed.

The kidney and liver were carefully excised, blotted dry and weighed. One section of each kidney and liver was used to assay the GPx, SOD and MDA activity. The tissues were homogenized (1:10 (w/v)) in cold 0.9% saline solution by using a motor-driven Teflon glass homogenizer. Homogenate was then centrifuged at 3500 rpm for 10 min at 4 °C. Another section of each kidney was frozen in liquid nitrogen and stored at -70 °C until the analysis of reverse transcription-polymerase chain reaction (RT-PCR).

2.5. Preparation of the polysaccharide

The polysaccharide (OJP1) was prepared as described previously (Chen et al., 2011b). Briefly, the roots of *O. japonicus* were soaked with 95% ethanol to remove the pigments and small lipophilic molecules. The residue was then extracted with 10 vol. of distilled water at 90 °C for 3 h thrice. All water-extracts were combined, filtrated, concentrated, and precipitated with 95% EtOH (1: 4 (v/v)) at 4 °C for overnight. The precipitate was collected by centrifugation, then deproteinated by Sevag method. Finally the supernatant was lyophilized to give crude polysaccharides. The crude polysaccharides were purified by DEAE-52 cellulose and Sephadex G-100, and the main polysaccharide fraction (OJP1) was collected and lyophilized. OJP1 was used for further study.

The average polysaccharide content of OJP1 was 98.5% as measured using the phenol-sulfuric acid assay, which is mainly comprised of arabinose, glucose and galactose with a relative molar ratio of 1:16:8 by GC analysis. The average molecular weight of OJP1 was determined as 35.2 kDa by HPGPC.

2.6. Measurement of glucose level and lipid profiles

Blood glucose was determined by an OneTouch® II micro blood glucose instrument (American Life Scan Company). Serum TC, TG, LDL-C and HDL-C concentrations were measured by enzymatic methods using an automated biochemistry analyzer. Assays were performed using commercially available kits following the manufacturer's instructions.

2.7. Assay of oxidative stress

Antioxidation capacity *in vivo* was studied by measuring the levels of SOD, GPx and MDA using commercially available kits. Briefly, the determination of SOD activity was based on the production of O_2^- anions by the xanthine/xanthine oxidase system. The amount of SOD that inhibits 50% the rate of reduction under the specified conditions was regarded as one enzyme unit.

GPx activity was estimated by the analysis of GSH in the enzymatic reaction. Reduced glutathione (GSH) is catalyzed by glutathione peroxidase (GPx) in the presence of hydrogen peroxide. One unit of enzyme activity represents a decrease in GSH concentration of 1 μM/min after subtracting the non-enzymatic mode.

Lipid peroxidation was determined by quantifying MDA concentrations, which was spectrophotometrically measured by the absorbance of a red-colored product with thiobarbituric acid.

2.8. Assay of ALT, AST, SCr, UA and UREA

Hepatic function was assessed by detecting the activities of AST and ALT according to reported methods (Pandikumar, Babu, & Ignacimuthu, 2009). Renal function was assessed by estimating SCr, UA and UREA according to reported methods (Arora, Reddy, & Balakuma, 2010). All the procedures were performed according to the kit manufacturer's instructions.

2.9. Histopathological observation of rat livers and kidneys

Processing of tissue samples for histology assessment followed established procedures. In brief, the tissues were fixed with 10% neutral paraformaldehyde, embedded in paraffin and then manually sections with a microtome to obtain 5 μm-thick sections. The tissues were stained using the hematoxylin and eosin (H&E) stains. The sections were dewaxed in two changes of xylene, hydrated in two changes of 100% ethanol, followed by 95% ethanol and 80% ethanol, rinsed with water and stained. The stained tissues were dehydrated with 80% ethanol followed by 95% ethanol, placed in two changes of 100% ethanol, and cleaned with two changes of xylene. Histological examinations were carried out by optical microscopy. The following parameters were used for histological description: 1) area of fat droplet in rats' liver; 2) diameter of glomerulus in rats' kidney. Then, a semi-quantitative analysis of these parameters was calculated. The histological assessment was performed in eight different sections by Image-Pro Plus.

2.10. Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was isolated using TRIZOL Reagent, followed by cDNA synthesis using a commercially available cDNA reverse transcription kit in a Bio-Rad thermocycler. The final volume of 20 μL consisted of 1 μL dNTPs, 1 μL oligo dT primer, 5 μL total RNA, 4 μL 5 × buffer, 1 μL retroviridase and 8 μL DEPC-H_2O. The reverse transcription conditions were as follows: 30 °C for 10 min, 42 °C for 30 min and 95 °C for 5 min. Then the obtained cDNA was amplified by PCR. PCR was performed in a final volume of 25 μL as follows: 2.5 μL cDNA, 2.5 μL 10 × PCR buffer, 1 μL dNTPs, 0.5 μL forward primer, 0.5 μL reverse primer, 0.25 μL Taq DNA polymerase and 17.75 μL DEPC-H_2O. The sequences used were as follows: transforming growth factor-β1 (TGF–β1), forward primer: 5'-TCTCCTCAGCCATGACCTCC-3', reverse primer: 5'-GTCCCATACCGTCCCTTCTTG-3', connective tissue growth factor (CTGF): forward primer, 5'-GTCCCGTTAGCCTCGCCTTGGT-3', reverse primer: 5'-TGCGGTCCTTGGGCTCATCAC-3', β–actin, forward primer: 5'-CACCCGCGAGTACAACCTTC-3', reverse primer: 5'-CCCATACCCACCATCACACC-3'. The PCR ampliformed as follows: an initial denaturing at 94 °C for 4 min, followed by 30 cycles of denaturing at 94 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 1 min, with a final extension at 72 °C for 10 min. The products of amplification were confirmed using electrophoresis in 1.0% agarose gels and visualized by staining with ethidium bromide (EB).

2.11. Statistical analysis of the data

All results were presented as mean ± S.D. Data were analyzed by one-way ANOVA using SPSS and Dunnett's test. *P* values less than 0.05 were considered significant.

3. Results

3.1. Effects of OJP1 on blood glucose and lipids levels in diabetic rats

In the present model, as shown in Table 1, blood glucose levels in normal rats were significantly ($P < 0.01$) lower than those of STZ-induced diabetic rats of the remaining four groups. The daily administration of OJP1 (150, or 300 mg/kg) in STZ-induced diabetic rats caused a significant reduction in the blood glucose levels when compared with the diabetic control group ($P < 0.05$ or $P < 0.01$). At the end of experimentation, the serum TG, TC and LDL-C levels in the diabetic control group were significantly higher than those in the normal control group ($P < 0.05$ or $P < 0.01$), whereas serum HDL-C levels in the diabetic control group were significantly lower ($P < 0.05$) (Table 1). After 28 days of oral administration, rats in the OJP1-150 group showed significant ($P < 0.05$ or $P < 0.01$) decreases in serum TG, TC, and LDL-C levels as well as significant ($P < 0.05$) increases in serum HDL-C levels when compared to rats of the model group. Both the OJP1-300 and metformin-treated groups had slightly reversed effects on lipids level, when compared with the diabetic control group.

3.2. Effects of OJP1 on SOD activity in STZ-induced diabetic rats

As shown in Table 2, the activity of SOD was significantly ($P < 0.01$) decreased in the serum, liver, and kidneys of the diabetic control rats. OJP1 treatment (150, 300 mg/kg) significantly ($P < 0.01$) increased SOD activity in diabetic rats. In fact, OJP1 treatment had a greater effect on SOD activity in the liver and kidneys than metfromin treatment, however, no difference was observed in serum. Metfromin treatment significantly ($P < 0.01$ or $P < 0.05$) increased the activity of SOD in the serum and kidneys, however, no significant difference was observed in the liver compared with rats in the diabetic control group.

3.3. Effects of OJP1 on GPx activity in STZ-induced diabetic rats

As shown in Table 2, the activity of GPx in diabetic control group was significantly ($P < 0.01$) decreased in the serum, liver and kidneys. OJP1 treatment significantly increased ($P < 0.01$) the activity of GPx in the serum and liver of diabetic rats, and treatment with 150 mg/kg OJP1 also significantly increased ($P < 0.01$) the activity of GPx in kidneys. Metformin treatment significantly increased ($P < 0.01$) the activity of GPx in the serum and kidneys of diabetic rats, yet no significant difference was observed in the liver when compared with the diabetic control group.

3.4. Effects of OJP1 on MDA level in STZ-induced diabetic rats

The levels of malondialdehyde (MDA), a secondary product of lipid peroxidation in the serum, liver and kidney tissue

Table 1
Effects of OJP1 on blood glucose and lipids levels in diabetic rats[a]

	Blood glucose (mmol/L)	TG (mmol/L)	TC (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)
Normal control	5.30 ± 0.41	1.45 ± 0.23	1.92 ± 0.34	1.61 ± 0.21	0.29 ± 0.04
Diabetic control	22.43 ± 2.18 [c]	3.45 ± 0.45 [c]	2.72 ± 0.49 [c]	1.27 ± 0.21 [b]	0.37 ± 0.08 [b]
OJP1-150	13.45 ± 2.69 [c,e]	2.18 ± 0.27 [c,e]	2.17 ± 0.26 [d]	1.53 ± 0.19 [d]	0.26 ± 0.11 [d]
OJP1-300	17.32 ± 3.83 [c,d]	3.04 ± 0.32 [c]	2.32 ± 0.25 [b]	1.41 ± 0.25	0.33 ± 0.16
Metformin	15.45 ± 3.09 [c,e]	1.74 ± 0.26 [e]	2.11 ± 0.25 [d]	1.50 ± 0.24 [d]	0.29 ± 0.12 [d]

[a] Data represent mean ± S.D. ($n = 8$ for each group).
[b] $P < 0.05$ compared with the normal control.
[c] $P < 0.01$ compared with the normal control.
[d] $P < 0.05$ compared with the diabetic control.
[e] $P < 0.01$ compared with the diabetic control.

Table 2
Effects of OJP1 on activities of SOD, GPx and levels of MDA in diabetic rat [a]

	Normal control	Diabetic control	OJP1-150	OJP1-300	Metformin
Serum					
SOD (U/mL)	309.17 ± 27.73	247.24 ± 24.87 [c]	319.66 ± 17.83 [e]	321.35 ± 22.34 [e]	321.33 ± 25.75 [e]
GPx (U/mL)	4206.9 ± 279.7	3286.7 ± 261.1 [c]	4077.6 ± 320.8 [e]	3981.4 ± 290.13 [e]	3922.1 ± 222.66 [e]
MDA (nmol/mL)	7.34 ± 0.60	11.36 ± 0.88 [c]	8.87 ± 0.51 [b,e]	10.05 ± 0.61 [c,d]	11.21 ± 0.68 [c]
Liver					
SOD (U/mg.protein)	292.51 ± 15.37	228.46 ± 18.03 [c]	322.45 ± 11.82 [b,e]	283.15 ± 11.90 [e]	247.08 ± 19.35 [c]
GPx (U/mg.protein)	533.72 ± 39.92	408.64 ± 38.93 [c]	609.78 ± 51.39 [b,e]	574.27 ± 45.34 [e]	447.69 ± 52.62 [c]
MDA (nmol/mg.protein)	1.21 ± 0.17	2.89 ± 0.30 [c]	1.86 ± 0.14 [c,e]	2.32 ± 0.21 [c,e]	2.76 ± 0.20 [c]
Kindey					
SOD (U/mg.protein)	364.16 ± 27.99	311.35 ± 29.44 [c]	393.88 ± 14.20 [b,e]	371.92 ± 21.01 [e]	353.96 ± 25.40 [d]
GPx (U/mg.protein)	710.92 ± 53.26	565.01 ± 49.53 [c]	694.88 ± 48.78 [e]	596.33 ± 36.01 [c]	702.58 ± 33.16 [e]
MDA (nmol/mg.protein)	1.71 ± 0.21	3.48 ± 0.30 [c]	2.11 ± 0.20[b,e]	2.36 ± 0.31 [c,e]	1.82 ± 0.25 [e]

[a] Data represent mean ± S.D. (n = 8 for each group).
[b] P < 0.05 compared with the normal control.
[c] P < 0.01 compared with the normal control.
[d] P < 0.05 compared with the diabetic control.
[e] P < 0.01 compared with the diabetic control.

Table 3
Effects of OJP1 on liver and kidney index, ALT and AST activities and SCr, UA and UREA levels in diabetic rat [a]

	Normal control	Diabetic control	OJP1-150	OJP1-300	Metformin
Liver index (mg/g)	27.27 ± 0.77	47.77 ± 1.47 [c]	35.56 ± 2.54 [c,e]	41.36 ± 1.01[c,d]	43.31 ± 2.78 [c]
Kindey index (mg/g)	5.77 ± 0.73	12.61 ± 0.54 [c]	11.45 ± 0.37 [c]	11.42 ± 0.18 [c]	11.13 ± 0.25 [c]
ALT (U/L)	41.37 ± 2.48	181.62 ± 12.49 [c]	80.48 ± 4.73 [b,e]	112.51 ± 7.62 [c,e]	89.29 ± 5.45 [c,e]
AST (U/L)	124.36 ± 3.41	544.62 ± 14.93 [c]	178.39 ± 6.96 [b,e]	291.67 ± 9.35 [c,e]	113.88 ± 7.32 [b,e]
SCr (μmol/l)	35.46 ± 1.38	61.5 ± 4.26[c]	45.17 ± 3.07 [c,e]	21.28 ± 2.74 [c,e]	21.99 ± 2.97 [c,e]
UA (μmol/l)	39.91 ± 2.35	122.35 ± 7.2 [c]	116.2 ± 5.24 [b,d]	74.98 ± 3.29 [c,e]	119.66 ± 6.91[c]
UREA (mmol/L)	6.52 ± 1.19	22.4 ± 4.13 [c]	12.6 ± 3.24 [c,e]	9.31 ± 1.74 [c,e]	17.85 ± 3.77 [c,d]

[a] Data represent mean ± S.D. (n = 8 for each group).
[b] P < 0.05 compared with the normal control.
[c] P < 0.01 compared with the normal control.
[d] P < 0.05 compared with the diabetic control.
[e] P < 0.01 compared with the diabetic control.

homogenate, are shown in Table 2. The levels of MDA in the diabetic control groups were significantly (P < 0.01) increased compared with the normal control group. OJP1 treatment significantly decreased (P < 0.01 or P < 0.05) the levels of MDA in the serum, liver and kidneys of diabetic rats. However, metformin treatment only significantly (P < 0.01) decreased the levels of MDA in the kidneys of diabetic rats.

3.5. Effects of OJP1 on hepatic and renal function in STZ-induced diabetic rats

To elucidate the effect of OJP1 on diabetic rats, the functions of liver and kidney were investigated. As shown in Table 3, the liver and kidney indices of the diabetic rats were increased significantly compared with those of rats in the normal control group. After OJP1 treatment, the liver index was significantly (P < 0. 01 or P < 0. 05) decreased compared with that in the diabetic control group, whereas the kidney index showed no significant difference between the OJP1-treated groups and diabetic control group.

Serum ALT and AST activities in the diabetic control group were significantly higher than those in the normal control group (P < 0.01) (Table 3), and abnormal behavior of the liver factors were observed. After treatment, the ALT and AST activities in the OJP1-150, OJP1-300 and metformin groups were significantly (P < 0.01) decreased, compared with those in the diabetic control group.

Serum creatinine, UA and UREA levels of rats in the diabetic control group were significantly (P < 0.01) increased (Table 3) compared with those of rats in the normal control group, indicating the renal functions of diabetic rats were deteriorated. In contrast to the SCr levels of the rats in the diabetic control group, the SCr levels in the OJP1-treated and metformin-treated groups were significantly (P < 0.01) decreased. Likewise, the serum UA and UREA levels of rats in the OJP1-treated groups were also significantly (P < 0.01 or P < 0.05) decreased compared with those of rats in the diabetic control group. Administration of OJP1 revealed a notable (P < 0.01 or P < 0.05) decline in SCr, UA, and UREA when compared with the diabetic control group. There was no significant difference between serum UA levels of rats in the metformin group and those of rats in the diabetic control group.

3.6. Histopathological observations of rat livers and kidneys

As shown in Fig. 1, the hepatic cell cords arranged in orderly manner and hepatocyte morphology was normal. Each hepatocyte had an abundant cytoplasm, distinct cell borders, and a round, central nucleus (Fig. 1A). In the diabetic group, the hepatic cords in liver were not arranged in an orderly manner, and large numbers of liver cells had undergone degeneration. Different sizes of fat droplets (Table 4) and fatty degeneration of the liver were observed in the cytoplasm of hepatocytes (Fig. 1B). After OJP1 treatment, the circular fat droplets in the cytoplasm were significantly decreased, and the degeneration of the hepatocyte was markedly decreased, in addition, the liver cell structure was similar to normal liver architecture (Fig. 1C). Semi-quantitative of the area of fat droplets in rats' liver showed that it decreased to 0.5-fold in OJP1-treated groups compared with the diabetic control group (Table 4). The histopathological analysis was consistent with data on ALT and AST activities, which are biochemical indicators of liver function.

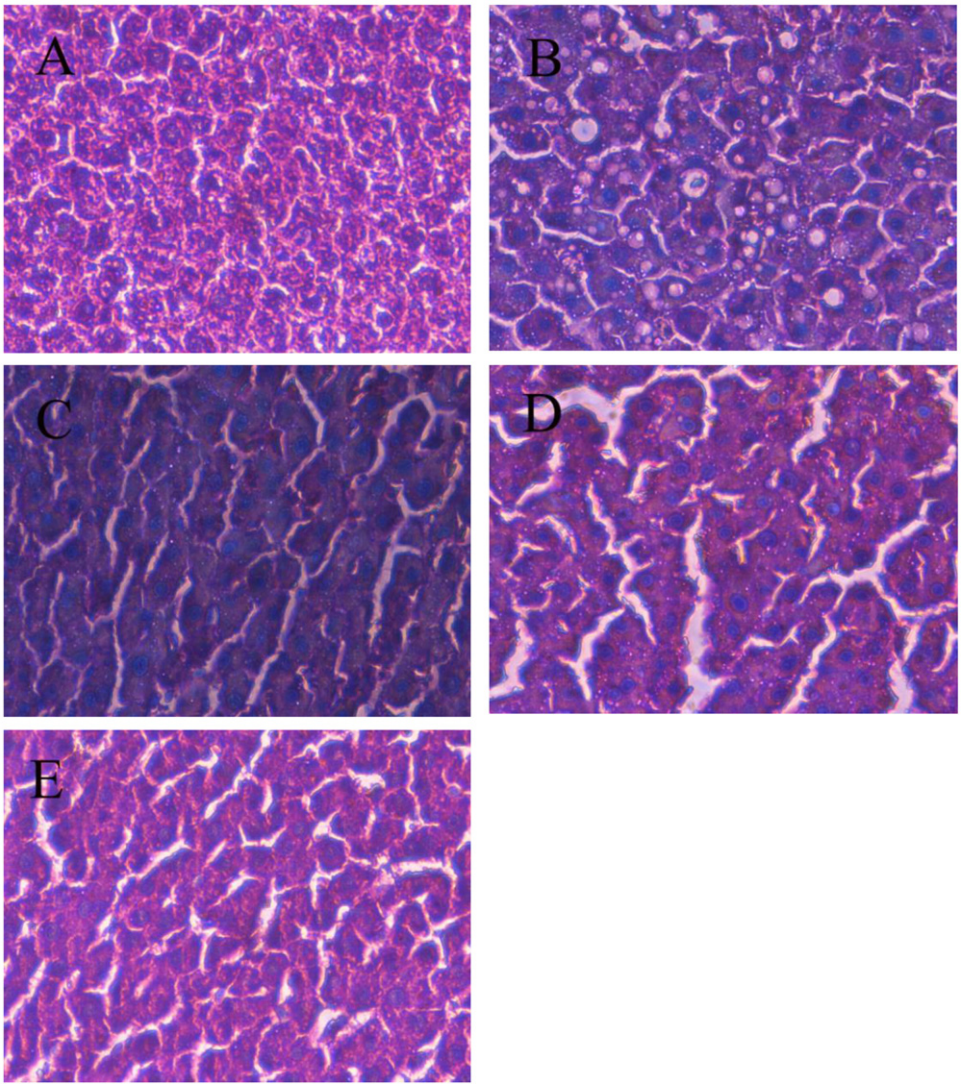

Fig. 1. Effects of OJP1 on livers of diabetic rats (H&E stain × 400). (A) Nondiabetic animals given saline as the negative control, (B) diabetic animals given saline as the diabetic control, (C) diabetic animals given OJP1 at a dose of 150 mg/kg, (D) diabetic animals given OJP1 at a dose of 300 mg/kg, (E) diabetic animals given Metformin at a dose of 200 mg/kg.

The changes in renal histology of the different groups are shown in Fig. 2. The sections from the normal control group were normal sizes without any abnormal phenomena by visual observation (Fig. 2A). Conversely, glomerular proliferation and mesangial matrix augmentation occurred in the diabetic group. These broad changes caused loading of the Bowman's capsule space and adhesion of capillaries to the wall (Fig. 2B). The structures of the kidney sections of OJP1-treated groups also changed in morphology, however, a decreased extent of the expansions in the glomerulus and the mesangial matrix were observed (Fig. 2C and D). When a semi-quantitative analysis was used, it became readily apparent that a difference in the histological change was present the diabetic control group and other groups. As shown in Table 4, the diameter of glomerulus nearly increased to 1.7-fold in diabetic control group compared with the normal control group, and it was observed that the diameter of glomerulus in OJP1-treated groups decreased to 0.7-fold of that in diabetic control group.

3.7. The expressions of TGF-β1 and CTGF

To further elucidate the effect of OJP1 on the kidneys of diabetic rats, the effects of OJP1 on renal expressions of TGF-β1 and CTGF were determined by RT-PCR. As shown in Fig. 3, the expression levels of β-actin in different groups was consistent. However, the expression levels of both TGF-β1 and CTGF were up-regulated in the diabetic control group compared with the normal control

Table 4
Semi-quantitative analysis of histological changes in the rats' liver and kidney.

	Normal control	Diabetic control	OJP1-150	OJP1-300	Metformin
Area of fat droplet (μm^2)	–	9682.4 ± 3851.3	4091.1 ± 2271.8 [a]	4837.1 ± 2373.0 [a]	4999.4 ± 2944.9[a]
Diameter of glomerulus (μm)	816.2 ± 155.8 [a]	1320.6 ± 100.6	909.1 ± 142.7 [a]	956.2 ± 257.1 [a]	920.8 ± 245.2 [a]

Data represent mean ± S.D.
[a] $P < 0.01$ compared with the diabetic control.

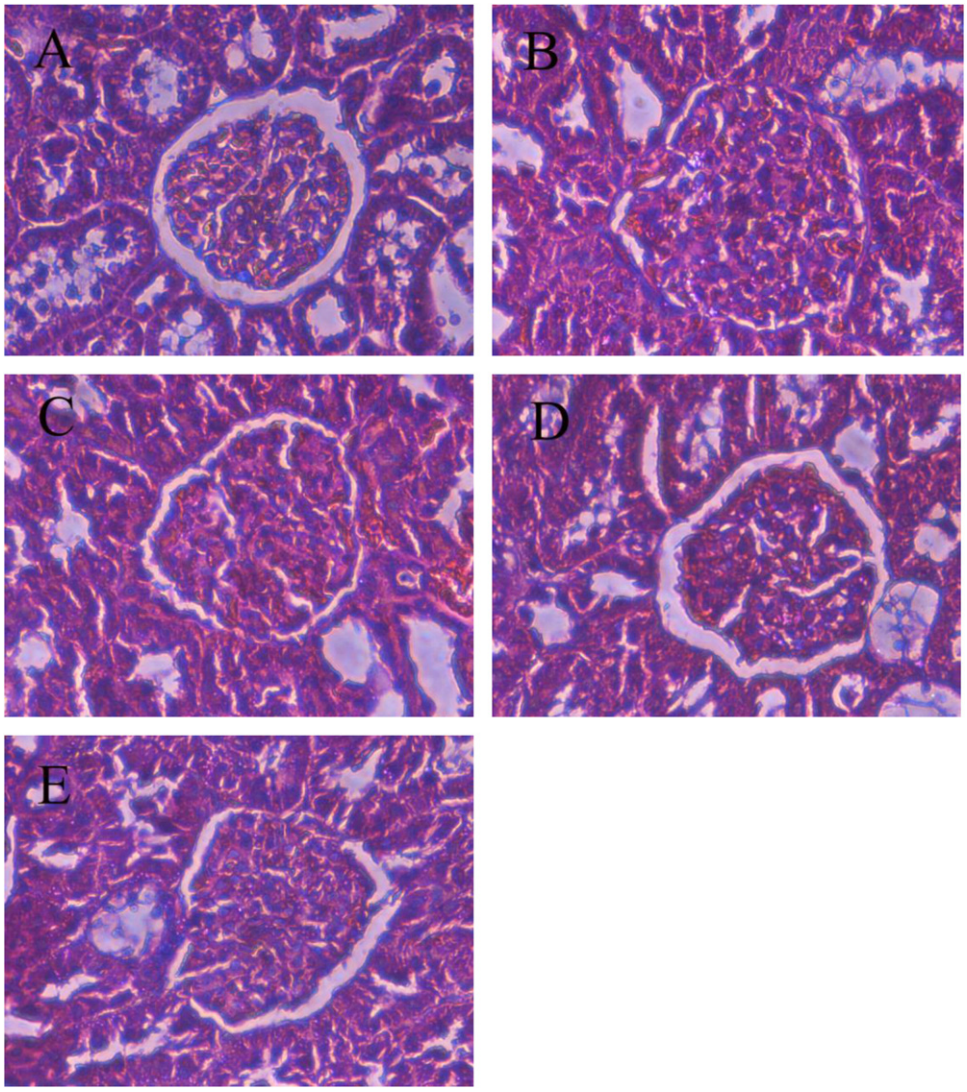

Fig. 2. Effects of OJP1 on kidneys of diabetic rats (H&E stain × 400). (A) nondiabetic animals given saline as the negative control, (B) diabetic animals given saline as the diabetic control, (C) diabetic animals given OJP1 at a dose of 150 mg/kg, (D) diabetic animals given OJP1 at a dose of 300 mg/kg, (E) diabetic animals given Metformin at a dose of 200 mg/kg.

group. Renal expression of mRNA for TGF-β1 and CTGF were both significantly increased in the diabetic group. OJP1 treatment decreased their expression in a dose-dependent manner.

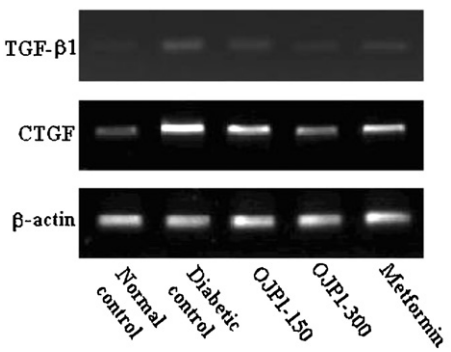

Fig. 3. Effect of OJP1 on the gene expressions of TGF-β1 and CTGF in diabetic rats. RT-PCR analysis of TGF-β1 and CTGF was performed as described in Materials and Methods.

4. Discussion

Diabetes mellitus is one of the most common human metabolic diseases, and derangements in lipid metabolism caused by diabetes are often important determinants of the course and status of the disease (Yu et al., 2009). In addition, diabetic patients have an increased risk of cardiovascular disease and stroke. Abnormal increases in serum TC, TG and LDL-C along with a decrease in HDL-C is an indicator of both coronary artery disease and artherosclerosis and is the main cause of cardiovascular disease worldwide (Lin et al., 2005). When compared with normal rats, the present results showed that the TC, TG and LDL-C levels in the serum were significantly increased ($P < 0.05$), whereas serum HDL-C level was significantly decreased ($P < 0.05$) in the STZ-induced diabetic rats. After administration of OJP1 for 28 days, the alteration in lipid metabolism was partially attenuated when compared with untreated diabetic rats, indicating that OJP1 can reduce the risk of artherosclerosis and cardiovascular diabetic complications.

Oxidation phenomena have been implicated in many illnesses, such as diabetes mellitus, arteriosclerosis, nephritis, Alzheimer's disease and cancer (Misthos et al., 2005; Pacher, Obrosova, Mabley, & Szabo, 2005; Sullivan & Brown, 2005). In diabetes mellitus,

chronic hyperglycaemia produces multiple biochemical sequelae, and diabetes-induced oxidative stress could play a role in the symptoms and progression of the disease. Diabetic patients as well as experimental animal models exhibit high oxidative stress due to persistent and chronic hyperglycemia, which may disrupt natural antioxidant defense mechanisms (Hong et al., 2004; Obrosova et al., 2002; Xue, Chen, Lu, & Jin, 2009). It was reported that reactive oxygen species (ROS) and free radicals were increased in both type of diabetes (Johansen, Harris, Rychly, & Ergul, 2005). Moreover, the onset of diabetes has been confirmed to be closely associated with oxidative stress in both clinical and experimental diabetes mellitus (Rosen et al., 2001). Under conditions of oxidative stress, the generation of free radicals such as superoxide radical, hydroxyl radical and other reactive oxygen species can lead to damage or destruction of a variety of tissues, which may contribute to the development of diabetic and its complications.

Major antioxidant enzymes, including SOD and GPx, are regarded as the first line of the antioxidant defense system against reactive oxygen species generated *in vivo* during oxidative stress. Their ability to decompose superoxide peroxide while blocking lipid peroxidation as well as their involvement in cellular defense mechanisms helps to protect tissues against oxidative damage (Yao et al., 2005). Our study showed that OJP1 treatment markedly restored the activity of SOD and GPx in the serum, liver and kidneys of STZ-induced diabetic rats. The enhanced activity of SOD and GPx can be very effective in scavenging the various types of oxygen free radicals and their products. Pancreatic β-cells may be protected from oxidative damage (induced by STZ) according to this reciprocal mechanism. Therefore, OJP1 has a direct or indirect preventive and protective effect in diabetes by decreasing oxidative stress and by preserving the integrity of pancreatic β-cell. These mechanisms may help explain why OJP1 has a protective effect in STZ-induced diabetic rats.

MDA, regarded as an index of cellular damage and cytotoxicity, is the end product of lipid peroxidation. It is generated by high levels of unsavanged free radicals and may result in protein damage and the inactivation of membrane-bound enzymes (Bagchi, Bagchi, Hassoun, & Stohs, 1995). Our study showed that the level of MDA in the serum, liver, and kidneys of STZ-induced diabetic rats is increased, yet OJP1 treatment decreased MDA levels and the level of lipid peroxides. The results may be associated with OJP1's potential protective effect on the organs in diabetic rats, and by improved glycometabolism and lipid metabolism in these rats.

ALT and AST are two of the most reliable markers of hepatocellular injury or necrosis. Their levels can be elevated in a variety of hepatic disorders. Of the two, ALT is thought to be more specific for hepatic injury, and is a sensitive index to measure the liver's function because it is present mainly in the cytosol of the liver and in low concentrations elsewhere (Giboney, 2005). The present study showed that the serum activities of AST and ALT in rats of the diabetic group were significantly ($P<0.01$) increased. This was attributed to damage to the structural integrity of the liver, since these factors were located in the cytoplasm and were released into circulation after cellular damage. OJP1 significantly ($P<0.01$) decreased the amount of ALT in the serum suggesting a reduction in hepatic injury. The biochemical analysis was consistent with the histopathological analyses of the livers.

Diabetic nephropathy (DN) is one of the major complications diabetes and it is known as the leading cause of mortality in diabetic patients due to a serious gradual decline in renal function (Chen, Lei, Liu, Xiong, & Ruan, 2011a). SCr, UA and UREA are the most commonly used indicators of renal function. In addition, SCr and UREA are noticeable indices for expressing the glomerular filtration rate. In this study, the SCr, UA and UREA levels were significantly increased in the diabetic group, and treatment with OJP1 likewise prevented this increase. This finding indicated that OJP1 may protect glomeruli from the injurious effects of diabetes. The results of the histopathological examination of the kidneys also showed that OJP1 could reduce the glomerular damage in diabetic rats.

TGF-β1 plays a crucial role in the development of pathological lesions in diabetic nephropathy. Two of the characteristic changes in diabetic nephropathy, glomerular basement membrane thickening and expansion of the mesangial-matrix, are mediated in large part by the TGF-β system (He, Li, Guo, Lin, & Lin, 2006). Connective tissue growth factor (CTGF), a novel fibrogenic protein induced by TGF-β1, is a potent inducer of extracellular matrix synthesis and increases in various clinical and experimental nephropathies (Zhou, Li, & Cai, 2004). In the present study, the expression levels of both TGF-β1 and CTGF were up-regulated in diabetic rats, and treatment with OJP1 decreased the expression of these factors in the rats of the diabetic group.

Based on our findings in this study, OJP1 was able to attenuate the diabetic nephropathy in rats. On one hand, the activities of antioxidant enzymes were enhanced and the oxidative stress was relived. On the other hand, the TGF-β1-dependent pathologic lesions of nephropathy were attenuated. The levels of SCr, UA and UREA were decreased. Finally, the renal function was improved. However, the exact mechanisms involved in DN are still unknown. Further studies are needed to investigate the exact mechanism of OJP1 on diabetic nephropathy.

In conclusion, the present investigation showed that OJP1 possess potent antioxidant activity, improves lipid metabolism, and protects the liver and kidney from the injurious effects of diabetes, however, there are no significant different between these two doses (150 mg/kg and 300 mg/kg). From the results, we hypothesize that OJP1 exerts protective effects in experimental diabetic animals, possibly by reducing oxidative stress, and hence, we assert that OJP1 protects the organism and its cells from oxidative damage and lipid abnormalities, and may reduce the development of diabetic complications. Above all, the results of this study may provide a mechanistic basis for using OJP1 as a potential natural plant-derived drug and functional food as an alternative means for the prevention and alleviation of diabetes and its complications.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (No. 20872113 and 21142014), and Yongjia Science and Technology Development Program (No. 2011310).

References

Adikwu, M. U., Yoshikawa, Y., & Takada, K. (2004). Pharmacodynamic-pharmacokinetic profiles of metformin hydrochloride from a mucoadhesive formulation of a polysaccharide with antidiabetic property in streptozotocin-induced diabetic rat models. *Biomaterials*, *25*, 3041–3048.

Arora, M. K., Reddy, K., & Balakuma, P. (2010). The low dose combination of fenofibrate and rosiglitazone halts the progression of diabetes-induced experimental nephropathy. *European Journal of Pharmacology*, *636*(1–3), 137–144.

Bagchi, D., Bagchi, M., Hassoun, E. A., & Stohs, S. J. (1995). *In vitro* and *in vivo* generation of reactive oxygen species, DNA damage and lactate dehydrogenase leakage by selected pesticides. *Toxicology*, *104*(1–3), 129–140.

Chen, J. L., Lei, Y. F., Liu, Y. J., Xiong, C. M., & Ruan, J. L. (2011). Extract of Cyclosorus acuminatus attenuates diabetic nephropathy in mice via modifying peroxisome proliferators activated receptor signalling pathway. *Food Chemistry*, *128*, 659–666.

Chen, X. M., Jin, J., Tang, J., Wang, Z. F., Wang, J. J., Jin, L. Q., et al. (2011). Extraction, purification, characterization and hypoglycemic activity of a polysaccharide isolated from the root of *Ophiopogon japonicus*. *Carbohydrate Polymers*, *83*, 749–754.

Fan, J., & Zhang, X. (2006). Research progress in pharmacology of *Ophiopogon japonicus* polysaccharides. *Chinese Archives of Traditional Chinese Medicine*, *24*(4), 626–627.

Giboney, P. T. (2005). Mildly elevated liver transaminase levels in the asymptomatic patient. *American Family Physician*, *71*(6), 1105–1110.

He, C. Y., Li, W. D., Guo, S. X., Lin, S. Q., & Lin, Z. B. (2006). Effect of polysaccharides from *Ganoderma lucidum* on streptozotocin-induced diabetic nephropathy in mice. *Journal of Asian Natural Products Research*, *8*(8), 705–711.

Hong, J., Bose, M., Ju, J., Ryu, J., Chen, X., Sang, S., et al. (2004). Modulation of arachidonic acid metabolism by curcumin and related ß-diketone derivatives: effects on cytosolic phospholipase A2, cyclooxygenases and 5-lipoxygenase. *Carcinogenesis*, *25*, 1671–1679.

Jia, W., Gao, W. Y., & Tang, L. D. (2003). Antidiabetic herbal drugs officially approved in China. *Phytotherapy Research*, *17*, 1127–1134.

Johansen, J. S., Harris, A. K., Rychly, D. J., & Ergul, A. (2005). Oxidative stress and the use of antioxidants in diabetes: Linking basic science to clinical practice. *Cardiovascular Diabetology*, *4*, 5.

Lin, J., Yang, R., Tarr, P. T., Wu, P. H., Handschin, C., Li, S., et al. (2005). Hyperlipidemic effects of dietary saturated fats mediated through PGC-1beta coactivation of SREBP. *Cell*, *120*, 261–273.

Maritim, A. C., Sanders, R. A., & Watkins, J. B., III. (2003). Diabetes, oxidative stress, and antioxidants: A review. *Journal of Biochemical and Molecular Toxicology*, *17*(1), 24–38.

Misthos, P., Katsaragakis, S., Milingos, N., Kakaris, S., Sepsas, E., Athanassiadi, K., et al. (2005). Postresectional pulmonary oxidative stress in lung cancer patients. The role of one-lung ventilation. *European Journal of Cardio-Thoracic Surgery*, *27*(3), 379–383.

Obrosova, I. G., Van Hysen, C., Fathallah, L., Cao, X., Greene, D. A., & Stevens, M. J. (2002). An aldose reductase inhibitor reverses early diabetes-induced changes in peripheral nerve function, metabolism, and antioxidative defense. *FASEB Journal*, *16*, 123–125.

Pacher, P., Obrosova, I. G., Mabley, J. G., & Szabo, C. (2005). Role of nitrosative stress and peroxynitrite in the pathogenesis of diabetic complications, emerging new therapeutical strategies. *Current Medicinal Chemistry*, *12*, 267–275.

Pandikumar, P., Babu, N. P., & Ignacimuthu, S. (2009). Hypoglycemic and antihyperglycemic effect of *Begonia malabarica Lam*. in normal and streptozotocin induced diabetic rats. *Journal of Ethnopharmacology*, *124*(1–6), 111–115.

Rosen, P. P., Nawroth, P., King, G., Moller, G., Tritschrev, H. J., & Packer, L. (2001). The role of oxidative stress in the onset and progression of diabetes and its complication. *Diabetes/Metabolism Research and Reviews*, *17*, 189–212.

Spinetti, G., Kraenke, N., Emanueli, C., & Madeddu, P. (2008). Diabetes and vessel wall remodeling: from mechanistic insights to regenerative therapies. *Cardiovascular Research*, *78*, 265–273.

Sullivan, P. G., & Brown, M. R. (2005). Mitochondrial aging and dysfunction in Alzheimer's disease. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *29*(3), 407–410.

Ugochukwu, N. H., & Babady, N. E. (2002). Antioxidant effects of *Gongronema latifolium* in hepatocytes of rat models of non-insulin dependent diabetes mellitus. *Fitoterapia*, *73*(7–8), 612–618.

Xue, S. X., Chen, X. M., Lu, J. X., & Jin, L. Q. (2009). Protective effect of Sulfated Achyranthes bidentata polysaccharides on streptozotocin-induced oxidative stress in rats. *Carbohydrate Polymers*, *75*, 415–419.

Yamac, M., Kanbak, G., Zeytinoglu, M., Bayramoglu, G., Senturk, H., & Uyanoglu, M. (2008). Hypoglycemic Effect of *Lentinus strigosus* (Schwein.) Fr. crude exopolysaccharide in streptozotocin-induced diabetic rats. *Journal of Medicinal Food*, *11*(3), 513–517.

Yao, D. C., Shi, W. B., Gou, Y. L., Zhou, X. R., Tak, Y. A., & Zhou, Y. K. (2005). Fatty acid-mediated intracellular iron translocation: a synergistic mechanism of oxidative injury. *Free Radical Biology and Medicine*, *39*, 1385–1398.

Yu, J., Cui, P. J., Zeng, W. L., Xie, X. L., Liang, W. J., Lin, G. B., et al. (2009). Protective effect of selenium-polysaccharides from the mycelia of Coprinus comatus on alloxan-induced oxidative stress in mice. *Food Chemistry*, *117*, 42–47.

Zhang, W., Zheng, L. J., Zhang, Z. M., & Hai, C. X. (2012). Protective effect of a water-soluble polysaccharide from *Salvia miltiorrhiz* Bunge on insulin resistance in rats. *Carbohydrate Polymers*, *89*, 890–898.

Zheng, Q., Feng, Yi., Xu, D. S., Lin, X., & Chen, Y. Z. (2009). Influence of sulfation on anti-myocardial ischemic activity of *Ophiopogon japonicus* polysaccharide. *Journal of Asian Natural Products Research*, *11*(4), 306–321.

Zhou, G. H., Li, C., & Cai, L. (2004). Advanced glycation end-products induce connective tissue growth factor-mediated renal fibrosis predominantly through transforming growth factor β-independent pathway. *American Journal of Pathology*, *165*(6), 2033–2043.

Zhou, Y. H., Xu, D. S., Feng, Y., Fang, J. N., Xia, H. L., & Liu, J. (2003). Effects on nutrition blood flow of cardiac muscle in mice by different extracts in radix *Ophiopogonis*. *China Journal of Experimental Traditional Medical Formulae*, *9*, 22–23.