A novel proteoglycan from *Ganoderma lucidum* fruiting bodies protects kidney function and ameliorates diabetic nephropathy via its antioxidant activity in C57BL/6 db/db mice

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**A R T I C L E   I N F O**

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**A B S T R A C T**

Diabetic nephropathy (DN) is the major cause of morbidity among diabetic patients. Thus, antidiabetic drugs with protection potential in the kidneys would have a higher therapeutic value. The effects of a novel proteoglycan, named FYGL, isolated from *G. lucidum* fruiting bodies, on the kidney function were investigated systematically in present work. FYGL (250 mg/kg) not only dose-dependently reduced the blood glucose concentration (23.5%, \(p < 0.05\)), kidney/body weight ratio (23.6%, \(p < 0.01\)), serum creatinine (33.1%, \(p < 0.01\)), urea nitrogen (24.1%, \(p < 0.01\)), urea acids contents (35.9%, \(p < 0.01\)) and albuminuria (30.7%, \(p < 0.01\)) of DN mice compared to the untreated DN mice but also increased the renal superoxide dismutase (75.3%, \(p < 0.01\)), glutathione peroxidase (35.0%, \(p < 0.01\)) and catalase activities (58.5%, \(p < 0.01\)) compared to the untreated DN mice. The decreasing of renal malondialdehyde content (34.3%, \(p < 0.01\)) and 8-hydroxy-2'-deoxyguanosine expression (2.5-fold, \(p < 0.01\)) were also observed in FYGL-treated DN mice compared to the untreated DN mice, along with an amelioration of renal morphologic abnormalities. We conclude that FYGL confers protection against the renal functional and morphologic injuries by increasing activities of antioxidants and inhibiting accumulation of oxidation, suggesting a potential nutritional supplement for the prevention and therapy of DN.

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**1. Introduction**

Diabetes mellitus, characterized by hyperglycemia and long term complications, is the most common endocrine disorder. Complications of diabetes mellitus can affect the function of kidneys, eyes, heart and nerves leading to nephropathy, retinopathy, cardiomyopathy and neuropathy, respectively. Among those complications, diabetic nephropathy (DN), a deterioration of renal function and glomerular structure, is the major cause of morbidity (Rossing, 2006). Thus, antidiabetic drugs with protection potential in the kidneys would have a higher therapeutic value.

Several studies have demonstrated that the ingestion of fresh fruits, vegetables or plants rich in nature has preventive effect of diabetes and its complications (Punithavathi et al., 2011). One of example is *Ganoderma lucidum* (*G. lucidum*), a white rot fungus, which has been widely used for the prevention and treatment of various human ailments in Asian countries (Paterson, 2006).

In insulin signaling pathway, there is one important phosphatase, protein tyrosine phosphatase 1B (PTP1B) considered to play an important role in the intracellular signal transduction process and metabolism (Asante and Kennedy, 2003). Previously, we successfully isolated a highly efficient PTP1B inhibitor, named FYGL, from the fruiting bodies of *G. lucidum*. FYGL was capable of decreasing the plasma glucose level and enhancing the insulin sensitivity in vivo. We have already investigated the dominant components, inhibition kinetics, pharmacology and toxicity of the efficient extract in vivo, and demonstrated FYGL being efficient for antidiabetic with high safety (LD\textsubscript{50} = 6 g/kg with 95% confidence limits of 4.8–7.4 g/kg). The results from our previous research indicated that FYGL can serve as a nutritional supplement or a health-care food for the diabetic therapy or protection (Teng et al., 2011, 2012; Wang et al., 2012; Pan et al., 2013). In addition to its hypoglycemic

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effects, preliminary research found that FYGL also could alter the antioxidant status and protect against functional and histopathology abnormalities in the kidneys of diabetic mice. However, the mechanisms underlying the protection effects on diabetic kidneys are still unclear due to the complex pathogenesis of DN. Since the pathogenesis of DN is closely associated with oxidative stress and many phytochemicals were reported to have multiple functions (Zhang et al., 2010), we hypothesized that FYGL can restore the kidney function via its antioxidant activity. To test this hypothesis, we evaluated whether different doses of FYGL exert protective effects on renal function and morphology in diabetic mice. In consideration of the natural property and widespread use of G. lucidum, results of the present study may provide an alternative for enhancing nutrition and diabetic control during DN.

2. Materials and methods

2.1. Materials

All the dried fruiting bodies of G. lucidum grown in north-eastern China were purchased from Leyunshang Pharmaceutical Co., Ltd., (Shanghai, China). All other agent used was of the highest available purity and purchased from Sigma–Aldrich (Shanghai, China).

2.2. Preparations of FYGL

The fractionation procedures were based on a previous work as shown in Fig. 1. Briefly, after the dried fruiting bodies of G. lucidum (160 g) was milled and defatted with boiling ethanol (3 L), the residues were decoted with boiling water (2 L) for 2 h. The supernatant of the decocted mixture was discarded, and the solid residues (12 g) were extracted by ammonia aqueous solution for 24 h at room temperature. The supernatant extracted from the aqueous solution was neutralized, concentrated, dialyzed and lyophilized successively; the crude extract (4.5 g) was then collected. Subsequently, the crude extract was dissolved in distilled water and further purified by Sephadex G-75 column chromatography with NaCl solution as the eluent. The eluted fractions were monitored by the phenol–sulfuric acid method with ultraviolet (UV) absorption at 490 nm and the main fraction, named as FYGL (1.54 g, yield of 0.8%), was collected.

2.3. Animals and induction of DN model

C57BL/6 mice and C57BL/6 db/db mice (male, 5 weeks old) were obtained from Shanghai Institute of Material Medical, Chinese Academy of Sciences. All the following animal trial procedures instituted by Ethical Committee for the Experimental Use of Animals in Center for Drug Safety Evaluation, Shanghai University of Traditional Chinese Medicine were followed. All animals were housed in plastic cages (4 mice/cage) with free access to drinking water and a pellet diet, under controlled conditions of humidity (50 ± 10%), light (12/12 h light/dark cycle) and temperature (23 ± 2°C). Ten C57BL/6 mice were set as Group I, normal mice. The C57BL/6 db/db mice were used as DN model. The DN mice were confirmed by the symptoms of polyphagia, polydipsia, and polyuria. Blood samples were collected and plasma glucose concentrations were measured using glucose oxidation method. After housed and acclimatized for 3 weeks, only those animals with plasma glucose higher than 11.1 mmol/L were selected as diabetic model for the following experiments. A total of sixty animals, including 10 normal C57BL/6 mice and 50 db/db diabetic mice, were divided into six groups (numbered as groups I–VI) with 10 mice in each group. Group 1 were normal mice treated with 0.9% saline solution (normal); Group II to VI were DN mice were treated with 0.9% saline solution (control), 75 mg/kg FYGL (low dosage), 250 mg/kg FYGL (middle dosage), 450 mg/kg FYGL (high dosage) and 200 mg/kg metformin (positive), respectively. FYGL and metformin were dissolved in 0.9% saline and administered orally for 8 weeks. Body weights, urinary volume, food taken, blood glucose level, etc. were measured weekly. The dosage was adjusted weekly according to the body weight to maintain the similar dose per kilogram of mice over the entire experiment. The glycosylated hemoglobin (HbA1c) level at 8 weeks treatments was measured with analyzer (Roche Diagnostics, Basel, Switzerland) using whole blood obtained from eye artery. The insulin levels in the separated serum were determined by radio-immunoassay (RIA) method.

2.4. Collection and preparation of samples

At the end of the experiment, all the mice were sacrificed, and blood samples were obtained and centrifuged at 4000 rpm/min for 10 min to separate serum which was then frozen at −70°C for the determination of serum biochemistry parameters. Urine samples which were used to calculate urine volume and urinary creatinine of 24 h were also collected. The kidneys were rapidly excised, weighed, and fixed in 2.5% glutaraldehyde for histology studies. Samples from the renal cortex were immediately frozen in liquid nitrogen and stored at −70°C before used.

2.5. Lipid profiles assay

Lipid profiles, including total cholesterol (TC), triacylglycerol (TG), low-density lipoprotein-cholesterol (LDL-c) and high-density lipoprotein-cholesterol (HDL-c), in serum were measured by the commercial enzymatic kits purchased from Nanjing Jianchen Bioengineering Institute (Nanjing, China). The atherogenic index (AI) was calculated according to the equations as following:

\[
AI = TC/\text{HDL-c}
\]

2.6. Determination of renal antioxidative activities and MDA content

Catalase (CAT) activity was measured as the decrease in H₂O₂ concentration by recording the absorbance at 240 nm (Aebi, 1984). Glutathione peroxidase (GSHPx) activity was assayed according to the method described previously (Paglia and Valentine, 1967). Superoxide dismutase (SOD) activity was assayed by measuring its inhibition of pyrogallol autoxidation for 10 min according to the method of Marklund and Marklund (1974). The Malondialdehyde (MDA) contents were measured by UV spectrometer on the method of Ohkawa et al. (1979).

2.7. Assay for renal function parameters

Samples from mice were collected for measurement of glucose, serum creatinine (Scr), urea nitrogen (BUN), uric acid (UA), urine creatinine (Ucr) and albuminuria. The detection was carried out using diagnostic kits (Jiangcheng, China). The kidney index was calculated as 1000 × kidney weight/body weight. Creatinine clearance (Ccr) was calculated according to the following formula:

\[
\text{Ccr} = \frac{\text{urinary creatinine(µM)}}{\text{urinary volume(ml/kg/min)}} \times \frac{\text{Scr(µM)}}{70}\text{ml/min/m²}
\]
2.8. Measurement of renal 8-hydroxy-2′-deoxy guanosine expression

The renal tissue was homogenized in a nine-time volume of ice-cold normal saline, and then the homogenates were centrifuged (4000 rpm/min, 4 °C, 10 min). The renal 8-hydroxy-2′-deoxy guanosine (8-OHdG) expression was determined using an ELISA assay kit (Jiangcheng, China). Extraction of renal 8-OHdG was performed using an extraction kit following the manufacturer’s protocol (Jiangcheng, China). The protein contents were measured by the method of Lowry et al. (1951) using bovine serum albumin as standard.

2.9. Assays for renal histology

The renal tissues were fixed in 10% (v/v) neutral buffered formalin and embedded in paraffin wax. Paraffin sections were cut into 5 μm thickness and de-paraffinized in xylene for 5 min dehydrated through the graded ethanol. The sections were stained with hematoxylin and eosin (H&E) for histopathological analysis by photomicroscope. The total operative procedures were complied with the standard protocols and the examination of slides was performed by a pathologist in a blind to the experimental profile.

2.9.1. Estimation of glomerular volume per kidney

Glomerular volume per kidney estimated with point counting rule as following: the images of microscopical fields from each section at total magnification of 400× were projected on point probe by video projector attached to the computer. Points that hit glomerular lobules or fall inside of the probe were both counted, respectively. Glomerular volume density (Vv) was estimated by Eq. (1)

\[
V_{\text{glomer}} = \sum_{i} \left( \sum_{j} P_i a_i \right)
\]

where \( \sum P_i \) represents the sum of points hitting glomerular lobules and \( \sum P_i \) represents the sum of points falling inside of the probe. Total glomerular volume per kidney was estimated from Eq. (2)

\[
V_{\text{glomer}} = V_{\text{glomer}} \times V_{\text{cortex}}
\]

Kidney weight (Vv) was used as kidney volume. The volume density of cortex per kidney (Vv/cortex) was also estimated by point counting rule from the kidney sections as the same way.

2.9.2. Estimation of glomerular number per kidney

Total glomerular number per kidney was estimated by physical dissector method. Five sections were selected from each kidney slice and the first one was treated as reference. Microscopic image of the reference and the investigated sections at the magnification of 400× were both projected on dissector frame a and frame b (both 10 cm × 10 cm on paper), respectively, in two projecting system. Glomeruli were counted if they presented in frame a but not on frame b and did not hit with hidden lines of dissector probe. The numerical density (Nv) of glomeruli per cortex was estimated from Eq. (3)

\[
N_{\text{glomer}} = \left( \sum Q \times M \right) / \left( \sum P \times a \times d \right)
\]

where \( \sum Q \) number of counted glomeruli; \( \sum P \) sum of studied fields from reference sections; a: dissector frame area; d dissector height (section thickness); m: magnification. Total glomerular number per kidney was estimated from Eq. (4)

\[
N_{\text{glomer}} = N_{\text{glomer}} \times V_{\text{cortex}}
\]

2.9.3. Study of glomerulosclerosis

Severity of the glomerulosclerosis in tissue sections was assessed semi-quantitatively by assigning a score 1–4 to each glomerulus according to the tuft demonstrating sclerosis: normal glomerulus = 0; up to 25% involvement = 1; 25–50% involvement = 2; 50–75% involvement = 3 and more than 75% involvement = 4.

3. Results

3.1. The basic properties of FYGL

GPC method and component analysis are used to detect the homogeneity and purity, indicating that FYGL is a water-soluble macromolecular proteoglycan with a protein to polysaccharide ratio of 17:7, a molecular weight of 2.6 × 10^9 g/mol and a relative purity of 91% (Teng et al., 2011). The polysaccharide moiety in FYGL consisted of 1,2-linked α-L-rhamose, 1,3,4-linked α-D-glucose and 1,6-linked α-D-glucose, 1,4-linked α-D-glucose, 1,2,4-linked α-D-glucose, 1,6-linked β-D-galactose, 1,3,6-linked β-D-galactose and 1 linked β-D-galactose. The analysis of amino acids in FYGL indicated that there were 16 common amino acids, among which aspartic acid, glycine, serine, alanine, glutamic acid and threonine were the dominant components. And more research demonstrates that the protein moiety in FYGL covalently linked to the 1,3-linked α-D-galactose and 1,6-linked β-D-glucuronic acid residue of polysaccharide in C-linkage type via both serine and threonine (Data unpublished and the detailed structure of FYGL in Supporting information for review only).

3.2. Effects of FYGL on physiological parameters

Physiological parameters including blood glucose concentration, Glycosylated hemoglobin level, serum insulin level, body weight, lipids profile and kidney/body weight ratio of the animals in different groups were summarized in Table 1 and Fig. 2. The blood glucose concentration decreases dose-dependently in all diabetic mice after 8 weeks of treatment with FYGL and metformin compared with the control group.

The HbA1c level significantly decreased from 8.8 ± 0.4% to 6.98 ± 0.2%, 6.82 ± 0.1%, 6.56 ± 1.5%, and 6.2 ± 0.8% (p < 0.05, p < 0.01, p < 0.05 and p < 0.05), respectively, for the mice treated with low, middle, high-dosage FYGL and metformin, compared with that of diabetic control group.

The body weight of diabetic mice treated with FYGL also decreased compared with untreated animals. Additionally, the final kidney/body weight ratios of untreated diabetic animals were significantly (p < 0.01) higher than those of normal animals, while

### Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal</th>
<th>Control</th>
<th>75 mg/kg FYGL</th>
<th>250 mg/kg FYGL</th>
<th>450 mg/kg FYGL</th>
<th>200 mg/kg Metformin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood glucose (mg/dL)</td>
<td>7.0 ± 0.3</td>
<td>32.3 ± 1.6</td>
<td>28.7 ± 4.2</td>
<td>24.7 ± 4.8</td>
<td>22.2 ± 7.8</td>
<td>20.7 ± 4.4</td>
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<tr>
<td>Glycosylated hemoglobin (%)</td>
<td>4.73 ± 0.2</td>
<td>8.80 ± 0.4</td>
<td>6.98 ± 0.7</td>
<td>6.82 ± 0.96</td>
<td>6.56 ± 1.46</td>
<td>6.20 ± 0.8</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>25.6 ± 0.4</td>
<td>60.5 ± 4.0</td>
<td>56.5 ± 3.7</td>
<td>57.5 ± 4.0</td>
<td>51.3 ± 1.6</td>
<td>52.6 ± 2.0</td>
</tr>
<tr>
<td>TC/HDL-c</td>
<td>1.73 ± 0.19</td>
<td>6.38 ± 1.19</td>
<td>4.04 ± 0.71</td>
<td>2.88 ± 0.65</td>
<td>2.58 ± 0.22</td>
<td>2.26 ± 0.22</td>
</tr>
<tr>
<td>LDL-c/HDL-c</td>
<td>0.35 ± 0.01</td>
<td>4.04 ± 1.23</td>
<td>2.38 ± 0.12</td>
<td>1.26 ± 0.06</td>
<td>0.68 ± 0.09</td>
<td>1.06 ± 0.06</td>
</tr>
<tr>
<td>Kidney/body weight (g/100g)</td>
<td>6.35 ± 1.2</td>
<td>10.23 ± 2.2</td>
<td>8.31 ± 1.5</td>
<td>7.81 ± 1.4</td>
<td>7.02 ± 1.3</td>
<td>6.89 ± 1.5</td>
</tr>
<tr>
<td>Albuminum (μg/24h)</td>
<td>20.53 ± 0.25</td>
<td>260.55 ± 20.8</td>
<td>220.8 ± 18.6</td>
<td>180.6 ± 13.5</td>
<td>160.5 ± 20.4</td>
<td>120.6 ± 16.2</td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>8.42 ± 0.70</td>
<td>11.41 ± 0.79</td>
<td>8.96 ± 1.97</td>
<td>8.66 ± 0.96</td>
<td>8.61 ± 1.36</td>
<td>7.59 ± 0.88</td>
</tr>
<tr>
<td>Scr (μmol/L)</td>
<td>96.71 ± 6.70</td>
<td>149.62 ± 8.31</td>
<td>121.70 ± 18.40</td>
<td>109.91 ± 14.23</td>
<td>93.6 ± 15.05</td>
<td>124.2 ± 15.40</td>
</tr>
<tr>
<td>Uric acid (μmol/L)</td>
<td>437.6 ± 25.2</td>
<td>1370.9 ± 108.7</td>
<td>953.8 ± 60.9</td>
<td>765.7 ± 49.6</td>
<td>615.5 ± 45.9</td>
<td>819.7 ± 72.8</td>
</tr>
<tr>
<td>UA (μmol/L)</td>
<td>59.59 ± 11.37</td>
<td>84.61 ± 11.37</td>
<td>69.62 ± 24.77</td>
<td>54.20 ± 13.71</td>
<td>56.79 ± 9.48</td>
<td>67.25 ± 7.65</td>
</tr>
</tbody>
</table>

* p < 0.05 By one-way ANOVA with Dunnett’s post-hoc test vs. control group.  
* p < 0.01 One-way ANOVA with Dunnett’s post-hoc test vs. control group.
FYGL-treated diabetic animals showed a significant reduction, which approached the levels of the metformin-treated group compared with the untreated animals.

As summarized in Table 1, the serum insulin level was increased in dose-dependent manner from 3.26 ± 0.24 mU/L for the diabetic control group to 3.59 ± 0.15, 3.69 ± 0.13, 3.74 ± 0.17 and 3.43 ± 0.11 mU/L for the mice treated with low, middle, high-dosage FYGL and metformin, respectively.

The serum TG and TC were significantly decreased by 47.8% (p < 0.01) and 41.0% (p < 0.01) for high dose of FYGL-treated db/db DN mice, compared with those for the control. Furthermore, the serum LDL-c levels for both middle and high-dosage of FYGL-treated mice were significantly (p < 0.01) lower than those for control group, whereas the serum HDL-c level for those nutritional supplement-treated groups were higher (26.2%, 34.3%, 29.6%, 2-folds and 12-folds) than those of normal group, also indicating that our model was successful at inducing DN. Administration of FYGL and metformin significantly (p < 0.05) reversed those changes in a dose-dependently manner. Especially, BUN, Scr, UA, Ccr, and albuminuria levels of medium and high dosage FYGL treated mice were reduced to near normal values.

3.4. Effects of FYGL on renal antioxidation status

As shown in Fig. 3, the renal SOD, CAT and GSH-px activities increased dose-dependently. For example, the renal CAT activities increased from 5.65 ± 0.93 U/mgprot for diabetic control group to 6.66 ± 1.07, 8.96 ± 1.07, 9.78 ± 0.91, and 8.02 ± 0.76 U/mgprot (p < 0.1, p < 0.05, p < 0.05 and p < 0.05), respectively, for FYGL and metformin-treated mice (Fig. 3B). However, the renal MDA contents decreased from 1.40 ± 0.07 nM for control mice to 1.11 ± 0.06, 0.92 ± 0.80, 0.51 ± 0.08 and 0.509 ± 0.08 nM (p < 0.05, p < 0.05, p < 0.05 and p < 0.05), respectively, for FYGL and metformin-treated mice (Fig. 3D).

3.5. Effects of FYGL on renal 8-OHdG expression

The renal 8-OHdG expression were significantly greater (2.5-fold, p < 0.01) in untreated diabetic mice than that in normal mice at 8 weeks after the onset of diabetes as shown in Fig. 4.
Administration of FYGL suppressed the increase of 8-OHdG in the diabetic mice to the same extent as metformin. In addition, the magnitude of these increases was reduced by FYGL treatment in a dose-dependent manner.

3.6. Effects of FYGL on morphologic changes in kidney

As shown in Fig. 5, no histological alterations in the kidney of normal mice was observed (Fig. 5A), however, pathological lesions in untreated db/db mice were objectified by necrosis in the epithelial cells of the proximal tubules, leucocytes infiltration, vascular congestion and tubular dilatation (Fig. 5B). Importantly, the kidney injury was remarkably reduced by the administration of FYGL (Fig. 5C–F). Moreover, as listed in Table 2, the value of glomerular volume in untreated diabetic mice was significantly higher than that of the normal animals. FYGL and metformin treatment significantly lower the level of glomerular volume compared with untreated diabetic group, indicating FYGL could inhibit the glomerular hypertrophy. Our researches also showed that glomerular numbers pre-kidney in control group were remarkably lower than that of normal group. The treatment of diabetic animals with different dosage of FYGL inhibited the glomerular numbers loss in comparison with the control group. Furthermore, glomerulosclerosis increased in the control group in comparison with the normal group. Treatment of diabetic animals with different dosage of FYGL and metformin could inhibit glomerulosclerosis significantly in comparison with the control group.

4. Discussion

Our previous investigation showed the potent antihyperglycemic activity of a novel proteoglycan, named FYGL, from G. lucidum.
fruiting bodies. In the present study, we attempted to figure out whether FYGL had more function such as hypolipidemic and anti-oxidant in diabetes. As we observed, FYGL significantly dose-dependently decreased the blood glucose at the doses ranging from 75 mg/kg to 450 mg/kg in db/db diabetic mice. Furthermore, the serum insulin level of FYGL-treated diabetic mice was significantly increased at the end of experiments. The variation caused by the insulin resistance in the early stage is the islet β-cell hyperplasia resulting in marked hyperinsulinemia. However, when the db/db mice reach at 12–24 weeks old, islet develops β-cell necrosis, hyperinsulinemia is diminished, and the mice manifest symptoms of insulin deficiency. We also observed that the islet architecture of FYGL-treated mice (data not shown) preserved relatively better. Thus, the present data suggested that the serum insulin level in the db/db mice may be gradually declined after reaching the peak point due to the reduction of β-cell mass, whereas FYGL is capable to slow the age-dependent insulin decline, i.e., FYGL can partly protect the pancreas from dysfunction which is associated with impaired insulin secretion and biosynthesis in the late stage of diabetes. Taken together, this study showed that the supplementation of FYGL for eight weeks improved the glucose homeostasis in db/db mice, in agreement with our previous results.

Table 2

<table>
<thead>
<tr>
<th></th>
<th>Normal Control</th>
<th>75 mg/kg FYGL</th>
<th>250 mg/kg FYGL</th>
<th>450 mg/kg FYGL</th>
<th>200 mg/kg Metformin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glomerular volume/kindey (mm³)</td>
<td>24.92 ± 3.25 †</td>
<td>60.35 ± 6.89</td>
<td>58.48 ± 8.25</td>
<td>42.65 ± 5.66</td>
<td>36.24 ± 3.67 †</td>
</tr>
<tr>
<td>Glomerular number/kindey</td>
<td>30,633 ± 1346 †</td>
<td>22,532 ± 856</td>
<td>23,565 ± 1052</td>
<td>26,536 ± 1256</td>
<td>27,466 ± 1028 †</td>
</tr>
<tr>
<td>Glomerular sclerosis (sore 0–4)</td>
<td>0.5 ± 0.05 †</td>
<td>3.5 ± 0.20</td>
<td>3.0 ± 0.25</td>
<td>2.5 ± 0.50 †</td>
<td>2.6 ± 0.50 †</td>
</tr>
</tbody>
</table>

† p < 0.05 by one-way ANOVA with Dunnett’s post-hoc test vs. control group.
‡ p < 0.01 one-way ANOVA with Dunnett’s post-hoc test vs. control group.

Fig. 5. (A) Micrograph from kidney of normal group, H&E, 50 ×; (B) Micrograph from kidney of diabetic untreated group demonstrated lymphocyte infiltration and vascular congestion, H&E, 50 ×; (C–F) diabetes group treated with 75, 250, 450 mg/kg FYGL and 200 mg/kg metformin, respectively: treatment with FYGL significantly attenuated the kidney injury, H&E, 50 ×.
It was reported that diabetes was associated with profound alterations in lipid and lipoprotein profiles. In addition to its hypoglycemic effect, FYGL had strong hypotriglyceridemic and hypocholesterolemic effects on db/db mice with a significant reduction in serum LDL-c levels and an increase in HDL-c levels. Furthermore, the atherogenic indexes (AI) of FYGL-treated mice were markedly decreased, leading to a reduction in LDL-c to HDL-c ratio. The increase in LDL-c/HDL-c ratio is one of the most important criteria for the anti-atherogenic agents. The relative high levels of HDL-c are associated with low incidences of vascular diseases and complications (Young, 2005). Thus, our results suggested that FYGL could be helpful for the prevention of diabetic complications.

Diabetic nephropathy (DN) is one of the major micro-vascular complications of diabetes mellitus. In the present study, the development of DN in db/db mice was confirmed by significant elevations of fasting blood glucose, kidney/body weight ratio, serum creatinine, urea nitrogen, and urea acid in diabetic rats, as previously reported (Xue et al., 2011).

Oxidative stress constitutes the key and common event in the pathogenesis of numerous diabetic complications; especially in DN. Reactive oxygen species (ROS), caused from oxidative stress, can react with polyunsaturated fatty acids leading to lipid peroxidation and irreversible tissue damage (Seifi et al., 2010). Typical markers of oxidative stress already have been reported in both diabetic human and animals, including the increased ROS, MDA, and reduced levels of antioxidants (Evans et al., 2002). Furthermore, in the present study, we also found that the kidney showed a markedly high expression of 8-hydroxy-2′-deoxyguanosine (8-OHdG) in the DN mice. DNA is susceptible to oxidation, resulting in the single-strand breaks and a relatively stable oxidation product, i.e. 8-OHdG, formation. Several studies also revealed that diabetic patients show an increased 8-OHdG expression when compared to non-diabetic individuals. Even pre-diabetic individuals in these studies showed an elevated 8-OHdG expression, suggesting that oxidative damage to DNA is present even before the clinical development of diabetes (Aubaidy and Jelinek, 2011). Thus, 8-OHdG expression can represent accurately the DNA damage within the body as a whole. Importantly, the present study revealed that such oxidative damage can be suppressed by the extract from G. lucidum, a Chinese folk medicine, in a dose-dependent manner at DNA level.

G. lucidum is an herb commonly used to treat diabetes in traditional Chinese medicine (TCM). An early study reported that G. lucidum could prevent STZ-induced activation of lipid peroxidation in the pancreas (Jia et al., 2009). In this study, we used extract (FYGL) from G. lucidum, identified to be consisted of proteoglycan, as a sample to investigate its antioxidant activity. We focused on the effects of FYGL on DN, a major cause of morbidity among diabetic patients. Significant amelioration on kidney of diabetic mice was observed after eight weeks treatment with different doses of FYGL. Thus, this findings also provided a valuable evidence for past reports on the antidiabetic action of G. lucidum (Chen et al., 2005).

The high level of oxidative stress is not only associated with the functional changes including the increased concentration of Scr, BUN, and UA, but also lead to a structural abnormalities of kidneys such as the increased glomerular filtration rate and glomerulosclerosis (Kitada et al., 2003). As we observed in the present work, the DN animal exhibited the severe symptoms of glomerular hypertrophy, glomerulosclerosis and glomerular number loss. However, eight weeks treatment of diabetic animals with FYGL effectively prevented the above functional and morphologic alterations.

Furthermore, an imbalance between the production of ROS and antioxidants is believed to be involved in renal failure. Induction of diabetes in the present study caused a significant elevation of MDA and a reduction of SOD, GSH-px and CAT in the kidneys, as compared with the normal mice. Administering FYGL to diabetic mice daily for eight weeks reversed these oxidant/antioxidant changes. Thus, the present results indicate that FYGL exerts its protective effects on the renal lesions of diabetic rats possibly via inhibiting the accumulation of oxidized DNA in the kidney (Vinod, 2012; Afsharia et al., 2007; Xue et al., 2011).

The present work indicated that FYGL treatment caused a significant improvement of DN in diabetic mice. We speculate that there were three possible pathways to explain the protective effects of FYGL based on present data and previous report (Xin et al., 2011): (1) FYGL directly removed ROS, suppressed lipid peroxidation of renal cells, protected cell membrane from oxidative stress, and maintained normal structure and functions; (2) FYGL indirectly scavenged the radicals by activating antioxidant enzyme systems in kidney tissues to alleviate oxidative injury; (3) FYGL chelate with metal ion by forming cross-bridge between carboxyl groups in galacturonic acid, leading to the decrease ROS generation. The proteoglycan compounds, which were also the dominant ingredient of FYGL, were effective antioxidants due to their unique redox properties and chemical structure. Previous studies have shown that some compound such as chitin and K-carrageenan oligosaccharides can inhibit low-density lipoprotein oxidative and have an overall positive effect on lipid metabolism (Ma et al., 2009). Such compounds could function as chain-breaking electron donors or as chelating metal ions or even as detoxificant of intermediary oxygen reactive products of lipo-peroxidation by increasing available GSH-px (Aherne and O’Brien, 1999). In fact, the increase in GSH-px levels in FYGL-treated mice supported these findings. In Conclusion, all present findings support our hypothesis that FYGL has a renal protective role against oxidative damage, which may be due to its antioxidant potential.

In addition, metformin was selected as a positive control in the present study because of its well-known hypoglycemia effect. Table 1 show that the hypoglycemia effect of medium and high dose of FYGL reversed the renal lesions and oxidant/antioxidant status to almost the levels of the metformin treated group. However, these synthetic drugs usually come with considerable side effects, such as hypoglycemia, drug-resistance, dyspsy and weight gain. Therefore, our results provide further insight into therapeutic strategies for diabetic kidney disease.

Conflict of Interest
The authors declare that there are no conflict of interest.

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Appendix A. Supplementary material
Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.fct.2013.10.046.

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