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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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ABSTRACT

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 dosedependently 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 acid 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_{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

Abbreviations: DN, diabetic nephropathy; *FYGL*, Fudan–Yueyang–*G. lucidum*; ROS, reactive oxygen species; MDA, malondialdehyde; 8-OHdG, 8-hydroxydeoxyguanosin; SOD, super oxide dismutase; CAT, catalase; GSH-px, glutathione peroxidase; TC, total cholesterol; TG, triacylglycerol; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein-cholesterol; Scr, serum creatinine; BUN, blood urea nitrogen; UA, uric acid; Ucr, urine creatinine; Ccr, Creatinine clearance.

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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 Leiyunshang 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 decocted 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 0.9%), was collected.

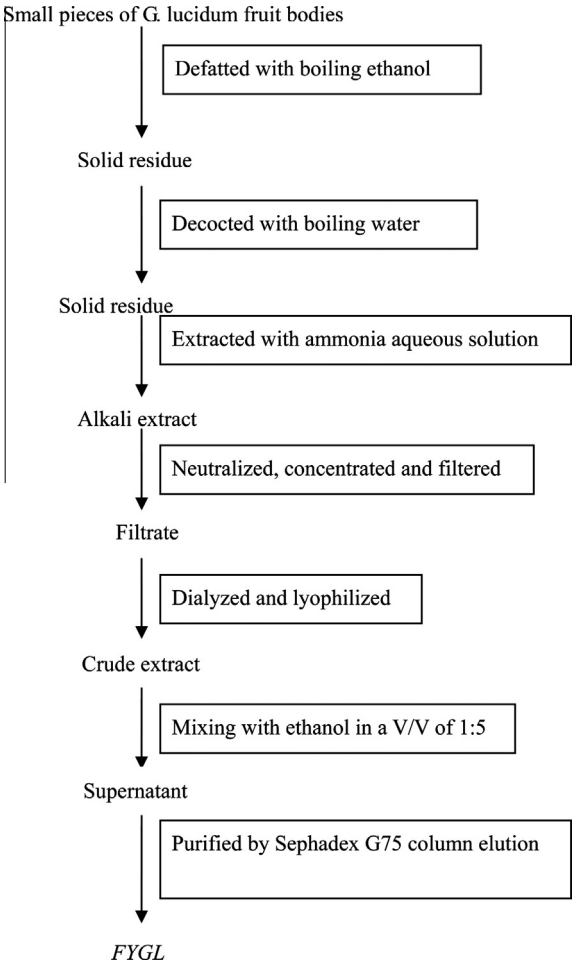

Fig. 1. Scheme for extraction of *FYGL*.

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 plasmas 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 I 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 (HbAlc) 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:

$$AI = TC/HDL\text{-}c$$

2.6. Determination of renal antioxidase activities and MDA content

Catalase (CAT) activity was measured as the decrease in H_2O_2 concentration by recording the absorbance at 240 nm (Aebi, 1984). Glutathione peroxidase (GSH-px) 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:

$$Ccr(ml/kg/min) = \text{Urinary creatinine}(\mu M) \times \text{urinary volume}(ml/kg/min)/Scr(\mu M).$$

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