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# Hypoglycemic effect and mechanism of a proteoglycan from *Ganoderma Lucidum* on streptozotocin-induced type 2 diabetic rats

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**Abstract. – *Background and Objectives:*** **Diabetes mellitus inducing a leading cause of morbidity are widespread in the entire globe. The present study was to investigate the antidiabetic potency and mechanism of a proteoglycan extract, named *FYGL* (Fudan-Yueyang-G. lucidum), from the fruiting bodies of *Ganoderma Lucidum* as published recently, using streptozotocin-induced type 2 diabetic mellitus (T2DM) rats.**

***Material and Methods:*** **The T2DM model rats were treated with *FYGL* as well as metformin and rosiglitazone. The levels of plasma glucose and insulin were measured, and the expression and activity of the protein tyrosine phosphatase 1B (PTP1B) and the tyrosine phosphorylation level of the insulin receptor (IR) β-subunit in the livers and skeletal muscles of the T2DM rats were analyzed by immunoprecipitation and Western blotting methods. In addition, the levels of free fatty acid and serum lipid profile were measured using commercial kits for those trailed rats.**

***Results:*** **The decrease in fasting plasma glucose and the increase in insulin concentration dose- and time-dependently in the T2DM rats treated by *FYGL*, comparable with that by the clinical drugs, metformin and rosiglitazone. The levels of the PTP1B expression and activity were decreased, and the tyrosine phosphorylation level of the IR β-subunit was increased in the skeletal muscles of the T2DM rats. Furthermore, *FYGL* significantly decreased the levels of free fatty acid, triglyceride, total cholesterol and low density lipoprotein-cholesterol as well as increased the level of high density lipoprotein-cholesterol.**

***Discussion:*** **It is suggested that the hypoglycemic mechanisms of *FYGL* are caused by inhibition of the PTP1B expression and activity, consequently, regulation of the tyrosine phosphorylation level of the IR β-subunit. As those results, *FYGL* also controlled the plasma biochemistry indexes relative to the type 2 diabetes-accompanied metabolic disorders. This is possibly the first report on the underlying mechanisms responsible for the antidiabetic effect of *Ganoderma lucidum*.**

*Key Words:*

*Ganoderma lucidum*, Proteoglycan, Protein tyrosine phosphatase 1B, Type 2 diabetes mellitus, Metabolic disorders.

## Introduction

Type 2 diabetic mellitus (T2DM) is a group of metabolic disorders induced by many etiologies, and characterized by the hyperglycemia. In recent years, T2DM has become a leading cause of morbidity. The main pathogenesis of diabetes is insulin resistance and lack of insulin secretion[1]. The insulin action is regulated by its receptor binding the surface of insulin-sensitive tissues, such as liver and skeletal muscle[2]. The biological effect of insulin is initiated with insulin binding to the α-subunit of insulin receptor (IR) and activating

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the intrinsic tyrosine kinase activity of the β-subunit of the receptor. The activation of IR results in the subsequent phosphorylation of intracellular substrates, leading to various biological processes including the regulation of glucose uptake[3].

One of important phosphatases, protein tyrosine phosphatase 1B (PTP1B), which is considered critical in the protein tyrosine phosphatases (PTPs) family, regulates the insulin signaling pathway. PTP1B can interact with and dephosphorylate the insulin receptor as well as insulin receptor substrate (IRS)[4]. The studies *in vivo* for the PTP1B-null mice have shown that PTP1B knocked-out resulted in the obesity resistance and the increase in the insulin sensitivity, and the mice were found to be very healthy[5]. Therefore, PTP1B has been considered promising as an insulin-sensitive drug target for the prevention and the treatment of insulin-based diseases[6].

*Ganoderma lucidum* (*G. lucidum*) is one of the oldest mushrooms used to treat many ailments in ancient Chinese medicine, and widely cultivated nowadays. During the past three decades, *G. lucidum* has also been found to have various biological activities, including immunomodulatory, antitumor, antioxidate and anti-inflammatory activities[7-9]. Effects of the active extracts of *G. lucidum* on the plasma glucose level *in vivo* have also been reported[10,11]. For instance, Mohammed et al[11] found that the doses of 500 and 1000 mg/kg of the aqueous extract of *G. lucidum* administered intraperitoneally significantly ($p < 0.05$) decrease the blood glucose levels of alloxan diabetic Wistar rats in 4, 8 and 24 h. As the important biologically active components in *G. lucidum*, polysaccharide and proteoglycans are exciting a great interests for their essential roles in many molecular processes[12,13]. The studies indicated that the polysaccharide of *G. lucidum* could promote the release of serum insulin, regulate the activity of various enzymes participating in the glucose metabolism and decrease the plasma glucose *in vivo*[13].

In our previous work[14], a novel PTP1B activity inhibitor, named *FYGL* (Fudan-Yueyang-*G. lucidum*), was screened from the fruiting bodies of *G. lucidum*. *FYGL* is a water-soluble macromolecular proteoglycan and shows an efficient PTP1B inhibitory potency with $IC_{50} = 5.12 \pm 0.05$ μg/mL *in vitro*. The type 2 diabetic mice treated orally by *FYGL* for 4 weeks showed a decrease in the plasma glucose level compared with that of the diabetic controls without drug treatment.

In the present work, the hypoglycemic mechanisms *in vivo* of the *FYGL* were investigated on the study of the expression and activity of PTP1B, and the tyrosine phosphorylation level of IR β-subunit in the livers and soleus muscles of the T2DM rats, in addition to the study of the plasma glucose level and the serum insulin concentration.

## Materials and Methods

### Materials

All the dried fruiting bodies of *G. lucidum* were purchased from Shanghai Leiyunshang Pharmaceutical Co. Ltd. (Shanghai, China). Sprague-Dawley rats were bought from the experimental animal center of Shanghai University of Traditional Chinese Medicine. The kit for the analysis of insulin was purchased from Beifang Biotech Research Center (Beijing, China). The kits for the analysis of free fatty acid (FFA), triglyceride (TG), total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C) and high density lipoprotein-cholesterol (HDL-C) were purchased from Nanjing Jianchen Bioengineering Institute (Nanjing, China). Streptozotocin (STZ), metformin, bovine serum albumin (BSA), porcine insulin, *p*-nitrophenyl phosphate (*p*NPP), Tris, nonidet P-40 and nitrocellulose (NC) membranes were purchased from Sigma Chemical Co. (St Louis, MO, USA). Rosiglitazone maleate was from GlaxoSmithKline (Guangzhou, China). The reagents for the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting experiments were purchased from BioVision (Palo Alto, CA, USA) and the necessary apparatus from Bio-Rad (Richmond, VA, USA). Protein A-Sepharose was from Pharmacia (Uppsala, Sweden). α-PTP1B polyclonal antibody, rabbit polyclonal anti-insulin receptor β-subunit (IRβ) antibody and monoclonal anti-phosphotyrosine antibody (PY99) were purchased from Santa Cruz Biotechnology Inc (Santa Cruz, CA, USA). The anti-rabbit immunoglobulin G (IgG) conjugated with the horseradish-peroxidase and the enhanced chemiluminescence (ECL) detection reagents were from GE Inc (USA). Bradford protein assay reagent was purchased from Bio-Rad (Richmond, VA, USA). All other chemicals were of the highest analytical grade.

### Preparation of FYGL from G. lucidum

*FYGL* was extracted from the fruiting bodies of *G. lucidum* as described in detail in our previous work[14]. Briefly, the dried fruiting bodies of *G. lucidum* (160 g) were milled and then degreased with boiling ethanol, and then decocted with boiling water. After filtration of the decocted mixture, the residues were treated with 2 L of 2 M aqueous ammonia solution for 24 h at room temperature, then the mixture was filtered, and the supernatant was neutralized by 2 M acetic acid and then dialyzed, concentrated and treated with 5-fold volumes of ethanol for the precipitation. The supernatant was subjected to a Sephadex G75 column chromatography with 2 M sodium chloride solution as the eluent. The eluates (6 mL/tube) were collected and characterized by the phenol-sulfuric acid method with ultraviolet (UV) absorption at wavelength of 490 nm[15]. There were 300 mL (50 tubes) of solutions collected in all, and the elution volumes of 91-126 mL which have relatively high UV absorption were combined together, and then dialyzed and lyophilized, and 1.54 g of *FYGL* (yield of 0.96%) was obtained. Gel permeation chromatography (GPC) analysis of *FYGL* with double detectors of UV and refractive index indicates that *FYGL* is formed covalently by polypeptide and polysaccharide, and the purity of *FYGL* is about 91%[14].

The previous work[14] demonstrated that the water-soluble *FYGL* is a macromolecular proteoglycan with viscosity-average molecular weight of $2.6 \times 10^5$. The major monosaccharide of *FYGL* is glucose, and major amino acids are aspartic acid, glycine, glutamic acid, alanine, serine and threonine. The ratio of protein to polysaccharide is 17:77.

### Type 2 Diabetic Rat Model and Treatment Protocol

Seventy male Sprague-Dawley rats, 8 weeks of age and approximately 200 g of weight, were selected for the trails. All animal trial procedures instituted by Ethical Committee for the Experimental Use of Animals in Shanghai University of Traditional Chinese Medicine were followed. Ten rats were fed normal foods under normal environment as a normal group, and the other 60 rats were housed 5 in a cage with a 12:12 hours of light/dark cycle at ambient temperature of 22~25°C and fed a high-fat diet. After 5 weeks, the 60 rats were fasted for 12 h, but had free access to water, and then injected with STZ (40 mg/kg in 0.1 M citrate-buffered saline, pH 4.5) into the intraperitoneal to induce type 2 diabetes. The STZ-treated rats had free access to high-fat foods and water for 1 week and were subjected to 12 h of fasting. A total of 50 among the 60 STZ-treated rats showed fasting glucose levels of ≥16.7 mmol/L and were considered to be type 2 diabetic rats.

Sixty animals, including 10 normal rats and 50 STZ-induced type 2 diabetic rats, were divided into six groups (numbered as groups 1-6) with 10 rats in each group. Group 1 are normal rats that were given 20 mL/kg physiological NaCl solution (vehicle); group 2 are T2DM rats (control), which were also given 20 mL/kg physiological NaCl solution (vehicle). However, the rats in group 3 and 4 were diabetic and orally treated with 40 and 120 mg/kg doses of *FYGL*, respectively, once a day. The rats in group 5 and 6 were diabetic and treated with 250 mg/kg and 3 mg/kg doses of the clinical oral drugs, metformin and rosiglitazone, respectively, once a day. All of the animals in the different groups were fed normal daily foods in addition to the given drugs during the trial. Before killed, half of the saline- and *FYGL*-treated animals administered intraperitoneally the insulin (5 U/kg) in saline with 0.1% bovine serum albumin (BSA). Ten minutes late, the livers and soleus muscles from all the killed animals were taken out and stored in refrigerator at −70°C for uses.

### Measurement of Plasma Glucose and Insulin Levels

The plasma samples for the plasma glucose analysis were obtained from the tail vein 2 h before the oral administration of the drugs in every 10 days. Glucose level in plasma was measured using glucose oxidase method (sensitivity of 0.1 mM, Sigma Diagnostics, St. Louis, MO). The last fasting plasma samples were obtained by celiac puncture under halothane anesthesia before the rats were killed and then centrifuged (3000 × g, 15 min) at 4°C for separating the serum, and the insulin levels in the separated serum were determined by radio-immunoassay (RIA) method.

### Lysate Preparation and Protein Assay in vivo

The frozen liver tissue (50 mg) was homogenized in 2 mL lysis buffer containing 20 mmol/L Tris-HCl (pH 7.4), 1% Triton X-100, 10% glycerol, 150 mmol/L NaCl, 2 mmol/L

edetic acid, 25 mmol/L β-glycerophosphate, 20 mmol/L sodium fluoride, 1 mmol/L sodium orthovanadate, 2 mmol/L sodium pyrophosphate, 10 mg/L leupeptin, 1 mmol/L benzamidine, 1 mmol/L 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride and 1 mmol/L microcystin, and rocked for 40 min at 4°C. The homogenate was centrifuged (12000 × g, 15 min) at 4°C. The muscle sample (50 mg) in 50 mmol/L ice-cold Hepes buffer (pH 7.4) containing 150 mmol/L NaCl, 10 mmol/L sodium pyrophosphate, 2 mmol/L $Na_3VO_4$, 10 mmol/L NaF, 2 mmol/L edetic acid, 2 mmol/L phenylmethylsulfonyl fluoride (PMSF), 5 mg/L leupeptin, 1% Nonidet P-40, and 10% glycerol was homogenized and the homogenate was centrifuged (12000 × g, 15 min) at 4°C.

The supernatant homogenate from the trailed rat tissue was collected individually, and the protein concentration in the homogenate was measured with Bradford protein assay reagent, using bovine serum albumin (BSA) as the standard.

### Western Blotting for PTP1B Expression Analysis in vivo

Liver or muscle supernatant homogenate containing 20 μg protein was run on SDS-PAGE (10% gel) and transferred electrophoretically onto the nitrocellulose (NC) membrane for 5 h. The NC membrane was then blocked for 2 h at room temperature with the block solution provided in the ECL kits. The NC membrane was incubated with anti-α-PTP1B polyclonal antibody overnight at 4°C. The NC membrane was then washed for 30 min with wash solution (provided in ECL kits), followed by 1 h incubation with anti-rabbit IgG conjugated with the horseradish-peroxidase in block solution. The NC membrane was washed for 30 min with wash solution, and the immunoreactive bands were detected by the enhanced chemiluminescence method.

### PTP1B Activity Analysis in vivo

The assay protocol of PTP1B activity in the tissue homogenate was followed on the instruction. Briefly, the PTP1B activity was measured by adding 50 μL of 1 mM *p*-nitrophenyl phosphate (*p*NPP, as substrate) buffer solution (pH 8.0, containing 50 mM Tris and 150 mM NaCl) into 50 μL of 100 μg/mL tissue supernatant homogenate. After incubation for 30 min at 37°C, the PTP1B enzyme reaction was terminated with 200 μL of 3 M NaOH. The amount of produced *p*-nitrophenol (*p*NP) was measured by UV absorption at wavelength of 405 nm with a microplate reader. The higher the UV value of *p*NP, the better the PTP1B activity is.

### Tyrosine Phosphorylation Level of IR β-subunit in vivo

Liver or muscle supernatant homogenate containing 1 mg protein was immunoprecipitated with 2 μg anti-IR β-subunit antibody coupled to protein A-Sepharose overnight at 4°C. The immune complex was washed three times by phosphate-buffered saline (PBS, pH 7.4) containing 1% Nonidet P-40 and 2 mmol/L $Na_3VO_4$, and resuspended in the Laemmli buffer, and boiled for 5 min. The protein was quantified and run on SDS-PAGE (10% gel), and then electrotransferred from the gel to the NC membrane. The NC membrane was incubated with 1 mg/L PY99 overnight at 4ºC. The following steps were performed as described in section of Western blotting for PTP1B expression analysis *in vivo*.

### Measurement of FFA and Lipid Profile in Serum

FFA, TG, TC, LDL-C and HDL-C levels in the serum from the last fasting blood samples were measured following the commercial kit's instructions.

### Statistical Analysis

Data are expressed as mean ± standard deviation (SD). The statistical significance in the behavioral and biochemical effects was determined by one-way analysis of variance (ANOVA). A possibility of the *p* value less than 0.05 or 0.01 was considered as the significant or very significant difference between the means.

## Results

### Characteristics of Trailed Animals

It is found that the antihyperglycemic potency of *FYGL* shows the dose- and time- dependency against T2DM control rats as shown in Figure 1. High dose (120 mg/kg) of *FYGL* and long duration (30 days) of treatment have the better drug potency, and show the comparable potency with the commercial clinical drugs of metformin and rosiglitazone which were as the positive controls. The trail results in 30 days of drug treatments are summarized in Table I.

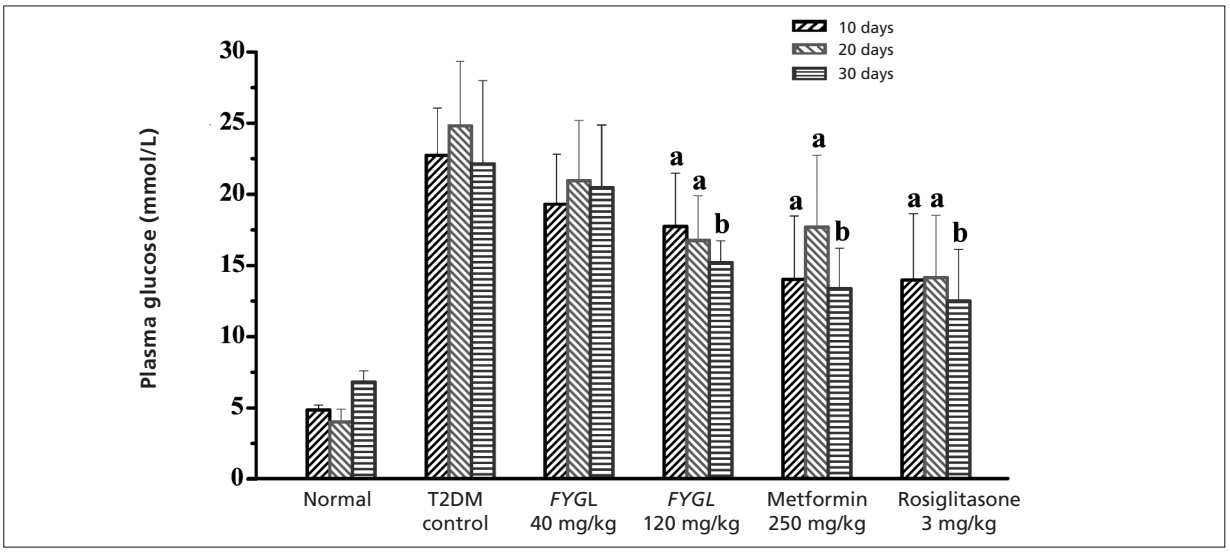


**Figure 1.** Potencies of *FYGL*, metformin and rosiglitasone on the plasma glucose *in vivo*. The drugs were administered orally once a day for 30 days. Data were expressed as mean ± standard deviation (n = 10), a: $p < 0.05$ vs T2DM control rats; b: $p < 0.01$ vs T2DM control rats.

Observed from Table I, the fasting plasma glucose level is significantly higher ($p < 0.01$) in the STZ-induced T2DM control rats (22±5 mmol/L) than that in normal rats (6.8±0.7 mmol/L). The level of fasting plasma glucose in T2DM control rats was significantly decreased (15±1 mmol/L, $p < 0.01$) after the rats were treated by *FYGL* in high dose for 30 days, compared with that (22±5 mmol/L) in the T2DM rats without the drug treatment. The decrease in the plasma glucose level in the *FYGL* treatment group (15±1 mmol/L) in high dose of 120 mg/kg is comparable statistically with that in the metformin treatment group (13±2 mmol/L) in dose of 250 mg/kg and that in the rosiglitazone treatment group (12±3 mmol/L) in dose of 3 mg/kg.

It is also noted in Table I that the serum insulin concentration in the high dose of *FYGL*-treated T2DM rats (105±12 $\mu$IU/mL) is significantly higher ($p < 0.01$) than that in the T2DM control rats (87±10 $\mu$IU/mL). In addition, there is no significant difference in the body weights among the T2DM control rats and the *FYGL*-, metformin- and rosiglitazone-treated T2DM rats.

### *Effects of FYGL on the PTP1B Expression in Livers and Muscles in vivo*

PTP1B levels in the livers and skeletal muscles of the T2DM rats were significantly increased by 29% ($p < 0.05$) and 35% ($p < 0.05$), respectively, compared with those of normal rats. While when the T2DM rats were treated by high

**Table I.** Characteristics of the trailed animals after 30 days of drug treatments[a].

| Group | Plasma glucose (mmol/L) | Insulin (μIU/mL) | Body weight (g) |
|---|---|---|---|
| Normal | 6.8 ± 0.7 | 121 ± 7 | 514 ± 23 |
| T2DM control | 22 ± 5[b] | 87 ± 10[b] | 347 ± 38[b] |
| FYGL 40 mg/kg | 20 ± 4b | 101 ± 12[b,c] | 343 ± 35[b] |
| FYGL 120 mg/kg | 15 ± 1[b,d] | 105 ± 12[b,d] | 363 ± 36[b] |
| Metformin 250 mg/kg | 13 ± 2[b,d] | 100 ± 14[b,c] | 373 ± 34[b] |
| Rosiglitazone 3 mg/kg | 12 ± 3[b,d] | 95 ± 9[b] | 361 ± 31[b] |

[a]The data were expressed as mean ± standard deviation (n = 10), [b]$p < 0.01$ vs normal group; [c]$p < 0.05$ vs T2DM control group; [d]$p < 0.01$ vs T2DM control group.

dose of *FYGL*, the PTP1B level in the livers was not affected (Figure 2-A, 2-A'), but significantly reduced by 19% ($p < 0.05$) in the skeletal muscles, compared with that of the T2DM control rats (Figure 2-B, 2-B').

### *Effects of FYGL on the PTP1B Activity in Livers and Muscles in vivo*

PTP1B activities in the livers and skeletal muscles of T2DM rats were increased by 30% ($p < 0.05$) (Figure 3-A) and 20% ($p < 0.05$) (Figure 3-B), respectively, compared with that of the normal rats. While when the T2DM rats were treated by high dose of *FYGL*, the PTP1B activities in the livers were not affected (Figure 3-A), but significantly decreased by 22% ($p < 0.05$) in the skeletal muscles (Figure 3-B), compared with that in the T2DM control rats.

### *Effects of FYGL on the Tyrosine Phosphorylation Level of IR β-subunit in vivo*

In general, the insulin stimulation *in vivo* results in the increase in the tyrosine phosphorylation level of the IR β-subunit, which is shown as "+" columns in Figure 4. Therefore, the tyrosine phosphorylation level of the IR β-subunit in the normal rats stimulated by the insulin (+) is referred as 100%. It is observed that the tyrosine phosphorylation levels of IR β-subunit are 62% in the livers (Figure 4-A, 4-A') and 41% ($p < 0.01$ *vs* normal rats) in the skeletal muscles (Figure 4-B, 4-B') of the T2DM control rats after stimulation with insulin. When the T2DM rats are treated by high dose of *FYGL*, in the liver, the tyrosine phosphorylation level (73%) of the *FYGL*-treated rats has no significant difference from that (62%) of the T2DM control rats, shown in Figure 4-A and 4-A', but in the skeletal muscle, the level (89%) of the *FYGL*-treated rats is significantly higher ($p < 0.01$ *vs* T2DM control rats) than that (41%) of the T2DM control rats, shown in Figure 4-B and 4-B'.

### *Effects of FYGL on the Serum Lipid Profile in vivo*

The effects of drugs including *FYGL*, metformin and rosiglitazone on the serum lipid profile *in vivo* after 30 days of treatment are summarized in Table II. FFA, TG, TC and LDL-C levels were significantly decreased by 30.1% ($p < 0.05$), 29.4% ($p < 0.05$), 26.2% ($p < 0.05$) and 39.9% ($p < 0.05$), while the HDL-C level was significantly increased by 112% ($p < 0.05$) for the 120 mg/kg dose of *FYGL*-treated T2DM rats after 30 days, compared with those for the T2DM control rats. It is also observed that the FFA, TG, TC and LDL-C levels were decreased by 15.5%, 21.7% ($p < 0.05$), 15.3% and 25.7% ($p < 0.05$) for the 250 mg/kg dose of metformin-

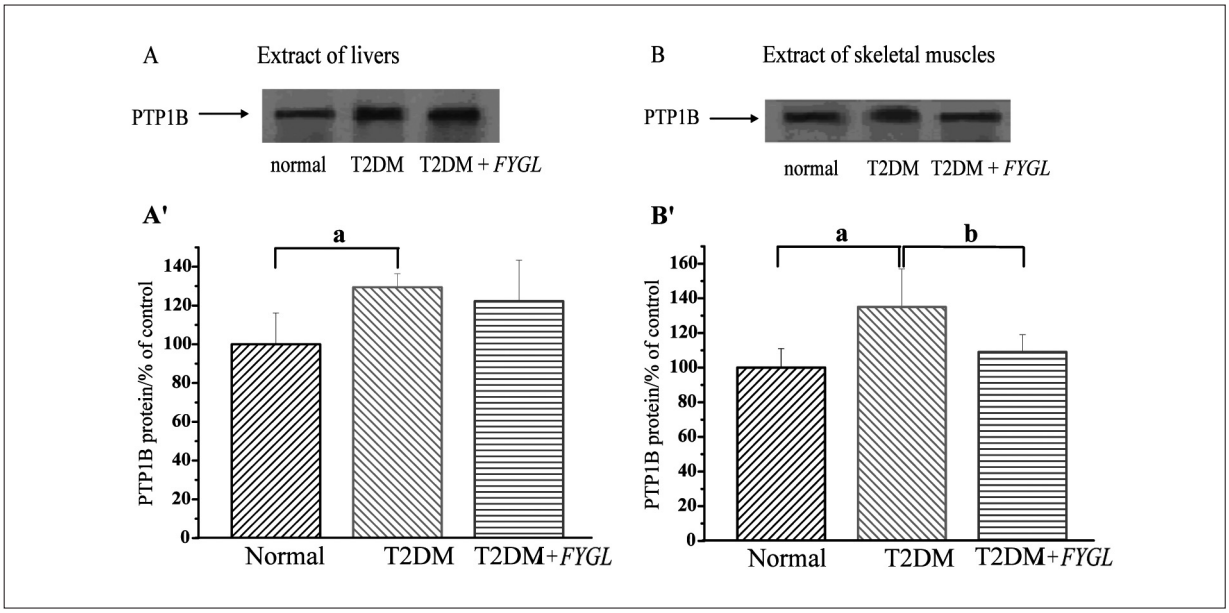


**Figure 2.** Effects of high dose of *FYGL* on PTP1B expression in the livers (*A*, *A'*) and skeletal muscles (*B*, *B'*) *in vivo*. The data were expressed as mean ± standard deviation (n = 10), referred to the normal rats as 100%, a: $p < 0.05$ vs normal rats; b: $p < 0.05$ vs T2DM control rats.

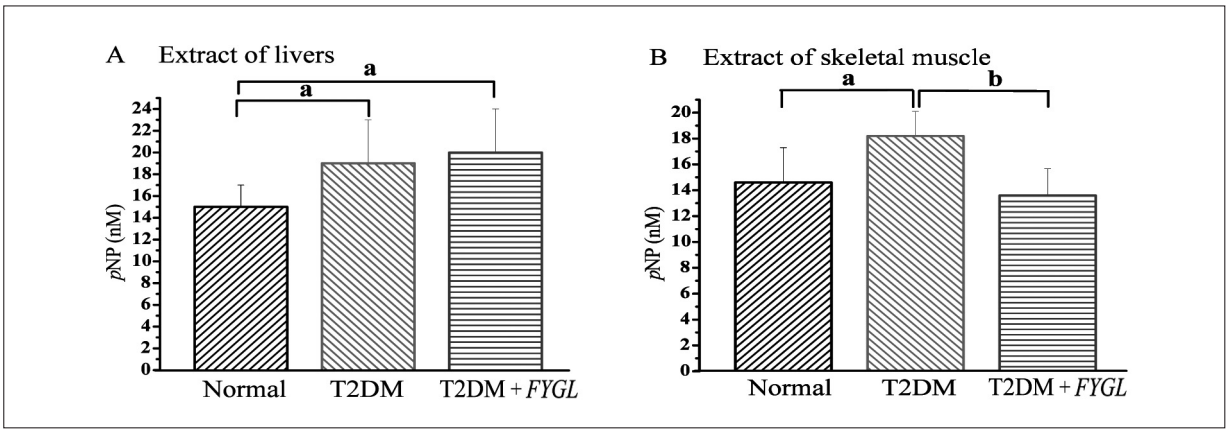


**Figure 3.** Effects of high dose of *FYGL* on PTP1B activities in livers (*A*) and skeletal muscles (*B*) *in vivo*. Data were expressed as mean ± standard deviation (n = 10), a: $p < 0.05$ vs normal rats; b: $p < 0.05$ vs T2DM control rats.

treated T2DM rats, and by 8.7%, 10.9%, 12.2% and 16.9% ($p < 0.05$) for the 3 mg/kg dose of rosiglitazone-treated T2DM rats, compared with those for the T2DM control rats. Meanwhile, the HDL-C levels were increased by 52% ($p < 0.05$) and 80% ($p < 0.05$) for the metformin- and rosiglitazone- treated T2DM rats, respectively, compared with that for the T2DM control rats.

## Discussion

### PTP1B Target and its Inhibitors and Functional Mechanism

In this study, we observed that PTP1B expression and activity were increased in the liver and skeletal muscle tissues of the STZ-induced T2DM rats. Similarly, Tagami et al[16] found that PTP1B

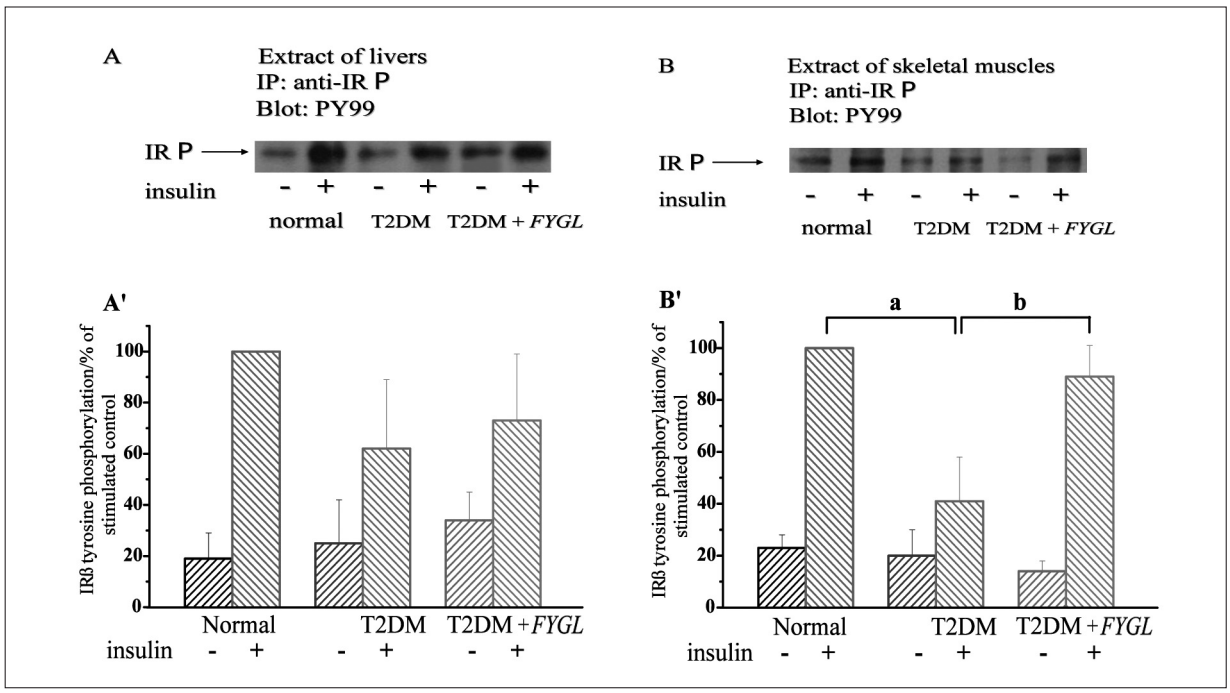


**Figure 4.** Effects of *FYGL* on the insulin-stimulated tyrosine phosphorylation levels of IR β-subunit in livers (*A*) and skeletal muscles (*B*). The quantitative analysis of the relative IR β-subunit tyrosine phosphorylation levels in livers (*A'*) and skeletal muscles (*B'*) of high dose of *FYGL*-treated rats are referred to that of the insulin-stimulated normal rats. '-': without insulin stimulation; '+': with insulin stimulation. Data were expressed as mean ± standard deviation (n = 5), a: $p < 0.01$ vs normal rats; b: $p < 0.01$ vs T2DM control rats.

**Table II.** Effects of the drugs on the FFA and serum lipid profile in vivo after 30 days of treatment[a].

| Group | FFA (mmol/L) | TG (mmol/L) | TC (mmol/L) | LDL-C (mmol/L) | HDL-C (mmol/L) |
|---|---|---|---|---|---|
| Normal | 0.69 ± 0.13 | 1.33 ± 0.34 | 1.62 ± 0.19 | 0.85 ± 0.07 | 0.78 ± 0.25 |
| T2DM control | 1.03 ± 0.27 | 3.13 ± 0.46 | 2.29 ± 0.38 | 1.83 ± 0.42 | 0.25 ± 0.11 |
| FYGL 40 mg/kg | 0.83 ± 0.26 | 2.85 ± 0.52 | 2.02 ± 0.34 | 1.50 ± 0.27 | 0.34 ± 0.14 |
| FYG 120 mg/kg L | 0.72 ± 0.17[b] | 2.21 ± 0.46[b] | 1.69 ± 0.25[b] | 1.10 ± 0.33[b] | 0.53 ± 0.12[b] |
| Metformin 250 mg/kg | 0.87 ± 0.24 | 2.45 ± 0.42[b] | 1.94 ± 0.33 | 1.36 ± 0.24[b] | 0.38 ± 0.11[b] |
| Rosiglitazone 3 mg/kg | 0.94 ± 0.22 | 2.79 ± 0.34 | 2.01 ± 0.2 | 1.52 ± 0.32[b] | 0.45 ± 0.09[b] |

[a]The data were expressed as mean ± standard deviation (n = 10); [b]$p < 0.05$ vs T2DM control group.

activity was increased in the skeletal muscles of the Otsuka Long-Evans Tokushima fatty (*OLETF*) rats. Ahmad and Goldstein[17] also reported that PTP1B activity was increased in the muscles of the genetic insulin-resistant obese (*fa*/*fa*) and diabetic (ZDF/Drt-*fa*/*fa*) Zucker rats. However, finding a selective small molecule phosphatase inhibitor with good cell permeability and oral bioavailability *in vivo* has been difficult[18]. Significant medicinal chemistry efforts have been carried out since the late 1990s, and a few of synthetic PTP1B inhibitors were found potential *in vivo*[18,19]. Fukuda et al[19] found the monosodium({[5-(1,1-dimethylethyl)thiazol-2-y-l]methyl}{[(4-{4-[4-(1-propylbutyl)phenoxy]methyl}phenl)thiazol-2-yl]meth-yl}amino)acetate (named JTT-551), a synthetic small molecule PTP1B inhibitor, showed an inhibitory effect on the PTP1B *in vitro*. The administration of JTT-551 for both genetic diabetic *ob*/*ob* and *db*/*db* mice enhanced the insulin receptor phosphorylation of the livers and decreased the glucose level and had no increase in the body weight. In addition, the small molecular PTP1B inhibitors extracted from the plants were also reported. Lu et al[20] found that total flavonoids of *Litsea Coreana* (*TFLC*), an alcohol extract from the dried leaves of the *Litsea Coreana*, could decrease the PTP1B expression in diabetic rat livers.

*FYGL*, a natural macromolecular product, could decrease PTP1B expression and inhibit PTP1B activity in the skeletal muscles of the T2DM rats, leading to the increase in the tyrosine phosphorylation level of the IR β-subunit in the skeletal muscles, therefore, decreasing the plasma glucose. The results suggest that the antihyperglycemic mechanism of *FYGL* is possibly that: *FYGL* changes PTP1B expression and activity in the skeletal muscles, therefore, regulating the tyrosine phosphorylation level of the IR β-subunit, and consequently controlling the plasma glucose. The similar results were also found from one PTP1B inhibitor extracted from *Astragalus*[21].

### *FYGL Effects on the Serum Insulin and Pancreatic β-cells*

In this work, the STZ-induced type 2 diabetic mellitus rat model with high fatty diets was used to evaluate the antihyperglycemic potency of *FYGL in vivo*. The HFD/STZ-induced T2DM rats are not extremely obese and have the metabolic characteristics of the type 2 diabetic patients[22]. STZ, a highly cytotoxic reagent of pancreatic β-cells, induces the diabetes by damaging the pancreatic β-cells, leading to the decrease in the insulin release[23]. From Table I, the serum insulin concentration is significantly higher in the high dose of *FYGL*-treated T2DM rats than that in the T2DM control rats, comparable with that in the rats treated by the commercial clinical drug of metformin which was reported capable of inhibiting the pancreatic β-cell apoptosis and preserving the β-cell function[24]. Therefore, *FYGL* is maybe able to recover the pancreatic β-cells, leading to the release of the serum insulin. Similarly, Eidi et al[25] found that the garlic extract significantly decreased serum glucose level, while increased serum insulin level in STZ-induced diabetic rats but not in normal rats ($p < 0.05$). Akhani et al[26] studied the effect of the juice of *Z. officinale* (4 mL/kg, p.o. daily) for 6 weeks on STZ-induced type 1 diabetic rats and found that treatment with *Z. officinale* produced a significant increase in insulin level and a decrease in fasting glucose level in diabetic rats. Shirwaikar et al[27] found that an aqueous extract from the bark of *Garuga pinnata* Roxb (GP) could significantly increase the serum insulin level and decrease the fasting blood glucose level, and recover the pancreas islet cells near to the normal values.

### More Functions of FYGL

From Table I, it is found that there is no significant difference in the body weight between T2DM control rats and the *FYGL*-treated T2DM rats. The STZ-induced T2DM rats are generally not obese due to the increase in the muscle waste and the tissue protein loss[28]. Similarly, we observed from Table I that the weights of STZ-induced T2DM rats are significantly lower than those of normal rats, while the *FYGL*-treated T2DM rats neither recover nor lose the body weights, which maybe imply that *FYGL* could either control the muscle waste and the tissue protein loss or inhibit the appetite. The similar result was also reported by Adeneye and Agbaje[29]. Their results showed the fresh leaf aqueous extract from *Cymbopogon citratus Stapf* lowering the fasting plasma glucose and inhibiting the appetite.

In general, for the diabetic patients, hyperglycemia is accompanied with the dyslipidemia[30], that is, FFA, TG, TC, LDL-C are increased and HDL-C is decreased, they are the important plasma biochemistry indexes for the metabolic disorders. The abnormal values of those indexes indicate the characteristics of metabolic disorder. From Table II, it is found that there are higher values of FFA, TG, TC, LDL-C and lower value of HDL-C in the T2DM control rats, while *FYGL* can decrease considerably not only the plasma glucose level *in vivo*, but also the FFA, TG, TC and LDL-C levels, as well as increase the HDL-C level. Although the commercial clinical drugs of metformin and rosiglitazone have also the similar results, the efficiency of *FYGL* is considerably better than that of those of clinical drugs. We noted that Saenz et al[31] reported that metformin could decrease clinically the TG, TC, LDL-C levels of the T2DM patients, but not affect the HDL-C. Miyazaki and DeFronzo[32] reported that rosiglitazone participated in the regulation of fatty acid metabolism and increased clinically the HDL-C and LDL-C levels of the T2DM patients, but had no effect on the TG. Our results are somewhat different from those reports possible due to the STZ-induced diabetic rats different from human beings.

In conclusion, *FYGL*, screened from *G. lucidum*, is an efficient PTP1B inhibitor *in vivo*, therefore can decrease the plasma glucose level through inhibiting the PTP1B expression and activity, consequently, regulating the tyrosine phosphorylation level of the IR β-subunit. In addition, *FYGL* can decrease FFA, TG, TC and LDL-C levels as well as increase the HDL-C level *in vivo*. *FYGL* is promising to be used as a drug candidate for the therapy of the type 2 diabetes and the accompanied metabolic disorders.

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