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# Antioxidation, anti-hyperglycaemia and renoprotective effects of extracellular polysaccharides from *Pleurotus eryngii* SI-04

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ABSTRACT

The present study was designed to investigate the anti-hyperglycaemia, hypolipidemia and renoprotective effects of two extracellular polysaccharides (EPS) from the broth of *Pleurotus eryngii* SI-04 (EPS1 and EPS2) in mice with streptozotocin (STZ)-induced diabetic nephropathy (DN). The results showed that both EPS1 and EPS2 exhibited potential anti-DN effects, including significantly decreasing GLU levels; suppressing ALB, BUN, CRE and UA levels; reducing serum lipid (TC, TG, VLDL-C and LDL-C) levels; improving renal antioxidant status (GSH-Px, SOD, CAT and MDA); and attenuating pathological nephropathy damage. These results demonstrated that both EPS1 and EPS2 exhibited potential antioxidant, anti-hyperglycaemic, and renoprotective effects against STZ-induced DN, indicating that *P. eryngii* SI-04 could be used as a functional food and natural drug. In addition, the monosaccharide compositions and bond types of these two polysaccharides were analysed.



## 1. Introduction

Diabetic nephropathy (DN), a common microvascular complication of diabetes mellitus (DM), affects approximately one-third of diabetes patients and increases the morbidity and mortality of diabetes patients worldwide [1,2]. The main pathology of DN is characterized by a series of renal abnormalities, including renal cell necrosis, basement membrane thickening, mesangial expansion, glomerulosclerosis and tubulointerstitial fibrosis [3]. Experimentally, streptozotocin (STZ) is the toxic substance used for establishing DN mouse models. The mechanism through which STZ induces hyperglycaemia may primarily be its bio-toxicity to pancreatic β-cells, which disturbs the homeostasis of blood glucose and leads to a series of complications [4,5].

The clinical therapeutic strategies for treating DN usually involve controlling the blood glucose levels and blood pressure; however, the results of treatments that aim to halt the progression of renal dysfunction have been clinically non-significant [6,7]. Moreover, the synthetic drugs used for the treatment of DN are not practical for long-term use due to side effects, drug dependence and exorbitant prices. Therefore, there is a need for natural and novel therapeutic agents that can be exploited for preventing and treating DN [8]. Previous studies have demonstrated that a complex interplay of numerous factors, including glucolipid metabolism derangements, renal hemodynamic changes, inflammation, oxidative stress and lipid mediators, are involved in the progress of DN, but the detailed mechanisms underlying these processes are still unclear [7,9]. Increasing evidence has shown that many abnormal physiological responses, including autoxidation of glucose, formation of advanced glycation end products (AGEs), and metabolic stress resulting from glycometabolic dysfunction in the kidneys, in the diabetic condition can boost the production of reactive oxygen species (ROS) [10]. Excessive ROS can directly reduce the activities of antioxidative enzymes, destroy membrane lipids, cause vascular endothelium abnormalities and enhance growth factor levels, thereby promoting renal cell proliferation and hypertrophy, which initiates and accelerates the development of DN [11]. Interestingly, appropriate antioxidant treatment has been shown to improve the damage caused by DN by scavenging ROS and alleviating oxidative stress [12]. Additionally, dyslipidaemia, a common pathological status in DN patients, is always considered to be a risk factor for the progression of DN [13]. DN patients clinically exhibit multiple lipid abnormalities during the development and progression of nephropathy, such as decreased levels of high-density lipoprotein (HDL-C) as well as increased levels of very low-density lipoprotein (VLDL-C), low-density lipoprotein

*Abbreviations:* AGEs, advanced glycation end products; ALB, albumin; Ara, arabinose; BUN, urea nitrogen; CRE, creatinine; CAT, catalase; DM, diabetic mellitus; DN, diabetic nephropathy; EPS, extracellular polysaccharides; ESRD, end-stage renal diseases; FT-IR, Fourier translation infrared spectrum; Gal, galactose; GC, gas chromatography; Glu, glucose; GLU, blood glucose; GSH-Px, GSH peroxide; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Man, mannose; MDA, malondialdehyde; NMR, nuclear magnetic resonance; Rha, rhamnose; Rib, ribose; ROS, reactive oxygen species; SOD, superoxide dismutase; STZ, streptozotocin; TC, total cholesterol; TFA, trifluoroacetic acid; TG, triacylglycerols; UA, uric acid; UV, ultraviolet; Xyl, xylose.

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(LDL-C), triglycerides (TC) and cholesterol (TG) [14,15]. Spencer et al. [13] demonstrated that hyperlipidaemia and hyperglycaemia act synergistically to exacerbate DN. Therefore, given the multifactorial causes of DN, it is necessary to find an alternative agent with abundant bioactivities, including antioxidation, anti-hyperglycaemia, anti-hyperlipidaemia and nephric protection, for the treatment of DN.

Edible mushrooms have become increasingly popular, not only due to their excellent taste but also due to their nutritional value and health-promoting properties [16]. Owing to their physiologically beneficial bioactive compounds, edible mushrooms have been regarded as a treasured source of natural medicine and functional food for thousands of years [16,17]. Polysaccharides from mushrooms have gained increasing attention for their various bioactivities, such as immunoregulation, antioxidant, anti-aging, anti-obesity, anti-diabetes, anticancer, antibiotic and hepatoprotective effects [16,18,19]. Hence, it seems practical to investigate edible mushrooms for the presence of potential anti-hyperglycaemic polysaccharide-derived substances that could be used to prevent and treat DN based on their antioxidant and pre-oxidant properties [18]. One type of edible mushroom, *Pleurotus eryngii* (*P. eryngii*), contains many biologically active substances, such as polysaccharides, lipids, peptides and sterols [20]. Several studies focusing on the biological activities of polysaccharides from the mycelia, residue and fruiting body by *P. eryngii* have been published. Ma et al. have demonstrated the antitumor activities of polysaccharides from *P. eryngii* residue [17]. Chen et al. have proved that the polysaccharide from the *P. eryngii* fruit body showed potential hypolipidaemic and hypoglycaemic activities [21]. Our previous work has demonstrated the polysaccharide from mycelia of *P. eryngii* exhibited antioxidant activity and hepatoprotective effects on mice with $CCl_4$-induced acute liver damage [22]. However, the extracellular polysaccharides (EPS) extracted from the fermentation broth of *P. eryngii* have been paid scare attention.

Based on previous studies on polysaccharides from *P. eryngii*, the present study was designed to investigate the antioxidant, anti-hyperglycaemia, anti-hyperlipidaemia and renoprotective effects of EPS from *P. eryngii* SI-04 in mice with STZ-induced DN. Moreover, the monosaccharide compositions and bond types of the EPS were also analysed.

## 2. Materials and methods

### 2.1. Ethics statement

All methods and experimental protocols were submitted to and approved by the ethics committee of Shandong Agricultural University, and all protocols were performed in accordance with the Animals (Scientific Procedures) Act of 1986 (amended in 2013).

### 2.2. Materials

The diagnostic kits for analysing superoxide dismutase (SOD) activity, glutathione peroxidase (GSH-Px) activity, catalase (CAT) activity and malondialdehyde (MDA) content were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The standard monosaccharide samples, including rhamnose (Rha), ribose (Rib), arabinose (Ara), xylose (Xyl), glucose (Glc), mannose (Man) and galactose (Gal), were provided by Merck Company (Darmstadt, Germany) and Sigma Chemical Company (St. Louis, USA). Other reagents and chemicals used in present work were analytical reagent grade and supplied by local chemical suppliers.

### 2.3. Preparation and purification of EPS

The strain of *P. eryngii* SI-04 was provided by the Fungi Institute of Academy of Agricultural Sciences (Tai'an, China). The liquid fermentation technology was described in our previous work [22]. After centrifugation for 15 min (3000 rpm), the fermentation broth supernatant was mixed with 3 volumes of 95% ethanol (v/v), then stirred sufficiently and stored at 4 °C overnight. The resulting precipitate was centrifuged (3000 rpm, 15 min) and lyophilized by vacuum freeze drying (Labconco, USA) to obtain EPS.

An EPS solution was prepared (1 g of EPS dissolved in 10 mL of distilled water at 80 °C) and filtered through a 0.22-μm membrane filter and infused into a DEAE-cellulose column (26 mm × 400 mm), which was eluted with NaCl solutions at concentrations of 0, 0.2, 0.3 and 0.5 mol/L at a flow rate of 1.0 mL/min. The carbohydrate contents were analysed spectrophotometrically using the phenol–sulfuric acid method [23]. The major eluate was collected separately and further purified by gel permeation chromatography (Sephadex G-100 column AQ3, 1.3 cm × 50 cm). The main fractions were lyophilized by vacuum freeze drying (Labconco, USA) for structural analysis and animal experiments.

### 2.4. Assays testing the inhibition of α-amylase and α-glucosidase activity

EPS1 and EPS2 were dissolved in phosphate buffer (0.2 mol/L, pH 6.6) and confected into different concentrations (1.0 to 6.0 mg/mL). The reaction mixture including α-amylase (0.2 mL, 6 U/mL) and the polysaccharide solution (0.2 mL) was activated by the addition of the starch solution substrate (0.4 mL, 1%, w/v) and processed at 37 °C for 10 min until the reaction was terminated by the addition of 2 mL of DNS reagents (1% 3,5-dinitrosalicylic acid and 12% sodium potassium tartrate in 0.4 mol/L sodium hydroxide) [24]. After the reaction mixtures were incubated in boiling water for 10 min and diluted with 15 mL of distilled water in an ice bath, the absorbance was measured at 540 nm, and the rate of α-amylase inhibition was calculated using formula 1. The IC50 value was defined as the concentration of polysaccharides resulting in a 50% inhibition rate.

$$\text{Inhibition rate}\ (\%) = (1-(A_1-A_2)/A_0) \times 100 \quad (1)$$

$A_0$ was the absorbance of the mixture including 0.2 mL of phosphate buffer, 0.4 mL of starch solution and 0.2 mL of α-amylase solution; $A_1$ was the absorbance of the mixture including 0.2 mL of sample, 0.4 mL of starch solution and 0.2 mL of α-amylase solution; and $A_2$ was the absorbance of the mixture including 0.4 mL of phosphate buffer, 0.2 mL of α-amylase solution and 0.2 mL of sample.

The inhibition of α-glucosidase was measured by the method described by Kim, Wang, & Rhee [25] with slight modification. EPS1 and EPS2 were prepared as polysaccharide solutions, dissolved in 0.2 mol/L phosphate buffer (pH 6.8) at various concentrations (1.0 to 6.0 mg/mL). The α-glucosidase (0.2 mL, 3.75 U/mL) was premixed with the polysaccharide solutions and 6 mmol/L *P*-nitrophenyl-α-D-glucopyranoside (dissolved in phosphate buffer). The reaction was incubated at 37 °C for 30 min and then terminated by the addition of 6 mL of sodium carbonate (0.1 mol/L). The absorbance was measured at 400 nm, and the rate of inhibition of α-glucosidase was calculated using formula 2.

$$\text{Inhibition rate}\ (\%) = (1-(A_1-A_2)/A_0) \times 100 \quad (2)$$

$A_0$ was the absorbance of the mixture including 0.2 mL of phosphate buffer, 0.2 mL of *P*-nitrophenyl-α-D-glucopyranoside solution and 0.2 mL of α-glucosidase solution; $A_1$ was the absorbance of the mixture including 0.2 mL of sample, 0.2 mL of *P*-nitrophenyl-α-D-glucopyranoside solution and 0.2 mL of α-glucosidase solution; and $A_2$ was the absorbance of the mixture including 0.2 mL of phosphate buffer, α-glucosidase solution and 0.2 mL of sample.