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Physicochemical properties and antidiabetic effects of a polysaccharide from corn silk in high-fat diet and streptozotocin-induced diabetic mice

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ABSTRACT

This study aimed to investigate the physicochemical properties and antidiabetic effects of a polysaccharide obtained from corn silk (PCS2). PCS2 was isolated and the physicochemical properties were characterized. The hypoglycemic effects were determined using the high-fat diet and streptozocin induced type 2 diabetic mellitus (T2DM) insulin resistance mice. The results showed that PCS2 was a heteropolysaccharide with the average molecular weight of 45.5 kDa. PCS2 was composed of D-galactose, D-mannose, D-(+)-glucose, D-(+)-xylose, L-arabinose and L-rhamnose. PCS2 treatment significantly reduced the body weight loss, decreased blood glucose and serum insulin levels, and improved glucose intolerance ($P < 0.05$). The levels of serum lipid profile were regulated and the levels of glycated serum protein, non-esterified fatty acid were decreased significantly ($P < 0.01$). The activities of superoxide dismutase, glutathione peroxidase and catalase were notably improved ($P < 0.05$). PCS2 also exerted cytoprotective action from histopathological observation. These results suggested that PCS2 could be a good candidate of functional food or medicine for T2DM treatment.

1. Introduction

As a public health problem, type 2 diabetes mellitus is an important endocrine and metabolic disorder. The worldwide prevalence of T2DM continues to rise at an alarming pace (Hartstra, Bouter, Backhed, & Nieuwdorp, 2015). It has been estimated that nearly 592 million adults become diabetic patients by the year 2035 due to aging, high prevalence of obesity and the spread of calorie rich, fatty and fast foods (Fronstin, 2010). Although many drugs are available for treating the diseases, many of them are out of reach for a significant proportion of the population and are beset with some adverse effects. The use of medicinal plants and their phytochemicals for treating diabetes is not just a search for safer alternatives to pharmaceuticals, which transiently lower the blood glucose and prevent heart disease, and also enhancing the antioxidant system, insulin action and secretion (Vinayagam & Xu, 2015).

Corn silk (*Maydis Stigma*) is frequently used in traditional Chinese herbal medicines. It is made from stigmas, the yellowish thread like strands from the female flower of maize with various therapeutic values (Bae, Kim, Lee, Kim, & Son, 2015). It is a by-product material from corn cultivation and it is available in abundance. Main chemical components of corn silk are crudefibre, polysaccharides, flavonoids, organic acid, saponins, alkaloids, etc (Ebrahimzadeh, Pourmorad, & Hafezi, 2008). It has been reported that corn silk extracts exhibit multiple pharmacological activities such as antioxidant (Chen et al., 2014), anti-diabetic (Guo, Liu, Han, & Liu, 2009), antitumor (Yang, Li, Xue, Wang, & Liu, 2014), anti-obesity (Chaiittianan, Chayopas, Rattanathongkom, Tippayawat, & Sutthanut, 2016), and neuroprotective effects (Choi, Kim, Choi, & Park, 2014). Subchronic toxicity study of corn silk with rats suggested that consumption of corn silk had no adverse effects and supported the safety for humans (Wang et al., 2011). Previous studies showed that *in vitro* aqueous extract of corn silk exhibited potent and moderate inhibitory potential against α-amylase and α-glucosidase, respectively (Sabiu, O'Neill, & Ashafa, 2016). *In vivo* analysis of crude corn silk polysaccharides showed that it could reduce the serum lipid level in streptozotocin (STZ)-induced diabetic rats (Zhao, Yin, Yu, Liu, & Chen, 2012) and inhibit the expression level of TGF-β1 of diabetic rat's kidney (Wen & Yue, 2015). However, no studies have examined the hypoglycemic and hypolipidemic effects of polysaccharide of corn silk with a specific molecular weight on T2DM mice.

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In this study, crude polysaccharides of corn silk were isolated and purified. The comparison *in vitro* antioxidant and α-amylase inhibitory assay was carried out on the different fractions. The physicochemical properties of the most effective polysaccharide PCS2 were characterized. The hypoglycemic and hypolipidemic effects of PCS2 were investigated in T2DM mice induced by combination of high-fat diet and STZ injection. Furthermore, the repairation ability of PCS2 on the organ injury was also studied. It was the first time that the hypoglycemic and hypolipidemic effects of PCS2 were investigated on T2DM insulin resistance mice.

2. Materials and methods

2.1. Materials and chemicals

Corn silk was purchased from the local market (Tianjin, China). STZ was purchased from Sigma-Aldrich Company. Blood glucose meters were purchased from ACON biotech (Hangzhou) Co., Ltd. Test kits for the following compounds were obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu Province, China): glycogen, total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), non-esterified fatty acid (NEFA), glycated serum protein (GSP), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and malondialdehvde (MDA). ELASA kits for insulin were obtained from Wuhan Huamei Biotech Co., LTD. (Wuhan, China). All the other chemicals used were of analytical grade.

2.2. Experimental animals

Male Qunming mice (certificate number: SXCK (Jing) 2014-0008) with 20 ± 2 g of body weight, high-fat diet and normal diet were purchased from Beijing HFK Bioscience Company. The composition of high-fat diet was of normal diet (crude protein ≥ 18.0%, crude fat ≥ 4.0%, crude fiber ≤ 5.0%, crude ash ≤ 6.5%, minerals 0.6–1.2%, and moisture content ≤ 8.0%) 77%, lard oil 15%, saccharose 5%, cholesterol 2%, sodium cholate 0.2% and salt 0.7%. Mice were housed in automatically controlled conditions with a 12 h light/dark cycle, 22 ± 1 °C, and 45–55% relative humidity, and had access to standard laboratory food and fresh water freely. All animal experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals.

2.3. Isolation of polysaccharides

Crude corn silk polysaccharides (CPCS) were obtained using the hot water extraction method according to our previous study (Chen et al., 2013). Then CPCS dissolved in distilled water and filtrated by ultrafiltrate membranes to get the polysaccharides which molecular weight were less than 30 kDa (PCS1), between 30 kDa and 80 kDa (PCS2), more than 80 kDa (PCS3), respectively. The ultrafiltrate was then lyophilized to produce the dried polysaccharides. The total neutral polysaccharides yield of PCS1, PCS2 and PCS3 was determined using the phenol-sulfuric acid method (Chen et al., 2013).

2.4. Determination of antioxidant and α-amylase inhibitory activity of PCS1, PCS2 and PCS3

The radical (DPPH) scavenging activities of PCS1, PCS2 and PCS3 were conducted by using the method before (Fu, Chen, Dong, Zhang, & Zhang, 2010). Ferric reducing antioxidant power (FRAP) potential of PCS1, PCS2 and PCS3 was determined according to a modified method (Nakajima, Sato, & Konishi, 2007). The α-amylase inhibitory activity was measured according to our previous study (Chen et al., 2013).

2.5. Structural characterization of PCS2

2.5.1. FT-IR analysis

For IR spectroscopy, polysaccharides powders were mixed with KBr, grounded, and pressed into a 1 mm pellet. FT-IR spectra were recorded on a Bruker TENSOR 27 spectrometer.

2.5.2. Monosaccharide composition analysis

Gas chromatography was used for identification and quantification of monosaccharides in PCS2 according to our previous studies (Zhang et al., 2013). Monosaccharides including L-rhamnose, L-arabinose, D-mannose, D-(+)-glucose, D-galactose and D-(+)-xylose (Sigma-Aldrich, Shanghai, China) were used as the standards.

2.5.3. Molecular weight determination

The molecular weight (Mw) of PCS2 were determined by gel permeation chromatography according to the method before (Zhang et al., 2014). Mw of PCS2 was calculated by the calibration curve obtained by using various standard dextrans with different Mw (5, 12, 25, 50, 80, 150, 410, 670 kDa).

2.5.4. Scanning electron microscope analysis (SEM) analysis

The morphological features of PCS2 were studied with S-4800 scanning electron microscope (Hitachi, Japan). The dried sample was mounted on a metal stub and sputtered with gold in a vacuum sputter coater.

2.6. Animal experimental design

After two days of adaptation, all mice were fed with a high-fat diet except for one normal control (NC) group containing 8 mice during the experiment. After 4 weeks, all mice were intraperitoneally injected of STZ (dissolving in saline) 90 mg/kg twice in 72 h. Saline of same volume was injected into the NC group. Blood samples were collected from the tail vein of overnight fasting mice 3 days after STZ injection. Fasting blood glucose levels were measured. The mice that were marked hyperglycemia (the blood glucose level >11.6 mmol/L) were used as the diabetic mice for further study.

The diabetic mice were randomly divided into 5 groups (8 mice per group). NC group consisted of 8 normal mice which were allowed to free access to a normal diet and treated with saline for 28 days. Diabetic control (DC) group consisted of 8 diabetic mice which were treated with saline for 28 days. Positive control (PC) group received 2 mg/kg body weight of rosiglitazone (RSG) for 28 days. The other three groups received 200, 500 and 800 mg/kg body weight of PCS2, respectively for 28 days. Water consumption, food intake and body weight of experimental mice were recorded every day until they were sacrificed.

On the last day of the experiment, the mice were fasted overnight and sacrificed by cervical dislocation. Blood sample was collected in EP tubes and immediately separated by centrifugation (3000 × *g*, 4 °C, 10 min). Livers, kidneys and pancreas were collected in microtubes. Samples were stored at −80 °C until assayed.

2.7. Estimation of fasting blood glucose (FBG)

During the experimental period, FBG levels were measured on day 7, 14, 21 and 28. Blood samples were collected from the tail vein after overnight fasting and FBG levels were measured using glucose meter.