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Anti-diabetic effects of *Inonotus obliquus* polysaccharides-chromium (III) complex in type 2 diabetic mice and its sub-acute toxicity evaluation in normal mice

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ABSTRACT

Polysaccharides are important bioactive ingredients from *Inonotus obliquus*. This study aimed to synthesize and characterize a novel *I. obliquus* polysaccharides-chromium (III) complex (UIOPC) and investigate the anti-diabetic effects in streptozotocin (STZ) induced type 2 diabetes mellitus (T2DM) mice and sub-acute toxicity in normal mice. The molecular weight of UIOPC was about 11.5×10^4 Da with the chromium content was 13.01% and the chromium was linked with polysaccharides through coordination bond. After treatment of UIOPC for four weeks, the body weight, fasting blood glucose (FBG) levels, plasma insulin levels of the diabetic mice were significantly reduced when compared with those of the diabetic mice ($p < 0.05$). The results on serum profiles and antioxidant enzymes activities revealed that UIOPC had a positive effect on hypoglycemic and antioxidant ability. Histopathology results showed that UIOPC could effectively alleviate the STZ-lesioned tissues in diabetic mice. Furthermore, high dose administration of UIOPC had no obviously influence on serum profiles levels and antioxidant ability of the normal mice and the organ tissues maintained organized and integrity in the sub-acute toxicity study. These results suggested that UIOPC might be a good candidate for the functional food or pharmaceuticals in the treatment of T2DM.

1. Introduction

Diabetes mellitus(DM) is a global health concern due to its increasing prevalence, serious chronic metabolic and disabling complications (Chen et al., 2009). According to the International Diabetes Federation (IDF) reports, the prevalence of diabetes is expected to increase to 366 million by 2030 (The IDF Diabetes Atlas, 2013). Clinically, around 90% diabetes patients are reported to be type 2 diabetes mellitus (T2DM), which is characterized by insulin resistance and dysfunction of β-cells (Zhang and Moller, 2000; Sharma et al., 2011). T2DM can cause impairment in glucose and carbohydrate metabolism, and deterioration in lipid metabolism in serum (Dey et al., 2003; Wang et al., 2014). In addition, the hyperglycemia is associated with generation of reactive oxygen species which subsequently cause the oxidative damages in liver, kidney and pancreas (Bagri et al., 2009). So the drugs or functional foods on T2DM are being the focus of the researchers.

Chromium is an essential mineral which is proved to be necessary for the human health (MacKowiak et al., 2010). Currently, two kinds of chromium have been found in nature. The trivalent chromium (Cr^{3+}) is considered to be the main biologically active form in the complex, while hexavalent chromium (Cr^{6+}) is proved to be toxic. Since the first publication that reported the chromium (III) as glucose tolerance factor (Schwarz and Mertz, 1959), enormous studies have been published on its potential role in maintaining homeostasis of glucose and lipid (Cefalu WT., 2004; Sushil et al., 2006). Laboratory and clinical studies proved that certain forms of trivalent chromium could improve insulin sensitivity, overcome insulin resistance, ameliorate diabetes, suppress free radical formation and decrease systolic blood pressure (Peng and Yang, 2015). The potential mechanism of modulating glucose was reported to be the enhancement of intracellular signaling which increased insulin binding to the cells and activated insulin receptor kinase to improve the utilization of glucose (Anderson, 2000). The trivalent chromium could enhance the translocation of GLUT4 and insulin responsiveness via AMPK (Adenosine Monophosphate

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Activated Protein Kinase) signaling pathway (Pattar et al., 2006; Hoffman et al., 2014). However, it should be noted that chromium might cause harms when the intake dose is too high. Thus, the administration dose and toxicity should be evaluated before the application.

Modern pharmacological studies have demonstrated that active ingredients such as polysaccharides and polyphenols from natural products possess significant bioactivities by inhibiting α-amylase or α-glucosidase activity, improving antioxidant effects, protecting the pancreatic β-cells against glucose toxicity and inhibiting the formation of advanced glycation end products (Zhong et al., 2009; Xie et al., 2016; Xiao and Högger, 2015). The mushroom *Inonotus obliquus* (*I. obliquus*), a white rot fungus, belongs to the family of Hymenochaetaceae, Basidiomycetes, has been used as a traditional folk remedy in China, Russia, Japan and other Asian countries for centuries (Zhang et al., 2013). Polysaccharides from *I. obliquus* are known to be the main components with great biological activities (Ma et al., 2013). Some studies had illustrated the hypoglycemic and hypolipidemic effects of *I. obliquus* polysaccharides. Diao et al. (2014) reported that crude polysaccharides from *I. obliquus* could ameliorate the diabetic symptoms induced by streptozotocin. Liang et al. (2009) reported that *I. obliquus* polysaccharides showed great antioxidant and hypolipidemic capacities in hyperlipidemic rats. The hot water extractions of *I. obliquus* were also reported to be beneficial in preventing obesity and related metabolic abnormalities in C57BL/6J mice (Soh et al., 2008). Besides, polysaccharides from the mycelium proved to reduce blood glucose level, lower the lipid in serum profile and enhance the antioxidant status (Cha et al., 2006). Therefore, polysaccharides from *I. obliquus* (IOPS) might be a good candidate as an anti-diabetic agent.

Ligand plays a significant role in improving the bioactivity of different kinds of chromium complex, and many complexes with different ligands have been synthesized and characterized including chromium propionate, chromium picolinate, chromium niacin, chromium D-phenylalanine, chromium dinicocysteinate, chromium histidinate and so on (Król and Krejpcio, 2010; Wang and Yao, 2009; Zambon et al., 2014; Yang et al., 2005; Jain et al., 2012; Dogukan et al., 2009). However, most chromium ligands reported are small molecules and no polysaccharides-chromium (III) complex has ever been synthesized and studied in STZ-induced diabetic mice till now.

In this study, a fraction of the ultrafiltration polysaccharide from *I. obliquus* (UIOPS) was prepared and used to synthesize a polysaccharides-chromium (III) complex (UIOPC) for the first time. Then the physicochemical properties of UIOPC were characterized and the anti-diabetic effects in high fat-diet and STZ-induced T2DM mice. The sub-acute toxicity properties of UIOPC in normal mice were also evaluated.

2. Materials and methods

2.1. Materials and chemicals

I. obliquus sclerotia was obtained from the Northeast Natural Products Trading Company (Haerbin, China) and identified by Prof. Haixia Chen and a voucher specimen was deposited in the Herbarium of School of Pharmaceutical Science and Technology, Tianjin University, Tianjin, P.R.China for further reference. STZ was purchased from Sigma (Saint Louis, Missouri, USA). Rosiglitazone was purchased from Hengrui pharmaceutical Co., Ltd (Chengdu, China). Test kits for the determination of glycogen, total cholesterol (TC), triglyceride (TG), low density lipoprotein-cholesterol (LDL-C), high density lipoprotein-cholesterol (HDL-C), glycosylated serum protein (GSP), nonesterified fatty acids (NEFA), malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) were obtained from the Jiancheng Bioengineering Institute (Nanjing, China). ELISA detection kit for insulin was acquired from Wuhan Huamei Biotech Co., LTD. (Wuhan, China). All other chemicals and reagents were of analytical grade.

2.2. Preparation of ultrafiltration polysaccharides and its chromium (III) complex

The *I. obliquus* polysaccharides (UIOPS) was extracted and purified according to our previous studies (Zhang et al., 2013). The polysaccharides-chromium (III) complex was synthesized according to the method of Tang et al. (2013) with minor modifications. Briefly, aqueous solutions of polysaccharides (UIOPS) purified by ultrafiltration (50 mg/mL) were incubated in a water bath at 70 °C. $CrCl_3$ solution (2 mol/L) and NaOH solution (2 mol/L) were added to the polysaccharides solution and the pH value was adjusted to 8.5. The reaction was continued at 70 °C for another 1 h. After cooling, 95% ethanol was added and maintained overnight to precipitate polysaccharides-chromium (III) complex. The precipitate was collected by centrifugation at 3000 × g for 10 min and then washed with acetone and petroleum ether in turns. The crude complex was freeze-dried using rotary vacuum evaporator and then dissolved, dialyzed and freeze dried. Polysaccharides-chromium (III) complex was obtained and named as UIOPC.

2.3. Characterization of UIOPS and its chromium (III) complex (UIOPC)

2.3.1. Components analysis

Total sugar contents of the polysaccharides were determined by the phenol-sulfuric acid method, using D-glucose as standard (DuBois et al., 1956). Uronic acid content was determined by the carbazole-sulfuric acid method, using galacturonic acid as standard (Bitter and Muir, 1962). Protein content was measured by the method of Bradford, using bovine serum albumin as standard (Bradford, 1976). The trivalent chromium content was determined as described by Stoyanova (2005), using chromium trichloride as standard.

The monosaccharide composition was determined according to the method reported by Chen et al. (2013a) with minor modification. Briefly, 10 mg of polysaccharide was dissolved in 3 M trifluoroacetic acid (3 mL) and hydrolyzed at 120°C for 6 h. The hydrolysate was evaporated to dryness under reduced pressure by co-evaporations with methanol and then converted to alditol acetates. A Shimadzu GC-14B Gas chromatography (Kyoto, Japan) with capillary column (HP-5, 30 m × 0.32 nm × 0.5 μm) was used for the identification and quantification of monosaccharides. The injection temperature and detector temperature were kept at 250°C and 260°C respectively. L-rhamnose, D-arabinose, D-xylose, D-mannose, D-galactose and D-glucose were used as the monosaccharide standards. N_2 was used as the carrier gas and maintained at 1.0 mL/min during the experiment.

2.3.2. Molecular weight distribution

The molecular weight (*Mw*) was measured by high performance gel permeation chromatography (HPGPC). It was performed on a Shimadzu LC-20A chromatograph equipped with a Shodex OH pak SB-804 column (7.8 × 300 mm, ShowaDenko, Kawasaki, Japan) and an IR detector (Polymer Lab. Ltd., Tokyo, Japan). A sample solution (20 μL) was injected in each run and eluted at a buffer (0.2 M NaAc-AcOH, pH 6.0) flow rate of 0.8 mL/min at 35 °C. T-series Dextran standards (T-10, T- 40, T-70, T-110, and T-500) were used to pre-calibrate the HPGPC system and establish a standard curve.