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Comparative investigation for hypoglycemic effects of polysaccharides from four substitutes of *Lonicera japonica* in Chinese medicine

Xiangmei Zhao [a], Dongying Wang [b,*], Lijie Qin [a], Xianzhi Yang [a], Chuanyu Gao [a]

[a] Department of Emergency, Zhengzhou University People's Hospital, Zhengzhou 450003, China
[b] College of Food Science and Technology, Henan University of Technology, Zhengzhou 450001, China

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ABSTRACT

The polysaccharide fractions were obtained from flower buds of the four substitutes of *Lonicera japonica*, *L. macranthoides* (LMPB), *L. hypoglauca* (LHPB), *L. fulvotomentosa* (LFPB) and *L. confuse* (LCPB), and their hypoglycemic effects were investigated. In study, streptozocin (STZ)-induced diabetic rats were orally administrated once daily with LMPB, LHPB, LFPB and LCPB (each 800 mg/kg) for 42 days. Reduction for food and water intake ($p < 0.05$, $p < 0.01$) and levels of sugar and insulin ($p < 0.01$, $p < 0.05$) in blood, as well as elevation for contents of liver and skeletal muscle glycogen ($p < 0.05$) and concentrations of hepatic pyruvate kinase and hexokinase ($p < 0.01$, $p < 0.05$) were observed. Together with significant decline of total cholesterol (TC, 45.8 51.0%, $p < 0.05$), total triglyceride (TG, 50.6–53.8%, $p < 0.01$), low-density lipoprotein-cholesterin (LDL-C, 71.2–76.3%, $p < 0.01$) and very-low-density lipoprotein-cholesterin (VLDL-C, 45.2–50.0%, $p < 0.01$), the significant rise of high-density lipoprotein-cholesterin (HDL-C, 21.6–24.3%, $p < 0.05$) were also demonstrated. Consequently, the four polysaccharide fractions displayed notable hypoglycemic effects, similar to that of the polysaccharide fraction from *L. japonica* (LJP), so that they can be also considered as ingredients of functional foods for type-2 diabetes mellitus (T2DM).

1. Introduction

Diabetes mellitus (DM) is a major endocrine disorder and a global public health concern characterized by the inability of the body to produce insulin (type-1 diabetes mellitus, T1DM) or by the defects in insulin secretion and action (type-2 diabetes mellitus, T2DM) [1]. Among them, T2DM has become an important threat to people's health by accounting for more than 90–95% of all diabetes, which was characterized by persistent hyperglycemia, hyperinsulinemia, insulin resistance, glucose intolerance and impaired insulin tolerance [2,3]. According to the epidemiological data from International Diabetes Federation (IDF), around 382 million people were affected in 2012; the prevalence is expected to rise beyond 592 million in less than 25 years [4]. The incidence of the disease has rapidly increased in China, as the social and economic environment has improved. Nowadays, China has become the largest country of T2DM all over the world and the control of the disease is an urgent task [3,5]. However, although plenty of anti-diabetic drugs have been used to treat and control T2DM in clinic, such as biguanides, sulfonylureas and α-glucosidase inhibitors, the long-term use of these drugs could lead to several side effects including hypoglycemia, liver and kidney dysfunction [4,6]. Therefore, with the increasing incidence of T2DM, search for new alternative therapies for its prevention and treatment is quite urgent.

Meanwhile, medicinal plants have been found to play an important role in the treatment of T2DM, especially in some developing countries where most of the people have limited medical resources and do not have an access to modern treatment [7]. In China, plenty of medicinal plants have been involved in T2DM's traditional therapeutic actions in Chinese medicine [8,9]. Gu et al. reported that both fresh and dried *Portulaca oleracea* possessed antidiabetic activities in insulin-resistant HepG2 cells and STZ-induced C57BL/6J diabetic mice [10]. Huang et al. reported that *Penthorum chinense* produced a moderated anti-hyperglycemic effect on STZ-induced diabetic rats and starch induced postprandial hyperglycemic mice [11]. Liu et al. demonstrated that polyphenols enriched extract of *Rosa rugosa*

* Corresponding author.
E-mail addresses: uyi_zhao@163.com (X. Zhao), dywang@haut.edu.cn (D. Wang), qinlijie1819@163.com (L. Qin), hnsrmyyyxz@163.com (X. Yang), hnsrmyygaocy@163.com (C. Gao).

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could reduce blood glucose in type 2 diabetic rats by improvement of insulin sensitivity, and the effect was likely achieved by inhibition of oxidative stress and α-glucosidase [3]. Weng et al. demonstrated that total saponins extracted from *Aralia taibaiensis* had excellent antihyperglycemic, hypolipidemic and antioxidant activities in T2DM rats and might be a promising drug in the therapy of diabetes mellitus and its complications [12]. Amazingly, in our previous article, one fraction of the crude polysaccharides extracted from *Jin Yin Hua* (LJP), the flower buds of *Lonicera japonica* Thunb. (LJ, Caprifoliaceae), was found to display its hypolipidaemic and hypoglycaemic activities by STZ-induced diabetic rats [13].

Before 2005, *L. japonica* and some related species were all described as the original plants of *Jin Yin Hua* because they were not distinguished in practical applications. In 2005, the flower buds from several species of *Lonicera*, including *L. macranthoides* Hand. Mazz. (LM), *L. hypoglauca* Miq. (LH), and *L. confusa* DC. (LC) were considered as substitutes of *Lonicera japonica* and listed in the Pharmacopoeia of the People's Republic of China (PPRC) with the name *Shan Yin Hua*, In the 2010 PPRC, *L. fulvotomentosa* Hsu. (LF) was also listed as one of the original plants of *Shan Yin Hua* [14,15]. However, to date, although the anti-diabetic effects of the polysaccharide extracted from *Lonicera japonica* have been reported in our previous article, the anti-diabetic effects of the polysaccharides extracted from its four substitutes of *L. japonica*, *L. macranthoides* (LMPs), *L. hypoglauca* (LHPs), *L. fulvotomentosa* (LFPs), and *L. confusa* (LCPs) against T2DM have never been systematically investigated [13].

Consequently, the study herein was conducted to explore the anti-diabetic effects of the polysaccharides extracted from the four substitutes of *L. japonica*, LMPs, LHPs, LFPs, and LCPs, not only to compare the anti-diabetic effects, but also to provide the theory basis to the clinical application of them.

2. Material and methods

2.1. Materials and chemicals

α-Glucosidase (EC 3.2.1.2, 100 U/mg) from *Saccharomyces cerevisiae*, α-amylase (EC 3.2.1.1, P3.70 U/mg) from *Bacillus subtilis* and STZ were purchased from Sigma-Aldrich Co. (St. Louis, Mo, USA). Assay kits for total cholesterol (TC), high-density lipoprotein-cholesterin (HDL-C) and total triglyceride (TG) were purchased from Changchun Huili Biotechnology Co., Ltd. (Changchun, China). The assay kits for pyruvate kinase, hexokinase, alanine transaminase (ALT), aspartate transaminase (AST), gamma-glutamyl transpeptidase (GGT), catalase (CAT) superoxide dismutase (SOD), glutathione (GSH) and the enzyme-linked immunosorbent assay (ELISA) kit for insulin were the products of Nanjing Biotechnology Co. Ltd. (Nanjing, China). All the other chemicals and reagents used in this study were of either analytical or HPLC grade.

2.2. Preparation of the polysaccharide fractions from LMPs, LHPs, LFPs, and LCPs

The flower buds of *L. macranthoides*, *L. hypoglauca*, *L. fulvotomentosa*, and *L. confusa* were harvested from the countryside region of Xiushan County (Chongqiu), Xincheng County (Guangxi), Anlong County (Guizhou) and Xuwen County (Guangdong) of South China, and bought from Yuzhou Chinese Medicine Market (Henan) of Middle China. All of the plant materials were identified by Prof. Fan Wenchang in Guangdong Food and Drug Vocational College according to the identification standard of Pharmacopeia of China (2015 edition), and the voucher specimens of the plant materials were deposited at the College of Food Science and Technology, Henan University of Technology, Zhengzhou, China. The polysaccharides were extracted and purified by the method of hot-water extraction and EtOH precipitation according to our previous procedure [16,17]. Briefly, the flower buds stripped from *L. macranthoides* were dried for 30 min in a domestic microwave oven at 900 W until a constant weight (500 g), crushed into powder using a multi-functional grinder (400Y, Yongkang Boou Machinery Co., Ltd. China). The powder was soaked in 95% EtOH (1:10, w/v) refluxing at 80 °C for 4 h as an ungreased treatment. After the mixture was filtered, the residues were dried in air and then were extracted in heat water (1:40, w/v) at 80 °C for 3 h. Three cycles later, the incorporate extraction solution was filtrated and concentrated to about 10% of the original volume with a rotary evaporator under reduced pressure, and then it was precipitated via adding five times of volume of 95% (v/v) EtOH by slowly stirring at 4 °C for 24 h. The separated precipitate was completely dissolved in appropriate volume of water, and intensively dialyzed for four days against distilled water (cut-off Mw 8000 Da) to remove the small molecular compounds (e.g. flavonoids or polyphenols). The retentate portion was deproteinized by the freeze-thaw process for repeating ten times, followed by filtration. Finally, the extracts were centrifuged at 3000 r/min for 10 min to remove insoluble material and the supernatant was lyophilized in the freeze-dry apparatus (FD-1, Henan Yuhua Instrument Co., Ltd. China) to obtain the refined polysaccharide, LMPs. By the same approach, LHPs, LFPs, and LCPs were all obtained and purified with some procedural adjustments one after another. After got these polysaccharides, according to Chen et al.'s method [18], the refined polysaccharides were directly dissolved in the distilled water and chromatographed by means of a DEAE-52 cellulose anionexchange chromatography column (300 mm × 26 mm, GE Healthcare, UK). After loading with sample, the column was eluted with distilled water, followed by stepwise elution with increased concentration of NaCl (0.2, 1.0 and 1.5 M, respectively) at 4 mL/5 min/1 tube. The eluted fractions LMPA, LMPB and LMPC from LMPs, LHPA, LHPB, and LHPC from LHPs, LFPA, LFPB and LFPC from LFPs, and LCPA, LCPB and LCPC from LCPs were all successively collected for subsequent experiments.

2.3. Assessment of eluted fractions on enzymes linked to carbohydrate hydrolysis

2.3.1. α-Amylase activity

The α-amylase inhibition was conducted by means of the previous method [19]. Concisely, 500 μL of starch solution (0.5%, 20 mM sodium phosphate buffer with 6.7 mM sodium chloride, pH 6.9) was pre-mixed with 10 μL test sample in the buffer at different concentrations. The reaction was started by adding 10 μL of 1 unit α-amylase in cold distilled water to the mixture. Following incubation at 65 °C for 5 min, the reaction was terminated by addition of 600 μL of the DNS reagent (1% 3,5-dinitrosalicylic acid, 12% sodium potassium tartrate in 0.4 M NaOH). The α-amylase inhibitory activity was determined at 540 nm, and the inhibitory activity was calculated by the following formula: α-amylase inhibitory activity (%) = $(1 - A_{sample}/A_{control}) \times 100$. Here the $A_{control}$ and A_{sample} are the absorbance values of the blank sample and the test sample checked after cooled down to ambient temperature, respectively. Acarbose was used as the positive control and all the tests were conducted in triplicate.

2.3.2. α-Glucosidase activity

The α-glucosidase inhibitory activities were carried out according to the reported previous method [19]. Briefly, 5 μL of α-glucosidase solution (10 units/mL, 0.1 M potassium phosphate buffer, pH 6.9) was pre-mixed with test sample at different concentrations in 1 mL of potassium phosphate buffer (0.1 M, pH 6.9). Following incubation at 37.5 °C for 20 min, 10 μL *p*-nitro phenyl glucopyranoside (*p*NPG, 10 mmol/L) as substrate was added to the mixture to start the reaction. The reaction was carried out