International Journal of Biological Macromolecules 101 (2017) 543–552

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Consumption of guava may have beneficial effects in type 2 diabetes: A bioactive perspective

Yukun Jiao[a,b,c], Mengliu Zhang[a], Shumei Wang[b,c,**], Chunyan Yan[a,*]

[a] School of Pharmacy, Guangdong Pharmaceutical University, Guangzhou 510006, PR China
[b] Key Laboratory of Digital Quality Evaluation of Chinese Materia Medica of State Administration of TCM, PR China
[c] Engineering & Technology Research Center for Chinese Materia Medica Quality of the Universities of Guangdong Province, PR China

ARTICLE INFO

Article history:
Received 23 December 2016
Received in revised form 13 March 2017
Accepted 19 March 2017
Available online 25 March 2017

Keywords:
Polysaccharides
Guava
Type 2 diabetes

ABSTRACT

The objectives of this study were to evaluate the anti-diabetic and anti-hyperlipidemic effects and relative mechanisms of guava polysaccharides (GPs) in rats with type 2 diabetic mellitus (T2DM). The chemical characterization and monosaccharide compositions of GPs, named as GP-1, GP-2, GP-3, and GP-4, were determined by PMP-HPLC and FT-IR. The results revealed that all GPs had the typical saccharide absorptions, and all were heteropolysaccharides. In addition, GPs efficiently decreased levels of fasting blood glucose, glucosylated serum protein, serum insulin, homeostasis model assessment of insulin resistance, total cholesterol, triglyceride and serum alanine transaminase, improved oral glucose tolerance, and increased insulin sensitivity in rats with T2DM. Histopathological observations suggested that GP-1, GP-3, and GP-4 could alleviate injury in pancreatic islet cells, and Western blot analysis showed that these GPs upregulated gene expression of the insulin receptor, insulin receptor substrate 2, Akt, and glucose transporter type 4. Taken together, these data suggest that GPs may be beneficial in treating T2DM and reducing the risk of hyperlipidemia, vascular disease, and cirrhosis via the PI3K/Akt signaling pathway.

1. Introduction

Diabetes mellitus (DM) is a metabolic disorder with a high prevalence worldwide, and is characterized by impaired glucose and lipid metabolism and associated complications [1]. The increase in life expectancy has correlated with an increase in stress and unhealthy lifestyles, which in turn, has led to an increase in DM. This disease now affects more than 415 million people worldwide, which is projected to increase by 10% by 2040 [2]. Type 2 diabetes mellitus (T2DM) is the most common form of diabetes, accounting for more than 90% of all diagnosed cases, and is characterized by increased blood glucose levels, insulin resistance (IR), and β-cell injury. In T2DM, the sensitivities of tissues and organs to insulin are decreased, resulting in elevated insulin and blood glucose levels and the associated impairment of glucose metabolism. IR can also lead to other complications such as hyperlipidemia, diabetic nephropathy, and liver impairment [3].

The PI3K/Akt pathway modulates insulin function and secretion to regulate glucose homeostasis, and its impairment is one of the main molecular mechanisms of diabetes [4]. Insulin acts through the insulin-like growth factor signaling system, with the InsR (insulin receptor) playing a crucial role [5]. Insulin receptor substrate-2 (IRS-2) phosphorylates and combines with PI3K, affecting the metabolism of phospholipids [6,7] Akt and Glut4 can strengthen the translation of glucose to enhance the utilization of glucose to reduce the blood glucose level [8,9]. The abnormal expression of genes in the PI3K/Akt pathway can result in increased IR and reduced insulin sensitivity. Currently, the major oral anti-diabetic drugs are metformin, sulfonylurea, rosiglitazone, and α-glucosidase inhibitors with metformin being the most well studied and one of the most effective. However, long-term treatment with these drugs can result in unfavorable effects and high rates of secondary failure. Therefore, it is essential to develop effective natural products that can decrease blood glucose levels and treat IR with minimal side effects.

Psidium guajava Linn of the family Mytraceae and genus *Psidium* is a tropical plant that is mainly known for its various bioactivities such as anti-hyperglycemic, hepatoprotection, anti-allergy, and anti-nociceptive activities. Guava, the fruit of *P. guajava*, is a

Abbreviations: ALT, serum alanine transaminase; AST, serum aspartate transaminase; AKT, protein kinase B; FBG, fasting blood glucose; Glut4, glucose transporter type 4; GSP, glucosylated serum protein; HOMA-IR, homeostasis model assessment of insulin resistance; INS, serum insulin levels; InsR, insulin receptor; IRS-2, insulin receptor substrates-2; ISI, insulin sensitivity index; OGTT, oral glucose tolerance test; STZ, streptozocin; PI3K, phosphatidylinositide 3-kinases.

* Corresponding author.
** Corresponding author at: Key Laboratory of Digital Quality Evaluation of Chinese Materia Medica of State Administration of TCM, PR China.
E-mail addresses: 2395903468@qq.com (S. Wang), ycybridge@163.com (C. Yan).

http://dx.doi.org/10.1016/j.ijbiomac.2017.03.130
0141-8130/

functional fruit that is widely used in folk medicine as an adjuvant therapy in diabetes in China and Mexico. Several studies have shown that this fruit is rich in biological active compounds such as carbohydrates, phosphoric, oxalic, and malic acids [10] with carbohydrates being one of the major active components [11]. In addition, studies have revealed that carbohydrates extracted from some traditional plants have anti-diabetic effects in diabetic rats [12]. However, the hypoglycemic effects of guava fruit polysaccharides *in vivo* have not been reported. Our previous studies detailed the structures of guava polysaccharides (GPs) and determined the effects of α-glucosidase inhibition and anti-oxidation *in vitro* [13,14]. However, the systematic investigation of the anti-diabetic activities of GPs *in vivo* and the underlying mechanisms were not evaluated.

Here, we report the anti-diabetic effects of guava fruit in T2DM rats and explore the possible underlying molecular mechanisms.

2. Materials and methods

2.1. Materials

Streptozotocin (STZ) was obtained from Aladdin Industrial Co. (Shanghai, China). All of the other chemicals and reagents were of analytical reagent grade. Guava fruits were purchased from a specialty guava orchard on Haiou Island, Guangzhou (Guangdong province, China). The polysaccharides tested in this study, GP-1, GP-2, GP-3, GP-4, were obtained with the method in our previous studies [13,14]. Briefly, the fresh guava fruits were cut into pieces, and extracted with hot water 10:1 (v/w) three times for 3 h each. The water extracts were combined, concentrated, and centrifuged after which the supernatant was treated with 95% ethanol to a final concentration of 50% at room temperature for 12 h. The precipitate was centrifuged at 3500 rpm for 15 min and named GP50. This process was repeated, and the supernatant was treated with ethanol to final concentrations of 70% and 90% that were labeled GP70 and GP90, respectively. The fruit residues were further extracted three times with 0.5 M NaOH solution, after which the alkaline extracts were filtered and neutralized with 0.5 M HCl solution. The aqueous fraction was concentrated and precipitated with ethanol to a final centration of 75%. The precipitate was named as GPB. The crude polysaccharides (GP50, GP70, GP90 and GPB) were deproteinized by using the sevag method [13]. After dialyzed and lyophilized, the polysaccharides were termed GP-1, GP-2, GP-3, GP-4, respectively.

2.2. Monosaccharide composition of GPs

The monosaccharide compositions of GP-1, GP-2, GP-3 and GP-4 were identified by HPLC with the method in our previous study [14]. Briefly, the samples (6 mg) were hydrolyzed with 2 mL of 3 M trifluoroactic acid (TFA) at 120 °C for 6 h. Then, the completed hydrolyzed samples were codistillated with methanol three times to remove the excessive acid. An improved 1-phenyl-3-methyl-5-pyrazolone (PMP) precolumn derivatization method of monosaccharide content was used. Briefly, the hydrolysate were mixed with 0.3 M NaOH and 0.5 M PMP-methanolic solution, and reacted at 70 °C for 30 min. The reactants were neutralized with 0.3 M hydrochloric acid and extracted with chloroform twice. Finally, the supernatant was analyzed by HPLC on an Agilent 1260 system (Agilent, USA) with a ZORBAX XDB-C_{18} column (Ø 4.6 mm × 250 mm, 5 μm) at 25 °C with UV detection. The mobile phase was composed of acetonitrile (83:17, v/v) and 0.1 M phosphate buffer solution (pH 6.9).

2.3. FT-IR spectrum analysis

The FT-IR spectrum of GPs (GP-1, GP-2, GP-3, GP-4) were detected using a PerkinElimer spectrometer. The samples (2 mg) were mixed with 200 mg dry KBr powder and then grounded into 1 mm pallets for FT-IR measurement in the region of 4000–400 cm^{-1}, respectively.

2.4. Animals and treatment

Adult male Wistar (220 ± 20 g) rats were provided by the Experimental Animal Center of Guangzhou University of Chinese Medicine (Certificate: SCXK20130034) and housed in the specific pathogen-free animal laboratory in the Experimental Animal Center of Guangdong Pharmaceutical University. The procedures described in this experiment were approved by the Animal Ethics Committee of Guangdong Pharmaceutical University (No. GDPU 2013033). The animals were maintained at 12 h light and dark cycles in a temperature range of 25 °C ± 1 °C and relative humidity ranging from 50% to 70%, and were supplied sufficient food and water. The rats were acclimatized to the environment for 1 week. Then they were randomly divided into two groups: one fed a normal diet (n = 10) and considered the normal control (NC) group, and the other fed a high-fat diet (HFD, Table 1, 32.10% of total energy from lipid and 55.94% of total energy from carbohydrates, most was sucrose. n = 50) for 4 weeks. And, the nutritional composition of the two different experimental diets were exhibited in Table 1. The rats in the HFD group were fasted overnight and induced with an intraperitoneal injection of freshly prepared STZ (40 mg/kg, b.w) in 0.1 M (pH = 4.5) citrate buffer [15]. A fasting blood glucose (FBG) test was taken 3 days after injection, and rats with a FBG greater than 11.1 mmol/L, polyuria and other diabetic features were considered diabetic [16]. A total of 48 diabetic rats were randomly divided into 6 groups (n = 8): diabetes control (DC), positive control (PC), GP-1, GP-2, GP-3, and GP-4 groups. The PC group had intragastric administration of metformin (200 mg/kg) and the GP-1, GP-2, GP-3, and GP-4 groups were administered GP-1, GP-2, GP-3, and GP-4 (400 mg/kg/d, b.w), respectively, based on the Human Rat Equivalent Dose Conversion Principle [17,18]. Rats in the NC and DC groups received equal amounts of distilled water. All of the rats were supplied free food and water, and the cages were cleaned daily.

2.5. Measurement of body weight and FBG levels

The body weight and FBG levels of all groups were measured once a week, and the rats were fasted overnight before each measurement. The FBG was measured using the One-touch Ultra Glucometer (Johnson & Johnson).

2.6. Oral glucose tolerance test

At the end of the experiment, the oral glucose tolerance test (OGTT) was given after oral administration of glucose (1.5 g/kg). The blood samples were collected from the tail of the rats and measured 0, 30, 60, 90, 120, and 150 min after glucose administration [19].

2.7. Collection of blood and tissue samples

After 5 weeks of GPs treatment, the animals were anesthetized by intraperitoneal injection of sodium pentobarbital, and blood samples were collected and centrifuged (4000 rpm/min for 15 min) to isolate serum for the measurement of biochemical parameters. The organs (*i.e.* liver and pancreas) were obtained and divided into two parts: the pancreas was stored in 10% formalin solution for subsequent histopathological observations and the livers was stored at −80 °C for biochemical analysis.