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Purification, characterization and anti-diabetic activity of a polysaccharide from mulberry leaf

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ABSTRACT

In the present study, a high-purity polysaccharide from mulberry leaf (MLP) was purified and characterized, and its anti-diabetic effects were investigated in streptozotocin (STZ)-induced diabetic rats. Our results showed that the obtained MLP (purity 99.8%) was determined to be composed of D-arabinose, D-xylose, D-glucose, D-rhamnose and D-mannose with molar ratio of 1:2.13:6.53:1.04:8.73. Oral administration of MLP at 50–200 mg/kg body weight daily for 5 weeks significantly reduced the levels of fasting blood glucose (FBG), glycosylated serum protein (GSP), serum total cholesterol (TC), and serum triglyceride (TG), and increased the body weight, fasting insulin (FINS), C-peptide (C-P), liver glycogen, liver glucokinase, and serum high-density lipoprotein cholesterol (HDL-C). Moreover, MLP promoted marked pancreatic β-cell regeneration and insulin secretion, and reduced liver fat accumulation in diabetic rats. The treatment effect of MLP on diabetes was similar to the effect of antidiabetic drug glibenclamide. These results clearly indicated that MLP may have a potential for the treatment of hyperglycemia and hyperlipidemia in diabetes.

1. Introduction

Diabetes mellitus, one of the most common health problems around the world, is characterized by metabolic disorders along with several symptoms, including polyuria, polydipsia, polyphagia, a selective loss of pancreatic islet β-cell mass, and a high blood glucose level (Amos et al., 1997). Currently available therapies for diabetes mellitus include insulin and various oral hypoglycemic drugs, such as metformin, α-glucosidase inhibitors, sulphonylureas, and rosiglitazone (Scheen, 2005). Although the oral drugs mentioned above have various hypoglycemic effect, most side effects and drug resistance occur after long-term use (Mukherjee et al., 2006). Therefore, it is necessary to develop new hypoglycemic agents with fewer/no side effects. In an effort to reduce side effects, there has been growing interest in herbal remedies (Grover et al., 2002; Li et al., 2004). The biologically active components of herbal remedies with hypoglycemic activity included polysaccharides (Chen et al., 2011b), alkaloids (Okonta and Aguwa, 2007), sterols (Fred-Jaiyesimi et al., 2009), saponins (Han et al., 2008), terpenoids (Mohamed et al., 2011), and flavonoids (Ahmad et al., 2000). Recently, many researchers have found that polysaccharides extracted from Chinese traditional plants and edible/medicinal fungi exhibit hypoglycemic activity (Chen et al., 2013; Fu et al., 2012; Wang et al., 2013; Xu et al., 2011; Zhang et al., 2013).

Mulberry (*Morus alba* L., family of Moraceae) leaf, one of the most widely used traditional Chinese medicines, has attracted much attention because of its multiple pharmacological effects, such as anti-diabetic, anti-inflammation, anti-cancer, and so on (Andallu et al., 2001; Andallu and Varadacharyulu, 2007; Naowaboot et al., 2009). Previous studies have confirmed that various alkaloids, including 1-deoxynojirimycin (DNJ), 1,4-dideoxy-1,4-imino-D-arabinitol (DAB), and 1,4-dideoxy-1,4-imino-D-ribitol (DRB), could have beneficial physiological effects on diabetes (Kimura et al., 2004; Sharma et al., 2010). In recent years, the anti-diabetic activity of crude polysaccharide extract (Chen et al., 2011a; Wang et al., 2010) and the mixture of DNJ and polysaccharides (Li et al., 2013, 2011) from mulberry leaf have been reported. However, most of mulberry polysaccharide used in the previous study was crude polysaccharide extract and could contain possibly other constituents which may contribute to the treatment of diabetes. To the best of our knowledge, limit research has been reported on the high-purity extraction of polysaccharide fraction from mulberry leaf and its anti-diabetic properties in streptozotocin-induced diabetic rats so far. In this study, we isolated and characterized a

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high-purity polysaccharide from mulberry leaf (MLP) and examined its hypoglycemic and hypolipidemic effects in STZ-induced diabetic rats.

2. Materials and methods

2.1. Ethics statement

The animals were treated in accordance with the Regulations of Experimental Animal Administration issued by State Committee of Science and Technology of the People's Republic of China on November 14, 1988. All animal experiments were carried out following protocols approved by the Institutional Animal Care and Use Committee of the Shandong Agriculture University. Rats were kept at an ambient temperature of 22 ± 2 °C and relative humidity of 55 ± 5% with fixed 12 h light–dark cycle and free access to standard rodent pellet diet and tap water. After the experiments, rats were sacrificed by CO_2 inhalation.

2.2. Isolation and purification of polysaccharide from mulberry leaf

Fresh mulberry (*Morus Multicaulis* Perr.) leaves were collected from the mulberry plantation of forestry department of Shandong Agricultural University (Taian, China). The 4th and the 5th leaves from the apex of healthy plants were plucked, washed thoroughly under running tap water, shade dried for 3 days and ground to a fine powder in an electric mixer. This mulberry leaf powder was used in the following experiment. The mulberry leaf polysaccharide was based on the water extraction and ethanol precipitation method, with some modification, as described previously (Gai et al., 2005). Briefly, 100 g of mulberry leaf powder was refluxed with 95% ethanol for 1 h to remove lipophilic compounds, and successively boiled in water 3 times (for 1.5, 1.5, and 1 h). The pooled filtrate was concentrated to a minimal volume in a rotary evaporator, and then was precipitated with ethanol (80% v/v). This precipitates was deproteinated by the Sevag method (Alam and Gupta, 1986) to obtain crude polysaccharide. Crude polysaccharide was dissolved in distilled water and further purified by ultrafiltration (membrane MW cut-off: 10,000) under pressure (0.5 MPa). Products with molecular weights estimated to be less than 10,000 were fractionated on DEAE Sepharose Fast Flow column and eluted with a linear gradient of NaCl (0–2 mol/L). Only one fraction, named MLP, was pooled, concentrated, and lyophilized. MLP was further purified using a Sephadex G-100 column eluted with distilled water, giving a white power.

2.3. Physicochemical properties of MLP

The average molecular weight of MLP was determined by high-performance size-exclusion chromatography (HPSEC) on an Agilent 1200 system as described by Zhu et al. (2010). Briefly, T-series dextrans standards (Sigma; St. Louis, MO, USA) and MLP were dissolved water at a concentration of 2.0 mg/mL. Then, the sample (20 μL) was injected and eluted with distilled water at a flow rate of 0.6 mL/min. The column and detector compartment were maintained at 40 °C.

The analysis of monosaccharides in MLP was determined by high performance liquid chromatography (HPLC). MLP was hydrolyzed into component monosaccharides and subsequently labeled with 1-phenyl-3-methyl-5-pyrazolone (PMP) as described by Honda et al. (1989), and then the labeled monosaccharide derivatives were analyzed on the Agilent 1100 HPLC system (Agilent Technologies Wilmington, USA) equipped with an Agilent four-unit pump, a 7125 injector and a G1314A UV detector. The analytical column used was a Zorbax Extend-C_{18} column (4.6 mm × 250 mm i.d., 5 μm, Agilent, USA). The wavelength for UV detection was 250 nm. Elution was carried out at a flow rate of 1.0 mL/min at room temperature. The mobile phase consisted of 50 mM sodium phosphate (KH_2PO_4–NaOH, pH 6.9) with (A) 15% and (B) 40% acetonitrile, using a gradient elution of 0–8–20–50% buffer B by a linear increase from 0–10–30–55 min. The injection volume was 20 μL.

The polysaccharides samples were analyzed by Fourier transform infrared spectroscopy (FTIR) as a film between two KBr plates on a FTIR spectrometer (Thermo Nickolet Nexus-870, USA). The recording was done from 4000 to 400 cm^{-1} wave number.

2.4. Acute toxicity study

Acute toxicological test of MLP in Wistar rat were performed. Three groups of fasted healthy rats (6 per group) were orally administered MLP at a dose of 1, 2 and 3 g/kg. The rats in all groups were allowed access to food and water, and behavioral changes were observed over a period of 24 h for sign of acute toxicity, such as writhing, gasping, palpitation and decreased respiratory rate or mortality.

2.5. Animals and induction of diabetes

Adult male albino rats of the Wistar strain weighing 140–160 g were obtained from the Experimental Animal Center in Shandong University, Jinan, Shandong Province.

Diabetes was induced in rats using high-fat diet and low dose of STZ. Briefly, rats were given high-fat diet consisting of 20% sucrose, 10% lard, 2.0% cholesterol, 0.5% cholate and 67.5% normal food. After 5 weeks of the high-fat diet, rats were fasted for 12 h (free access to water) and each rat was injected intraperitoneally with 35 mg/kg streptozotocin (STZ; Sigma, St. Louis, MO, USA) in 0.1 M citrate buffer, pH 4.5, whereas rats in the normal control group, fed with normal food, were injected with only the buffer solution. After 3 days, the STZ-injected rats were checked for fasting blood glucose (FBG) level $\geqslant$ 7.8 mmol/L to confirm the status of diabetes. FBG levels were assessed on blood collected from the tail vein using an One-Touch Ultra blood glucose meter (LifeScan, Milpitas, CA, USA).

2.6. Experimental design

From the above high fat diet treated rats, 40 rats that have developed diabetes were randomly divided into five groups with 8 rats in each group: diabetic control group; diabetic rats treated with MLP (50 mg/kg b.w./d); diabetic rats treated with MLP (100 mg/kg b.w./d); diabetic rats treated with MLP (200 mg/kg b.w./d); diabetic rats treated with glibenclamide (5 mg/kg b.w./d; Sigma, St. Louis, MO, USA). The MLP-treated diabetic rats received the MLP dissolved in 2 mL of distilled water and glibenclamide-treated diabetic rats received the standard antidiabetic drug glibenclamide suspended in 2 mL of distilled water, while rats in the normal control group and diabetic control group received an equal volume of distilled water.

The treatment was given daily through oral gavage for 5 weeks. Individual body weight and FBG levels of rats were measured at the end of each week. At the end of the experiment, rats were fasted for 12 h (free access to water), and trunk blood were collected on tubes treated with 0.1 M EDTA as anticoagulant. The serum was prepared by centrifugation (3000*g*, 10 min, 4 °C). After blood withdrawal, the liver was excised immediately and rinsed in ice-chilled normal saline, and then stored at −80 °C for estimation of glycogen. Pancreas and liver were preserved in 10% neutral buffered formalin and then embedded in paraffin for observation of