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Saponin rich fractions from *Polygonatum odoratum* (Mill.) Druce with more potential hypoglycemic effects

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ABSTRACT

Aims: The root of *Polygonatum odoratum* (*YuZhu*), also a medicinal food has long been used for the treatment of diabetes. The objective of the study was to characterize the anti-diabetic active fractions or compounds in this herb.
Materials and methods: Fractions with a different polarity were prepared by solvent extraction and macroporous absorptive resin (D101) column and their anti-diabetic potentials were evaluated by glucose uptake in HepG2 cells and STZ-induced diabetic rats. In addition, α-glycosidase inhibitory activities of active fractions were measured *in vitro* and chemical compositions including saponin, total flavonoids and total sugar in the fractions were determined.
Results: The n-buthanol fraction, a saponin-rich fraction obtained by partitioning the ethanol extract with n-buthanol after petroleum ether and acetic ether showed the highest anti-diabetic potential in glucose uptake in HepG2 cells followed by acetic ether fraction which was rich in flavonoids. Further fractionation the saponin-rich fraction using macroporous resin column (D101), polysaccharide, flavonoid and saponin rich fractions were obtained by elution with water, 40% and 60% ethanol, respectively and their anti-diabetic potentials proved by glucose uptake test in HepG2 cells and STZ-induced diabetic rats were in the order of saponin rich fraction > flavonoid rich fraction > polysaccharide rich fraction. Long-term therapy test (60 d) in severe diabetic rats indicated that saponin-rich fraction significantly ameliorated clinical symptoms of diabetes including the elevated blood glucose, body weight loss as well as the increased food and water intake while flavonoid-rich fraction was more potential than saponin-rich fraction to increase superoxide dismutase (SOD) activity and decrease malondialdehyde (MDA) level in rat plasma. Additionally, saponin-rich fraction and flavonoid-rich fraction showed α-glycosidase inhibitory activity with IC_{50} value of 2.05 ± 0.32 and 3.92 ± 0.65 mg/ml, respectively.
Conclusion: The results suggested that saponin in this herb was more important than flavonoid in exhibiting anti-diabetic activity and flavonoid contributed more to anti-oxidant activity *in vivo*.

1. Introduction

Diabetes mellitus (DM) is a group of chronic metabolic disorders characterized by hyperglycemia resulting from defects in insulin secretion, action or both and the chronic hyperglycemia causes serious body damage such as blood vessels and nerves damages (O'Connell et al., 2008). Current knowledge strongly supports that control of hyperglycemia is critical in the treatment of not only diabetic patients but also individuals with impaired glucose tolerance (Laakso, 1999). So far a number of hypoglycemic agents including insulin injection and oral drugs such as glycosidase inhibitors, biguanides and sulphonylureas have been clinically used to maintain blood glucose level (Scheen and Lefebvre, 1998). Unexpectedly, some adverse effects had been seen in the use of those agents. Pioglitazone, for instance, may induce hepatocellular-cholestatic liver injury (May et al., 2002) and metformin should be stopped for the therapy of diabetic nephropathy when glomerular filtration rate (GFR) is lowered than 60 ml/min (Phillips and Braddon, 2002).

Abbreviations: AUC, areas under the curve; CAT, catalase; DM, diabetes mellitus; DMSO, dimethyl sulfoxide; FBG, fasting blood glucose; FBS, fetal bovine serum; GFR, glomerular filtration rate; MDA, malondialdehyde; MTT, methylthiontrazole; OGTT, oral glucose tolerance test; PBG, postprandial blood glucose; RPO, rhizomes of *Polygonatum odoratum*; ROS, reactive oxygen species; SOD, superoxide dismutase; STZ, streptozotocin; pNPG, p-nitrophenyl-α-D-glucopyranoside.

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Therefore, it is believed that herbal medicine is a valuable reservoir for novel drugs to deal with this disease due to its few side effects (Li et al., 2004; Jung et al., 2007).

The rhizome of *Polygonatum odoratum* (Mill.) Druc (RPO), an edible medicinal herb has long been used to treat various diseases including DM (China Pharmacopoeia Committee, 2010). Studies had shown that RPO had the beneficial effects on hyperglycemia. Chen et al. (2001) reported that the water extract of RPO decreased the blood glucose level in starch loaded mice. Meanwhile, the n-butanol fraction from the methanol extract of this herb exhibited dramatic hypoglycemic effects in STZ-induced diabetic mice (Kato and Miura, 1994). Although a few studies had revealed that flavonoid, saponin and polysaccharide were crucial compounds (Choi and Park, 2002; Shu et al., 2009), there was no definite conclusion about the active constituents. The objective of this study is to characterize the anti-diabetic constituents or fractions by evaluation anti-diabetic potential of different fractions prepared systematically.

2. Materials and methods

2.1. Materials and chemicals

The rhizomes of *Polygonatum odoratum* were purchased from Tongjunge Drugstore of Chongqing (Chongqing, China). The taxonomic identification of the plant material was confirmed by a group of professors in pharmacognosy laboratory of the college of pharmaceutical sciences, Southwest University and a voucher of specimen (No. 20090031) was deposited there.

Streptozotocin (STZ) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma (St. Louis, MO, USA). RPMI-1640 culture medium and fetal bovine serum (FBS) were obtained from Gibco Life Technologies (Burlington, Ontario, Canada). Superoxide dismutase (SOD), malondialdehyde (MDA) and glucose assay kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China). Metformin was purchased from Beijing Coway Pharmaceutical Factory (Beijing, China). α-Glucosidase and p-nitrophenyl-α-D-glucopyranoside (pNPG) were purchased from Sigma Chemical Co. All other reagents were of the highest available purity and were used as purchased.

2.2. Experimental animals

Male Sprague-Dawley rats (200 ± 20 g) and diets were purchased from Animal Breeding Center of the Third Military Medical University (Chongqing, China) and the use and care of animals were in accordance with National Research Council's guidelines for the use of experimental animals and permitted by Chongqing Animal Care Committee (SYXK-(YU), 2009-0002). Animals were housed under standard environmental conditions (24 ± 2 °C, 55 ± 10% relative humidity, 15 times air changes in 1 h, 12 h light-dark cycle) and adapted to diet for one week before the experiment. Diabetes was induced by single injection of STZ (40 mg/kg for mild diabetes and 55 mg/kg for severe diabetes) in citrate buffer (0.1 M sodium citrate and 0.1 M citric acid, pH 4.5) in overnight fasted rats (Brosky and Logothetopoulos, 1969; Dias et al., 2010). Fasting blood glucose (FBG) level was evaluated 3 d after STZ administration using glucose assay kits and rats with FBG value of 120–250 mg/dl were included in mild diabetic group and with FBG value of 300 mg/dl or above were included in severe diabetic groups (Gupta et al., 2005; Kesari et al., 2006).

2.3. Preparation RPO fractions

The dried RPO (5 kg) were extracted with 80% ethanol (40 L × 3) at 80 °C for 2 h, and then ethanol was evaporated under vacuum condition to give a brown residue (25.00% yield, labeled as Fr.0). The residue was suspended in water and successively partitioned with petroleum ether (0.47% yield, labeled as Fr.1), acetic ether (0.31% yield, labeled as Fr.2), n-buthanol (2.08% yield, labeled as Fr.3). The remaining aqueous fraction was labeled as Fr.4 (21.09% yield). The fractions, then, were subjected to glucose uptake test in HepG2 cells. Fr.3 with the highest ability to enhance glucose uptake in HepG2 cells was further fractionated with D101 macroporous resin column in which water and ethanol at various concentrations (20%, 40%, 60% and 80%) were used as eluent. Sequentially, Fr.3-1 (1.58%), Fr.3-2 (0.12%), Fr.3-3 (0.08%), Fr.3-4 (0.09%) and Fr.3-5 (0.03%) were obtained. The detailed process was shown in Fig. 1.

2.4. Test of glucose uptake in HepG2 cells

HepG2 cells were seeded into 48-well plates in 1640-culture medium supplemented with 10% heat-inactivated FBS and penicillin-streptomycin (100 U/ml, each) and cultured in an incubator (5% CO_2) at 37 °C for 24 h. Then the medium was replaced by 1640-culture medium without FBS and extracts with different concentrations as well as metformin (0.01 mg/ml) were added to corresponding wells. After 24 h incubation, the glucose concentrations in the medium were determined using commercially available kits. Glucose uptake was calculated by subtracting the glucose concentration of control groups from treated groups.

MTT assay (van de Loosdrecht et al., 1991; Zheng et al., 2011) was subsequently carried out after the glucose uptake test to estimate the influence of extracts on cell survival. Briefly, 15 μl MTT (5 mg/ml in DMEM) was added to each well and incubated at 37 °C for 4 h. The corresponding supernatant, then were discarded and cells were washed. DMSO (200 μl) was added to each well to extract the dye and the microplate was placed on a shaker for well dissolution. After 10 min, the optical density (OD) of each well was measured at 490 nm on a microplate reader.

2.5. Chemical analysis

Total saponin content was determined using a spectrophotometric method and oleanic acid was used to prepare the calibration curve (Hiai et al., 1976). Total sugar was determined by phenol-sulfuric acid method (Rao and Pattabiraman, 1989). Meanwhile, total flavonoids content was determined by the aluminum chloride colorimetric method and rutin was used to prepare the calibration curve (Chang et al., 2002).

2.6. Oral glucose tolerance test

The mild diabetic rats were divided into seven groups ($n = 6$) and oral glucose tolerance test (OGTT) was carried out after overnight fasting. Control group was given vehicle (distilled water) only and positive group received metformin (300 mg/kg). Meanwhile, other groups received different fractions (Fr.3-1, Fr.3-2, Fr.3-3, Fr.3-4 and Fr.3-5) suspended in distilled water at a dose of 500 mg/kg. After 30 min of administration, each rat received orally a glucose solution (2 g/kg). The blood glucose levels were measured before and 30, 60 and 120 min after glucose loading using commercially available kits. Total glycemic responses to OGTT were calculated from respective areas under the curve (AUC) of glycemia during the 120 min observation period.