Hypoglycaemic and hypolipidaemic properties of peach gum polysaccharides

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Abstract Hyperglycaemia and hyperlipidaemia are major risk factors for coronary artery diseases and atherosclerosis. Peach gum polysaccharides (PGPs) possess various bioactivities. In this study, PGPs were extracted with thermostable α -amylase and investigated in terms of hypolipidaemic and hypoglycaemic activities. KKAY mice were gavaged once daily with either PGPs or distilled water (control group) for 3 weeks. Oral administration of PGPs decreased the levels of serum triglyceride, cholesterol low-density lipoprotein cholesterol, fasting blood glucose, plasma insulin, C-peptide, and HbA1c in mice. Moreover, treatment with PGPs increased the insulin sensitivity index in KKAY mice. Results indicated that PGPs possess significant hypoglycaemic effects and could be developed as a drug for preventing hyperlipidaemia and hyperglycaemia.

Keywords Peach gum · Polysaccharide · Hypolipidaemia · Hypoglycaemia

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Introduction

Diabetes mellitus is a common, chronic, hereditary disease caused by insulin deficiency or impaired insulin action and is the leading cause of death (Liu et al. 2014). More than 90% of diabetic patients are diagnosed as non-insulin-dependent (type 2 diabetes); this type is characterised by hyperglycaemia, dyslipidaemia, and hyperlipidaemia, which are caused by defects in either insulin secretion or action (Huang et al. 2015). Different synthetic drugs, e.g., biguanides and sulfonylureas, have been developed for treatment of type 2 diabetes; however, these drugs exhibit certain adverse effects, contraindications, and high prices, restricting their use (Grover et al. 2002). Therefore, alternative drugs and natural therapies with low toxicity and high efficiency must be developed.

Peach, which belongs to the subfamily Prunoideae and the family Rosaceae, secretes abundant gum from their trunks following mechanical injury and microbial attacks (Simas et al. 2008). Peach gum consists of polysaccharides, which possess various bioactivities, i.e., antioxidant (Yao et al. 2013b) and antibacterial (Yao et al. 2013a). Nevertheless, data regarding hypolipidaemic and hypoglycaemic activities of PGPs are limited.

In this study, PGPs were extracted by thermostable α -amylase-assisted methods. The hypolipidaemic and hypoglycaemic activities of the extract were then evaluated in KKAY mice.

Materials and methods

Ethics statement

This study was approved by the Ethics Committee of Huaihai Institute of Technology, China.

Materials

Dried peach gum was purchased from Shandong Dingli Rubber Industry Co., Ltd. (Shandong, China). The thermostable α -amylase from *Thermococcus* sp. HJ21, with an enzymatic activity of 20,000 U/g, was prepared in our laboratory according to the method described by Wang et al. (2009). Standard monosaccharides such as glucose, fucose, rhamnose, galactose, arabinose, xylose, and mannose were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents were of analytical grade.

PGP preparation

The dried peach gum was pulverised and sifted through a 60-mesh sieve to obtain fine powder. The powder was soaked in distilled water under agitation at room temperature ($\sim 25^\circ\text{C}$) for 10 min to yield a suspension with a concentration of $\sim 1\%$ (w/v). The pH of the suspension was adjusted to 5, and 6000 U/g of thermostable α -amylase was added. The reactor was maintained in a thermostatic water bath at 95°C for 30 min and the thermostable α -amylase was inactivated by incubating at 120°C in a sterilizer for 10 min. Proteins in the resulting supernatant were separated using the Sevag method, precipitated with six volumes of absolute ethanol, filtered through a Whatman GF/A filter paper, and freeze dried.

PGP characterisation

Total sugar, protein, and ash contents in the products were determined using phenol–sulphuric acid colorimetric method, Kjeldahl method, and a method described by Hou (2004). Monosaccharide composition of the PGPs was analysed according to the method of Sheng et al. (2007). The Fourier transform infrared (FTIR) spectra of representative product samples were obtained in KBr pellets using a Nicolet Nexus FTIR 470 spectrophotometer over a wavelength range of $400\text{--}4000\text{ cm}^{-1}$. The UV spectra were recorded on a UV spectrometer (Spectra Test, German).

Test animals

KKAy mice were used to develop type 2 diabetes models. Twenty-four KKAy mice with similar initial weights and blood glucose levels were divided into control and three PGP groups (50, 100, and 150 mg/kg PGPs). Mice in all groups were fed with a high-fat diet containing 10% (w/w) lard, 15% (w/w) egg yolk powder, 1% (w/w) cholesterol, and 76% (w/w) basic diet, with composition conforming to AIN 76 (Shanghai SLAC Laboratory Animal Co. Ltd.,

Shanghai, China). Mice in the PGP group were gavaged twice daily with 50, 100, and 150 mg/kg PGPs, and mice in control group were gavaged twice daily with equal volumes of distilled water.

Biochemical measurement

After 3 weeks of treatment with PGPs (treatment group) or distilled water (control group), the mice were starved for 12 h and anaesthetised. Blood samples were collected, placed into pre-chilled tubes, and immediately centrifuged at $3000\times g$ for 5 min. The separated serum was analysed. The levels of plasma serum triglyceride (TG), cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), C-peptide, and HbA1c were determined using commercial assay kits. Serum insulin level was determined using a mouse insulin enzyme-linked immunosorbent assay kit. Insulin sensitivity index (ISI) was calculated according to the following equation:

$$\text{ISI} = 1/(\text{fasting blood glucose} \times \text{fasting insulin}). \quad (1)$$

Statistical analysis

Data are presented as mean $\pm$ SD, and Student–Newman–Kuels multiple range test was performed to compare the means of the two groups. Statistical significance at the 95% probability level was set at $p < 0.05$.

Results and discussion

Product characterisation

Ash, moisture, protein, and total sugar contents in the PGP sample were 0.4, 2.9, 6.2, and 90.1% (w/w), respectively, indicating that PGPs could be glycoproteins. PGP samples were water-soluble white powders. Analysis of monosaccharide composition through gas chromatography indicated that the PGPs consisted of arabinose ($\sim 52\%$), galactose ($\sim 31\%$), and uronic acid (~ 13); this finding is consistent with the report of Qian et al. (2011). The FTIR spectra of the PGPs peaked at $\sim 3400\text{ cm}^{-1}$ (O–H), $\sim 1420\text{ cm}^{-1}$ (symmetrical deformation of $-\text{CH}_3$ and $-\text{CH}_2$), $\sim 1040\text{ cm}^{-1}$ (stretching vibration of the C–O–C in glucose circle), and $\sim 1705\text{ cm}^{-1}$ (special absorbance peaks of aldehyde in PGPs) (Fig. 1), indicating that the PGP product was mainly composed of polysaccharides. The UV spectra of the PGP samples displayed two peaks at 200–280 nm (Fig. 2), indicating that the PGP product contained protein.

Fig. 1 FT-IR spectra of peach gum polysaccharides

Fig. 2 Ultraviolet visible spectrum of peach gum polysaccharides

Serum lipid levels in KKAY mice

Dyslipidaemia is one of main characteristics of KKAY mice. Table 1 lists the effects of PGP treatment on serum lipid levels in KKAY mice. Low doses of PGPs (50 mg/kg) did not significantly affect the levels of serum TG, TC, LDL-C, and HDL-C in KKAY mice ($p > 0.05$). High doses of PGPs (100 and 150 mg/kg) significantly decreased the levels of serum TG, TC, and LDL-C ($p < 0.05$), except for HDL-C, in KKAY mice ($p > 0.05$). Hence, PGP treatment dose-dependently decreased the lipid levels in KKAY mice. Similarly, the previous studies reported that serum levels of TG, TC, and LDL-C in mice were decreased by chitosan

(Zhang et al. 2011, 2012, 2013) and polysaccharides from *Porphyra yezoensis* (Qian et al. 2014), *Enteromorpha prolifera* (Teng et al. 2013) and pumpkin (Zhao et al. 2014).

Fasting blood glucose levels

The initial fasting blood glucose levels were not significantly different among all groups (Table 1, $p > 0.05$). Treatment with 100 and 150 mg/kg PGPs decreased the fasting blood glucose level in KKAY mice ($p < 0.05$), whereas treatment with 50 mg/kg PGPs did not change the fasting blood glucose level in KKAY mice ($p > 0.05$). Similarly, polysaccharides prepared from *Enterobacter cloacae* decreased the fasting blood glucose level in mice (Huang et al. 2015; Liu et al. 2014) (Table 2).

Effect of PGP administration on insulin, C-peptide, ISI, and HbA1c levels in KKAY mice

The effects of PGP treatment on insulin, C-peptide, ISI, and HbA1c levels in KKAY mice were investigated (Table 3). Treatment with 100 and 150 mg/kg PGPs decreased the serum levels of insulin, C-peptide, and HbA1c in KKAY mice compared with those in the control group ($p < 0.05$). Consequently, these treatments increased the ISI in KKAY mice compared with that in the control group ($p < 0.05$). Serum levels of insulin, C-peptide, and HbA1c were not significantly different between treatment with 50 mg/kg PGP and control ($p > 0.05$). The previous studies reported that other polysaccharides exhibit hypoglycaemic activities; these polysaccharides include those derived from *Enterobacter cloacae* (Huang et al. 2015), *Trichosanthes* peel (Chen et al. 2016), bamboo shoots (*Leleba oldhami* Nakal) shells (Zheng et al. 2016), *Lycium barbarum* L (Tang et al. 2015), loach (Zhou et al. 2015a), the fruiting bodies of the shaggy ink cap medicinal mushroom (Zhou et al. 2015b), and *Grifola frondosa* (Xiao et al. 2015). However, the mechanism underlying the hypoglycaemic effect of PGPs is difficult to explain because of their different structures.

Conclusions

PGPs were extracted with thermostable α -amylase-assisted methods and investigated in terms of hypolipidaemic and hypoglycaemic activities. PGPs showed dose-dependent hypoglycaemic and hypolipidaemic activities in KKAY

Table 1 Effect of peach gum polysaccharides (PGPs) on serum TG, TC, LDL-C, and HDL-C levels in KKAY mice

Group	TG (mmol/L)	TC (mmol/L)	LDL-C (mmol/L)	HDL-C (mmol/L)
Control	2.09 ± 0.71	2.39 ± 0.38	0.98 ± 0.41	1.13 ± 0.38
50 mg/kg PGPs	1.92 ± 0.63	2.34 ± 0.32	0.92 ± 0.38	1.09 ± 0.36
100 mg/kg PGPs	0.89 ± 0.51*	1.81 ± 0.18*	0.41 ± 0.16*	1.07 ± 0.35
150 mg/kg PGPs	0.75 ± 0.51*	1.76 ± 0.17*	0.31 ± 0.14*	1.04 ± 0.33

Values are expressed as mean ± SD ($n = 3$)

* Significant difference compared with control group ($p < 0.05$)

Table 2 Effect of peach gum polysaccharides (PGPs) on fasting blood glucose level in KKAY mice

Group	Initial (mmol/L)	Treatment (mmol/L)
Control	17.26 ± 4.18	23.31 ± 5.16
50 mg/kg PGPs	17.33 ± 4.21	22.86 ± 5.03
100 mg/kg PGPs	17.67 ± 4.19	11.34 ± 6.16*
150 mg/kg PGPs	17.18 ± 4.09	9.52 ± 3.15*

Values are expressed as mean ± SD ($n = 3$)

* Significant difference compared with control group ($p < 0.05$)

Table 3 Effect of peach gum polysaccharides (PGPs) on insulin, C-peptide, ISI, and HbA1c levels in KKAY mice

Group	Insulin (mU/L)	C-peptide (μg/L)	ISI (μg/L)	HbA1c (%)
Control	24.09 ± 3.71	1.53 ± 1.49	−6.37 ± 0.42	10.57 ± 3.27
50 mg/kg PGPs	23.74 ± 6.63	1.49 ± 0.82	−6.22 ± 0.38	10.02 ± 3.34
100 mg/kg PGPs	17.81 ± 7.52*	0.81 ± 0.68*	−5.24 ± 0.61*	8.07 ± 2.36*
150 mg/kg PGPs	14.73 ± 1.59*	0.31 ± 0.12*	−4.36 ± 0.17*	6.08 ± 2.43*

Values are expressed as mean ± SD ($n = 3$)

* Significant difference compared with control group ($p < 0.05$)

mice. Therefore, PGPs may be developed as a promising drug for prevention of hypoglycaemia and hypolipidaemia. Nevertheless, the mechanisms underlying the hypoglycaemic and hypolipidaemic activities of PGPs must be further investigated.

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Compliance with ethical standards

Conflict of interest The authors have declared that no competing interests exist.

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