Polysaccharide from *Plantago asiatica* L. attenuates hyperglycemia, hyperlipidemia and affects colon microbiota in type 2 diabetic rats

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**A B S T R A C T**

Type 2 diabetes is a complex metabolic and endocrine disorder worldwide, which causes severe health and economic problems. The effects of polysaccharide from *Plantago asiatica* L. (PLP) on high-fat diet and streptozotocin-induced type 2 diabetic rats were examined. Administration of PLP caused significant decreases in the concentrations of blood glucose, insulin, total cholesterol, triglyceride, non-esterified fatty acid and maleic dialdehyde, and significant increases in the levels of high density lipoprotein-cholesterol and the activities of antioxidant enzymes compared with diabetic rats after 4 weeks' treatment. The concentrations of short-chain fatty acids (SCFA) were significantly higher in the feces of diabetic rats after treatment with PLP. Moreover, colon bacterial diversity and abundance of bacteria, including *Bacteroides vulgatus*, *Lactobacillus fermentum*, *Prevotella loescheii* and *Bacteroides vulgates* were significantly increased by PLP treatment. These results indicated that the anti-diabetic effect of PLP in type 2 diabetic rats may be associated with regulation of gut microbiota and increased levels of SCFA.

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### 1. Introduction

Type 2 diabetes (T2D) is a complex metabolic disorder characterized by hyperglycemia, low-grade inflammation, insulin resistance and β-cell failure and mainly affects glucose, lipid and protein metabolism (Farzaei, Rahimi, Farzaei, & Abdollahi, 2015). According to the International Diabetes Federation, 380 million or more individuals suffer from diabetes worldwide, and this number was projected to increase to 592 million by 2035, among which T2D accounts for at least 90% (Guariguata et al., 2014). The chronic metabolic disorder are believed to be caused by a combination of genetic and environmental interactions, such as diet, age, gut microbiota, lifestyle and obesity (Karlsson et al., 2013). A number of anti-diabetic agents have been synthesized for the treatment of diabetes, such as biguanides, α-glucosidase inhibitors and thiazolidinediones, but the side-effects including flatulence, discomfort and sometimes diarrhea were presented in patients after administration (Krentz & Bailey, 2005; May, Lefkowitch, Kram, & Rubin, 2002). The potential anti-diabetic effects of phytochemicals from natural resources, such as polysaccharide might have comparatively low side-effects and low cost (Y. G. Li et al., 2011).

Some polysaccharides have been reported to have anti-diabetic effects through a variety of mechanisms, such as targeting β-cell dysfunction, insulin enhancement and inhibiting α-amylase and α-glucosidase (Wu, Shi, Wang, & Wang, 2016). A polysaccharide from corn silk was found to have hypoglycemic and hypolipidemic effects in high-fat diet and STZ-induced diabetic mice, and the organ injury accompanied by diabetes were repaired at the same time (Pan et al., 2017). Soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) from deoiled cumin significantly improved glucose control and insulin resistance in type 2 diabetic rats, and IDF may have better anti-diabetic effects than IDF (Ma & Mu, 2016). Furthermore, physicochemical properties of polysaccharide, such as viscosity and solubility are important for its hypoglycemic effect, viscous dietary fibers may reduce glycemia to a greater extent than non-viscous fibers (Chutkan, Fahey, Wright, & McRorie, 2012).

Increasing evidence indicates the strong association between gut microbiota and diabetes development (Haro et al., 2016). Gut microbial dysbiosis always occurred in diabetic individuals, such as a greater abundance of opportunistic pathogens (*Betaproteobacteria, Clostridiunum botleae,* and a relatively low abundance of some universal butyrate-producing bacteria (*Fusabacterium prausnitzii*) were found in the gut of diabetic patients compared with healthy controls (Qin et al., 2012; Xu et al., 2015), which may result in a decreased level of short chain fatty acids and increased levels of harmful fermented products (such as ammonia and indol) (Zhu...
et al., 2016). Especially the increase of gram-negative bacteria in diabetes resulted in accumulation of lipopolysaccharides (LPS), which was considered as a major factor in onset and progression of metabolic endotoxemia (Munoz-Garach, Diaz-Perdigones, & Tihanones, 2016). Consequently, polysaccharide-evoked structure change of gut microbiota has become a new target for diabetes treatment.

Plants of the Plantago family are a traditional Chinese medicine which have been used as folk medicine throughout the world (Samuelsen, 2000). Our research group has isolated and purified a novel polysaccharide from the seeds of P. asiatica L. (PLP) with a molecular weight of 1894 kDa, which was a arabinoxylan characterized by high viscosity and rich in xylose (60%) and arabinose (32%) (Yin, Nie, Zhou, Wan, & Xie, 2010). It was found that PLP had notable influence on slowing down glucose diffusion and inhibiting α-amylase activity in vitro (Hu, Nie, Li, & Xie, 2013). PLP administration significantly reduced serum total triglyceride, cholesterol, atherogenic index and improved the structure of colon microbiota in normal mouse. (Hu, Nie, Min, & Xie, 2012; Hu et al., 2014). However, the effects of PLP on type 2 diabetes have not been investigated. Accordingly, the present study was undertaken to investigate the hypoglycemic and hypolipidemic effects and regulation of gut microbiota of PLP in high-fat diet and STZ-induced type 2 diabetic rats.

2. Materials and methods

2.1. Polysaccharide preparation

The seeds of P. asiatica L. were purchased from Ji'an, Jiangxi Province, China. Polysaccharide from P. asiatica L. seeds was prepared using our previous method (Yin et al., 2010). Briefly, the seeds of P. asiatica L. were defatted with ethanol (m:v/1:20) at room temperature for 24 h and then extracted with doubly distilled water (m:v/1:10) at 100 °C for 2 h. Aqueous extract of P. asiatica L. seeds was separated through a cotton cloth bag and the residue was re-extracted. The combined aqueous extract (a gel of high viscosity) was concentrated by rotary evaporator to yield P. asiatica L. water extract. The concentrated water extract was precipitated by adding 4 volumes of anhydrous ethanol at 4 °C for 12 h, precipitate was re-dissolved by distilled water and solution was deproteinized with 1/3 volume of CHCl3/n-BuOH (4:1, v/v) to remove protein. The resulting aqueous solution was extensively dialyzed against doubly distilled water for 72 h, and lyophilized to yield the polysaccharide.

2.2. Animals and experimental design

Seven-week-old male inbred Wistar rats (180–200 g) were obtained from Vital River Laboratories (VRL, Beijing, China). All animals were acclimatized under conditions at 22 ± 2 °C, 55 ± 10% relative humidity, and 12/12 h light/dark cycle for 1 week before commencement of the animal experiment.

After acclimation, 20 rats fed a standard normal chow diet and the others were fed a high fat diet (HFD, 66.5% normal chow diet, 10% lard, 20% sucrose, 2.5% cholesterol and 1% sodium cholate) (C. Li et al., 2014). After 8 weeks of dietary manipulation, all rats were fasted for 12 h. Rats fed on HFD were injected with streptozotocin [STZ, 30 mg/kg body weight (BW)] into the tail vein to induce type 2 diabetes and those fed on basal diet received an equivalent volume of saline (Zhu et al., 2013). Rats were considered diabetic when the fasting blood glucose (FBG) level exceeded 16.7 mmol/L. The experimental design was shown in Fig. 1. Food intake and water consumption were measured weekly and body weights were weighed daily to adjust gavage dose. All animals used in this study were approved by the Animal Care and Use Committee, Nanchang University.

2.3. Measurement of FBG, serum GSP, insulin level and QUICKI

During the experimental period, FBG levels were measured weekly. The FBG levels in the rats were determined by Accu-Chek Performa (Roche Diagnostics, Mannheim, Germany) from the tail vein after overnight fasting. At the end of the 4-week experimental period, the rats were humanly anesthetized with isoflurane, blood samples were obtained by eyeball exteriorping and centrifuged at 1,000 g for 10 min to collect serum. The concentrations of insulin and glycated serum protein (GSP) in serum were determined using commercially available kits (Nanjing Jianchen Bioengineering Institute, Jiangsu, China). Furthermore, quantitative insulin sensitivity check index (QUICKI) was calculated from fasting glucose and fasting insulin levels according to the formula:

\[\text{QUICKI} = 1/[(\text{fasting insulin}) + \log(\text{fasting glucose})]\]

2.4. Measurements of serum lipid, kidney function and NEFA

Levels of serum lipids including total cholesterol (TC), triacylglycerols (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C), and kidney function parameters (serum creatinine, uric acid and urea nitrogen) were determined by using an Automatic Biochemical Analyzer (Mindray BS-380, Shengzheng, China). Non-esterified fatty acid (NEFA) content in the serum was determined by an assay kit (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China) as the manufacturer's instructions.

2.5. Determination of oxidative stress parameters in the serum

Levels of malondialdehyde (MDA), activities of superoxide dismutase (SOD) and catalase (CAT), and total antioxidant capacity (T-AOC) were determined following the instructions of the kits. MDA and CAT kits were obtained from Beyotime (Shanghai, China), and SOD and T-AOC assay kits were purchased from Nanjing Jiancheng (Jiangsu, China).

2.6. DGGE analysis

The total DNA was extracted from colon content samples (QIAamp DNA Stool Mini Kit, QIAGEN, Shanghai, China) and used as a template for PCR amplification. Amplicons of the V3 regions of bacterial 16S rDNA and denaturing gradient gel electrophoresis (DGGE) analysis were performed as described previously (Xie et al., 2016). DGGE image were normalized and analyzed with Bio-Rad Quantity One 4.4.0 software and Canoco 4.5, and clustering was done with Pearson correlation and the unweighted pair group means average (UPGMA) method. Identification of DGGE DNA bands was according to the previous method (Hu et al., 2014).

2.7. Analysis of fecal SCFA and pH

SCFA of feces and fecal pH value were measured as described in our previous report (Hu et al., 2012). Briefly, 200.0 mg of feces were diluted with deionized water at a ratio of 1: 9, vortexed for 2 min, and then subjected to ultrasound for 5 min. The sample was allowed to stand in the ice bath for 20 min and then centrifuged at 4,800 g for 20 min. The procedure was repeated and the
Supernatant were collected for the gas chromatography analysis and pH measurement.

2.8. Statistical analysis

Statistical analysis was determined by SPSS 17.0 (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) for multiple comparisons followed by Tukey’s test were used to determine significance of differences between groups. A difference with $p < .05$ was considered significant.

3. Results and discussion

3.1. Effects of PLP on body weight, food intake and water consumption

STZ-induced diabetic rats always showed gradual emaciation, exhibited decreased BW, increased food intake and water consumption compared with the normal rats. These symptoms were alleviated by treatment of PLP. The food intake and water consumption were elevated significantly in type 2 diabetic rats (from 10.88 to 17.05 g/100 g BW and 57.95 to 72.26 mL/100 g BW in DM group, Table 1) compared with non-diabetic controls. PLP treatment significantly decreased the polydipsia in diabetic rats when compared with un-treated diabetic rats.

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>1 week</th>
<th>2 week</th>
<th>3 week</th>
<th>4 week</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Food intake (g/100 g BW)</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>DM</td>
<td>10.88 ± 0.20</td>
<td>13.15 ± 0.09</td>
<td>14.55 ± 0.30</td>
<td>17.05 ± 0.73</td>
</tr>
<tr>
<td>LP + DM</td>
<td>11.73 ± 0.17</td>
<td>12.33 ± 0.05</td>
<td>11.78 ± 0.03**</td>
<td>13.07 ± 1.00**</td>
</tr>
<tr>
<td>MP + DM</td>
<td>13.37 ± 0.11</td>
<td>12.66 ± 0.68</td>
<td>13.56 ± 0.03</td>
<td>13.94 ± 1.05**</td>
</tr>
<tr>
<td>HP + DM</td>
<td>11.27 ± 0.15</td>
<td>13.90 ± 0.04</td>
<td>12.81 ± 0.26**</td>
<td>13.18 ± 1.02**</td>
</tr>
<tr>
<td>Met</td>
<td>11.26 ± 1.27</td>
<td>11.35 ± 1.65</td>
<td>12.72 ± 0.60**</td>
<td>13.51 ± 0.96**</td>
</tr>
<tr>
<td>CON</td>
<td>5.94 ± 0.53**</td>
<td>4.98 ± 0.52**</td>
<td>5.50 ± 0.10**</td>
<td>6.18 ± 0.12**</td>
</tr>
<tr>
<td>MP</td>
<td>6.64 ± 0.13**</td>
<td>6.60 ± 0.05**</td>
<td>7.71 ± 0.21**</td>
<td>7.07 ± 0.24**</td>
</tr>
<tr>
<td><strong>Water consumption (mL/100 g BW)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>57.95 ± 0.11</td>
<td>65.04 ± 1.90</td>
<td>68.14 ± 1.61</td>
<td>72.26 ± 1.12</td>
</tr>
<tr>
<td>LP + DM</td>
<td>54.74 ± 2.48</td>
<td>58.09 ± 0.69**</td>
<td>58.00 ± 1.33**</td>
<td>54.54 ± 1.96**</td>
</tr>
<tr>
<td>MP + DM</td>
<td>57.00 ± 0.50</td>
<td>58.77 ± 0.15**</td>
<td>61.55 ± 2.62**</td>
<td>55.58 ± 0.35**</td>
</tr>
<tr>
<td>HP + DM</td>
<td>53.61 ± 0.55**</td>
<td>59.32 ± 0.45**</td>
<td>64.82 ± 0.61</td>
<td>56.33 ± 1.69**</td>
</tr>
<tr>
<td>Met</td>
<td>55.48 ± 0.68</td>
<td>53.40 ± 0.58**</td>
<td>58.96 ± 1.23**</td>
<td>53.85 ± 2.50**</td>
</tr>
<tr>
<td>CON</td>
<td>10.04 ± 1.23**</td>
<td>11.21 ± 2.01**</td>
<td>9.49 ± 0.81**</td>
<td>9.65 ± 0.73**</td>
</tr>
<tr>
<td>MP</td>
<td>9.76 ± 0.58**</td>
<td>9.10 ± 0.53d**</td>
<td>11.49 ± 1.21**</td>
<td>8.72 ± 0.61**</td>
</tr>
</tbody>
</table>

*indicates significant difference compared with DM groups, $^*p < .05$, $^{**}p < .01$. 

The initial BW (0 week) of diabetic rats was less than the normal rats because STZ injection could result in the loss of BW (Fig. 2) (Pan et al., 2017). Control group showed steadily increased BW and DM group showed opposite trend, while the BW loss was significantly reduced by PLP and metformin treatment after 4 weeks. Furthermore, BW of MP group (normal rats treated with 200 mg/kg PLP) was significantly lower than that of control group (p < .05), which indicated that PLP treatment might have potential effect on BW control for the normal rats.

3.2. Effects of PLP on levels of FBG, GSP and insulin

T2D was characterized by increased FBG level, insulin resistance and higher insulin level (S. Q. Li et al., 2015). Levels of FBG, GSP, and insulin in each group were measured at the end of the experimental period (Fig. 3A). DM group reached the highest FBG (31.2) and LP + DM group exhibited the lowest glucose levels (19.8). FBG levels in LP + DM, MP + DM, HP + DM and Met group were decreased by 36.5%, 17.9%, 25.3% and 32.1% compared with DM group, respectively (Fig. 3A). The levels of GSP, an effective indicator of the blood glucose level, were reduced by PLP and metformin treatments compared with DM group, the data were in agreement with FBG but only low-dose PLP administration exhibited significance (p < .05) (Fig. 3B). Ou, Kwok, Li, and Fu (2001) have reported that the diffusion rate of glucose could be reduced by polysaccharides due to their viscosity, and polysaccharides could absorb the glucose and prevent its diffusion. PLP is a kind of gel with high viscosity, and our previous study reported that PLP had notable influence on slowing down the diffusion of glucose and could inhibit the activity of α-amylase (Hu et al., 2013; Yin et al., 2012). Therefore, the reduction of the FBG in diabetic rats may be explained by glucose adsorption and viscosity of PLP.

Insulin resistance and higher insulin level in serum of T2D is essential to cope with the glucose load (Pan et al., 2017). Insulin level was significantly increased (53.41%) in DM group compared with the control group, indicating insulin resistance (Fig. 3C). The insulin level significantly decreased from 54.7 ± 2.6 to 44.8 ± 2.6, 45.1 ± 1.1, 48.0 ± 4.0 and 46.0 ± 3.9 (p < .05 or p < .01), respectively, for the diabetic rats treated with low, middle, high-dose PLP and metformin, compared with that of diabetic rats. QUICKI is an essential indicator to estimate insulin resistance, diabetic rats were found to be the lowest in QUICKI level, indicating the significant insulin resistance occurred (Fig. 3D).

3.3. Effects of PLP on serum lipid profile and kidney function

It is well known that diabetic patients often had dyslipidemia, the disorder of lipid metabolism always leading to accumulation of plasma TC, TG, LDL-C and decreased HDL-C. Dyslipidemia is considered as the major risk factor for cardiovascular diseases, such as coronary heart disease and atherosclerosis, and lipid profile is an essential factor of cardiovascular risk in T2D (Rangikia, Dayananda, & Peiris, 2015). The effects of PLP on serum TC, TG, LDL-C, and HDL-C concentrations of the animals in different groups are summarized in Table 2. The levels of TC, TG, and LDL-C were increased 0.5, 4.7 and 1.7 fold and HDL-C level was decreased 0.3 fold in DM group compared with control group, respectively. PLP administration significantly reduced levels of TC (except middle-dose PLP), TG and increased level of HDL-C compared with diabetic rats (p < .05). LDL-C level in diabetic rats was also partially improved by PLP administration. Notably, levels of TC, TG and LDL-C tend to be lower in the MP group (normal rats treated with 200 mg/kg PLP), with the difference between the control group and the MP group being significant (p < .05). These results were in agreement with those of Hu et al. (2014) that PLP appeared to play a role in lowering the serum TC and TG levels in normal mice. The beneficial effects of PLP on serum lipid profile in diabetic rats may attribute to relatively high level of propionate acid after PLP administration (Fig. 5A). Diets supplemented with propionate acid exhibited hypocholesterolemic effects in animals, and increased serum HDL-C also found in a human study (Chen, Anderson, & Jennings, 1984; Venter, Vorster, & Cummings, 1990). Furthermore, physiological concentrations of propionic acid may attenuate hepatic cholesterol synthesis in rat liver cells (Anderson & Bridges, 1984). Thus, the potential regulatory effect of PLP on lipids profile in type 2 diabetes was beneficial for health.

NEFA originates in the adipocyte and has ability to modulate insulin action, which is associated with metabolic disorders, such as insulin resistance and obesity (Delarue & Magnan, 2007). The levels of NEFA were found to be higher in diabetic rats (0.92 mmol/mL) compared with normal rats (0.61 mmol/mL), and NEFA levels in diabetic rats were significantly reduced to 0.72, 0.72, 0.63 and 0.71 mmol/mL by three doses of PLP and metformin treatments, respectively. The result of NEFA is in agreement with QUICKI (Fig. 3D) that excess NEFA is primarily responsible for the insulin resistance (Delarue & Magnan, 2007).

Diabetic nephropathy is one of serious complications of diabetes and is a major contributing cause of morbidity and mortality in patients with diabetes. The parameters including BUN, UA and CRE have been widely used in clinics to reflect the physical status of kidney (Rippin, Barnett, & Bain, 2004; Stevens & Levey, 2005). There were significantly higher levels of renal functional parameters, CRE (3.0 fold), UA (1.9 fold) and BUN (1.5 fold) in diabetic rats compared with normal rats (Table 2), indicating that our model was successful at inducing diabetic nephropathy. Administration of PLP and metformin improved kidney function parameters, especially the PLP at the dose of 200 mg/kg and 400 mg/kg (p < .01 or p < .05) (Table 2). Notably, the regulation effects of PLP on CRE and UA were better than metformin. The results suggested that PLP may have protective effects on kidney in diabetic rats.

3.4. Effect of PLP on MDA level and activities of antioxidant enzymes

Glucotoxicity characterized by chronic hyper-glycemia is a common feature of T2D, which is a cause of impairment of insulin biosynthesis and secretion by β-cells and in turn, gradually
deteriorates β-cell function (Jonas et al., 1999). The mechanism of glucotoxicity is mediated by oxidative stress which has been considered as a major risk factor for the onset and progression of T2D (Wu et al., 2016). Free radicals are generally formed disproportionately by glucose oxidation, lipid per-oxidation, non-enzymatic glycation in diabetes, and antioxidant enzymes, such as SOD, CAT, and GSH-Px scavenge free radicals and increase the response of antioxidant defense systems (Yu et al., 2009). Moreover, MDA (product of chain reaction of lipid peroxidation) is also associated with the diminution of the activities of antioxidant enzymes.

As shown in Table 3, MDA level (7.72 μmol/L) was significantly increased (p < 0.01) whereas activities of SOD and CAT (66.19 and 95.10 U/mL, respectively) were significantly decreased (p < 0.01) in diabetic rats when compared with normal rats. The MDA level significantly decreased (from 7.72 ± 1.53 to 3.22 ± 0.70, 3.28 ± 0.32, 3.22 ± 0.51 and 2.81 ± 0.75 (p < 0.01), respectively), for the diabetic rats treated with low, middle, high-dosage PLP and metformin, compared with that of diabetic rats. Activities of SOD and T-AOC (except MP + DM group) were significantly increased by PLP treatment but no dose-related effect, and high-dose PLP showed the strongest T-AOC. Furthermore, the activities of CAT in PLP group also tended to increase compared with diabetic rats. Oxidative stress impaired the living cell membrane that result in the injury to the organs like langerhans and kidney. Antioxidant activity of polysaccharides might have played an important role in the treatment of diabetes, and polysaccharide appeared to be more effective to boost antioxidant status (Huang, Korivi, Chaing, Chien, & Zeng, 2011). Overall, the results indicated that PLP could suppress oxidative stress by improving the activities of antioxidant enzymes.

### 3.5. Changes in colon microbiota composition

Composition of gut microbiota may be directly responsible for inducing a low-grade inflammatory state closely associated with T2D disorder. Abundance of Gram-negative bacteria that specifically belong to the phylum Bacteroidetes and Proteobacteria (which main compounds of outer membranes are LPS) were relatively high in patients with T2D (Larsen et al., 2010). Diversity indices of the DGGE bacterial profiles (Fig. 4A) are shown in Table 4. The richness for the DM group (20.2 ± 1.5) was significantly lower than for the control group (26.0 ± 1.4), which was significantly increased by PLP and metformin treatments compared with un-treated diabetic rats (p < 0.01). In addition, a significant increase was observed in the Shannon-Wiener index in LP + DM and Met group relative to the DM group (p < 0.01). Maximum Shannon-Wiener index of the PLP group was higher than that of DM group (p < 0.05) but the evenness of the DM group was not significantly different from those of the other groups.

The effects of PLP on the bacterial communities in rats were analyzed by DGGE after 4 weeks' oral administration. UPGMA cluster analysis for the DGGE image showed that the colonic bacterial community structure had a relatively low similarity between DM group and other groups. A majority of PLP treatment in diabetic rats were clustered with control group in a same unit, which means PLP administration may be a main factor affecting the bacterial community. RDA (Redundancy Analysis, correlational analysis between independent variable and dependent variable) was used to analyze the correlation between the banding patterns of different treatments. A Monte Carlo permutation analysis indicated that the factors (PLP, diabetes and metformin) had a significant influence on the colon bacterial community structure (p < 0.01). Fifteen bands were observed from the RDA (Fig. 4B–D) that was significantly correlated with PLP, diabetes and metformin. Six, 8 and 3 strains had positive correlations with PLP, DM and metformin, respectively, three strains including Bacteroides vulgaris, Lactobacillus fermentum and Prevotella loechei had positive correlations with PLP and metformin, while Alistipes obesi, Eubacterium fissicatena, Clostridium bolteae and Ruminococcus bromii were increased in diabetic rats. Furthermore, Alistipes obesi had positive correlations with DM but negative correlations with PLP and metformin, abundance of Bacteroides ovatus was only increased by PLP treatment (Table 5).

Prebiotics (including polysaccharide) are promising agents for improvement of gut health, the macromolecular substances are resistant to digestion by human enzymes and rely on microbial enzymes for their digestion. The fermentation procedure yield energy for the microbial growth, leading to SCFA production which exert profound effects on the health of the host. Abundance of Bacteroides vulgaris, Lactobacillus fermentum and Prevotella loechei was increased by PLP and metformin administration.

### Table 2

Effect of PLP on serum lipid and kidney function in rats with type 2 diabetes.

<table>
<thead>
<tr>
<th>Group</th>
<th>TC (mmol/L)</th>
<th>TG (mmol/L)</th>
<th>HDL-c (mmol/L)</th>
<th>LDL-c (mmol/L)</th>
<th>NEFA (mmol/mL)</th>
<th>Creatinine (μmol/L)</th>
<th>Uric acid (μmol/L)</th>
<th>Urea nitrogen (μmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM</td>
<td>3.43 ± 0.32</td>
<td>3.62 ± 0.86</td>
<td>1.21 ± 0.04</td>
<td>1.38 ± 0.28</td>
<td>0.92 ± 0.09</td>
<td>68.96 ± 9.73</td>
<td>119.27 ± 14.15</td>
<td>6.81 ± 0.43</td>
</tr>
<tr>
<td>LP + DM</td>
<td>2.65 ± 0.57*</td>
<td>1.60 ± 0.78**</td>
<td>1.61 ± 0.22**</td>
<td>1.03 ± 0.31</td>
<td>0.72 ± 0.02**</td>
<td>23.53 ± 6.54**</td>
<td>95.60 ± 23.52</td>
<td>5.35 ± 0.41*</td>
</tr>
<tr>
<td>MP + DM</td>
<td>2.75 ± 0.73</td>
<td>1.67 ± 0.80**</td>
<td>1.47 ± 0.08**</td>
<td>1.01 ± 0.45</td>
<td>0.72 ± 0.13**</td>
<td>24.32 ± 5.90**</td>
<td>83.00 ± 24.56**</td>
<td>5.45 ± 1.03*</td>
</tr>
<tr>
<td>HP + DM</td>
<td>2.57 ± 0.39</td>
<td>2.35 ± 0.77**</td>
<td>1.43 ± 0.22**</td>
<td>0.82 ± 0.25**</td>
<td>0.63 ± 0.10**</td>
<td>17.93 ± 1.93**</td>
<td>87.33 ± 15.24**</td>
<td>5.43 ± 0.77**</td>
</tr>
<tr>
<td>Met</td>
<td>2.73 ± 0.35</td>
<td>1.10 ± 0.32**</td>
<td>1.70 ± 0.43**</td>
<td>0.72 ± 0.05**</td>
<td>0.71 ± 0.08**</td>
<td>39.53 ± 2.45**</td>
<td>73.00 ± 5.68**</td>
<td>6.01 ± 0.72**</td>
</tr>
<tr>
<td>CON</td>
<td>2.90 ± 0.26</td>
<td>1.84 ± 0.19**</td>
<td>0.51 ± 0.11**</td>
<td>0.61 ± 0.04**</td>
<td>0.61 ± 0.04**</td>
<td>22.93 ± 8.35**</td>
<td>63.80 ± 5.82**</td>
<td>4.52 ± 0.53**</td>
</tr>
<tr>
<td>MP</td>
<td>1.31 ± 0.14**</td>
<td>0.34 ± 0.05**</td>
<td>0.86 ± 0.15**</td>
<td>0.30 ± 0.05**</td>
<td>0.65 ± 0.04**</td>
<td>21.40 ± 3.48**</td>
<td>70.33 ± 14.58**</td>
<td>4.81 ± 0.55**</td>
</tr>
</tbody>
</table>

*indicates significant difference compared with DM group, *p < 0.05, **p < 0.01.

### Table 3

Effect of PLP on activities of antioxidant enzymes and MDA level.

<table>
<thead>
<tr>
<th>Group</th>
<th>MDA (μmol/L)</th>
<th>CAT (U/mL)</th>
<th>SOD (U/mL)</th>
<th>T-AOC (mmol/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM</td>
<td>7.72 ± 1.53</td>
<td>95.10 ± 14.23</td>
<td>66.19 ± 10.37</td>
<td>24.92 ± 8.37</td>
</tr>
<tr>
<td>LP + DM</td>
<td>2.32 ± 0.70*</td>
<td>130.71 ± 25.62</td>
<td>87.02 ± 7.61*</td>
<td>40.07 ± 4.30*</td>
</tr>
<tr>
<td>MP + DM</td>
<td>2.38 ± 0.32**</td>
<td>137.00 ± 18.12</td>
<td>93.28 ± 9.10**</td>
<td>38.19 ± 11.41</td>
</tr>
<tr>
<td>HP + DM</td>
<td>3.22 ± 0.51**</td>
<td>108.08 ± 33.89</td>
<td>87.84 ± 7.41**</td>
<td>44.25 ± 10.48**</td>
</tr>
<tr>
<td>Met</td>
<td>2.81 ± 0.75**</td>
<td>131.70 ± 14.22</td>
<td>94.12 ± 6.04**</td>
<td>48.67 ± 8.53**</td>
</tr>
<tr>
<td>CON</td>
<td>2.55 ± 0.73**</td>
<td>170.93 ± 21.57**</td>
<td>104.10 ± 1.36**</td>
<td>59.10 ± 5.15**</td>
</tr>
<tr>
<td>MP</td>
<td>2.64 ± 0.57**</td>
<td>220.73 ± 17.84**</td>
<td>102.60 ± 2.98**</td>
<td>58.69 ± 2.03**</td>
</tr>
</tbody>
</table>

*indicates significant difference compared with DM group, *p < 0.05, **p < 0.01.


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**Bacteroides vulgatus** was a cholesterol-reducing bacterium in human feces (Gerard et al., 2007); *Lactobacillus fermentum* has been shown to lower serum lipids and reduce the progression of atherosclerotic lesions in hypercholesterolemic hamsters (Bhathena et al., 2009); *Prevotella loescheii* was able to produce propionic acid (main metabolite) and dietary fiber-induced improvement in glucose metabolism was associated with increased abundance of *prevotella* (Kovatcheva-Datchary et al.).

**Table 4**

Diversity indices calculated from the DGGE banding profiles.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Con</th>
<th>DM</th>
<th>LP + DM</th>
<th>MP + DM</th>
<th>HP + DM</th>
<th>Met</th>
<th>MP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Richness (S)</td>
<td>26.0 ± 1.4**</td>
<td>20.2 ± 1.5</td>
<td>26.2 ± 1.6**</td>
<td>23.8 ± 0.4**</td>
<td>25.2 ± 1.9**</td>
<td>26.4 ± 3.3**</td>
<td>27.8 ± 1.1**</td>
</tr>
<tr>
<td>$H^a$</td>
<td>3.0 ± 0.05</td>
<td>2.9 ± 0.15</td>
<td>3.1 ± 0.08**</td>
<td>3.0 ± 0.05</td>
<td>3.1 ± 0.09</td>
<td>3.2 ± 0.13**</td>
<td>3.1 ± 0.05**</td>
</tr>
<tr>
<td>$H_{\text{max}}^b$</td>
<td>3.3 ± 0.05**</td>
<td>3.0 ± 0.07</td>
<td>3.3 ± 0.06**</td>
<td>3.1 ± 0.02**</td>
<td>3.2 ± 0.07**</td>
<td>3.3 ± 0.12**</td>
<td>3.3 ± 0.04**</td>
</tr>
<tr>
<td>Evenness (E)</td>
<td>0.92 ± 0.03</td>
<td>0.95 ± 0.04</td>
<td>0.96 ± 0.01</td>
<td>0.96 ± 0.01</td>
<td>0.96 ± 0.01</td>
<td>0.96 ± 0.01</td>
<td>0.95 ± 0.02</td>
</tr>
</tbody>
</table>

*a* indicates significant difference compared with DM group, *p < .05, **p < .01.

$^a$ $H$, Shannon–Wiener index.

$^b$ $H_{\text{max}}$, maximum Shannon–Wiener index.

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Fig. 4. Microbiota analysis of the colon contents in rats. (A) DGGE of V3 region of bacterial 16s rDNA amplifications and UPGMA cluster analysis of DGGE profiles; (B, C and D) RDA for PLP, DM and Met. The circles indicate Van Dobben circles. The red circle is the positive correlation circle and the blue one is the negative correlation circle. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2015; Ochiai, Kuritaochiai, & Fukushima, 1995). In addition, Bacteroides ovatus, reported to play an important role in the degradation of β-1,4-linked xylose unit (PLP is characterized by this link type) (Whitehead, 1995) was only enriched after PLP treatment, and thus, the cholesterol-lowering effect and increase of SCFA (Section 3.6) may be associated with increased abundance of these microbes. Alistipes obesi, Eubacterium fissaicatenae, Clostridium bolteae and Ruminococcus bromii were enriched in diabetic rats, among which Alistipes obesi and Clostridium bolteae may be two important indicators for diabetes. Alistipes obesi is a newly reported species that was isolated from the feces of a morbidly obese individual, and its genome is enriched in amino acids and energy conversion pathways (Hugon et al., 2013). A great abundance of Clostridium bolteae was found in diabetes as opportunistic pathogens, and Clostridium are the common commensal bacteria in the colon and not good for health in a large amount (Qin et al., 2012). Taken together, the administration of PLP can modulate the composition of gut microbiota and improve intestinal metabolism by increasing the concentrations of SCFAs.

### 3.6. Effects of PLP on concentrations of SCFA and pH of feces

Gut microbiota and gut health seems to be important to the pathophysiology of T2D, associated with inhibition of the prevalence of obesity, diabetes and other diseases, are reflected by changes of SCFA and other metabolites in the intestine (Munoz-Garach et al., 2016). SCFAs (dominated by acetate, butyrate and propionates) play important roles in energy metabolism, glucose homeostasis, lipogenesis regulation and immune regulation processes. Diabetes lowered the level of SCFAs and altered metabolic activity of microbe, conversely, reduction of SCFAs may exacerbates dysbiosis of intestinal microecology (Zhu et al., 2016).

Acetate, butyrate and propionate were the main fermentation products in fecal (Fig. 5A). DM group contained significantly lower concentrations of acetic acid (2.41 ± 0.43 mmol/L), propionate acid (0.48 ± 0.05 mmol/L), valeric acid (0.028 ± 0.005 mmol/L) and total SCFA (3.48 ± 0.49 mmol/L) compared with the control group (p < .05). Acetic acid was significantly increased from 2.41 ± 0.43 to 3.90 ± 0.30, 3.78 ± 0.20, 3.67 ± 0.14 and 4.30 ± 0.44 (p < .01), respectively, for the diabetic rats treated with low, middle, high-dose PLP and metformin, compared with that of diabetic rats. Significant difference was also observed between the DM group and PLP treatment group in the amount of propionic acid and butyric acid produced (except HP + DM group, p < .05). Treatment of low-, middle-, and high-dose PLP led to increases in the concentrations of propionate acid (35%, 38% and 19%), butyric acid (79%, 68% and 32%) and total SCFA (61%, 57% and 44%) respectively, compared with the DM group. In particular, the concentrations of acetic acid, butyric acid and total SCFAs reached maximum levels in low-dose PLP group. There were not significant difference in the concentration of valeric acid between PLP group and DM group (except MP + DM group). The levels of acetate acid, propionate acid and total SCFAs in Met and MP groups were significantly higher than control group (p < .05).

SCFA plays a key role in the prevention and treatment of metabolic syndrome. Acetic acid can alter hepatic and skeletal glucose metabolism by reducing xylulose-5-phosphate accumulation in liver and phosphofructokinase-1 activity in skeletal muscle, then the metabolic disturbances of fasting glucose were alleviated by altering glycolysis/gluconeogenic cycle in diabetic individuals (Russhimi et al., 2001; White & Johnston, 2007). But the result was inconsistent with those of Ma & Mu, 2016 who reported that acetic acid in diabetic rats were significantly higher compared with normal rats. This difference may be caused by the different methods of animal model establishment because high-fat diet was given to rats before STZ injection in our study, and high-fat diet was reported to disturb the balance of intestinal microflora and significantly reduce the levels of acetic acid and total SCFAs (Wang et al., 2017).

Propionic acid has direct effects on glucose-stimulated insulin release and maintaining β-cell mass through inhibition of apoptosis, which also mediates the release of gut hormones and improves insulin resistance (Chambers et al., 2015; Pingitore et al., 2017). Propionic acid was mainly produced by the fermentation of glucose, xylose and arabinose (Mortensen, Holtug, & Rasmussen, 1988), and arabinoxylan with high amounts of branches and single xylose units would likely be tolerable and completely fermentable, possibly reaching to the distal colon (Rumpagaporn et al., 2015). The highly branched PLP that is rich in xylose (60%) and arabinose (32%) may be responsible for the relatively high amount of propionic acid. Butyric acid could improve insulin sensitivity and increase the

---

**Table 5**

Band identification for searching the bacterial identity in DGGE using bacterial V3 primers.

<table>
<thead>
<tr>
<th>Band</th>
<th>Closest relative</th>
<th>DM</th>
<th>PLP</th>
<th>Met</th>
<th>Identity</th>
<th>Accession no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>r3</td>
<td>Alistipes obesi</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>89%</td>
<td>NR_133025.1</td>
</tr>
<tr>
<td>r5</td>
<td>Eubacterium fissaicatenae</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>89%</td>
<td>NR_117142.1</td>
</tr>
<tr>
<td>r7</td>
<td>Clostridium bolteae</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>100%</td>
<td>NR_113410.1</td>
</tr>
<tr>
<td>r10</td>
<td>Bacteroides ovatus</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>91%</td>
<td>NR_112940.1</td>
</tr>
<tr>
<td>r17</td>
<td>Bacteroides vulgatus</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>95%</td>
<td>NR_074515.1</td>
</tr>
<tr>
<td>r24</td>
<td>Lactobacillus fermentum</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>99%</td>
<td>NR_104927.1</td>
</tr>
<tr>
<td>r25</td>
<td>Ruminococcus bromii</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>93%</td>
<td>NR_025930.1</td>
</tr>
<tr>
<td>r31</td>
<td>Prevotella loescheii</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>95%</td>
<td>NR_113109.1</td>
</tr>
</tbody>
</table>

+ positive correlation; − negative correlation.

---

**Fig. 5.** Concentration of fecal SCFA (A) and pH value (B) in different groups.
energy expenditure in dietary-obese mice (Gao et al., 2009). Metabolic benefits of butyric acid on glucose control were also improved by activating intestinal gluconeogenesis via a gut-brain neural circuit (Venter et al., 1990). The increase of butyric acids might be due to the fermentation of xylose in PLP (Hu et al., 2012), and this change may indirectly indicate the improvement of gut microbiome in diabetic rats.

Interestingly, diabetic rats treated with high-dose PLP exhibited the lowest amount of individual SCFA and total SCFAs. It is well-known that the fermentation of SCFA depends on physico-chemical characteristics of the dietary fiber, such as monomeric composition, type of linkages, molecular weight and solubility. High-dose PLP has the strongest gel-forming ability and the lowest solubility compared with low- and middle-dose PLP, the two characteristics might influence the degree of fermentation based on steric hindrance (Lindstrom, Holst, Hellstrand, Oste, & Andersson, 2012). In addition, high viscosity of the intestinal contents may impair the absorption of nutrients and other compounds (Kerckhoffs, Brouns, Hornstra, & Mensink, 2002). Consequently, the relatively lower amount of SCFA was presented in high-dose PLP group. These results suggested that PLP treatment increases the production of SCFA in the colon of diabetic rats, which may exert beneficial effects on T2D.

As shown in Fig. 5B, the pH in feces of diabetic rats (7.95 ± 0.12) was significantly higher compared with normal rats (7.63 ± 0.11), which was significantly decreased by administration with low, middle-dose PLP and metformin compared with diabetic rats. T2D was associated with compositional changes in intestinal microbiota, a relatively low level of colonic pH value was confirmed to restrain the growth of some pathogens and which may be beneficial in improving gastrointestinal health. The significantly lower pH values in LP + DM, MP + DM and Met group might result from the production of SCFA by PLP and metformin administration.

4. Conclusions

The polysaccharide isolated from the seeds of P. asiatica L. (PLP) may have a potential for the treatment of hyperglycemia and hyperlipidemia in type 2 diabetes. Administration of PLP reduced the body weight loss, improved polydipsia, decreased blood glucose and serum insulin levels in diabetic rats. PLP also decreased the levels of serum lipid and oxidative stress and increased fecal SCFA content. In addition, structure of gut microbiota was significantly changed and abundance of Bacteroides vulgatus, Lactobacillus fermentum, Prevotella loescheii and Bacteroides ovatus in diabetic rats were increased by PLP treatment, which may be associated with lipid metabolism and increase of SCFA. Thus, these results indicated that the health benefits of PLP in type 2 diabetes may be associated with its effects on gut microbiota and its metabolites.

Acknowledgments

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expression of glucokinase, phosphoenolpyruvate carboxykinase and glucose-6-


fibres from deoiled cumin in low-dose streptozotocin and high glucose-fat diet-

cholangiocarcinoma liver injury after pioglitazone therapy (vol. 136, pg 449, 2002).

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