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Anti-diabetic effect of *Ornithogalum caudatum* Jacq. polysaccharides via the PI3K/Akt/GSK-3β signaling pathway and regulation of gut microbiota

Xue-liang Zhao [a], Guang-yu Lin [b], Tong Liu [a,*]

[a] *Key Laboratory for Metabolic Regulation and Activity Research of Medicinal Plants, Baicheng Medical College, Baicheng, Jilin 137000, China*
[b] *Jilin animal husbandry information center, Changchun, Jilin 130000, China*

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ABSTRACT

This study evaluated the anti-diabetic effect of polysaccharides isolated from *Ornithogalum caudatum* and their underlying mechanisms. To achieve this, a type 2 diabetes mellitus mouse model was established using a combination of a high-fat diet and low-dose streptozotocin injection. The mice were treated with *Ornithogalum caudatum* polysaccharides (OCPs) for 4 weeks. OCPs treatment significantly decreased body weight loss, fasting blood glucose levels, and plasma insulin levels in diabetic mice. Additionally, compared with the untreated group, OCPs treatment significantly decreased total cholesterol, triacylglycerol, and low-density lipoprotein-cholesterol levels, but increased those of high-density lipoprotein-cholesterol in diabetic mice. Moreover, antioxidant enzyme activity and histopathology results revealed that OCPs effectively alleviated oxidative stress and streptozotocin-induced lesions by increasing antioxidant enzyme activity. Results from mechanistic studies showed that OCPs treatment significantly increased the expression of p-PI3K, *p*-Akt, and *p*-GSK-3β in the liver. Moreover, OCPs optimized the gut microbiota composition of diabetic mice by significantly decreasing the *Firmicutes/Bacteroidetes* ratio and increasing the levels of beneficial bacteria (*Muribaculaceae_norank, Prevotellaceae*_UCG-001 and *Alloprevotella*). Overall, these findings suggest that OCPs exert anti-diabetic effects by triggering the PI3K/Akt/GSK-3β signaling pathway and regulating the gut microbiota.

1. Introduction

Diabetes mellitus (DM) is a chronic, heterogeneous, life-threatening disease mainly characterized by polydipsia, polyuria, polyphagia, and weight loss, accompanied by high blood and urine glucose levels [1]. Type 2 DM (T2DM; also known as non-insulin-dependent DM) is characterized by insulin resistance and β-cell dysfunction, which may cause hyperglycemia or hyperlipidemia [2]. Accumulating evidence suggests that insulin resistance plays a major role in the development of diabetes [3]. Moreover, the consistently high blood glucose levels associated with T2DM can cause the generation of reactive oxygen species, which may induce oxidative damage in the pancreas, liver, and kidneys [4].

Over the years, several synthetic hypoglycemic agents, as well as insulin, have been used for the prevention and treatment of DM with varying results; however, these treatment modalities are associated with harmful side effects, such as nausea, vomiting, and

* Corresponding author. No. 27 Mianfang Road, Baicheng, Jilin 137000, China.
E-mail address: 569204208@qq.com (T. Liu).

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diarrhea [5]. Thus, there is a growing need to identify novel anti-diabetic agents in natural materials. Accordingly, several studies have shown that compounds extracted from plants, such as *Liriope spicata* [6], *Cecropia pachystachya* [7], and *Momordica charantia* [8], exhibit considerable anti-diabetic effects in the treatment of T2DM.

Ornithogalum caudatum Jacq. Is a perennial herb belonging to the Liliaceae family, which has been used as a traditional treatment for detoxification, liver cancer and hepatitis [9]. Polysaccharides are one of the most potent active compounds in *O. caudatum*, and exhibit multiple biological activities, including antioxidant, anti-inflammatory, anticancer, and anti-diabetic effects [10]. Some studies have shown that *Ornithogalum caudatum* polysaccharides (OCPs) have good hypoglycemic effects [11]. For instance, Qin et al. [12] reported that OCPs can reduce blood glucose levels and improve blood lipid metabolic disorders in diabetic mice. Additionally, OCPs can be used to synthesize the polysaccharide-chromium complex, which has considerable anti-diabetic effects in diabetic mice [13]. Despite promising results, the pharmacological properties and anti-diabetic mechanisms of OCPs have not yet been elucidated. Therefore, the aim of this study was to elucidate the anti-diabetic effect and mechanisms of OCPs in diabetic mice.

2. Materials and methods

2.1. Plant materials and reagents

Ornithogalum caudatum bulbs were purchased from Jilin Lvbo Traditional Chinese Medicine Pharmaceutical Co., Ltd. (Baicheng, China) and were identified by Prof. Xianwen Yue. Rabbit polyclonal antibodies against PI3K, p-PI3K, total Akt, *p*-Akt, GSK-3β, and *p*-GSK-3β were purchased from Cell Signaling Technology, Inc (Shanghai, China). Horseradish peroxidase-conjugated goat anti-rabbit IgG was purchased from Hangzhou HuaAn Biotechnology Co. Ltd. (Hangzhou, China). Reagent kits for the determination of insulin, glycogen, total cholesterol (TC), triacylglycerol (TG), low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C), catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) were acquired from the Jiancheng Bioengineering Institute (Nanjing, China). All other chemicals and reagents used were of analytical grade.

2.2. Preparation of OCPs

Ornithogalum caudatum bulbs were dried at 60 °C for 96 h. To remove the liposoluble components, the dried bulbs were then extracted by reflux with 95 % ethanol at 70 °C for 3 h. The organic solvent was volatilized, and a pretreated dry powder was obtained. Subsequently, 200 g of dry powder was extracted with 5L of water at 90 °C for 4 h and the extract was sieved through a filter paper, followed by two rounds of further extraction of the residue. Proteins were removed from the crude polysaccharides using five cycles of deproteinization with 5:1(v/v) CHCl3-*n*-BuOH, according to the Sevag method. The precipitates were redissolved in distilled water and further dialyzed (MWCO3500) in water for 48 h. Then the crude material was purified with the Dicthylaminoethyl Sepharose Fast Flow media and eluted with 3 times the column volume of 0.4 M sodium chloride solution. Finally, OCPs were freeze-dried and collected. The OCPs yield was calculated using the following formula:

Yield (%, w/w) = Weight of OCPs/Weight of dried material × 100.

The total sugar content of the OCPs was determined using the phenol-sulfuric acid method and total protein was quantified using a bicinchoninic acid assay kit (Pierce, Rockford, IL, USA).

2.3. Animals

Male Kunming mice weighing 20 ± 2 g were purchased from Beijing Huafu Kang Biotechnology Co., Ltd. (Beijing, China), and housed in a controlled environment (23 ± 2 °C, 55 ± 5 % humidity) under a 12 h light/dark cycle. All experiments were performed in accordance with the guidelines for the care and use of laboratory animals, and were approved by the Animal Ethics Committee of Jilin Agricultural University (permit no. ECLA-JLAU-2022-0519-002).

2.4. Induction of T2DM mice

The mice were adapted to the diet for 1 week and then randomly divided into two groups: the normal (n = 10) and high-fat (n = 40). Mice in the normal group were fed a regular diet, while those in the high-fat group were fed a high-fat diet (77 % regular diet, 15 % lard oil, 5 % white sugar, 2 % cholesterol, 0.7 % salt, and 0.3 % sodium cholate) for 4 weeks. Additionally, mice in the high-fat group were intraperitoneally injected with 60 mg/kg freshly prepared streptozotocin (STZ) solution in 0.1 mol/L citrate buffer (pH 4.5) twice within 72 h, while the normal group were injected with the same volume of citrate buffer. Blood glucose levels were determined one week after the last injection. Mice with blood glucose levels >11.1 mmol/L were considered diabetic and selected for further pharmacological studies.

2.5. Experimental design

The experimental groups were as follows:

normal control (NC): normal mice + saline; diabetic control (DC): diabetic mice + saline; positive control (PC): diabetic mice + rosiglitazone (2 mg/kg/day); low-dose (LD): diabetic mice + OCPs (200 mg/kg/day); high-dose (HD): diabetic mice + OCPs (400 mg/kg/day).

Mice in the OCPs groups received intragastric administration of OCPs once per day. The normal and diabetic groups were administered the same dose of saline. All mice were fed their respective diets until the end of the study, and body weights were recorded daily. Blood glucose levels were measured once a week using blood collected from the tail vein of overnight food-deprived mice.

2.5.1. Oral glucose tolerance test

Oral glucose tolerance test was performed on the last day of the experiment to assess the effect of OCPs on glucose tolerance. Mice in all groups were administered aqueous glucose solution (2 g/kg body weight) after overnight fasting, and blood glucose levels were measured at 30, 60, 90, and 120 min. The area under the curve (AUC) was calculated using the following formula:

$AUC = [(C0 + C1) \times (t1 - t0) \times 1/2] + [(C1 + C2) \times (t2 - t1) \times 1/2] \ldots$, where t represents the time point and c represents the plasma glucose level.

2.6. Biochemical analysis

At the end of the experimental period, blood samples were collected from the retro-orbital vein of the mice after overnight fasting for biochemical analysis. Serum was obtained by centrifuging the blood samples at 4000×*g* for 10 min at 4 °C. Blood glucose, insulin, TG, TC, HDL-C, and LDL-C levels were determined using ELISA kits (Jiancheng, Nanjing, China)), according to the manufacturer's instructions. A portion of the liver tissue was homogenized, centrifuged at 3000×*g* for 10 min at 4 °C, and the supernatant was collected for the determination of CAT, SOD, and GSH-Px activities and hepatic glycogen levels.

2.7. Histopathological analysis of the liver and pancreas

The pancreas and liver were fixed in 10 % neutral buffered formalin for 24 h, dehydrated using graded levels of alcohol, and embedded in paraffin. Subsequently, the paraffinized tissues were cut into 5 μm-thick sections using a microtome (Leica RM2235, Weztlar, Germany) and stained with hematoxylin and eosin for histopathological observation under an optical microscope.

2.8. Western blot

Proteins were extracted from mouse liver tissue using RIPA lysis buffer and quantified using a bicinchoninic acid assay kit (Pierce, Rockford, IL, USA). Thereafter, 20 μg of protein was separated via 15 % sodium dodecyl sulfate–polyacrylamide gel electrophoresis

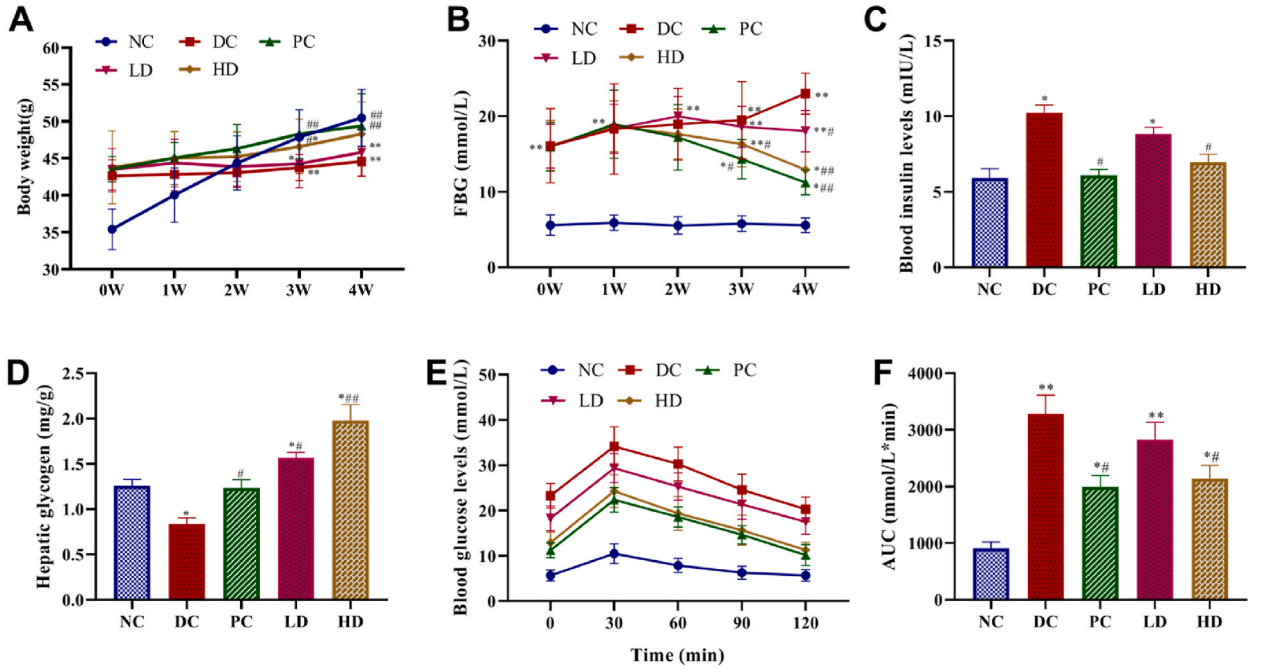

Fig. 1. (A) Body weight, (B) fasting blood glucose, (C) blood insulin, (D) hepatic glycogen, (E) oral glucose tolerance and (F) area under the curve in different groups during the experimental period. NC, normal control group; DC, diabetic group (diabetic mice with saline); PC, positive control group (diabetic mice with 2 mg/kg/day rosiglitazone); LD, low dose group (diabetic mice with 200 mg/kg/day *Ornithogalum caudatum* polysaccharides [OCPs]; HD, high dose group (diabetic mice with 400 mg/kg/day OCPs). Values are expressed as the mean ± standard deviation ($n = 10$). $^{*}p < 0.05$ and $^{**}p < 0.01$ compared with the normal control group, $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ compared with the diabetic group.

and then transferred onto a polyvinylidene fluoride membrane. The membranes were incubated with antibodies against PI3K (1:1000), p-PI3K (1:1000), total Akt (1:1000), *p*-Akt (1:1000), GSK-3β (1:1000) and *p*-GSK-3β (1:1000) overnight at 4 °C. After washing in phosphate-buffered saline with Tween 20, the membranes were incubated with horseradish peroxidase-conjugated sheep anti-rabbit (1:3000) at room temperature (18–25 °C) for 1 h. Finally, the enhanced chemiluminescence reagent was added and protein bands were observed using an enhanced chemiluminescence detection system (Amersham Pharmacia Biotechnology, Tokyo, Japan).

2.9. Gut microbiota DNA extraction and sequencing

Feces were collected from the normal control, diabetic control ，and high-dose groups, flash frozen in liquid nitrogen and stored at −80 °C. Total genomic DNA was extracted from the samples using the CTAB/SDS method. DNA concentration and purity was monitored on 1 % agarosegels. The 16 S rDNA V3–V4 region was amplified with the following primers: 341 F: CCTACGGGNGGCWGCAG; 806 R: GGACTACHVGGGTATCTAAT. 16 S rDNA sequencing was performed on an Illumina PE250 platform by bioprofile biotechnology Co., Ltd. (Shanghai, China). The raw data had the primer sequence cut and the low-quality data eliminated to obtain clean Tags. A similarity of 97 % was set as operational taxonomic units, and species annotation was conducted. After that, Rstudio (Rstudio, Boston, MA, USA) and Simca 14.1 (Umetrics, Sweden) were used to calculate and visualize the results.

2.10. Statistical analysis

Statistical analysis was performed using SPSS 11.0 (Chicago, IL, USA). All data were presented as ± standard error of the mean. The differences between experimental groups were determined using analysis of variance followed by a *t*-test. Pearson correlation analysis was implemented using the Chiplot online tool (https://www.chiplot.online/). $p < 0.05$ was considered statistically significant.

3. Results

Effect of OCPs on body weight and fasting blood glucose level.

A total of 7.92 % ± 0.84 % of OCPs was isolated and purified, with total sugar and protein contents of 76.56 % ± 8.14 % and 1.14 % ± 0.16 %, respectively. T2DM mice models were established using a combination of a high-fat diet and low-dose STZ injection. Diabetic mice administered OCPs (LD and HD groups) for 4 weeks had a significantly higher ($p < 0.05$) body weight than untreated diabetic mice (Fig. 1A). Additionally, mice fed a high-fat diet and injected with STZ had significantly higher ($p < 0.05$) fasting glucose levels (16.09 ± 4.91 mmol/L) than those fed a normal diet (5.59 ± 1.34 mmol/L), indicating the successful establishment of a T2DM model (Fig. 1B). OCPs treatment significantly decreased ($p < 0.05$) the fasting glucose levels of mice in the LD and HD groups compared with those in the DC group; however, blood glucose levels did not return to normal. Overall, these results indicated that OCPs may alleviate hyperglycemia in diabetic mice but may not completely restore impaired glucose metabolism.

3.1. Effect of OCPs on insulin and glycogen levels

Insulin resistance is a characteristic of diabetes; therefore, we examined the effect of the treatments on the insulin and glycogen levels of the mice. Mice in the DC group had significantly higher ($p < 0.05$) insulin levels (10.23 ± 0.51 mU/L) than those in the NC group (5.93 ± 0.60 mU/L) (Fig. 1C). However, OCPs treatment (400 mg/kg/day) significantly reduced ($p < 0.05$) insulin levels to 6.97 ± 0.52 mU/L, which was similar to that of the PC group (6.11 ± 0.38 mU/L).

Glycogen plays an important role in the maintenance of blood glucose homeostasis; however, diabetes can lead to decreased glycogen storage. Mice in the DC group had significantly lower ($p < 0.05$) hepatic glycogen levels than those in the NC group (Fig. 1D). OCPs treatment restored hepatic glycogen levels back to normal. Overall, these results indicated that the antihyperglycemic activity of OCPs was partly attributed to increased glycogen synthesis.

3.2. Effect of OCPs on glucose tolerance in diabetic mice

Compared with the NC group, the blood glucose levels of mice in the experimental groups increased rapidly after glucose administration (Fig. 1E and F), with the blood glucose level of mice in the DC group peaking 30 min after glucose administration and decreasing gradually afterwards. However, OCPs treatment significantly reduced the diabetes-induced increase in blood glucose levels 30–90 min after glucose administration, after which the blood glucose level continued to decrease gradually. Specifically, mice in the OCPs and PC groups had significantly lower ($p < 0.05$) glucose levels than those in the STZ model group 30–90 min after glucose administration.

3.3. Effect of OCPs on serum lipid and lipoprotein levels

DM is caused by an absolute or relative lack of insulin, which may lead to abnormal lipid metabolism. Compared with the NC group, the lipid profiles of STZ-induced T2DM mice were significantly altered (Table 1). However, OCPs treatment for 4 weeks significantly decreased ($p < 0.05$) TC, TG, and LDL-C levels and increased HDL-C levels in diabetic mice, especially in the HD group.

3.4. Effect of OCPs on the activity of antioxidant enzymes in the liver

Oxidative stress plays an important role in diabetes development and progression; therefore, the effect of OCPs on antioxidant enzymes in the liver of diabetic mice was examined. Compared with the NC group, there was a significant decrease ($p < 0.05$) in CAT, SOD, and GSH-Px activity in the livers of mice in the DC group (Table 1). However, OCPs treatment for 4 weeks significantly increased ($p < 0.05$) CAT, SOD, and GSH-Px activity, compared with those of the untreated diabetic group. Overall, these results indicated that OCPs can significantly alleviate oxidative stress by increasing antioxidant enzyme activity.

3.5. Morphological changes after OCPs treatment

Histopathological examination of the liver and pancreas of the experimental mice was performed using hematoxylin and eosin staining. The livers of mice in the control group were healthy, with distinct hepatic cells (Fig. 2A). In contrast, several histopathological changes were observed in the hepatic cells of diabetic mice, including focal necrosis and muscular hepatic cords. However, OCPs and rosiglitazone treatments ameliorated diabetes-induced liver injuries.

DM is caused by an absolute or relative insulin deficiency, and the ability of islets to produce insulin depends on their structure and the number of islet cells in the pancreas. In the NC group, the islets were regular ellipsoids and regular in structure, with uniform boundaries and ovoid and regularly arranged nuclei. In contrast, in DC group, there was a decrease in the number of islet cells and the distribution of the cells was disordered, with irregular shaped nuclei. However, OCPs and rosiglitazone treatments ameliorated the histological damage to varying degrees (Fig. 2B), with the pancreatic histology of mice in the HD group almost recovering to normal. Overall, these results indicated that OCPs had the ability to repair liver and pancreatic damage.

3.6. Effect of OCPs on the PI3K/Akt/GSK3β pathway in diabetic mice

To further understand the mechanism underlying the hypoglycemic effect of OCPs, Western blot was performed on mouse liver tissues (Fig. 3 and Supplementary Materials S1). Compared with the NC group, there was a decrease in p-PI3K and *p*-Akt expression in the livers of mice in the DC group. However, treatment with OCPs and rosiglitazone increased p-PI3K and *p*-Akt expression to varying degrees. Moreover, OCPs treatment for 4 weeks also increased GSK3β phosphorylation in the liver, indicating that OCPs played an important role in glycogen synthesis.

3.7. Effect of OCPs on gut microbiota

To explore the effect of OCPs on the intestinal microbiota of diabetic mice, 16s rDNA sequence assays were performed. The alpha (Fig. 4A) and beta (Fig. 4B) diversity index at the OUT level were studied. As shown in Fig. 4A, the Good's coverage index was > 0.99, with no significant differences ($p < 0.05$) among the groups, indicating that the sequencing depth of this experiment was reasonable, and that the subsequent data were accurate and reliable. The ACE index and Chao1 were significantly higher in the DC group than in the NG and HD groups ($p < 0.05$), indicating that community richness in the DC group was significantly increased. However, the Shannon index representing the community diversity showed no differences among the three groups ($p < 0.05$). This indicated that the community richness in diabetic mice was increased and that OCPs treatment could effectively reverse community richness.

PCA and PCoA were performed to attain a global view of the gut bacterial profiles of all samples. As shown in Fig. 4B, the three groups of samples were well separated from the first component of the PCA and PCoA map. Notably, the distances between NC and HD samples were relatively close. However, the DC samples differed slightly from the NC and HD samples. These results show that the gut bacterial differences between the NC and HD samples were small, and that the gut microbiota of the DC samples differed from those of the other groups.

The bacterial abundance in each group was determined to investigate composition alterations in the gut microbiota. At the phylum level (Fig. 5A), *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Campylobacterota*, *Desulfobacterota*, and *Actinobacteriota* were detected in all

Table 1
Effects of *Ornithogalum caudatum* polysaccharides (OCPs) on serum lipid profiles and antioxidant enzyme activities in T2DM mice.

Group	NC	DC	PC	OCPs	
				LD (100 mg/kg)	HD (200 mg/kg)
TC (mmol/L)	3.54 ± 0.63	7.29 ± 0.83**	4.79 ± 0.42$^{\#\#}$	6.34 ± 0.72**$^{\#}$	5.32 ± 0.98*$^{\#\#}$
TG (mmol/L)	2.23 ± 0.25	4.24 ± 0.52**	2.82 ± 0.56$^{\#\#}$	3.45 ± 0.31*$^{\#}$	2.79 ± 0.33$^{\#\#}$
HDL-C (mmol/L)	1.83 ± 0.24	0.89 ± 0.14**	1.95 ± 0.23*$^{\#\#}$	1.35 ± 0.23**$^{\#}$	1.64 ± 0.17*$^{\#\#}$
LDL-C (mmol/L)	0.63 ± 0.24	1.72 ± 0.33**	0.83 ± 0.21$^{\#\#}$	1.32 ± 0.45**$^{\#}$	0.92 ± 0.17*$^{\#\#}$
CAT (U/mg)	68.43 ± 4.21	48.23 ± 4.36*	62.85 ± 5.23$^{\#}$	55.62 ± 6.54*$^{\#}$	60.51 ± 5.89$^{\#}$
SOD (U/mg)	312.46 ± 34.58	203.87 ± 23.46**	263.48 ± 24.65*$^{\#\#}$	228.94 ± 34.56**	264.76 ± 29.47*$^{\#}$
GSH-Px (U/mg)	680.47 ± 62.48	424.78 ± 45.95**	560.31 ± 49.74*$^{\#\#}$	515.64 ± 51.32**$^{\#}$	553.64 ± 61.85*$^{\#\#}$

Note: NC, normal control group; DC, diabetic group (diabetic mice with saline); PC, positive control group (diabetic mice with 2 mg/kg/day rosiglitazone); LD, low dose group (diabetic mice with 200 mg/kg/day OCPs); HD, high dose group (diabetic mice with 400 mg/kg/day OCPs). Values are expressed as the mean ± standard deviation ($n = 10$). *$p < 0.05$ and **$p < 0.01$ compared with the normal control group, $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ compared with the diabetic group.

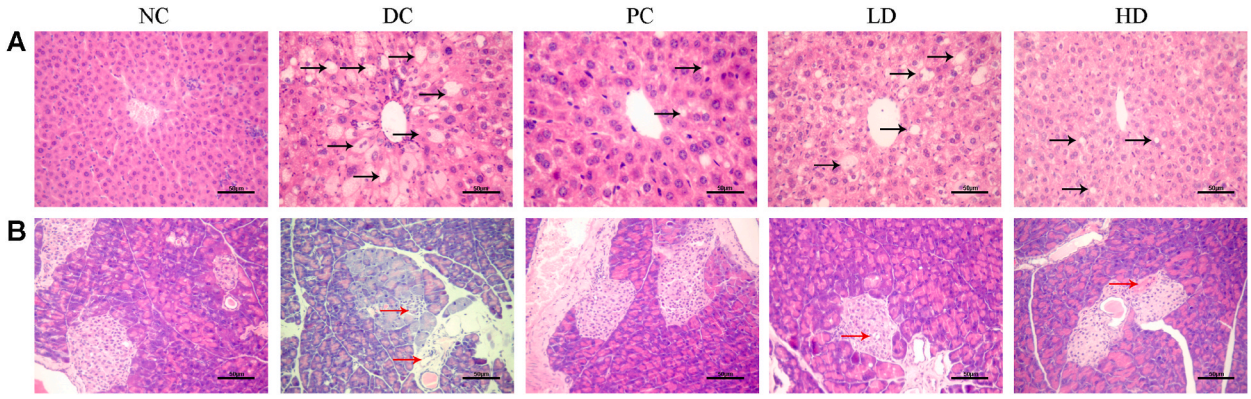

Fig. 2. Histological analysis of the liver and pancreas against damages in type 2 diabetes mellitu. (A) Histopathological examinations of the liver (hematoxylin and eosin [H&E] stain × 200); (B) histopathological examinations of the pancreas (H&E stain × 200). Black arrows, vacuolar degeneration in liver; red arrows, vacuolar degeneration and inflammatory infiltration in pancreas. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

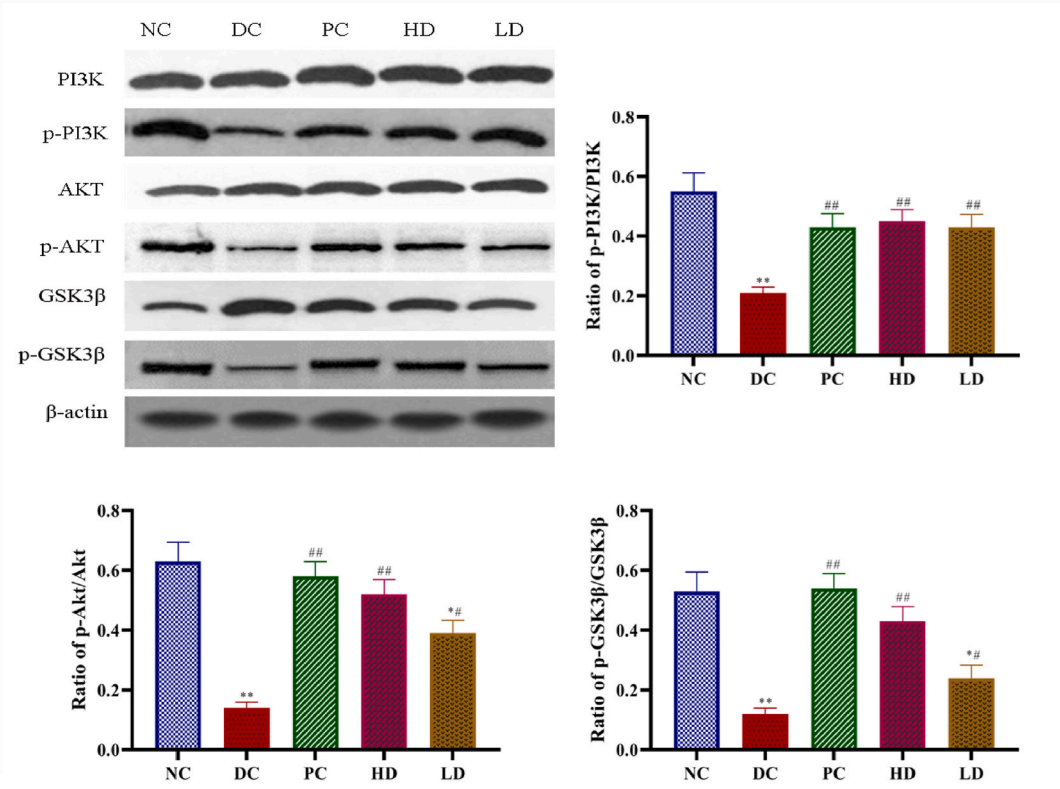

Fig. 3. Effects of *Ornithogalum caudatum* polysaccharides on the PI3K/Akt/GSK3β pathway in type 2 diabetes mellitu mice.Values are expressed as the mean ± standard deviation ($n = 3$). *$p < 0.05$ and **$p < 0.01$ compared with the normal control group, $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ compared with the diabetic group.

groups. Among the six phyla, the *Firmicutes* and *Bacteroidetes* were the dominant phyla. In the DC group, the relative abundance of *Firmicutes* was increased ($p < 0.05$), and *Bacteroidetes* was decreased ($p < 0.05$) compared to the NC group (Fig. 5A1, A2), and the Firmicutes/Bacteroidetes ratio was increased (Fig. 5A3). After OCPs intervention for 4 weeks, the relative abundance of *Firmicutes* decreased from 70.85 % to 56.74 % and that of *Bacteroidetes* increased from 25.04 % to 33.51 %. At the genus level (Fig. 5B), *Ligilactobacillus*(Fig. 5B1), *Lactobacillus*, *Muribaculaceae*_norank, *Bacteroides* (Fig. 5B4), *Prevotellaceae*_UCG-001, and *Alloprevotella* were the dominant genera in all groups. In the DC group, the relative abundance of *Muribaculaceae*_norank (Fig. 5B3), *Prevotellaceae*_UCG-001(Fig. 5B5), and *Alloprevotella* (Fig. 5B6) were decreased, and that of *Lactobacillus* (Fig. 5B2) was increased compared with the NC

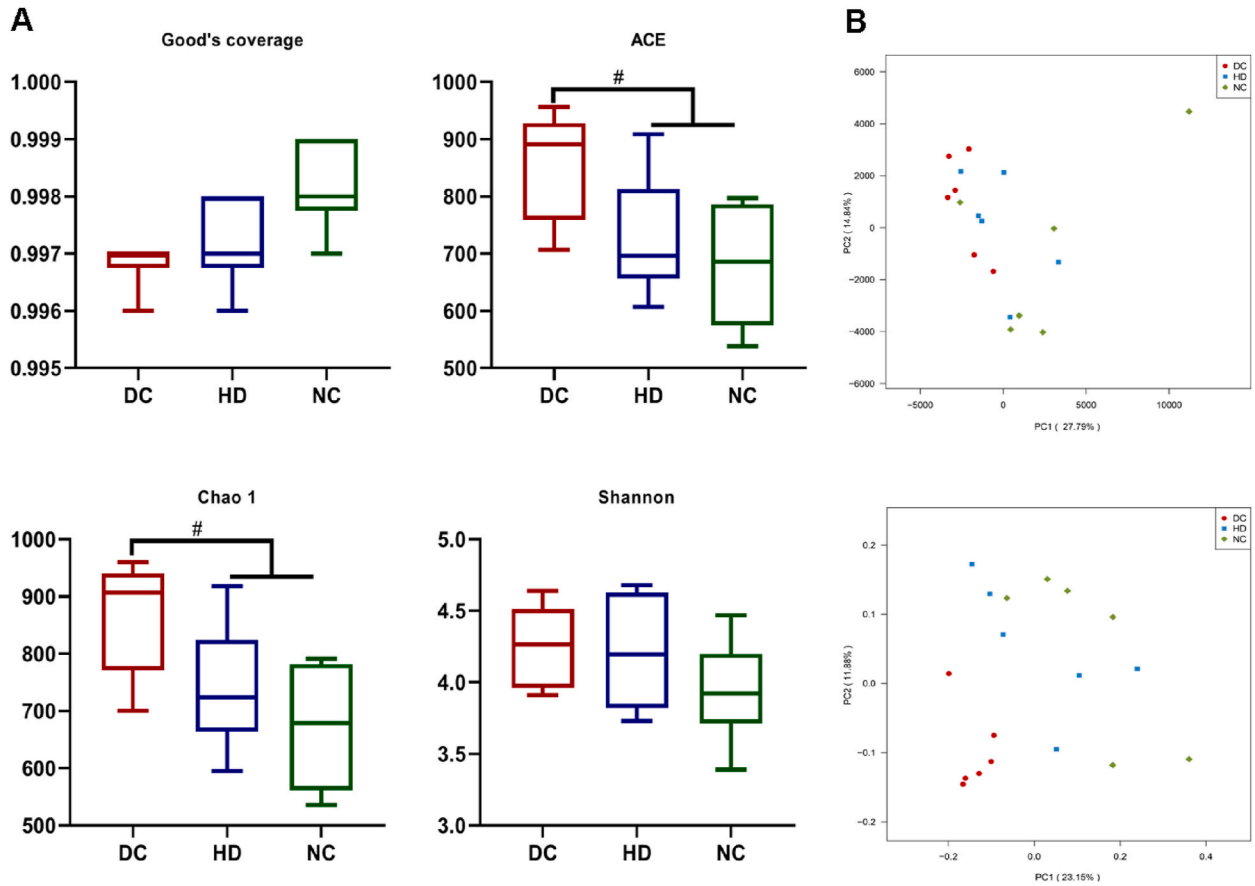

Fig. 4. Analysis of α and β-diversity analysis of the gut microbiota in mice. (A) α diversity evaluated base on Good's coverage, ACE, Chao1 and Shannon indices; (B) PCA and PCoA score plots. $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ compared with the diabetic group.

group ($p < 0.05$). After OCPs intervention for 4 weeks, the gut components were partly reversed. These results confirmed that OCPs regulated the gut components to achieve anti-diabetic effects.

3.8. Pearson heatmap analysis

To further explore potential correlations between gut microbiota and T2DM-related factors, Pearson heatmaps were constructed. At the phylum level (Fig. 6A), *Patescibacteria* was positively related to *Desulfobacterota* ($p < 0.05$ or $p < 0.01$), and *Actinobacteriota* was positively related to *Patescibacteria* and *Desulfobacterota* ($p < 0.05$). *Proteobacteria* was positively related to *Campylobacterota,* but negatively related to *Desulfobacterota* ($p < 0.05$ or $p < 0.01$). *Campylobacterota* was negatively related to *Desulfobacterota* ($p < 0.05$). Furthermore, *Firmicutes* was positively associated with TC, but negatively correlated with SOD and GSH-Px ($p < 0.05$ or $p < 0.001$). *Patescibacteria* and *Campylobacterota* were positively associated with HDL-C and *p*-GSK3β/GSK3β, but negatively correlated with TG ($p < 0.05$ or $p < 0.01$), and *Desulfobacterota* was positively associated with LDL-C and insulin levels, but negatively correlated with HDL-C and *p*-GSK3β/GSK3β ($p < 0.05$ or $p < 0.001$). At the genus level (Fig. 6B), *Ligilactobacillus* was positively related to TG, LDL-C and insulin levels, but negatively related to p-IRS1/IRS1, p-PI3K/PIK and *p*-GSK3β/GSK3β ($p < 0.05$ or $p < 0.01$). *Lachnospiraceae_ unclassified* was positively related to TC, but negatively related to CAT and SOD ($p < 0.05$ or $p < 0.01$).

4. Discussion

T2DM is a glucose metabolism disorder characterized by insulin resistance-induced hyperglycemia [14]. Insulin resistance can cause weight loss in diabetic mice, which may be due to abnormal metabolism of structural proteins [15]. In the present study, treatment with OCPs, especially at 400 mg/kg/day, significantly increased the body weight of diabetic mice, indicating that OCPs treatment can alleviate insulin resistance. Additionally, OCPs treatment significantly decreased the fasting glucose and insulin levels of the mice. These results indicate that OCPs can improve glucose homeostasis and insulin resistance in T2DM mice.

Diabetes is often accompanied by dyslipidemia [16]; therefore, we examined the effect of OCPs on serum biochemical indices. OCPs treatment for 4 weeks decreased the serum TC, TG, and LDL-C levels of diabetic mice; however, it increased the HDL-C levels compared

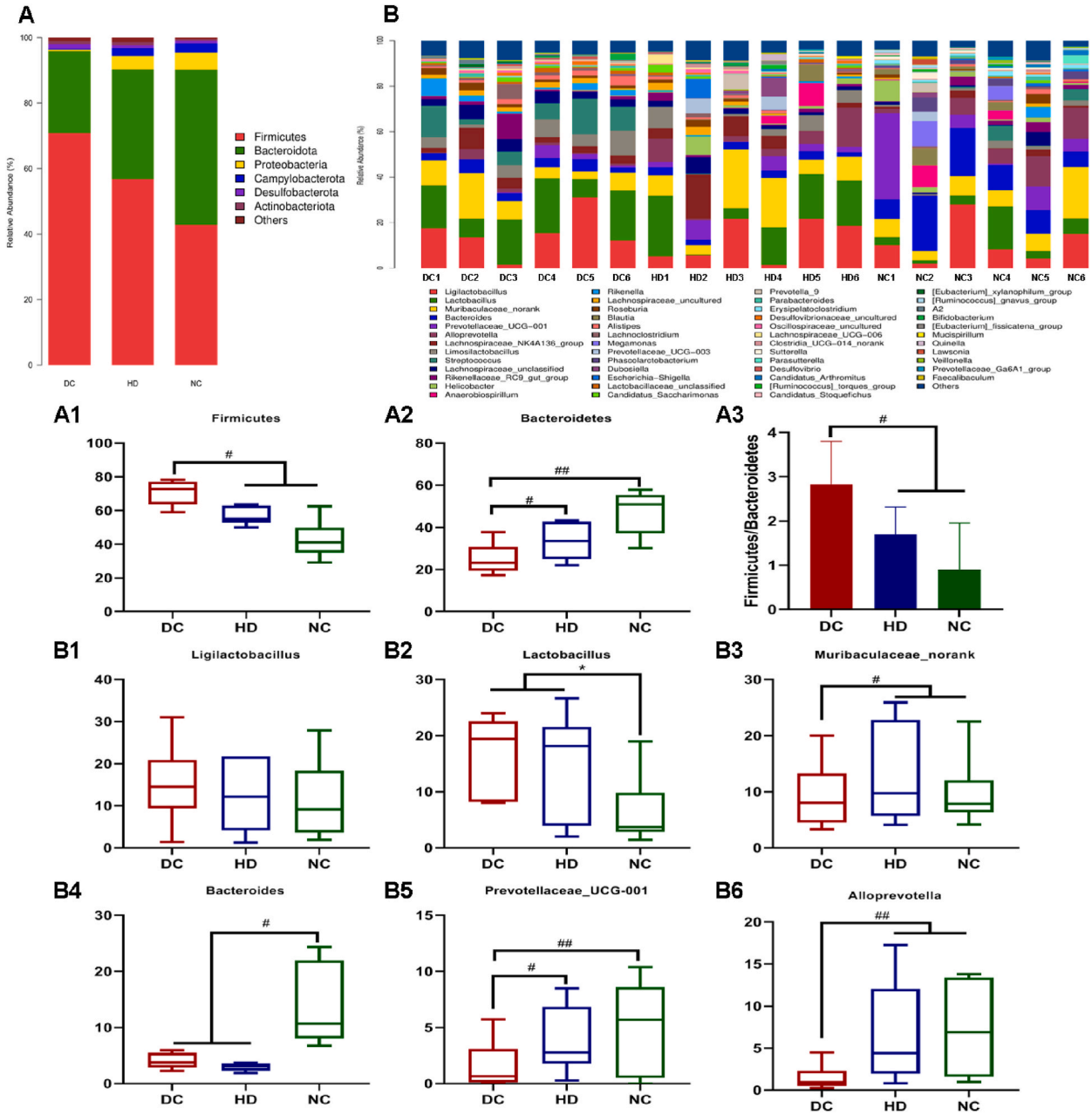

Fig. 5. Components of the gut microbiota at the phylum (A) and genus (B) levels. The relative abundances of *Firmicutes* (A1), *Bacteroidetes* (A2), *Ligilactobacillus* (B1), Lactobacillus (B2), *Muribaculaceae_norank* (B3), *Bacteroides* (B4), *Prevotellaceae*_UCG-001 (B5) and *Alloprevotella* (B6) were compared among groups. The Firmicutes/Bacteroidetes ratio was compared among three groups (A3). $^{*}p < 0.05$ and $^{**}p < 0.01$ compared with the normal control group, $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ compared with the diabetic group.

with those in the DC group. HDL-C plays an important role in cholesterol transport from the periphery to the liver via the "reverse cholesterol transport" pathway [17]. Therefore, the decrease in serum TC and TG levels observed in the present study might be partially due to the increase in HDL-C levels. The variation in lipid levels suggest that OCPs could be a promising candidate for modulating lipid metabolism in diabetic mice.

A consistently high blood glucose level is associated with the generation of reactive oxygen species, which may cause oxidative damage to the pancreas and liver [18]. In the present study, OCPs treatment increased the activity of CAT, SOD, and GSH-Px in the liver; additionally, histopathological analysis showed that OCPs ameliorated diabetes-induced injuries in the liver and pancreas. These results suggest that OCPs can alleviate oxidative damage to the liver and pancreas by increasing antioxidant enzyme activity.

As the primary intracellular storage form of glucose, glycogen is mainly stored in the liver [19]. Glycogen synthesis and

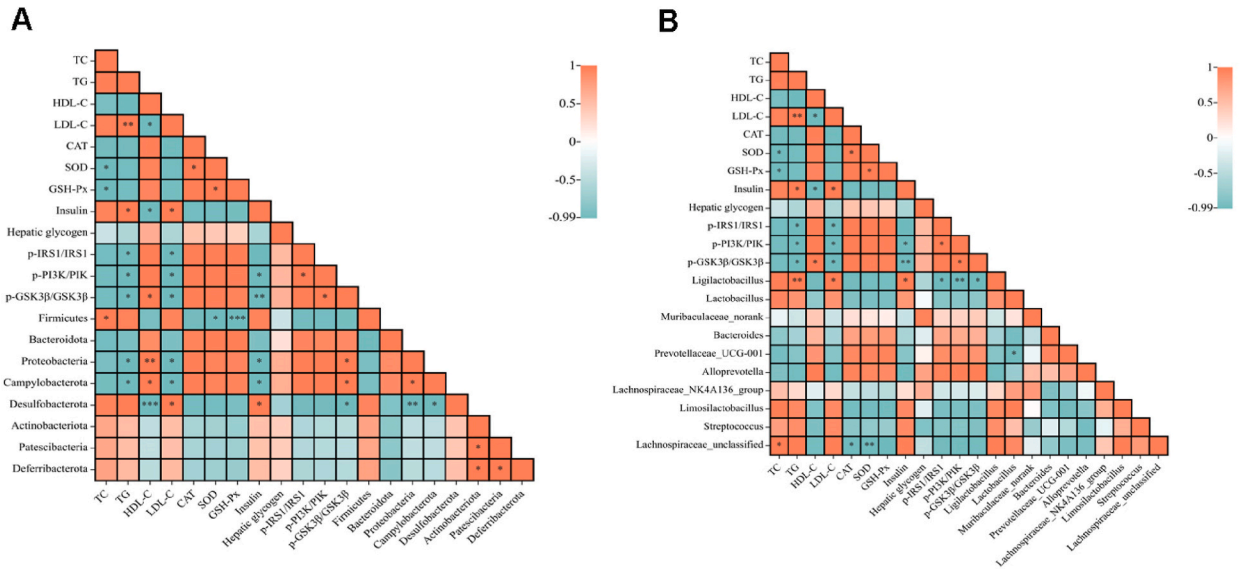

Fig. 6. Correlation analysis of intestinal flora and type 2 diabetes mellitu-related factors. (A) Correlation analysis at the phylum level, and (B) the genus level. $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$.

decomposition is necessary to maintain glucose homeostasis; however, diabetes can cause a decrease in glycogen storage, which is associated with a lack of insulin [20]. In the present study, treatment with OCPs successfully restored hepatic glycogen levels compared with those in DC group, indicating that the antihyperglycemic activity of OCPs may be partly attributed to improved insulin sensitivity in the liver and increased glycogen synthesis.

The PI3K/Akt signaling pathway plays a crucial role in insulin signal transduction and is involved in glucose and lipid homeostasis [21]. The stimulation of the PI3K/Akt signaling pathway is always accompanied by the alleviation of insulin resistance [22]. GSK-3 is a key downstream protein of the PI3K/Akt pathway, and its activity can be inhibited by Akt-mediated phosphorylation of GSK-3β at Ser 9 [23]. Under normal conditions, insulin can induce GSK-3β phosphorylation; however, GSK-3β inactivation promotes the synthesis of hepatic glycogen and reduces blood glucose levels [24]. In contrast, insulin resistance, a characteristic of diabetes, can inhibit GSK-3β phosphorylation, which can suppress hepatic glycogen synthesis, leading to increased blood glucose levels [25]. Accordingly, the effect of OCPs treatment on the PI3K/Akt signaling pathway was examined in the present study via Western blot. OCPs treatment significantly increased the expression of p-PI3K and *p*-Akt without altering the total Akt protein levels in diabetic mice. Additionally, OCPs treatment increased the *p*-GSK-3β/GS-3β ratio in the liver, indicating that the PI3K/Akt/GSK-3β signaling pathway was partly regulated by OCPs.

As the largest microecosystem in the human body, the intestinal microbiota participates in material and energy metabolism and has a significant impact on human health [26]. Diabetes leads to intestinal microbial dysbiosis and component changes, which reduces diversity and balance. Intestinal dysbiosis can promote the development of diabetes through the reduction of intestinal microbial metabolites, inflammatory responses and insulin resistance [27]. In this study, 16s rDNA sequence assays were conducted to explore the effect of OCPs on the intestinal microbiota of diabetic mice. At the phylum level, DC groups showed a significant increase in the *Firmicutes/Bacteroidetes* ratio, which is reportedly correlated to a high fasting blood glucose level and inflammation status [28]. After OCPs intervention for 4 weeks, a decrease in *Firmicutes* and an increase in *Bacteroidetes* was observed. At the genus level, OCPs intervention increased the relative abundances of *Muribaculaceae_norank, Prevotellaceae*_UCG-001, and *Alloprevotella*. *Muribaculaceae_norank,* inhibited harmful bacteria and oxidative stress, and improved the inflammation of the intestinal mucosa [29]. The presence of *Prevotellaceae_UCG-001* indicates that there is a close relationship between mediating glucose and lipid metabolism and energy metabolism-related signal regulation [30]. Moreover, *Alloprevotella* belongs to short-chain fatty-acid-producing bacteria and plays a positive role in alleviating inflammation and non-alcoholic fatty liver [31]. Pearson heatmaps further confirmed that there were positive or negative relationships between different bacteria, and these bacteria were generally more likely to be associated with serum lipid profiles, antioxidant enzyme activities, and anti-diabetic proteins [32].

This study found that OCPs can alleviate T2DM in multiple ways, such as protecting pancreatic tissue, improving oxidative stress, and regulating intestinal flora. However, its internal connections among various pathways are still unclear. Therefore, it is necessary to further explore the hypoglycemic mechanism of OCPs by combining multi-omics analysis techniques such as proteomics and transcriptomics.

5. Conclusions

In the present study, we examined the hypoglycemic effects of polysaccharides isolated from *O. caudatum* in high-fat diet-fed and

STZ-induced T2DM mice. OCPs treatment exerted significant antihyperglycemic effects by promoting blood glucose homeostasis, relieving abnormal lipid metabolism, alleviating oxidative stress, ameliorating insulin resistance, and balancing the abundance of intestinal flora. Specifically, OCPs alleviated insulin resistance partly by promoting the activation of the PI3K/Akt/GSK-3β signaling pathway. Moreover, OCPs increased the abundance of beneficial bacteria (*Muribaculaceae_norank, Prevotellaceae*_UCG-001 and *Alloprevotella*) in T2DM mice, further improving their glucose metabolism. Overall, these results indicate that OCPs could be used as a low-cost antihyperglycemic drug and be further applied in functional and health-care foods due to their hypoglycemic effects.

Author contributions

Tong Liu and Xueliang Zhao: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

Guangyu Lin: Performed the experiments; Analysed and interpreted the data; Contributed reagents, materials, analysis tools or data.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e20808.

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