Characterization of Polysaccharides from *Lycium ruthenicum* Murray and its Regulatory Effect on the Intestinal Flora of T2DM Mice

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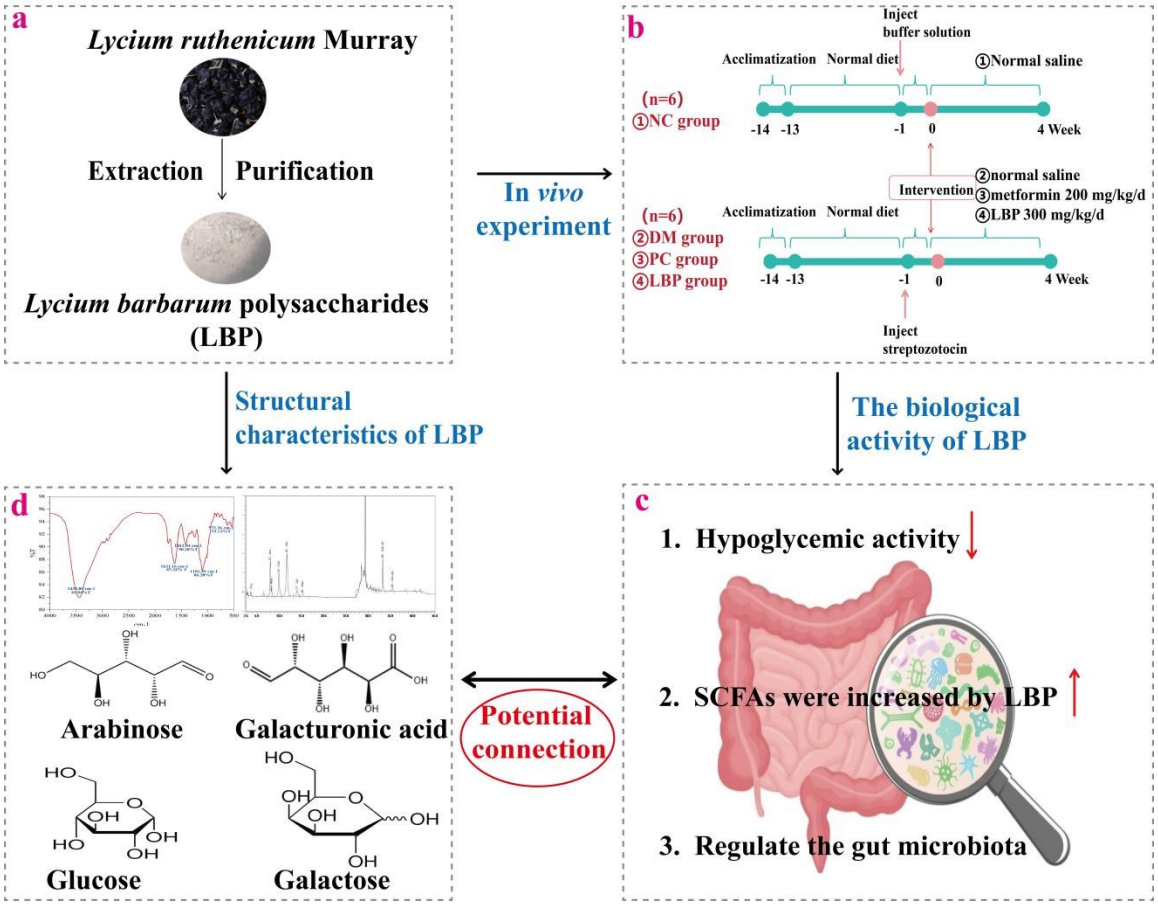
GRAPHICAL ABSTRACT



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Lycium barbarum polysaccharides (LBP), primary active components in the fruits of *Lycium barbarum* Murray, are effective bioactive compounds in the treatment of type 2 diabetes mellitus (T2DM). Microbiota, as the second genome, has been reported to be involved in the development of T2DM. Therefore, mechanisms of regulating the gut microbiota also involve its intestinal metabolites that might exist. This study examined the hypoglycemic effect and improvement of gut microbiota of LBP on T2DM mice for further investigation of potential mechanisms. Compared with the DM group, the LBP could not only increase the production of short chain fatty acids, especially the content of acetic acid, but also the community richness and diversity of the gut microbiota. In addition, the relative abundances of *Bacteroidetes* and *Firmicutes* was improved in the LBP group compared with the DM group, accompanied with decreased relative abundances of *Verrucomicrobia* and *Actinobacteria* at the phylum level. Additionally, at the family level, the relative abundances of *Lactobacillales* and *Bifidobacteriales* in the DM group sharply increased and was significantly reduced in the LBP group. These effects may be attributed to its monosaccharides components (arabinose, galactose, glucose, galacturonic acid), suggesting LBP as a potential T2DM intervention *via* gut microbiota regulation.

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Keywords: Lycium ruthenicum Murray; *Polysaccharides*; *Gut microbiota*; *Type 2 diabetes mellitus*

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INTRODUCTION

Polysaccharides are a class of abundant natural macromolecules in nature, widely derived from animals, plants, algae, and microorganisms (Song *et al.* 2021). Owing to their broad sources, low toxicity, and diverse biological activities, natural polysaccharides have been extensively studied worldwide. The biological activities of polysaccharides are determined by their molecular weights and monosaccharide compositions (Mirzadeh *et al.* 2021). The structural diversity and complexity endow them with the capability to exert functions in different biological systems (Silva *et al.* 2024). As dietary fibers, plant-derived polysaccharides are mostly degraded by polysaccharide-degrading enzymes secreted by gut microbiota in the intestine, exhibiting potential prebiotic activities in both non-diseased and diseased states. The prebiotic activity of polysaccharides is typically reflected in their role as specific substrates for beneficial microorganisms, promoting these microbes to produce beneficial functional metabolites (Wang *et al.* 2024a).

Lycium barbarum polysaccharides (LBP) – potential prebiotic mainly composed of key monosaccharide components including glucose, arabinose, and galactose – can regulate gut microbiota and may improve type 2 diabetes-related symptoms (Feng *et al*. 2024). A study isolated a high-purity polysaccharide (LBP-W) from *L. barbarum*, then characterized its structure and assessed the anti-obesity effect on the mice model. It was found that LBP-W might be a potential prebiotic active ingredient for obesity and its complications (Yang *et al*. 2021). According to literature reports, compared with the control group, mice in the LBP group exhibited higher abundances of *Lactobacillus* and *Bifidobacterium*, but lower abundances of *Enterobacteriaceae* and *Enterococcus*. These findings suggest that LBP ameliorate the gut micro-environment and possesses prebiotic potential (Yun *et al.* 2022). Whereas, a number of studies have shown that LBP cannot be digested by the gastrointestinal tract and can safely reach the large intestine, where it is fermented and produces many important beneficial metabolites, such as SCFAs, thus regulating the composition of gut microbiota (Zhou *et al.* 2024b; Dong *et al.* 2025). Another study also reported that LBP might regulate immune response by regulating gut microbiota (Lin *et al.* 2024). It is hypothesized that these monosaccharide components in LBP may improve type 2 diabetes-related symptoms by regulating the structure and function of the intestinal microbiota. Nevertheless, it remained unclear whether the hypoglycemic effects of these components in *Lycium barbarum* polysaccharides were related to the regulation of the intestinal microbiota.

Diabetes is a metabolic disease, characterized by high blood glucose, among which type 2 diabetic mellitus (T2DM) is the most common type. In recent decades, the prevalence of T2DM has increased rapidly around the world. According to the latest version of Diabetes Epidemic Map published by the International Diabetes Federation in 2022, about 828 million adults are suffering from diabetes (Zhou *et al.* 2024a), which poses a great threat to human health and is one of the ten leading causes of death in adults. Research shows that the gut microbiota is altered in diabetic patients, indicating its significant role in the pathophysiology of T2DM (Wang *et al.* 2024b). Moreover, oral antidiabetic medications also have a tremendous effect on the gut microbiota. However, most hypoglycemic agents are associated with unpredictable side effects, usually related to extra-targeted effects, contraindications, sustainability, safety, and tolerability (DeMarsilis *et al.* 2022), which render them unsuitable for long-term use.

LBP has a significant hypoglycemic effect, and the long-term toxicity of natural extract is far less than that of biosynthetic drugs, so it can be used as a drug substitute for the prevention or treatment of diabetes. However, there are few studies on the relationship between T2DM and LBP, especially those from Delingha in Qinghai province. To further explore the effect, polysaccharides in *Lycium ruthenicum* Murray (LRM) from Delingha were extracted, characterized, and given to the diabetic mice to study the hypoglycemic effect and regulation of gut microbiota of LBP. This study will provide scientific basis for the promotion and application of LRM and its polysaccharides and thus lay a solid theoretical foundation for the development of adjuvant drug for T2DM.

EXPERIMENTAL

Materials

Metformin hydrochloride sustained-release tablets were purchased from Jiangsu Merck Pharmaceutical Co. LTD. Streptozotocin (STZ) and 4-methylpentanoic acid were

purchased from Sigma-Aldrich Co., Ltd (Shanghai, China). Isobutyl chloroformate, n-hexane, pyridine, isobutyl alcohol, acetic acid, propionic acid, n-butyric acid, isobutyric acid, isovaleric acid, citric acid, and sodium citrate were acquired from Shanghai Aladdin Reagent Co., Ltd. The high-fat diet (HFD, formula: 67% normal diet + 10% lard + 20% sucrose + 2.5% cholesterol + 0.5% sodium cholate) was obtained from Beijing Keao Xieli Feed Co., Ltd. The feed quality certificate number was SCXK (Beijing) 2019-0003.

Preparation of LBP

The extraction of LBP was performed based on the previous research and slightly modified (Masci *et al.* 2018). LRM was dried at 60 ℃ for 4 h in the oven, then crushed and screened through 20 mesh. It was then degreased at 85 ℃ for 2 h and the residue was heated at 80 ℃ for 2 hours. Ultrapure water was added to this residue in a round-bottomed flask keeping the liquid-material ratio of 15:1 mL/g, followed by heating in a water bath at 100 ℃ for 2 hours. Then water was removed under the conditions of 60 ℃ and 120 r/m and mixed with the Sevag reagent in a ratio of 5:1 (v/v) into a pear-shaped funnel. After a period of standing, the crude polysaccharide extract was obtained. About four times the volume of anhydrous ethanol was then added and left overnight in a refrigerator at 4 ℃. The sediment formed was collected, washed, and freeze-dried to obtain LBP. It was ground into a powder and stored at -20 ℃ for future use.

The carbohydrate content in LBP was determined by the phenol-sulfuric acid method with D-glucose as a standard (Yang *et al.* 2018). The monosaccharide composition of LBP was determined using a Thermo ICS5000 ion chromatography system (ICS5000, Thermo Fisher Scientific, USA) equipped with an electrochemical detector and a Dionex™ CarboPac™ PA20 column (150 mm × 3.0 mm, 10 μm). The characterization of LBP was conducted by ultraviolet visible absorption spectrophotometry, infrared spectroscopy, and thermogravimetric analysis.

LBP and metformin were dissolved in normal saline before being intragastrically administered. Streptozotocin (STZ) was dissolved in sodium citrate-citric acid buffer (10 mg/mL, pH = 4.2 ~ 4.5) before using it.

Animal Experiments

About 40 four-week-old male C57BL/6 mice (No. 410975211100017576) were purchased from Experimental Animal Center of Zhengzhou University (No. SCXK (Henan) 2017-0001). The animal experiment was carried out in the specific-pathogen-free animal laboratory of College of Public Health, Zhengzhou University (SYXK (Henan) 2018-0005). The mice were fed at 50 ± 15% relative humidity, 22 ± 2 ℃ temperature and 12 h dark-light cycle. Five mice lived in a cage and had free access to sterile water and feed. This animal experiment was approved by the Life Science Ethics Review Committee of Zhengzhou University (ZZUIRB 2021-03). All experimental procedures were carried out in accordance with the guidelines of the Animal· Protection and· Use. Committee of Zhengzhou University and the. National Research Council's Guide for the Care and Use of Laboratory Animals., and every effort was made to reduce the suffering of the animals.

After one week of acclimatization, 40 mice were randomly stratified into 2 groups: the normal control group (NC, n=10) fed with normal diet (340 kcal/100 g, 65.08% from carbohydrates, 11.85% from fat, 23.07% from protein) and the diabetic group (n=30) fed with HFD (391 kcal/100 g, 53.3% from carbohydrates, 31.1% from fat and 15.6% from protein). After being fed for 12 weeks, the diabetic group was not fed for 12 h and then injected intraperitoneally with STZ (150 mg/kg bw) for 2 consecutive days. The NC group

was injected with the same volume of sodium citrate-citric acid buffer solution. After 3 days, blood samples were obtained from the caudal vein to detect FBG with a portable glucose meter (Sinocare Inc., Changsha, China). The mice in the diabetic group with FBG > 11.1 mmol/L were regarded as successful modeling mice. Finally, 26 mice met the criteria, and 4 mice were excluded. 18 mice were then included and randomly divided into diabetic model (DM) group, positive control (PC) group, and LBP intervention (LBP) group. The remaining 8 mice were used for pre-experiment. Similarly, 6 mice were randomly selected from the NC group for the follow-up experiments to achieve the matching degree among each group.

Based on the weight of the mice, the LBP group and the PC group were intragastric administrated with 300 mg/kg LBP and 200 mg/kg metformin daily, respectively. In addition, the NC group and the DM group were given with the equal volume of normal saline intragastrically per day. The pharmacological interventions were sustained for 4 weeks, and the body weight and FBG of each mouse were recorded every week.

Measurement of SCFAs

The SCFAs in the colon contents were quantitatively analyzed by GC-MS (Agilent, 7890B-5977A, USA) with some modifications of the method in a previous study (Ueyama *et al.* 2020).

Gut Microbiota Analysis

Gut microbiota analysis of colon contents was performed by Shanghai Zhongke New Life Biotechnology Co., Ltd. Briefly, the steps of the analysis were extraction of genomic DNA samples, and purity and concentration detection. According to the selection of sequencing regions, specific primers with Barcode and high-fidelity DNA polymerase were used for PCR amplification of selected V3-V4 variable regions. PCR products were tested by 2% agarose gel electrophoresis, and target fragments were cut and recycled by AxyPrepDNA Gel recovery kit (AXYGEN). According to the preliminary quantitative results of electrophoresis, PCR amplification and recovery products were detected and quantified using QuantiFluor™-ST blue fluorescence quantification system (Promega), and the corresponding proportion of mixing was performed according to the sequencing quantity requirements of each sample. Library construction was performed using the NEB Next® Ultra™DNA Library Prep Kit. Quality inspection was carried out on the constructed library, and sequencing was carried out on the machine after passing the test.

Statistical Analysis

GraphPad Prism 7.0 (Inc., San Diego, CA, USA) and RStudio 4.2.2 software were used for statistical analysis and graphical presentation. All values were expressed as means ± SD. Student's *t*-test was used to evaluate the differences between two groups, and one-way ANOVA was performed to investigate alterations among three or more groups. $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Characterization of LBP

The SEM results for LRM and LBP are presented in Fig. 2A-B. As can be observed from the micrographs, the two substances exhibit distinct morphological features with

varying degrees of surface smoothness. The retention times and contents of the monosaccharides in LBP were presented in Table 1. Figure 2C showed the chromatogram of monosaccharide composition of LBP. The results indicated that glucose, galacturonic acid, and arabinose are the predominant monosaccharides in LBP. The typical absorbance peak characteristics of acidic polysaccharides are shown in Fig. 2D. The decomposition of LBP was mainly endothermic reaction, and the thermal decomposition stage was mainly between 130 and 300 ℃ (Fig. 2E-F.).

Table 1. Retention Time and Content of Monosaccharides and Uronic Acids in LBP

Monosaccharides	Retention Time (min)	Content (μg/mg)
Fucose	3.725	0.85
Arabinose	7.967	61.03
Rhamnose	8.300	19.71
Galactose	9.892	46.04
Glucose	11.717	86.86
Xylose	13.992	12.63
Mannose	15.209	9.38
Galacturonic Acid	33.234	66.07
Glucuronic Acid	35.475	5.13

Effect of LBP in T2DM Mice

The therapeutic effect of LBP in T2DM mice was found in this study. The dynamic weight and fasting blood glucose (FBG) recorded every week are depicted in Figs. 3A-B. Compared to before model establishment, the weight significantly decreased, and FBG significantly increased in T2DM mice in the 0 week, indicating that the T2DM mice model was successfully established ($P < 0.01$). From the figure, it can be inferred that the weight started to be restored on the third week, and the FBG started to decrease on the second week. Compared with the 0 week, the weight of DM, PC, and LBP groups on the fourth week decreased by 2.8 g, 1.9 g, and 2.3 g, respectively. Meanwhile, the FBG of DM, PC, and LBP groups on the fourth week decreased by -0.5 mmol/L, 7.0 mmol/L, 5.4 mmol/L, respectively. These results all showed that metformin or LBP intervention could effectively improve the symptoms of weight loss and hyperglycemia of T2DM mice. To further estimate its therapeutic effect, the insulin level was determined, and the HOMA-IR index (Homeostatic Model Assessment for Insulin Resistance) was calculated, which increases with insulin resistance level (Song *et al*. 2021). Compared with the DM group, the insulin level in the LBP group recovered 0.53 mIU/L and the HOMA-IR index was decreased, but the differences were not statistically significant (Figs. 3C-D).

Effect of LBP on SCFAs

Studies have shown that appropriate supplementation of SCFAs can reduce FBG level in T2DM patients (Zhao *et al.* 2018; Sanna *et al.* 2019), and it might provide a novel approach for T2DM management. LBP can reach the distal digestive tract and generate SCFAs. Therefore, the concentrations of the five main short-chain fatty acids (SCFAs)—acetic acid, propionic acid, n-butyric acid, isobutyric acid, and isovaleric acid—were quantified by gas chromatography–mass spectrometry (GC–MS). The concentrations of each SCFA and total SCFAs in colon contents of the DM group were lower than those of the NC group, and acetic acid and total SCFAs were recovered or even higher in the LBP

group than that of the NC group (Table 2). However, none of these differences were statistically significant.

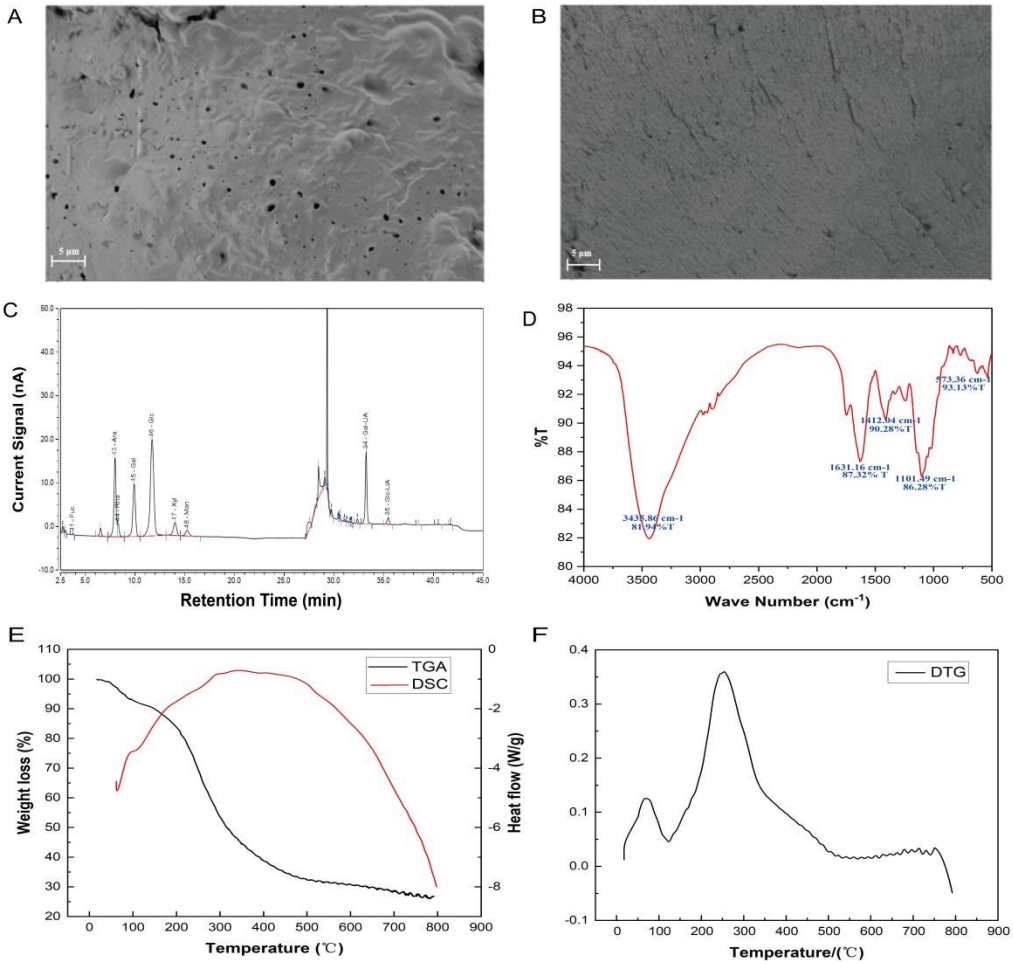

Fig. 2. Characterization of LBP: A: SEM of LRM; B: SEM of LBP; C: Chromatogram of monosaccharide composition of LBP; D: Infrared spectrum of LBP; and E-F: Thermogravimetric analysis of LBP

LBP Modulated Gut Microbiota of T2DM Mice

As the gut microbiota is highly associated with the development of T2DM, the presence of gut microbiota in colon contents from mice was assessed *via* the Illumina MiSeq platform. In total, 9402808 effective sequences were detected. Alpha diversity analysis mainly includes Shannon index and Simpson index, which was shown in Fig. 5. As can be seen, they were all smaller in the DM group than in the NC group, and the differences were statistically significant, indicating that the Alpha diversity in T2DM mice was significantly decreased. Compared with the DM group, these indexes in the PC group were further lower than those in the DM group; however, they were all higher in the LBP group than those in the DM group. It could be speculated that LBP could increase the diversity of gut microbiota at the OUT (Operational Taxonomic Units) level.

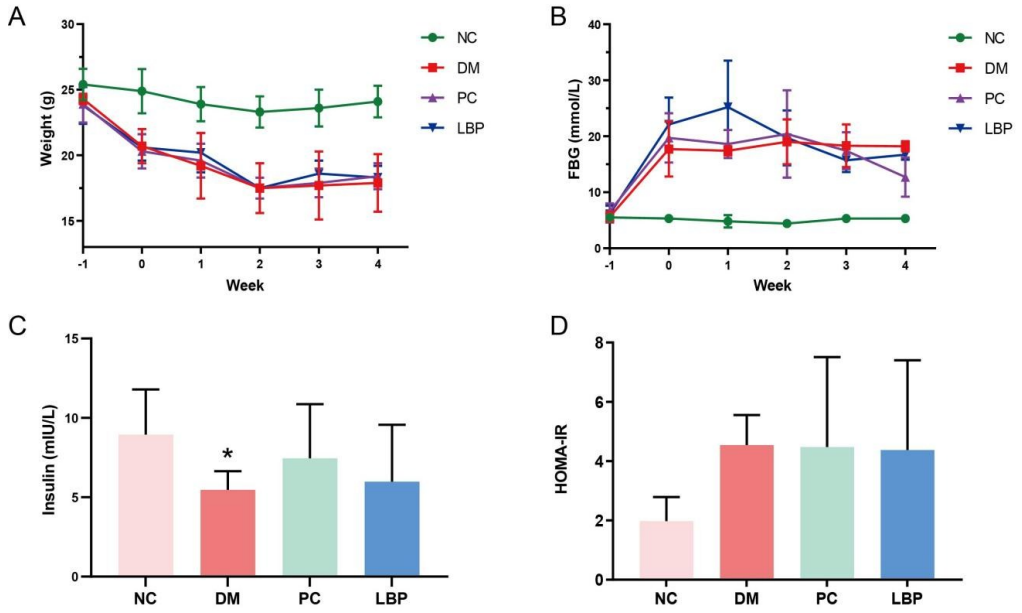

Fig. 3. A-B: Dynamic body weight and fasting blood glucose throughout the intervention. A: Weight; B: FBG; C-D: LBP recovered the level of insulin and HOMA-IR of T2DM mice; C: Insulin; and D: HOMA-IR. *P < 0.05, compared to the NC group

Table 2. Concentration of SCFAs in Colon Contents from Mice (mg/g, n = 6)

SCFA	NC	DM	PC	LBP
Acetic Acid	25.92 ± 2.09	23.82 ± 5.41	19.06 ± 4.78	28.00 ± 10.75
Propionic Acid	2.77 ± 1.02	2.43 ± 1.15	1.94 ± 0.57	1.39 ± 0.30*
n-butyric Acid	3.75 ± 1.71	3.46 ± 0.96	2.24 ± 0.78	1.69 ± 0.39
Isobutyric Acid	0.37 ± 0.07	0.35 ± 0.01	0.34 ± 0.03	0.32 ± 0.04
Isovalerate Acid	0.40 ± 0.06	0.38 ± 0.04	0.33 ± 0.03	0.31 ± 0.03*
Total SCFAs	33.22 ± 2.84	30.81 ± 5.94	24.13 ± 3.36	33.76 ± 8.88

*P < 0.05, compared to the NC group

To further investigate the gut microbial composition, the results were described at the phylum level (Fig. 5A). *Bacteroidetes*, *Firmicutes*, *Verrucomicrobia*, and *Actinobacteria* were the main phyla. The dominant bacteria in the NC, DM, and LBP groups were *Bacteroidetes*, while the abundance of *Bacteroidetes* in the PC group decreased and the dominant bacteria changed to *Verrucomicrobia*, indicating that metformin intervention might change the composition of gut microbiota largely and LBP intervention could recover the dysbiosis. Similar results were also found at the class level (Fig. 5B). The relative abundance with significant differences at phylum level is shown in Fig. 6. In the heat map, a redder color indicates a higher relative abundance of the bacterial phylum, while a bluer color indicates a lower relative abundance. After modeling, the composition of gut microbiota in the DM group was significantly different from the NC group. Thus, there was an apparent decrease in the relative abundances of *Bacteroidetes* and *Firmicutes*, and an increase in the relative abundances of *Verrucomicrobia* and *Actinobacteria*. Furthermore, the gut microbiota composition of the LBP group was markedly altered compared with the DM group, but a similar tendency was noted with the NC group. It was suggested that HFD feeding combined with STZ intraperitoneal injection could significantly disrupt the microbiota composition of normal mice, and LBP could repair this maladjustment to a certain extent.

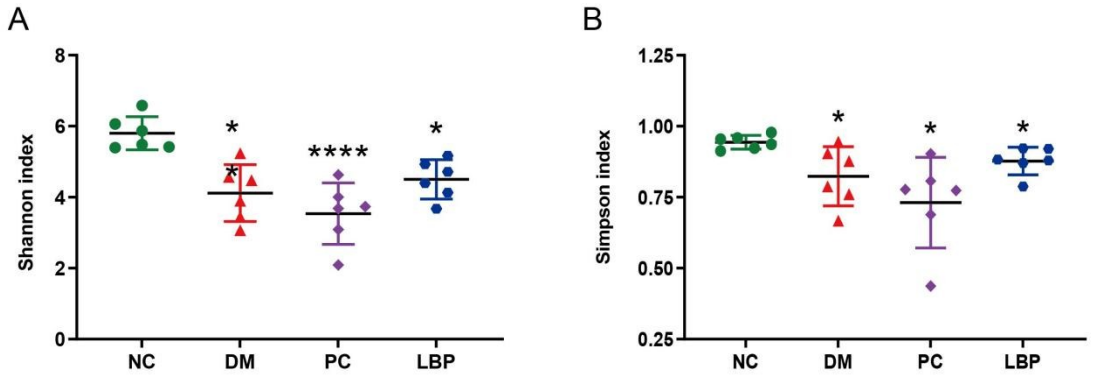

Fig. 4. The results of alpha diversity analysis in the colon contents. A: Shannon index; and B: Simpson index. *P < 0.05, **P < 0.01, ****P < 0.0001, compared to the NC group

In addition, a novel result was found. The relative abundance of Akkermansiaceae was increased in the DM group compared to the NC group. Furthermore, the level was significantly decreased in the LBP group compared to the DM group, and close to that in the NC group (Fig. 7). These results were contrary to previous findings.

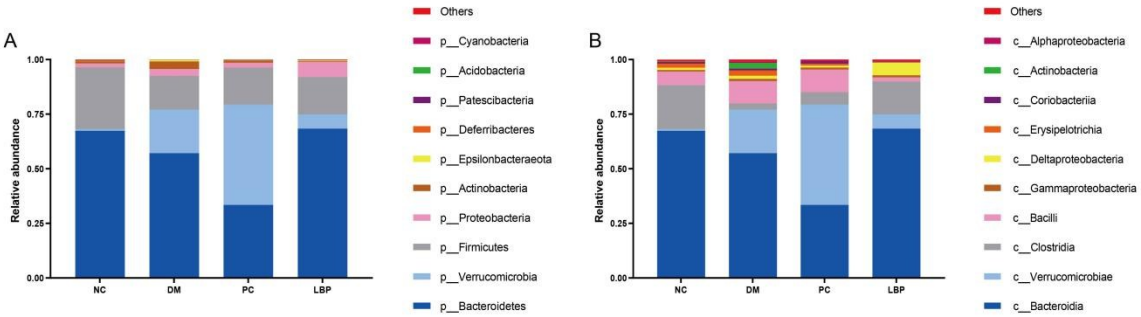

Fig. 5. Microbiota composition (top 10) at phylum (A); and class (B) level

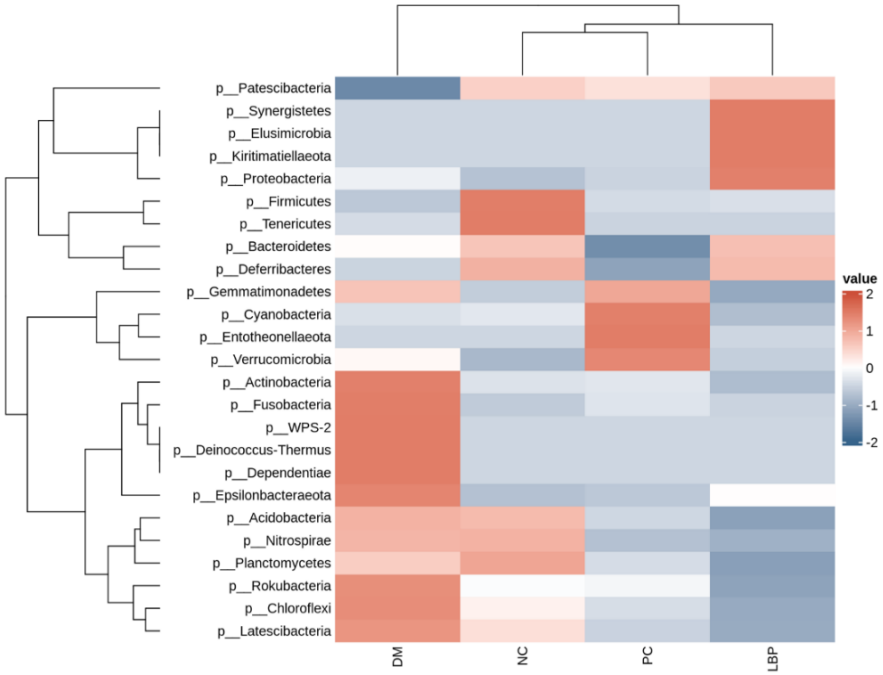

Fig. 6. Clustering heat-map (top 25) in the colon contents at phylum level

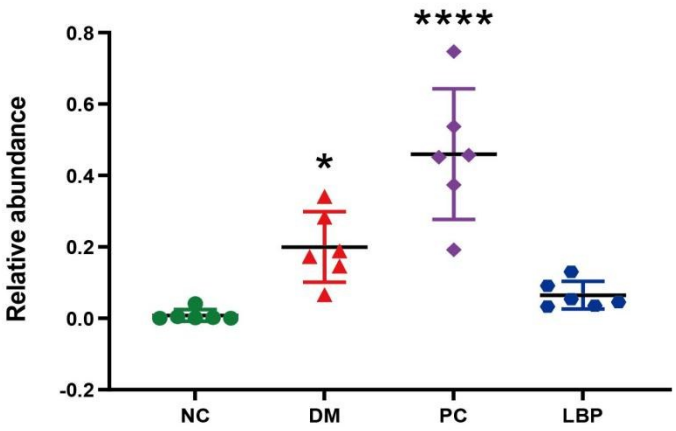

Fig. 7. The relative abundance of Akkermansiaceae in the colon contents
*P < 0.05, ****P < 0.0001, compared to the NC group.

DISCUSSION

The LBP are the primary active components from *Lycium barbarum*, which is composed of multiple monosaccharides linked by glycosidic bonds. Their monosaccharide composition mainly includes arabinose, galactose, glucose, mannose, *etc*. Recent studies have demonstrated that these monosaccharides regulate the gut microbiota through distinct mechanisms. Notably, the structural characteristics of LBP—such as monosaccharide composition and glycosidic linkage—are influenced by the extraction methods employed (Liu *et al.* 2020; Zhao *et al.* 2021), which may further affect their biological activity. Some research has indicated that LBP composed of neutral monosaccharides exhibit remarkable colitis-relieving effects, in which arabinose, galactose, and glucose play key roles (Li *et al.* 2023; Zhang *et al.* 2025). Additionally, this study identified arabinose, galactose, glucose, and galacturonic acid as the predominant monosaccharides in LBP, aligning with literature reports. This suggests their potential significance in gut microbiota modulation.

A large number of studies have shown that the diversity of gut microbiota in T2DM patients was reduced, and increasing diversity was beneficial to the treatment of T2DM (Qin *et al.* 2012; Bouter *et al.* 2017). In this study, the results of 16S rRNA sequencing analysis showed that the community diversity at the OTU level in the LBP group was increased and the composition of the gut microbiota was notably altered compared with the DM group. Another similar study also found that the composition and abundance of gut microbiota were significantly different between persons with T2DM and healthy ones (Hendijani and Akbari 2018). It was reported that the gut microbiota in normal human consisted of two main phyla, namely *Bacteroidetes* and *Firmicutes* (Wozniak *et al.* 2022). In this study, these two bacteria accounted for 95.6% in the NC group, 72.6% in the DM group, 50.3% in the PC group, and 85.6% in the LBP group, indicating that LBP could improve the disorders of gut microbiota in T2DM mice, making the composition more similar to the normal mice. However, the potential mechanism remains to be explored comprehensively. According to previous reports, the abundance of *Bacteroidetes* in the gut microbiota of T2DM patients was significantly reduced, and gradually recovered after treatment (Letchumanan *et al.* 2022), which is consistent with the results in this study.

CONCLUSIONS

These findings strongly suggest that LBP can reduce weight loss and blood glucose, promote insulin secretion in type 2 diabetic mice, increase the diversity of gut microbiota, and repair its structure and composition. The arabinose, galactose, glucose, and galacturonic acid present in LBP likely constitute the material basis for their prebiotic effects. Collectively, these findings provide a theoretical basis for the exploitation and utilization of *Lycium ruthenicum* Murray and its polysaccharides.

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Ethical Statement

This study and included experimental procedures were approved by the Life Science Ethics Review Committee of Zhengzhou University (ZZUIRB 2021-03). All experimental procedures were carried out in accordance with the guidelines of the Animal Protection and Use Committee of Zhengzhou University, and every effort was made to reduce the suffering of the animals.

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