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Dendrobium officinale polysaccharide attenuates type 2 diabetes in mice model by modulating gut microbiota and alleviating intestinal mucosal barrier damage

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ABSTRACT

The purpose of this study was to investigate the hypoglycemic effect and mechanism of *Dendrobium officinale* polysaccharide (DOP) on type 2 diabetes mellitus (T2DM) mice established by high-fat diet and streptozotocin. The results showed that DOP improved glycolipid metabolism and serum inflammation levels, and inhibited intestinal-derived lipopolysaccharide (LPS) translocation, suggesting that inhibiting LPS-mediated intestinal barrier damage may be a key target for DOP to alleviate T2DM. Interestingly, the study found that DOP reduced intestinal inflammation and oxidative stress levels, significantly up-regulated the mRNA expression of tight junction proteins *Claudin-1*, *Occludin* and zonula occluden-1 (*ZO-1*), and ameliorated intestinal epithelial damage. In addition, DOP strongly inhibited the intestinal pathogenic bacteria and LPS-producing bacteria *Helicobacter*, *Enterococcus* and *Desulfovibrio* with a reduction rate of 95%, 73% and 9%, respectively, and promoted the proliferation of anti-inflammatory bacteria *Bifidobacterium* and *Lactobacillus* by 139% and 8%, respectively. Taken together, the hypoglycemic effect of DOP was related to the protection of intestinal mucosal barrier, and its underlying mechanism lied in its excellent anti-inflammatory and gut microbiota-modulatory effects, providing a theoretical basis for developing DOP as a novel prebiotic in functional food for diabetes.

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1. Introduction

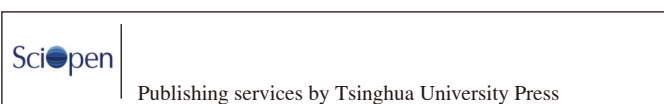
Type 2 diabetes mellitus (T2DM) is a chronic metabolic disease characterized by elevated blood glucose, accompanied by systemic low-level inflammation, insufficient insulin secretion or dysfunction caused by a variety of pathogenic factors such as genetic factors, epigenetic modifications and environmental factors, which leads to metabolic disorders such as sugar, fat, protein, water and electrolyte,

affecting the normal physiological activities of the body^[1-3]. In recent years, with the improvement of living standards and lifestyle changes, the incidence of T2DM is increasing year by year, which has become a serious public health problem endangering people's health^[4-7]. Numerous studies have shown that intestinal dysbacteriosis plays a key role in the occurrence and development of T2DM, which can induce chronic low-level inflammation, insulin resistance, and then trigger abnormal glucose and lipid metabolism^[8-9]. Therefore, targeted regulation of gut microbiota by means of lifestyle intervention (such as diet, exercise, etc.), supplement of microecological preparations (probiotics, prebiotics and synbiotics, etc.), hypoglycemic drugs, and microbial transplantation has become a new strategy for the prevention and treatment of T2DM^[10-11].

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Gut microbiota not only helps the host maintain normal digestion, absorption and metabolism, but also maintains the functional homeostasis of intestinal epithelial cells by interacting with the intestinal mucosa^[12]. The intestinal mechanical barrier composed of intestinal epithelial cells and their tight junctions is the key to inhibit ectopic intestinal bacteria and lipopolysaccharide (LPS) leakage^[13–14]. LPS is an important component of the outer membrane of intestinal Gram-negative bacteria, also known as endotoxin^[15]. When intestinal permeability increases, excessive endotoxin can enter the blood circulation and trigger inflammation cascade reaction and disrupt homeostasis of intestinal barrier function^[16]. Recent studies have confirmed that increased intestinal permeability and intestinal-derived LPS translocation are important causes of metabolic inflammation in T2DM^[17–18]. Therefore, targeting intestinal barrier is a promising strategy for the treatment of T2DM. As a high-quality prebiotic, polysaccharide can effectively stimulate the proliferation of intestinal beneficial bacteria and inhibit the growth of potentially harmful bacteria^[19]. Especially, plant-derived natural polysaccharides, have safe, green and stable effects, showing great advantages in regulating gut microbiota and protecting intestinal health^[18,20–21].

Dendrobium officinale Kimura et Migo (*D. officinale*) is a perennial herb of Orchidaceae family, widely distributed in Southeast Asia and Australia, and has been proved to have various physiological effects, such as hypoglycemic, anti-inflammatory, promote intestinal health, anti-aging, anti-oxidation and anti-tumor^[22–25]. In addition to its medicinal application in Asian traditional medicine with its stems, *D. officinale* is also a non-toxic and harmless new food resource in China, which has been included in the catalogue of medicinal and edible products and the catalogue of new food raw materials^[26]. *D. officinale* contains a variety of nutrients, including polysaccharides, phenanthrene compounds, alkaloids, glycosides, etc^[27]. Among them, *D. officinale* polysaccharide (DOP), the highest content of functional components, has been identified as one of the main components responsible for its beneficial effect, especially against diabetes, but its hypoglycemic mechanism remains unclear^[24,26,28].

Therefore, from the perspective of anti-inflammatory and intestinal microbial regulation, this study used high-fat diet (HFD) and streptozotocin (STZ) induced T2DM model mice to study the hypoglycemic effects of DOP and reveal its potential mechanism.

2. Materials and methods

2.1 Preparation of DOP

D. officinale was obtained from Zhixin Chinese Herbal Pieces Co., Ltd. (Guangzhou, China). DOP was prepared according to the previous method with slight modifications^[29]. Briefly, *D. officinale* powder was degreased by refluxing with 95% ethanol for 4 h at 70 °C, and then extracted 3 times with deionized water at 90 °C for 2 h each. After centrifugation, anhydrous ethanol was added to 80% (*V/V*) to precipitate the supernatant (24 h at 4 °C), and the precipitate was deproteinized with Sevag reagent (*V*(chloroform):*V*(butyl alcohol) = 4:1), then decolorized with AB-8 macroporous resin. Ultimately, the DOP was obtained after dialysis (48 h, cut-off M_w : 3 000 Da) with distilled water.

2.2 The chemical properties and composition of DOP

The neutral sugar, uronic acid and total protein contents of DOP were determined by phenol-sulfuric acid method, *m*-hydroxydiphenyl colorimetric method and Coomassie brilliant blue method, respectively^[7]. Furthermore, the molecular weight and monosaccharide composition of DOP were analyzed by high performance gel permeation chromatography and ion chromatography with reference to the previously reported method^[5], and the chemical properties and composition of DOP were shown in Table S1.

2.3 Animals and experimental design

A total of 36 male C57BL/6J mice ((20 ± 2) g, 6 weeks old) were purchased from Guangdong Sijiajingda Biotechnology Co., Ltd. (Guangzhou, China) with a production license number of SYXK Yue 2017–0125, and the experimental procedures were reviewed by the Chinese Experimental Animal Ethics Committee (approval number: gdpulacspf2017505). Mice were housed under SPF conditions (20–22 °C and (45 ± 5)% humidity for 12 h/12 h light-dark cycle) with free access to food and sterile drinking water. The specific experimental procedures and grouping were shown in Fig. 1. Specifically, After 1 week of adaptive feeding, 12 mice were randomly selected to be fed with standard diet as the normal (NC) group, and the rest were fed with 60% HFD (Cat. No.: TP23400; Trophic Animal Feed High-tech Co., Ltd., Nantong, China) until sacrifice. After 4 weeks of HFD feeding, mice were fasted for 12 h and then injected intraperitoneally once with 120 mg/kg STZ (Beijing Boaigang Biotechnology Co., Ltd., Beijing, China), and the fasting blood glucose (FBG) level greater than 11.1 mmol/L indicated successful T2DM model preparation^[30]. The successful T2DM modeling mice were randomly divided into 2 groups ($n = 12$), one group (DOP) was administered with 400 mg/(kg·day) DOP, and the another group (model (MC) group) was administered with equal volume of saline. Similarly, mice in the NC group were also administered with equal volume of saline for 6 weeks. During the experimental period, the food intake and water consumption of mice were monitored daily, and the body weight and FBG levels were monitored weekly.

2.4 Oral glucose tolerance test

After 6 weeks of administration, overnight-fasted mice were orally gavaged with glucose (1 g/kg), and the oral glucose tolerance test (OGTT) was measured according to the method previously described^[11].

2.5 Biological sample collection and preparation

At the end of the intervention, the feces of the mice were collected by stimulating the anus of mice and quickly kept at –80 °C before analysis. After a 12 h fast, the blood of the mice was drawn from the abdominal aorta and immediately centrifuged (7 104 × *g*, 10 min) to obtain serum for the analysis of biochemical indicators. Fresh colon tissues were collected, some were stored in 4% paraformaldehyde solution for pathological examination, and the rest were immersed in liquid nitrogen and stored at –80 °C for analysis.

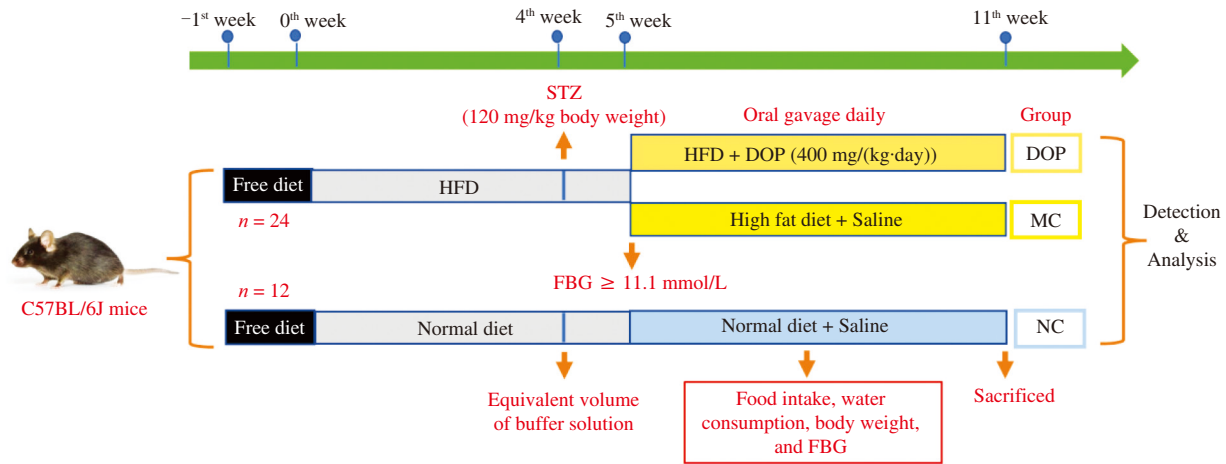


Fig. 1 Schematic diagram of experimental design.

2.6 Biochemical assays and ELISA test

Glycosylated serum protein (GSP); lipid metabolism indicators such as total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C); and oxidative stress factors such as superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) and malondialdehyde (MDA) were detected using biochemistry kits (Nanjing Jiancheng Bio-Engineering Institute Co., Ltd., Nanjing, China). Fasting insulin (FINS), LPS, diamine oxidase (DAO), *D*-lactate (*D*-LA), and metabolic inflammatory factors such as tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), interleukin-1 β (IL-1 β) and interleukin-10 (IL-10) were measured by ELISA kits (Jiangsu Meimian Industrial Co., Ltd., Jiangsu, China). The specific experimental steps were performed in strict accordance with the manufacturer's instructions. The homeostatic model assessment of insulin resistance (HOMA-IR) was calculated using the following formula^[31]:

$$\text{HOMA-IR} = \frac{\text{FBG (mmol/L)} \times \text{FINS (\mu U/mL)}}{22.5}$$

2.7 Histopathological examination

At the end of the experiment, the mice were anesthetized with ether and then executed, and fresh colon tissues were collected and preserved in 4% paraformaldehyde solution for hematoxylin and eosin (H&E) staining analysis according to our previously published method^[18].

2.8 Real-time quantitative polymerase chain reaction (qPCR) assay

The total RNA in the colon tissue was extracted using a TRIzol RNA extraction kit (ComWin Biotech Co., Ltd., Beijing, China) and reverse transcribed into cDNA using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Waltham, MA, USA). The levels of cDNA encoding zonula occluden-1 (*ZO-1*), *Occludin* and *claudin-1* were quantified using the Bio-Rad CFX96 Touch Real-time PCR Detection System (Bio-Rad, Hercules, CA, USA) and normalized by the housekeeping gene β -actin. The primers used in this study were listed in Table S2.

2.9 16S rRNA sequencing

The fecal samples were collected and the 16S rRNA V3-V4 hypervariable region was subjected to high-throughput sequencing by Shanghai Personalbio Biotechnology Co., Ltd. (Shanghai, China). First, genomic DNA was extracted from fecal samples using the OMEGA Soil DNA Kit (Omega Bio-Tek, Norcross, GA, USA). Second, the V3-V4 region of the bacterial 16S rRNA gene was amplified using the 338F/806R primer pairs (338F: 5'-ACTCCTACGGGAGGCAGCAG-3'; 806R: 5'-GGACTACHVGGGTWTCTAAT-3'). The PCR amplification products were then purified with VAHTSTM DNA Clean Beads (Vazyme Biotech Co., Ltd., Nanjing, China) and quantified using Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA). In the end, the sequencing library was constructed and sequenced on the Illumina MiSeq platform (Illumina, San Diego, CA, USA).

2.10 Statistical analysis

Data were presented as mean $\pm$ SD from more than three experimental replicates. Statistical differences were performed by one-way analysis of variance (ANOVA) followed by post-hoc Duncan's test using SPSS 26.0 (IBM Corp, Armonk, NY, USA), and $P < 0.05$ was considered statistically significant.

3. Results

3.1 DOP improved the symptoms of T2DM mice

In this study, a T2DM model was established using HFD feeding and STZ injection. As shown in Fig. S1, compared with the NC group, the symptoms of the MC group mice were increased food and water consumption ($P < 0.001$) and weight loss ($P < 0.001$), while DOP intervention significantly improved the symptoms of polyphagia ($P < 0.01$), polydipsia ($P < 0.001$) and body weight loss ($P < 0.05$) in T2DM mice. In addition, the FBG value of mice in the MC group (23.75 ± 4.11 mmol/L) was much higher than that in the NC group (6.02 ± 0.77 mmol/L), and DOP treatment significantly decreased the FBG level ($P < 0.01$, Fig. 2A), but there was still a gap compared with the NC group.

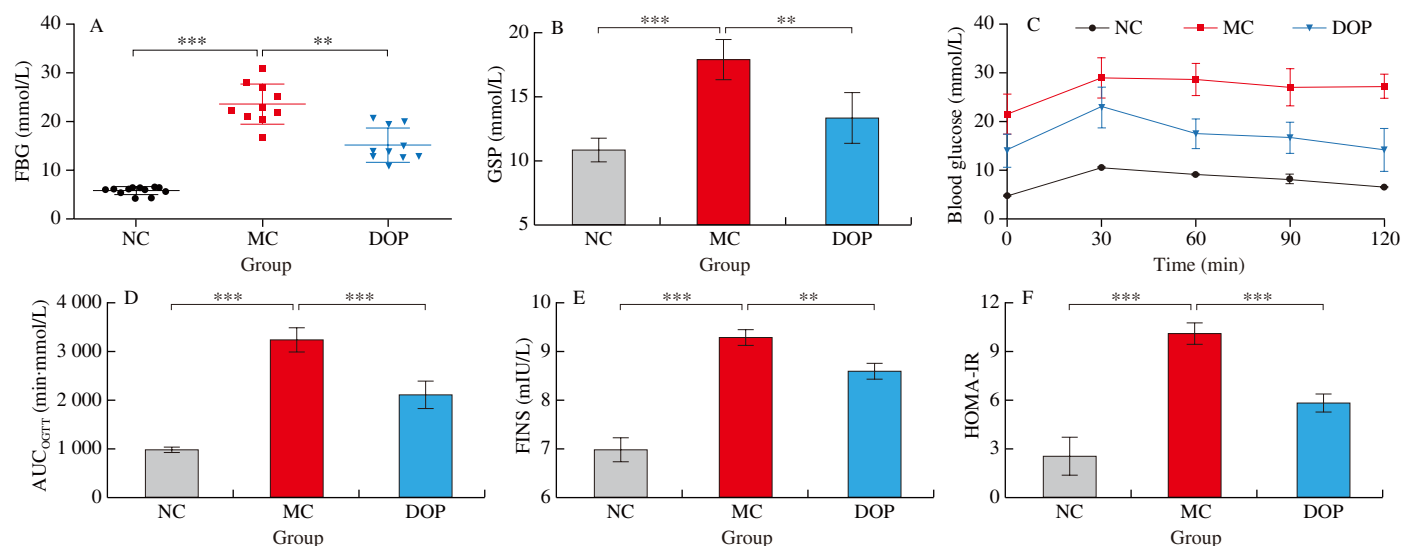


Fig. 2 DOP relieved glucose intolerance and insulin resistance. The levels of (A) FBG, (B) GSP, (C) OGTT, (D) the area under the curve (AUC) of the OGTT, (E) FINS, and (F) HOMA-IR of experimental mice treated with DOP for 6 weeks were studied. Data were presented as means $\pm$ SD. **, *** indicate significant difference ($P < 0.01$, $P < 0.001$, respectively). The same below.

3.2 DOP relieved glucose intolerance and insulin resistance

To elucidate the role of DOP in controlling glucose homeostasis and insulin sensitivity, in addition to the FBG level, the GSP, OGTT, and insulin levels were measured. Relative to MC group, DOP intervention significantly reduced the GSP value of T2DM mice by 25.66%, which meant that DOP could effectively inhibit the elevation of blood glucose in T2DM mice in the past 1–3 weeks (Fig. 2B). The oral glucose tolerance of the mice in DOP-treated group was better than that in the MC group at individual time point, and the AUC decreased by 35.04% within 120 min (Figs. 2C–D), indicating that DOP could enhance the body's ability to regulate elevated blood glucose level. Similarly, compared with the MC group, serum insulin level was also significantly reduced by 7.63% after DOP intervention (Fig. 2E), while HOMA-IR was decreased by 42.59% (Fig. 2F), suggesting that DOP could partially reverse glucose intolerance and insulin resistance in T2DM mice.

3.3 DOP regulated serum lipids metabolism

As a chronic metabolic disease, diabetes mellitus is accompanied by lipid metabolism disorder and induces hyperlipidemia, which is manifested as an obvious decrease in HDL-C and an significant increase in LDL-C, TC, and TG^[32]. Therefore, this study examined the effect of DOP on serum lipid levels in T2DM mice. Compared with the MC group, DOP administration substantially decreased TC, TG, and LDL-C levels (Figs. 3A–C, $P < 0.01$), accompanied by elevated HDL-C level (Fig. 3D, $P < 0.05$), indicating that DOP could effectively improve hyperlipidemia in T2DM mice.

3.4 DOP inhibited serum metabolic inflammatory levels in T2DM mice

Multiple evidences have shown that T2DM is often accompanied by intestinal mucosa damage, which may lead to bacteria and their

derived toxins entering important human tissues and organs through blood circulation, inducing metabolic inflammation, which in turn further promotes the development and changes of T2DM^[2,33]. Thus, we further detected the levels of intestinal permeability serological indicators LPS and serum inflammatory cytokines TNF- α , IL-1 β , IL-6 and IL-10. The results showed that compared with the NC group, the LPS content of the mice in MC group was significantly increased (Fig. 4A, $P < 0.001$), indicating that there was obvious intestinal barrier damage and LPS leakage in T2DM mice induced by HFD feeding and STZ injection. However, the upward trend was reversed by DOP treatment ($P < 0.001$). In addition, the study found that DOP effectively inhibited the expression of serum pro-inflammatory factors such as TNF- α ($P < 0.05$), IL-1 β ($P < 0.001$) and IL-6 ($P < 0.001$), but had no significant effect on the expression of anti-inflammatory IL-10 (Figs. 4B–E).

3.5 Correlation analysis of serum LPS with glycolipid metabolism and inflammatory indexes in T2DM mice

In order to explore whether there was a correlation between serum LPS and glycolipid metabolism and inflammatory indicators, the Spearman correlation analysis was performed. The results showed that LPS was positively correlated with FBG, GSP, AUC_{OGTT}, FINS, HOMA-IR, TC, TG, LDL-C, TNF- α , IL-6, and IL-1 β , and negatively correlated with HDL-C (Table 1). To sum up, LPS was significantly correlated with glucose and lipid metabolism and the levels of inflammatory factors, suggesting that inhibiting LPS-mediated inflammatory response and protecting the integrity of intestinal mucosa may be the key target of DOP in the treatment of T2DM. Therefore, in the follow-up experiments, we focused on the effect of DOP on the intestinal mucosal barrier and gut microbiota, and studied the mechanism of DOP in improving diabetes from the perspective of the intestine.

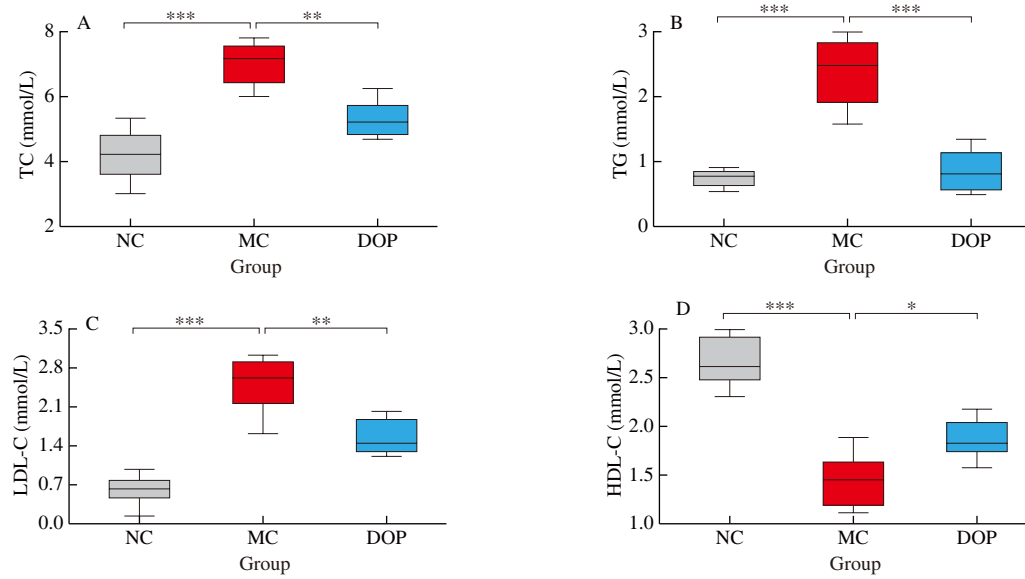


Fig. 3 DOP regulated serum lipids metabolism. (A) TC, (B) TG, (C) LDL-C, and (D) HDL-C of experimental mice treated with DOP for 6 weeks were studied. * indicates significant difference ($P < 0.05$). The same below.

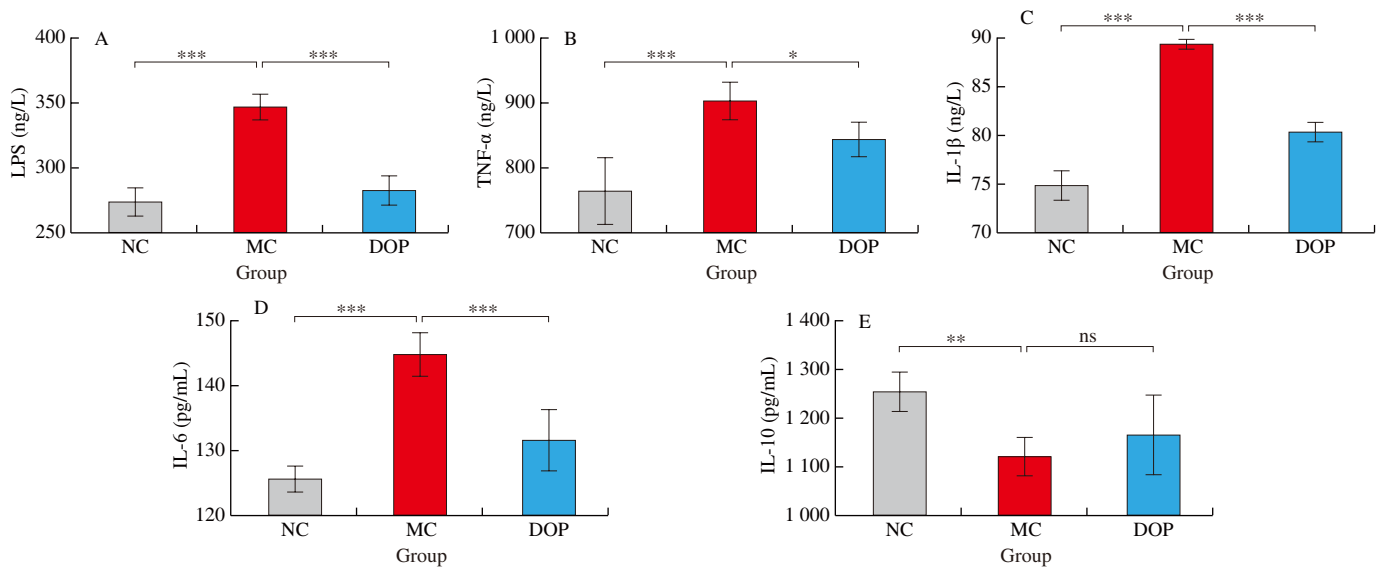


Fig. 4 DOP inhibited serum inflammation levels in T2DM mice. The serum levels of (A) LPS, (B) TNF-α, (C) IL-1β, (D) IL-6, and (E) IL-10 of experimental mice were analyzed after 6 weeks of DOP treatment. ns indicates not statistically significant ($P > 0.05$). The same below.

Table 1

Spearman correlation analysis of serum LPS with glycolipid metabolism and inflammatory indexes in T2DM mice.

Variable 1	Variable 2	<i>r</i>	<i>P</i>
LPS	FBG	0.611	0.015 5*
LPS	GSP	0.658	0.007 7**
LPS	AUC _{OGTT}	0.707	0.003 0**
LPS	FINS	0.699	0.003 7**
LPS	HOMA-IR	0.789	0.000 5***
LPS	TC	0.686	0.004 8**
LPS	TG	0.650	0.008 7**
LPS	LDL-C	0.686	0.004 7**
LPS	HDL-C	-0.574	0.025 3*
LPS	TNF-α	0.814	0.000 2***
LPS	IL-6	0.572	0.025 7*
LPS	IL-1β	0.803	0.000 3***
LPS	IL-10	-0.277	0.317 5

Note: *, **, *** indicate significant correlation ($P < 0.05$, $P < 0.01$, $P < 0.001$, respectively).

3.6 DOP inhibited the expression of serum markers of intestinal mucosal injury and intestinal inflammatory factors in T2DM mice

To investigate the effect of DOP on the intestinal mucosal barrier, serological markers associated with intestinal mucosal injury, including DAO and D-LA, as well as the intestinal inflammatory cytokines TNF-α, IL-1β, IL-6, and IL-10 were examined. The data showed that the levels of DAO and D-LA in the serum of the mice in the MC group were significantly increased, suggesting that there was intestinal mucosal damage in the T2DM mice, but the two metrics were significantly reduced by 17.34% and 5.53% after DOP intervention, respectively (Fig. 5A). Additionally, as shown in Figs. 5B-E, DOP supplementation significantly reduced intestinal TNF-α ($P < 0.01$), IL-1β ($P < 0.05$), and IL-6 ($P < 0.01$) levels, and

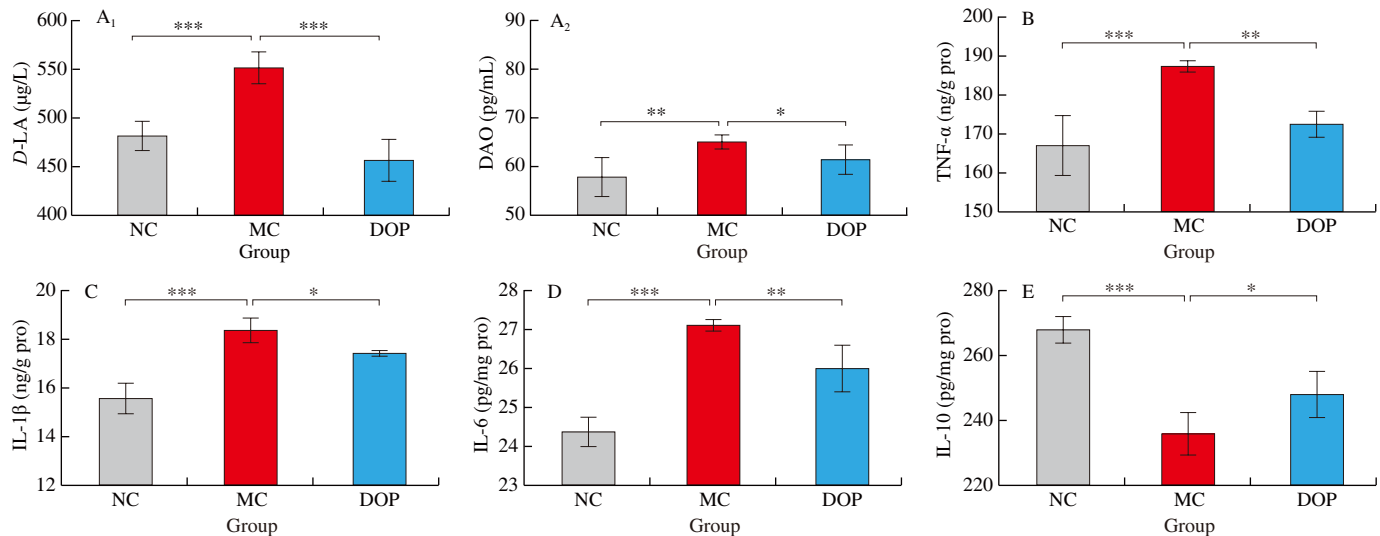


Fig. 5 DOP inhibited the expression of serum markers of intestinal mucosal injury and intestinal inflammatory factors in T2DM mice. The following indicators including (A) *D*-LA and DAO, (B) TNF-α, (C) IL-1β, (D) IL-6, and (E) IL-10 levels in the colon of experimental mice were analyzed.

promoted the IL-10 level ($P < 0.05$) relative to the MC group. In conclusion, the above results suggested that DOP has a good repairing effect on intestinal mucosal injury in T2DM mice by inhibiting intestinal inflammation.

3.7 DOP inhibited the intestinal oxidative stress levels in T2DM mice

Intestinal intraepithelial oxidative stress is also considered to be an important factor leading to intestinal mucosal damage^[34-35], so this study further detected oxidative stress-related indicators in the colon tissue of experimental mice. The data showed that T2DM mice had severe intestinal oxidative stress, which was manifested by significantly decreased SOD, CAT, and GSH levels ($P < 0.001$), and markedly increased MDA levels ($P < 0.001$). After DOP intervention, the oxidative stress levels in the colon of mice showed a trend towards the NC group, in which the levels of SOD, CAT and GSH were

significantly increased by 41.79%, 29.79% and 24.26%, respectively, while the level of MDA was significantly decreased by 29.54% (Fig. 6), meaning that DOP alleviated oxidative stress damage in colon of T2DM mice. Taken together, DOP played a protective role in the intestinal barrier by suppressing the levels of intestinal inflammation and oxidative stress. Additionally, it was essential to highlight that correlation analysis indicated that the inhibition of intestinal LPS leakage by DOP played a key role in reducing the elevation of intestinal inflammatory factors and oxidative stress (Table S3).

3.8 DOP regulated the expression of tight junction proteins in T2DM mice

Studies have confirmed that phenotypic characteristics of abnormal tight junction proteins expression were observed in both HFD and STZ-induced T2DM mouse model and spontaneous T2DM mouse model^[36-37], which was consistent with our study, that is, RT-qPCR

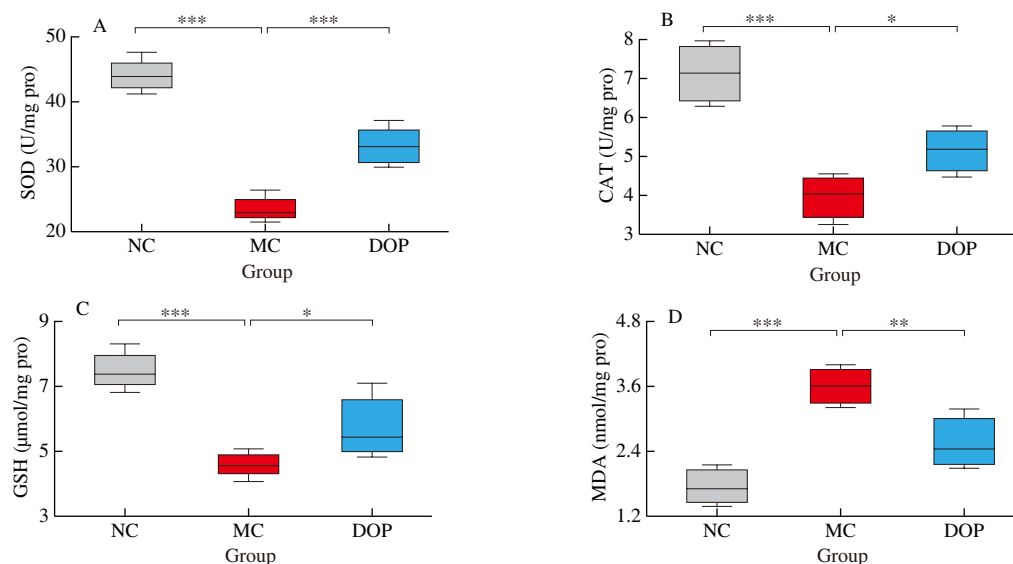


Fig. 6 DOP inhibited the intestinal oxidative stress levels in T2DM mice. Oxidative stress relevant indicators including (A) SOD, (B) CAT, (C) GSH, and (D) MDA were measured in the colon after 6 weeks of DOP treatment.

assay revealed that the mRNA expressions of representative intestinal tight junction proteins *ZO-1*, *Occludin*, and *Claudin-1* were decreased significantly ($P < 0.001$) in T2DM mice. In contrast, the mRNA expression levels of *ZO-1*, *Occludin*, and *Claudin-1* showed an upward trend after DOP treatment, and increased by 28.45%, 38.34% and 26.99%, respectively (Fig. 7A), indicating that DOP has a strong ability to regulate the expression of intestinal tight junction proteins.

3.9 DOP alleviates intestinal histopathological damage in T2DM mice

As shown in Figs. 7B–C, the colon length of mice in the MC group was significantly shorter than that in the NC group ($P < 0.01$). Furthermore, DOP administration attenuated the effect of colon shortening in T2DM mice, and the colon length increased by 29.54% compared with MC mice. To explore the intestinal pathological changes in T2DM mice and the intestinal protective effect of DOP, we proceeded to observe H&E staining images of colon tissue (Fig. 7D). The results showed that the MC group mice exhibited intestinal glands atrophy, crypts structural deformation, and the epithelial cells had a certain degree of degeneration or even necrosis, accompanied by inflammatory cell infiltration and enhanced transmittance. After DOP intervention, the above pathological changes were alleviated to varying degrees. In short, DOP exerted a favorable restorative and protective effect on the intestinal barrier by suppressing intestinal inflammation and ameliorating oxidative stress, indicating that the protective effect of intestinal barrier for DOP may be the fundamentals in the treatment of T2DM.

3.10 Analysis of the bacterial community composition

The fecal bacterial community of T2DM mice was detected by 16S rRNA gene amplicon sequencing. Alpha diversity indices, including Chao1, Observed species, Simpson, and Shannon, were applied to assess bacterial richness and diversity. Among them,

the Chao1 and Observed species indexes evidenced the abundance of bacteria, while the Shannon and Simpson indexes reflected the community diversity^[18]. As presented in Fig. 8, the bacterial richness and diversity of the MC group were significantly increased compared with the NC group, while after DOP intervention, the richness of bacteria significantly decreased by about 30% ($P < 0.001$), and the community diversity also decreased greatly and tended to the NC group, manifesting that DOP exhibited a significant impact on the gut microbiota of T2DM mice.

At the phylum level, Firmicutes, Actinobacteria, and Proteobacteria were the dominant phylum detected in all experimental groups (Figs. 9A–B), and their relative abundance accounted for more than 90% of the total bacterial community. Compared with the NC group, the intestinal bacteria in the MC group displayed a 750.09% increase in the relative abundance of Proteobacteria as well as 16.98% and 42.49% decreases in the relative abundance of Firmicutes and Actinobacteria, respectively. However, DOP treatment could restore the above phylum levels. Remarkably, the taxonomic tree in packed circles and the hierarchical clustering analysis further indicated that DOP had a modulating effect on bacterial community composition in T2DM mice induced by HFD and STZ (Figs. 9C–D).

Overall, at the genus level, the abundance of *Allobaculum*, *Bifidobacterium*, and *Lactobacillus* in the NC group mice was overwhelming and up to 84.07%. Among them, compared with the NC group, T2DM mice exhibited an obvious shift in the gut microbiome, with a reduction in the abundance of genera *Bifidobacterium* and *Lactobacillus*; and an increase in genera *Helicobacter*, *Bacteroides*, *Desulfovibrio*, *Odoribacter*, *Enterococcus*, *Flexispira*, *Adlercreutzia*, *Parabacteroides*, *Anaeroplasm*, AF12, *Akkermansia*, and *Halomonas* (Figs. 9E–F). Conversely, these genera were restored after DOP treatment, especially, the relative abundances of *Bifidobacterium* and *Lactobacillus* were increased by 139% and 8%, respectively, while the relative abundances of *Helicobacter*, *Desulfovibrio*, and *Enterococcus* were decreased by 95%, 9% and 73%, respectively.

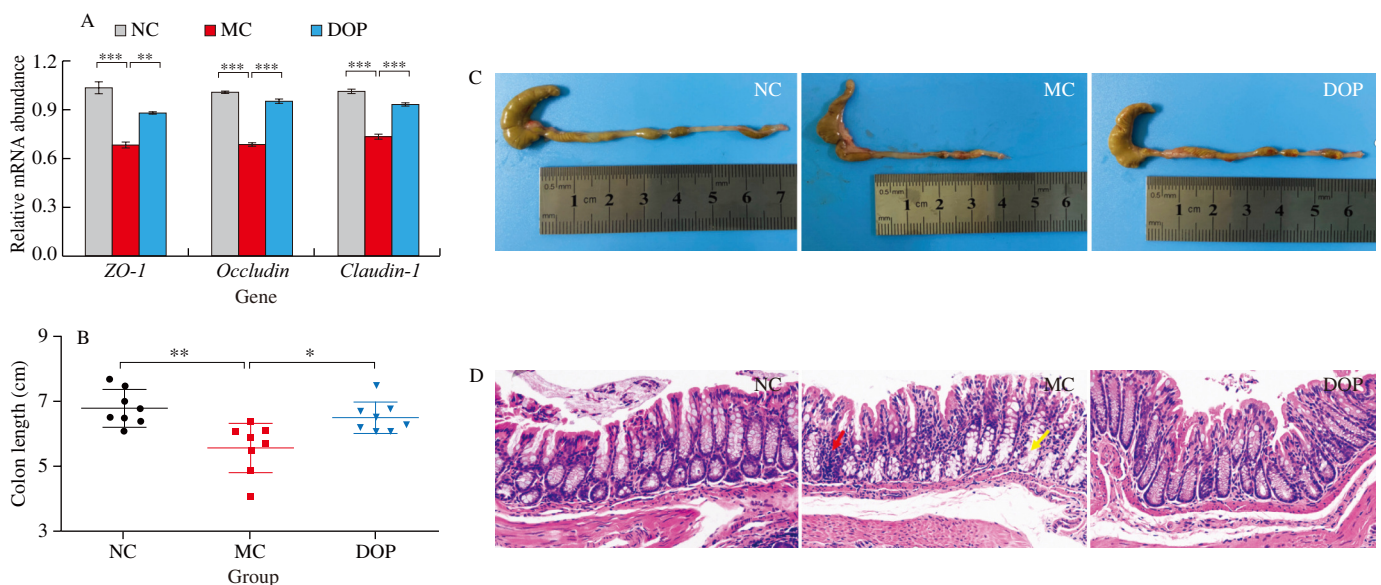


Fig. 7 (A) Effects of DOP on the mRNA expression of intestinal tight junction proteins (including *ZO-1*, *Occludin*, and *Claudin-1*) of T2DM mice. (B) Colon length of experimental mice. (C) Representative pictures of colonic anatomical tissue of experimental mice. (D) H&E staining of colon tissue of experimental mice (200×). Red arrow: inflammatory cell infiltration; yellow arrow: vacuolar degeneration of cells.

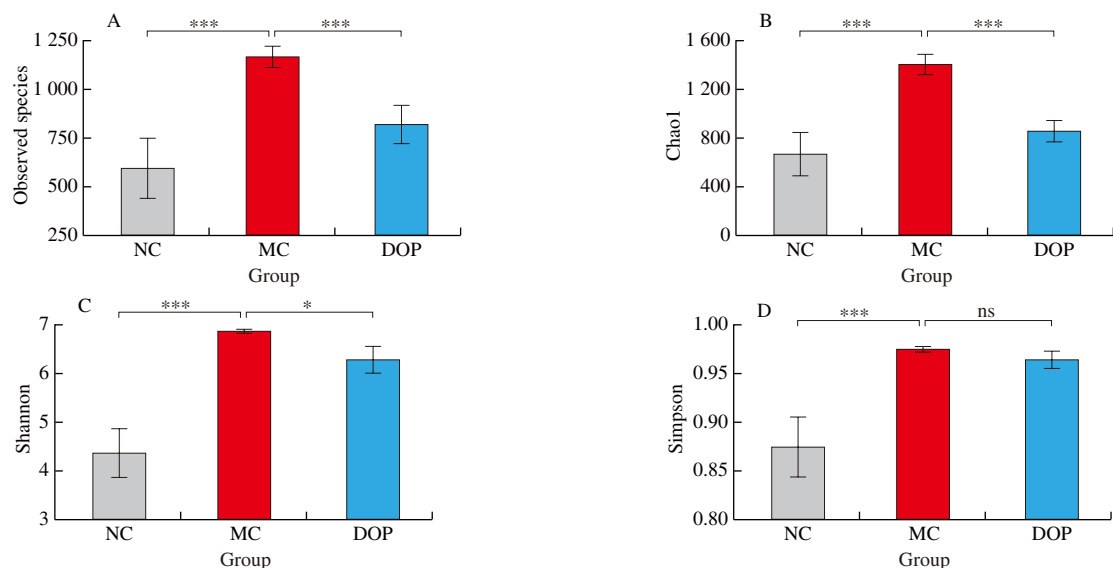


Fig. 8 Effects of DOP on alpha-diversity of fecal bacterial community in T2DM mice, including the (A) Observed species, (B) Chao1, (C) Shannon, and (D) Simpson indexes.

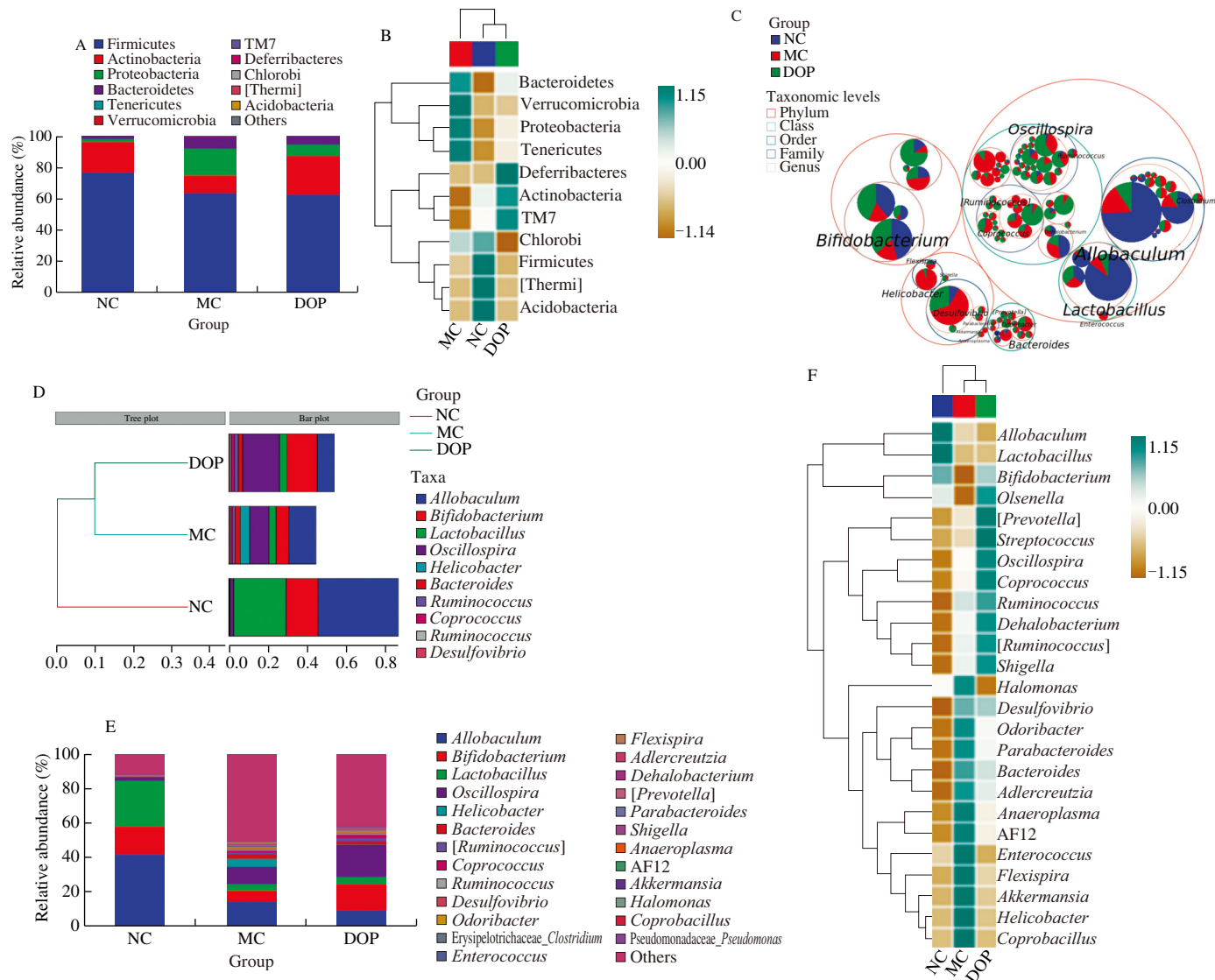


Fig. 9 Effects of DOP on the composition and abundance of intestinal microbiota in T2DM mice. (A) Microbiota composition at the phylum level in mice of each group (top 10); (B) The heatmap showed changes in the relative abundance at the phylum level; (C) Based on the taxonomic differences of 16S rRNA sequences, the largest circle represented the phylum level, and the inner circle represented the class, order, family and genus in turn; (D) Hierarchical clustering analysis of each group at the genus level, showing the similarity between groups in the form of hierarchical trees, and measuring the clustering effect by the branch length of the clustering tree; (E) The genus-level microbiota composition in each group of mice (top 25); (F) The heatmap showed the change of relative abundance of bacterial genera.

3.11 Analysis of the key bacterial genera

We have proved that DOP could modulate the gut microbiota in T2DM mice, and had a notable enriching effect on the *Bifidobacterium* and *Lactobacillus*, but a suppressing effect on *Helicobacter*, *Desulfovibrio*, and *Enterococcus*. To further explore the potential relationship between gut microbiota variation and T2DM-related indicators, the correlations (Table S4) between the 5 major genera obtained in the current study and the related indices of intestinal inflammation and glucolipid metabolism were calculated based on Spearman correlation analysis^[38]. As shown in Fig. 10, the genera decreased in the DOP group (*Helicobacter*, *Desulfovibrio*, and *Enterococcus*) were positively correlated with serum LPS and intestinal proinflammatory cytokines, while the genus enriched in the DOP group (*Bifidobacterium* and *Lactobacillus*) exhibited a negative relationship with the intestinal inflammatory status, indicating that DOP notably attenuated endotoxemia and intestinal inflammation in diabetic mice by remodeling intestinal microbiota structure. Similarly, the correlations analysis of the 5 major genera with glucolipid metabolism indexes displayed that the decreased bacteria *Helicobacter*, *Desulfovibrio*, and *Enterococcus* in the DOP group could promote the pathogenesis of T2DM, and the bacteria *Bifidobacterium* and *Lactobacillus* enriched in the DOP group could block the pathogenesis of T2DM. Generally, the above results indicated that DOP could improve intestinal inflammation and mucosal barrier function by modulating the abundance and composition of specific gut microbes, manifested as an increase in the abundance of beneficial bacteria *Bifidobacterium* and *Lactobacillus*, and a decrease in the production of harmful bacteria *Helicobacter*, *Desulfovibrio*, and *Enterococcus* in the feces of T2DM mice, thereby alleviating the symptoms of T2DM mice.

4. Discussion

The aim of this study was to investigate the mechanism of DOP in improving T2DM from the perspective of intestinal microbiota and intestinal mucosa. On the one hand, DOP improved the glucose and lipid metabolism in HFD and STZ-induced T2DM mice. On the other hand, DOP relieved intestinal mucosa damage, inhibited LPS

leakage, alleviated intestinal inflammation and oxidative stress, and regulated the gene expression of intestinal tight junction proteins *ZO-1*, *Occludin*, and *Claudin-1* in T2DM mice. 16S rRNA high-throughput sequencing results showed that DOP significantly reduced the abundance of intestinal conditioned pathogens *Helicobacter*, *Desulfovibrio* and *Enterococcus*, and promoted the proliferation of probiotics *Bifidobacterium* and *Lactobacillus*. Further studies have shown that the therapeutic effect of DOP on diabetes was attributable to changes in gut microbiota and regulation of intestinal barrier function. To our knowledge, this study is the first to demonstrate that DOP can effectively alleviate HFD and STZ-induced diabetes by reversing metabolic imbalances of gut microbiota and modulating gut barrier function.

It is well known that intestinal mucosa is the body's first line of defense against pathogenic microorganisms, and intestinal epithelial cells and tight junction proteins, including Occludin, Claudins, and ZOs (ZO-1, ZO-2, and ZO-3) together constitute the intestinal mucosal barrier^[14]. Once "Intestinal leak" occurs, bacteria and their metabolites, such as LPS and *D-LA*, invade the intestine and blood, leading to the release of various cellular inflammatory factors and inducing intestinal inflammation^[39]. Multiple evidences has shown that T2DM is often accompanied by chronic metabolic inflammation and intestinal mucosal damage, which in turn further promotes the development of T2DM^[40]. Because of its small molecular weight, LPS can enter the blood circulation through the mucosal barrier through the portal vein even though the intestinal permeability is slightly increased, so it is one of the ideal serological indexes to evaluate the intestinal permeability^[41]. Excessive LPS in the circulation activates Toll-like receptor 4 (TLR4) in a receptor-dependent manner and activates downstream transcription factors, including myeloid differentiation factor 88 (MyD88) and nuclear factor κ B (NF- κ B), increasing the synthesis and secretion of inflammatory cytokines, resulting in abnormal expression of a series of proteins closely related to insulin and glucose metabolism, thereby affecting the core aspects of human blood glucose regulation^[42]. Our study demonstrated that DOP inhibited the disturbance of glucose and lipid metabolism and the production of pro-inflammatory factors (LPS, IL-6, IL-1 β and TNF- α) in the serum of mice in the MC group. Importantly, by calculating the Spearman correlation coefficient, it was found that LPS was significantly correlated with glucose and lipid metabolism

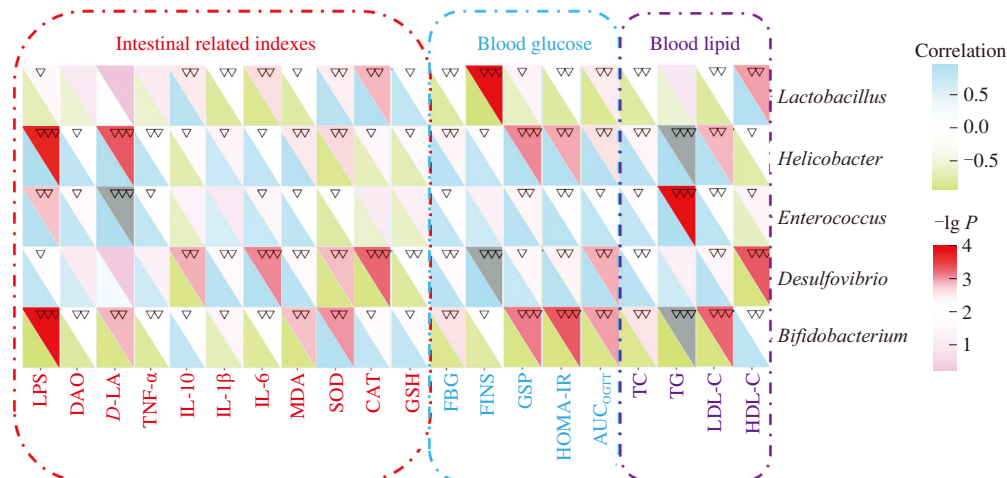


Fig. 10 Correlation analysis between T2DM-related parameters and the relative abundance of gut microbiota at genus level. Statistical significance was calculated by Spearman correlation analysis. ∇, ∇∇, ∇∇∇ indicate significant correlation ($P < 0.05$, $P < 0.01$, $P < 0.001$, respectively).

and serum inflammatory factor levels, suggesting that inhibition of LPS-mediated inflammatory response may be a key target for DOP treatment of T2DM.

Based on above findings, the present study was followed by an assessment of the repair effects of DOP on intestinal barrier damage by intestinal barrier serum markers, histological staining, and intestinal tight junction proteins expression levels. DAO is a highly active intracellular enzyme in the upper villi of human and mammalian small intestinal mucosa, and its activity is closely related to the synthesis of nucleic acid and protein in mucosal cells, which can reflect the integrity and damage of intestinal mechanical barrier^[43]. D-LA is a fermented product of intestinal bacteria and is often used as a serum marker of intestinal permeability because it is rarely absorbed and difficult to degrade rapidly^[44]. Tight junctions are the main connections between intestinal epithelial cells and play a crucial role in maintaining the normal structure and function of the intestinal barrier^[41]. This study found that compared with NC group, MC group mice showed higher circulating DAO, D-LA activities, indicating that HFD and STZ increased intestinal mucosal permeability and destroyed the intestinal mucosal barrier, which was consistent with previous studies^[12,40], but was reversed by DOP treatment. In addition, DOP had a significant upregulation effect on gene expression of tight junction proteins *ZO-1*, *Occludin*, and *Claudin-1*. Similar to the study of serum inflammatory levels, we further found that DOP inhibited the production of pro-inflammatory factors (LPS, IL-6, IL-1 β and TNF- α) in the colon of T2DM mice, but different from that, DOP also promoted the release of anti-inflammatory factor IL-10, further revealing that the anti-inflammatory effect of DOP may begin in the intestine, and the intestine was its main therapeutic target. Studies have proved that there is a close relationship between oxidative stress and immune inflammation, and intestinal intraepithelial oxidative stress is also considered to be an important factor leading to intestinal mucosal injury^[18,45]. In-depth study found that DOP achieved the therapeutic effect of protecting the intestinal barrier by reducing the oxidative stress response of intestinal tissue, which was manifested by increasing the levels of SOD, CAT and GSH and reducing the level of MDA. These results indicated that DOP reversed the intestinal mucosal injury induced by HFD and STZ to some extent and had protective effect on intestinal mucosal barrier.

The interaction between gut microbiota and intestinal mucosa is a key factor in maintaining intestinal barrier homeostasis^[12]. In this study, 16S rRNA high-throughput sequencing was used to detect intestinal microbial communities. Proteobacteria is considered to be a potential microbial marker for the diagnosis of intestinal epithelial dysfunction, and its increased abundance is mutually reinforcing with intestinal inflammatory response^[46], which was consistent with our study, that is, DOP reduced the abundance of Proteobacteria by 57.05% compared with MC group. At the genus level, DOP was found to suppress potential intestinal pathogens such as *Helicobacter*, *Desulfovibrio*, and *Enterococcus*. Interestingly, Aydemir et al.^[47] found that *Helicobacter* promotes insulin resistance by inducing chronic inflammation and affecting insulin regulation of gastrointestinal hormones, and confirmed that *Helicobacter* infection is closely related to the pathogenesis of T2DM and the general activation of the autoimmune system. As reported by Zhang et al.^[48], *Desulfovibrio* is a Gram-negative pathogenic bacterium that produces hydrogen sulfide and LPS, which disrupt barrier

function and lead to inflammation. In addition, studies have found an increase in *Enterococcus* in diabetic rodent models/patients, and its enrichment can also damage the epithelial barrier and lead to intestinal inflammation^[49]. As noted above, DOP strongly inhibits the proliferation of these potential LPS-producing bacteria and pro-inflammatory bacteria, hindering the activation of immune inflammation and thereby ameliorating intestinal epithelial damage and metabolic disorders. On the other hand, anti-inflammatory bacteria such as *Bifidobacterium* and *Lactobacillus* were promoted by DOP. Interestingly enough, *Bifidobacterium* have been shown to reduce intestinal endotoxin levels and improve mucosal barrier function, and restoring the quantity of *Bifidobacterium* in T2DM mice can regulate metabolic endotoxemia, inflammatory response and the development of diabetes^[8,13]. Additionally, *Lactobacillus* is an indispensable beneficial bacterium in the gut, which not only plays an antibacterial role by secreting lactic acid, hydrogen peroxide and bacteriocins, but also improves intestinal barrier function by competing for adhesion sites and regulating immune inflammation^[50]. Taken together, the regulation of intestinal microbiota by DOP may be one of the important reasons for its intestinal barrier protection and anti-inflammatory effects.

In summary, DOP played a role in the treatment of T2DM by regulating the intestinal microbiome and protecting the intestinal epithelial mucosa, specifically involving inhibition of LPS-mediated inflammatory response and reduction of oxidative stress levels (Fig. 11). In terms of molecular mechanism research, NF- κ B, as a key molecule closely related to oxidative stress and immune inflammation and regulated by both, may be a potential target for DOP pharmacological effects, which is the focus of our subsequent research.

5. Conclusions

This research showed that DOP attenuated the body weight loss, improved glucose tolerance deficit and insulin resistance, modulated lipid metabolism, and had ameliorating effects on LPS release and metabolic inflammation in T2DM mice induced by HFD and STZ. Then, correlation analysis revealed that the alleviating effect of DOP on intestinal permeability was the key to reduce the elevation of inflammatory factors and glucolipid metabolism disorder. Furthermore, DOP could protect the intestinal barrier by suppressing intestinal inflammation and oxidative stress levels and promoting the gene expression of intestinal tight junction proteins (*ZO-1*, *Occludin*, and *Claudin-1*). 16S rRNA analysis indicated that the above-mentioned beneficial effects of DOP were achieved through remodeling the gut microbiota, including inhibition of the potential intestinal pathogens *Helicobacter*, *Desulfovibrio*, and *Enterococcus* and restoration of the beneficial bacteria of *Bifidobacterium* and *Lactobacillus*, which was further confirmed by Spearman correlation analysis. In conclusion, DOP supplementation directly affected the integrity of the intestinal mucosal barrier, ultimately improving metabolic parameters in T2DM mice, most likely through its superior anti-inflammatory and gut microbiota-modulatory effects. These results provided a rationale for DOP as a potential prebiotic in diabetic food and drug applications.

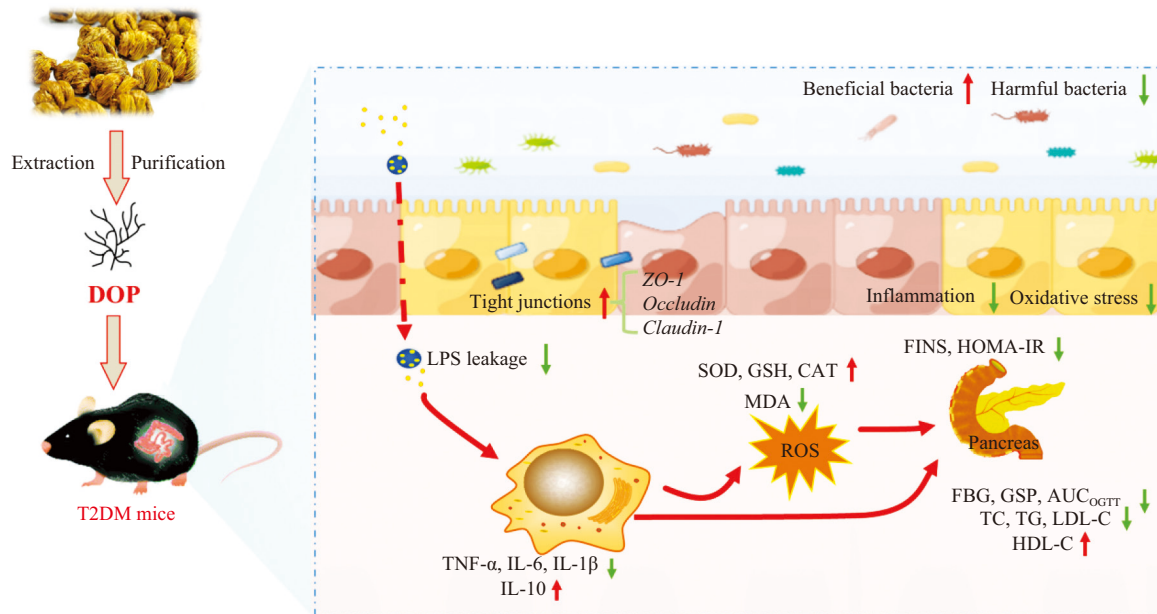


Fig. 11 Many beneficial changes occurred in T2DM mice after DOP administration, including normalizing gut microbiota structure, reducing intestinal permeability, repairing intestinal mucosa by inhibiting intestinal inflammatory response and oxidative stress, and improving glucose and lipid metabolism. ROS: reactive oxygen species.

Disclosure statement

Chun Chen and Changyang Ma are the editorial board members for *Food Science and Human Wellness*, and Wenyi Kang is an associate editor, neither of them was involved in the editorial review or the decision to publish this article. No potential conflict of interest was reported by the authors.

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Data availability statement

All data generated in this study are included in this article and supplementary information or are available from the corresponding author on reasonable request.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://doi.org/10.26599/FSHW.2024.9250007>.

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