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Lablab purpureus (L.) Sweet alleviated rhubarb induced diarrhea in a gut microbiota-dependent manner

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ABSTRACT

Lablab purpureus (L.) Sweet (*L. purpureus*) has been reported to alleviate diarrhea, although the precise mechanism remains unclear. This study identified the primary active components of *L. purpureus* utilizing ultra-performance liquid chromatography/tandem mass spectrometry (UPLC-MS/MS). A rhubarb-induced diarrhea model in rats was utilized to assess the therapeutic efficacy of *L. purpureus*. Alterations in gut microbiota and fecal metabolism were analyzed *via* 16S rDNA analysis and targeted metabolomics. Flora elimination and fecal transplantation techniques were employed to deepen understanding of the role of intestinal flora in *L. purpureus* treatment. The study findings indicated that the main constituents of *L. purpureus* included trigonelline, piperidinic acid, and *L*-(–)-malic acid, among others. *L. purpureus* treatment significantly alleviated all diarrhea symptoms in rats, encompassing reduced fecal water content, weight loss, shortened colon length, diminished histological damage, and decreased inflammatory factors. Furthermore, *L. purpureus* significantly enhanced the expression of tight junction markers and restored the dysregulated intestinal flora in diarrheic rats by increasing *Prevotella* and reducing *Lactobacillus*. Additionally, the production of propionic acid and other short-chain fatty acids (SCFAs) increased in diarrheic rats treated with *L. purpureus*, suggesting a substantial alteration in the intestinal environment. Crucially, the protective efficacy of *L. purpureus* diminishes in the absence of gut flora. Subsequent fecal transplantation tests demonstrated that feces from the *L. purpureus*-treated group alleviated rhubarb-induced diarrhea, emphasizing the pivotal role of gut microbiota in the antidiarrheal efficacy of *L. purpureus*. In conclusion, our findings elucidate the underlying mechanisms of *L. purpureus*' antidiarrheal action and its beneficial impact on intestinal microflora. Moreover, these results provide compelling evidence supporting the therapeutic use of *L. purpureus* for the treatment of diarrhea and its associated complications.

1. Introduction

Diarrhea, a prevalent gastrointestinal disorder, is characterized by increased bowel frequency, loose stool, and abdominal pain, presenting a significant public health challenge[1]. Clinical symptoms of diarrhea include phenomena such as weight loss, fatigue, and sensations of coldness[2-3]. Recent research has correlated diarrhea with various conditions, including disorders of the digestive system, imbalances in gastrointestinal hormones, immune deficiencies, and disruptions in energy metabolism[2,4]. The current frontline medications used to address diarrhea include loperamide, atropine, and montmorillonite. However, these medications, while providing partial relief from diarrhea, are associated with adverse effects such as increased heart rate and alterations in mental state[5]. Consequently, there is an immediate need for the exploration of novel treatments for managing diarrhea.

The seeds of *Lablab purpureus* (L.) Sweet (*L. purpureus*) hold significant importance across Asia and Africa, serving as both human sustenance and medicinal resources with the potential to contribute

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to the development of nutraceuticals and pharmaceuticals[6]. Additionally, the pods have the potential to serve as emergency food globally in regions where malnourishment and disease are prevalent. The National Academy of Sciences has recognized *L. purpureus* as a potential protein source[7]. Notably, *L. purpureus* has a historical role in traditional Chinese and Korean medicine as a remedy for gastrointestinal disorders[8]. In India, boiled *L. purpureus* pods are consumed to alleviate symptoms such as diarrhea, nausea, vomiting, and loss of appetite[9]. Despite these uses, the potential of *L. purpureus* for treating diarrhea and its relationship to intestinal flora remains to be investigated. *L. purpureus* is rich in polysaccharides, polyphenols, and flavonoids[9]. However, due to their low bioavailability, it is challenging for these substances to directly exert their effects on the body[10]. Recent studies have found that these compounds can improve intestinal health by modulating the gut microbiota[11-12]. Therefore, the gut microbiota can be considered a therapeutic target for treating intestinal inflammation, along with a new perspective on low bioavailability herbal medicines.

In recent years, dysregulation of gut flora in diarrhea has been demonstrated and modulation of gut flora is emerging as a new therapy for diarrhea[13]. Imbalance in its composition leads to disruption of motilin and gastrin secretion, inflammation, increased intestinal permeability, impaired tight junctions (TJs) and thus diarrhea[14]. In addition, short-chain fatty acids (SCFAs) synthesized by the gut microbiota through fermentation play a key role in maintaining host health and mitigating the onset of diarrhea and intestinal mucositis[15]. Increased production of SCFAs is thought to promote colonic fluid secretion and ameliorate dehydration associated with acute diarrhea[16]. In this study, rhubarb was used to create a model of diarrhea and rhubarb was chosen because it is inexpensive, easy to add, and clinically effective[17]. Sennosides, rheinosides and anthraquinone aglyconesa are considered to be the main bioactive components of *Rheum officinale*, the diarrhea-causing effect of rhubarb is a complex process involving multiple levels of chemical composition, biological activity, intestinal microbial metabolism, electrolyte balance and neuromuscular system regulation[18-20]. In summary, the induction of diarrhea models using rhubarb is a well-established and widely documented method in the scientific literature[21-23].

In conclusion, intestinal flora can enhance the bioavailability of herbal medicines and is closely linked to diarrhea. Thus, this study commenced from the perspective of intestinal flora to investigate whether the therapeutic effect of *L. purpureus* is contingent upon gut microbiota. Initially, we validated its therapeutic efficacy on diarrhea. Subsequently, we proceeded to deplete the intestinal flora by administering antibiotics to explore the potential impact of gut microbiota on the diarrhea-alleviating properties of *L. purpureus*. Furthermore, fecal microbiota transplantation (FMT) was employed to confirm the role of gut flora in the mitigation of rhubarb-induced diarrhea by *L. purpureus*.

2. Materials and methods

2.1 Preparation of L. purpureus and rhubarb

L. purpureus was obtained from China Beijing Tong Ren Tang (Group) Co., Ltd. (Beijing, China). Initially, 100 g of *L. purpureus* was soaked in distilled water at a ratio of 1:8 (*m*/*V*) and allowed to rest for 30 min. Subsequently, the *L. purpureus* was extracted twice with equal volumes of distilled water, with each extraction lasting 40 min. These extracts were combined, heated to 70 °C, and condensed to a final volume of 100 mL. The formulation of rhubarb followed the methodology detailed in our previous investigation[24]. In brief, rhubarb was first sliced into segments, soaked in water, and left in an oven maintained at a temperature range of 60–80 °C overnight. The resulting juice was extracted, sieved, and further condensed by heating to produce a liquid solution with a concentration of 2 g/mL.

2.2 Analysis of the main components of L. purpureus

HPLC-MS analysis was conducted using an ACQUITY UPLC HSS T3 column (2.1 mm × 100 mm, 1.8 μm), with an injection volume of 10 μL. The mobile phase, consisting of aqueous formic acid (0.1%, *V*/*V*) and acetonitrile (0.1% formic acid), enabled analysis at a flow rate of 0.3 mL/min. Specific analysis conditions are provided in Table S1. Compound identification methods can be found in previous literature[25].

2.3 Ultra-performance liquid chromatography/tandem mass spectrometry (UPLC-MS/MS) detection

UPLC-MS/MS was used to determine the amounts of three ingredients in *L. purpureus*, including trigonelline, pipecolic acid, and *L*-(–)-malic acid. The column temperature was 40 °C, and the flow rate was 0.2 mL/min. The gradient elution was carried out according to the following procedure (Table S2). The analyst software version 1.6.3 was employed for data acquisition of compound information, while the MultiQuant 3.0.3 software was utilized for the quantitative analysis of these compounds. The content of each compound in the samples was calculated through the application of a standard curve, the standard curve is shown in the Fig. S1.

2.4 Animal models and treatment

The experimental protocol encompassed the subsequent 3 phases:

1) Male Sprague-Dawley rats, aged 8 weeks and of SPF grade, were procured from SPF Biotechnology Co., Ltd. (Beijing, China) (SYXK 2019-0010) to evaluate the impact of *L. purpureus* on diarrhea. Following a oe-week adaptation period at conditions of 25 °C, 45%–50% humidity, and a 12-h light/dark cycle, we allocated the 30 rats into 5 groups ($n = 6$) in a random manner: control, diarrhea, *L. purpureus*-H (2.16 g/kg), *L. purpureus*-M (1.08 g/kg), and *L. purpureus*-L (0.54 g/kg). The moderate dose of 1.08 g/kg was determined by converting the human dosage to an equivalent based on body surface area. To thoroughly evaluate the therapeutic effects of varying dosages of *L. purpureus* on diarrhea, we established additional groups: a low-dose group at half the moderate dose and a high-dose group at twice the moderate dose. Except for the control group, the remaining groups were dedicated to diarrhea model treatment. Rats in these groups received rhubarb-derived liquid orally, twice daily (morning and evening), at 20 mL/kg dose with an 8-h interval, lasting for 10 days[24]. Following this, the *L. purpureus* group received varying doses of *L. purpureus* aqueous extract *via* gavage, while the control and model groups were administered saline for 5 days.

2) To explore the involvement of gut microbiota in *L. purpureus*'s protective action against rhubarb-induced diarrhea, we categorized 30 rats into 4 distinct groups: control, model, antibiotic cocktails (ABX), *L. purpureus*-M, and ABX-*L. purpureus*. The ABX and ABX-*L. purpureus* group was exposed to broad-spectrum antibiotics (ampicillin, metronidazole, neomycin at 1 g/L; and vancomycin at 0.5 g/ L) in their drinking water, supplemented with 1% glucose for a duration of 1 week[26]. Meanwhile, the control, model and *L. purpureus*-M groups received purified water. The antibiotics, obtained from Shanghai Yuanye Bio-Technology Co., Ltd. (purity exceeding 95%), were administered following the outlined protocols. *L. purpureus* administration and diarrhea induction were conducted accordingly. Rats were euthanized on the 16th day under anesthesia.

3) To further validate the influence of gut microbiota in *L. purpureus*'s favorable impacts on diarrhea, we executed experiments involving FMT. Rats were randomly divided into 3 groups: FMT-Con, FMT-Model, FMT-*L. purpureus*, and FMT- *L. purpureus*-treated sterile fecal filtrate (SFF) transplantation group. The induction of diarrhea in rats followed the methodology outlined in Part I. The methodology for microbiome elimination was conducted in accordance with the procedures outlined in the second phase.

The source of the FMT material was donor rats, offering fresh feces diluted with sterile phosphate buffered solution (PBS). This fecal suspension was meticulously prepared by immersing the feces in PBS, followed by centrifugation and filtration. For SFF, the supernatants were collected and passed through 70 and 0.22 μm filters. The resultant fecal suspension was blended with sterile glycerol and preserved at –80 °C until transplantation[27]. Every recipient rat was administered 2 mL of the bacterial suspension (10^8 CFU/mL) daily *via* oral gavage for a continuous span of 10 days[28].

At the end of the experiment, the colons were extracted, rinsed using PBS, and their dimensions were recorded. Serum samples were gathered and conserved at a temperature of –80 °C. For subsequent analysis, the colons and feces were cryogenically preserved at –80 °C. The animal trials strictly adhered to the regulations stipulated by the Institutional Animal Care and Use Committee of China Agricultural University (AW010203202-2-3).

2.5 Recording body weights and fecal moisture content

The weights and fecal moisture of rats were assessed, and fecal samples were gathered and weighed on day 8 and day 15. Afterward, the fecal samples were subjected to a 24-h drying process at 60 °C before being reweighed. Fecal moisture content was calculated by the equation:

$$\text{Fecal moisture content (\%)} = \frac{\text{Weight before drying (g)} - \text{Weight after drying (g)}}{\text{Weight before drying (g)}} \times 100$$

2.6 Hematoxylin&eosin (H&E) and Alcian blue-periodic acid-Schiff staining (AB-PAS) staining

The colon tissues were preserved in a 4% paraformaldehyde solution for a week, followed by paraffin embedding, sectioning into 4 μm thick slices, and H&E staining for microscopic assessment. Pathological alterations were observed *via* a light microscope. For AB-PAS staining, the samples underwent staining using an AB solution (Solarbio, Beijing, China), followed by treatment with periodate and Schiff's reagent, and then lightly counterstained with hematoxylin for nuclei. Differentiation with acidic alcohol and rinsing with Scott's tap water were performed. Subsequently, dehydration, washing, and mounting of the samples on glass slides with coverslips were carried out. The goblet cells were enumerated using a light microscope.

2.7 Immunohistochemistry analysis

The paraffin sections underwent treatment with 3% hydrogen peroxide to nullify inherent peroxidase activity. Following this, primary antibodies (Claudin 1 (1:100, Proteintech, Wuhan, China, Occludin (1:100, Abcam, Cambridge, UK)) were applied and allowed to incubate overnight at 4 °C after blocking with 5% bovine serum albumin. The sections were rinsed using 0.01 mol/L PBS and then exposed to biotinylated goat anti-rabbit IgG (1:400, Dako_K5007; Denmark) for 2 h at room temperature. After a wash, the tissues were treated with streptavidin-horseradish peroxidase. Immunoreactivity was rendered visible by subjecting the tissue sections to a solution containing 3,3'-diaminobenzidine and H_2O_2. Subsequent hematoxylin staining was performed. The images were captured through a light microscope, and quantification was carried out using ImageJ software. The outcomes were presented as the mean integral optical density of positive cells.

2.8 Enzyme-linked immunosorbent assay (ELISA) analysis

Serum samples underwent analysis to measure the levels of inflammatory markers (TNF-α, IL-1β, and IL-6), utilizing an ELISA kit obtained from Elabscience Biotechnology Co., Ltd. (Wuhan, China). The procedures of the assays adhered to the guidelines provided by the manufacturer.

2.9 Assessment of serum antioxidant markers in rats

Quantification of glutathione peroxidase (GSH-Px), total antioxidant capacity (TAOC), malondialdehyde (MDA), and superoxide dismutase (SOD) activities in serum was performed using commercially available assay kits (Jiancheng, Nanjing, China) in accordance with the manufacturer's guidelines.

2.10 Transmission electron microscopy (TEM) analysis

The colon tissue sections are promptly sliced into small fresh tissue fragments and rapidly submerged in fixative for subsequent utilization in TEM. The colon tissues were maintained in darkness and subjected to post-fixation with a 1% OsO_4 solution in 0.1 mol/L PBS (pH 7.4) at room temperature for a duration of 2 h. Upon removal of OsO_4, the tissues were subjected to three consecutive rinses of 15 min each in 0.1 mol/L PBS (pH 7.4). Subsequently, the samples were subjected to dehydration with ethanol at room temperature, followed by embedding in Epon-812 resin, and then allowed to polymerize for a duration exceeding 48 h at 65 °C. Ultrathin sections (ranging between 60–80 nm) were then meticulously prepared, followed by staining using 2% uranyl acetate and 2.6% lead citrate. These samples were examined employing a Hitachi-HT7800 electron microscope (Hitachi, Naka, Japan), with the resultant images captured for further analysis.

2.11 Permeability test of intestinal mucosal barrier

Intestinal barrier permeability assessment was conducted using 4 kDa fluorescein isothiocyanate (FITC)-dextran (FD4, Sigma-Aldrich, St. Louis, MO, United States). A solution of FD4 in PBS, with a concentration of 200 mg/mL, was meticulously prepared and stored within a black Eppendorf tube. The administration of the FD4 solution, dosed at 200 mg/kg body weight, was carried out orally for all rat groups. To prevent the possibility of regurgitation, the rats were held in an upright position for 30 s. Plasma samples were systematically collected and transferred to black 96-well microplates. The concentration of FD4 within these samples was subsequently quantified using a microplate spectrophotometer, with the excitation wavelength set at 485 nm and the emission wavelength at 530 nm.

Concurrently, colon tissue was obtained and then embedded in Tissue-Tek O.C.T compound (Sakura, Tokyo, Japan), followed by immediate freezing on dry ice. Utilizing the CM1950 cryostat (Leica Biosystem, Wetzlar, Germany), sections were adeptly prepared at a thickness of 10 μm. Subsequently, these sections were meticulously inspected under a fluorescence microscope (Olympus CKX53, Tokyo, Japan), with images being systematically captured for subsequent analysis[29].

2.12 Quantitative real-time polymerase chain reaction (qRT-PCR) for the gene expression

Colon tissues were used for the isolation of total RNA, utilizing an RNA extraction kit (Promega, Shanghai, China), subsequently subjected to reverse transcription *via* the GoScript™ Reverse Transcription System (Takara, Beijing, China). qRT-PCR was executed to accurately quantify the gene expression levels. The primer sequences for the designated genes can be located in Table 1. The relative expression of the target genes was meticulously evaluated using the $2^{-\Delta\Delta CT}$ method.

Table 1
Primers and probes for qPCR.

Gene	Primer sequence (5′–3′)	Gene accession number
ZO-1	F: CCATCTTTGGACCGATTGCTG R: TAATGCCCGAGCTCCGATG	NM_001106266.1
Ocln	F: GTCTTGGGAGCCTTGACATCTTG R: GCATTGGTCGAACGTGCATC	NM_031329.3
Claudin-1	F: CTAGTTTCAGAAGTTTGGCCTGGTA R: CTGCCAGCTTCCATTAGACTTTG	NM_031699.3
Gapdh	F: GCAAGTTCAACGGCACAG R: GCCAGTAGACTCCACGACAT	NM_017008.4

2.13 Determination of fecal flora diversity

Utilizing the E.Z.N.A.® soil DNA kit (Omega BioTek, Norcross, GA, USA), DNA extraction from colon contents was meticulously performed in accordance with the manufacturer's guidelines. The extracted genomic DNA underwent comprehensive assessment for integrity, purity, and concentration, which included 1% agarose gel electrophoresis. The outsourcing of this DNA to Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China) facilitated high-throughput 16S rDNA sequencing. To compare bacterial classification at the genus level between the two groups, the Wilcoxon rank sum test was employed. Additionally, the dominance of bacterial communities among the groups was assessed using linear discriminant analysis (LDA) effect size (LEfSe), where an LDA score (lg) of 3 served as the threshold.

2.14 SCFAs analysis

The standards for SCFAs encompassed acetate, propionate, butyrate, isobutyrate, valerate, isovalerate, and hexanate. Fecal samples (100 mg) underwent supplementation with 100 μL of internal standards (2-ethylbutyric acid, in the configuration of methanol, at a concentration of 1 000 μg/mL), along with 900 μL of methanol. The extraction procedure was executed following the guidelines stipulated by the manufacturer (Majorbio Bio-Pharm Technology Co., Ltd., Shanghai, China). For GC-MS analysis, a 8890B-5977B GC-MS system (Agilent Technologies, Santa Clara, CA, USA) was utilized, coupled with an HP FFAP capillary column (30 m × 0.25 mm × 0.25 μm, Agilent J&W Scientific, Folsom, CA, USA). The methods of SCFAs analysis can be found in previous literature[30].

2.15 Statistical analysis

The results were presented as mean ± standard deviation and analyzed through SPSS 26.0 software (IBM Co., Armonk, NY, USA). Statistical significance was evaluated using one-way analysis of variance (ANOVA), followed by SNK post-test, considering $P < 0.05$ as statistically significant. GraphPad Prism 8.0 software (GraphPad, CA, USA) was employed for generating the graphical representations.

3. Results

3.1 Compnents of L. purpureus

For the chemical analysis of *L. purpureus*, we employed UHPLC-QE Plus-MS/MS analysis, illustrating the MS chromatogram of *L. purpureus* in both positive and negative ion modes (Fig. 1). By comparing it with relevant literature, we determined the retention behavior of chromatographic peaks, precise molecular weight, and MS^E fragment information for *L. purpureus*. This comprehensive analysis led to the identification of a total of 157 components (Table S3). The findings underscore the significant presence of trigonelline, piperidinolic acid, *L*-(–)-malic acid, as the predominant active constituents within the formulation. Based on these observations, we chose to quantify the substances trigonelline, pipecolic acid, *L*-(–)-malic acid, and Table 2 lists the detailed contents of the 3 identified compounds. UPLC-MS/MS and MS2 mass spectrum are shown in Figs. S1 and S2.

Table 2
Results of the content of the three components in *L. purpureus*.

Component	Content (mg/g)
Trigonelline	1.58
Piperidinolic acid	1.84
L-(–)-Malic acid	0.70

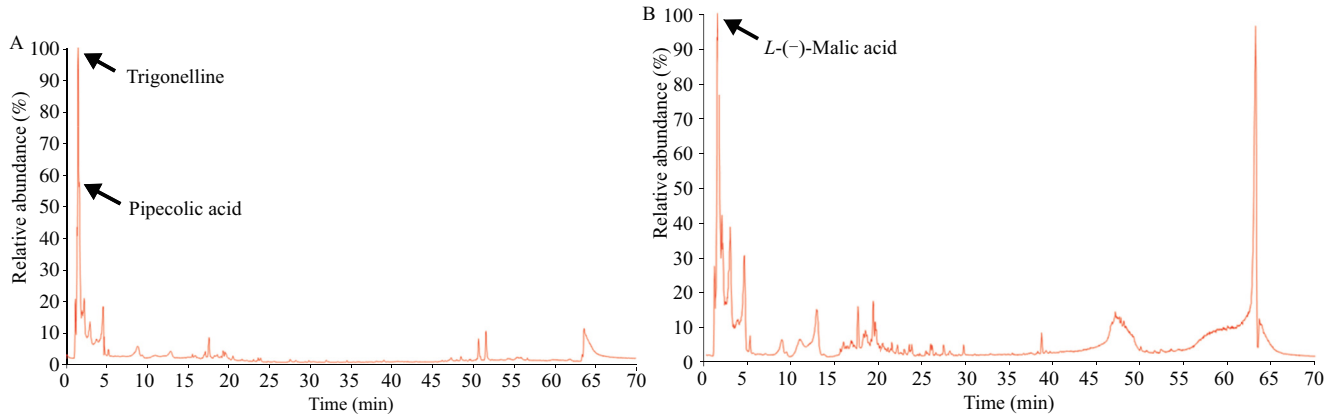

Fig. 1 UHPLC-MS fingerprint chromatogram of *L. purpureus*. (A) Positive mode. (B) Negative mode.

3.2 L. purpureus treatment ameliorated rhubarb-induced diarrhea

The *L. purpureus* treatment group received various doses of *L. purpureus* starting from the 11th day (Fig. 2A). The impact of *L. purpureus* on body weight, fecal moisture, colon length, and biochemical markers associated with diarrhea in rats was evaluated. Notably, *L. purpureus* significantly mitigated the severity of diarrhea, as evidenced by improvements in body weight ($P < 0.01$) (Fig. 2B), fecal moisture ($P < 0.05$) (Figs. 2C and D), and colon length ($P < 0.01$) (Fig. 2E) following continuous rhubarb treatment for 10 days. The impact of *L. purpureus* on gut morphology and the composition of intestinal epithelial cells was investigated. Colon tissues from the diarrhea group displayed signs of inflammation infiltration and disrupted cell arrangement compared to the control group. Conversely, the application of *L. purpureus* exhibited a safeguarding impact on diarrhea in rats, leading to the restoration of typical mucosal structure characterized by intact epithelial cells in both intestinal and colonic tissues (Fig. 2F). These results strongly imply that *L. purpureus* plays a defensive role against diarrhea and maintains the structural integrity of the mucosa.

3.3 L. purpureus suppresses inflammation and inflammation-induced oxidation in diarrhea rats

Diarrhea is commonly linked with intestinal inflammation. To gauge the inflammatory response, the serum concentrations of the inflammatory cytokines TNF-α, IL-1β, and IL-6 were measured, revealing a significant reduction upon *L. purpureus* treatment ($P < 0.01$) (Figs. 3A-C). Evaluating markers of oxidative stress, we investigated the levels of TAOC and the activities of MDA, GSH-Px, and SOD in the serum. The outcomes indicated that diarrhea considerably diminished TAOC levels and GSH-Px and SOD activities ($P < 0.01$), concurrently elevating MDA levels ($P < 0.01$). Nevertheless, *L. purpureus* intervention effectively counteracted the oxidative stress induced by diarrhea (Figs. 3D-G). Within the *L. purpureus*-treated group, there was a marked elevation in TAOC levels and GSH-Px and SOD activities, while the MDA level displayed a significant reduction compared to the control group ($P < 0.01$). These observations underscore *L. purpureus*'s capacity to attenuate both inflammation and inflammation-induced oxidative harm.

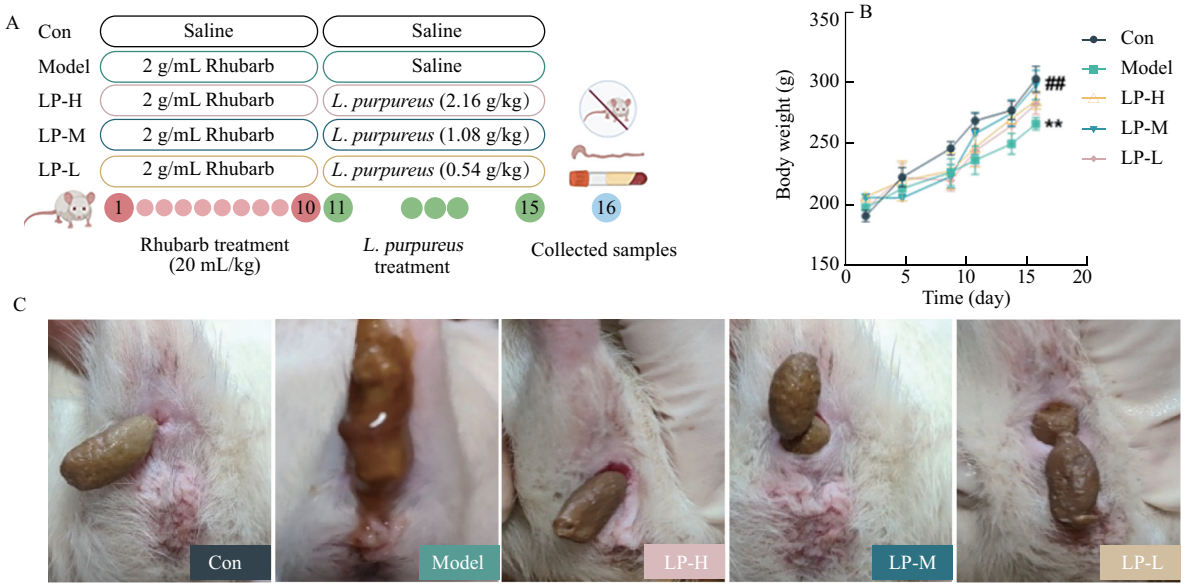

Fig. 2 *L. purpureus* treatment ameliorated rhubarb-induced diarrhea group. (A) The flow chart of rats treatments. (B) Body weight curve during the experiment. (C) Representative images of feces. (D) Fecal moisture content. (E) Representative images of rat colon and colon length. (F) Representative histopathologic changes in rat colon tissue. Scale bar = 250 μm Black arrows point to inflammatory cells and disorganized cells. Con, control group; Model, diarrhea group; LP-H, *L. purpureus* high dose group; LP-M, *L. purpureus* middle dose group; LP-L, *L. purpureus* low dose group. $^{*}P < 0.05$, $^{**}P < 0.01$ compared to the control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared to the model group.

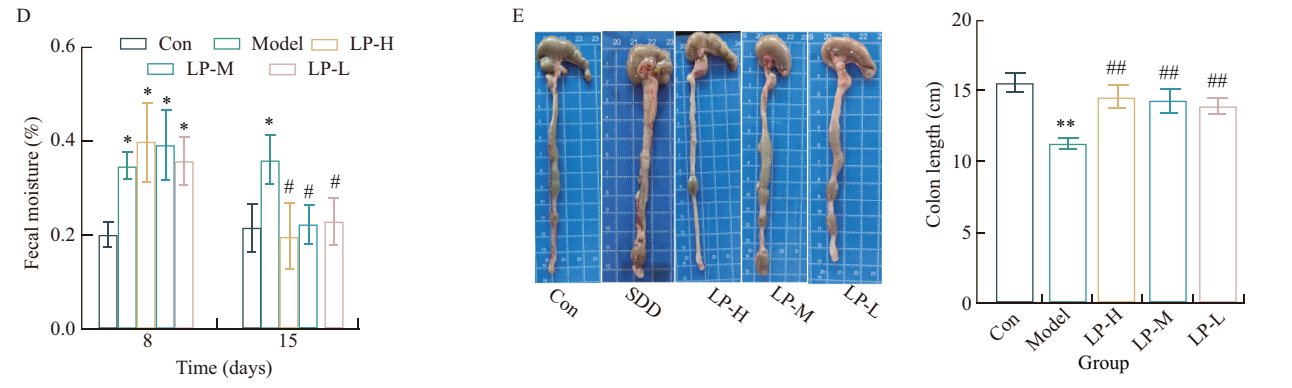

Fig. 2 (*Continued*)

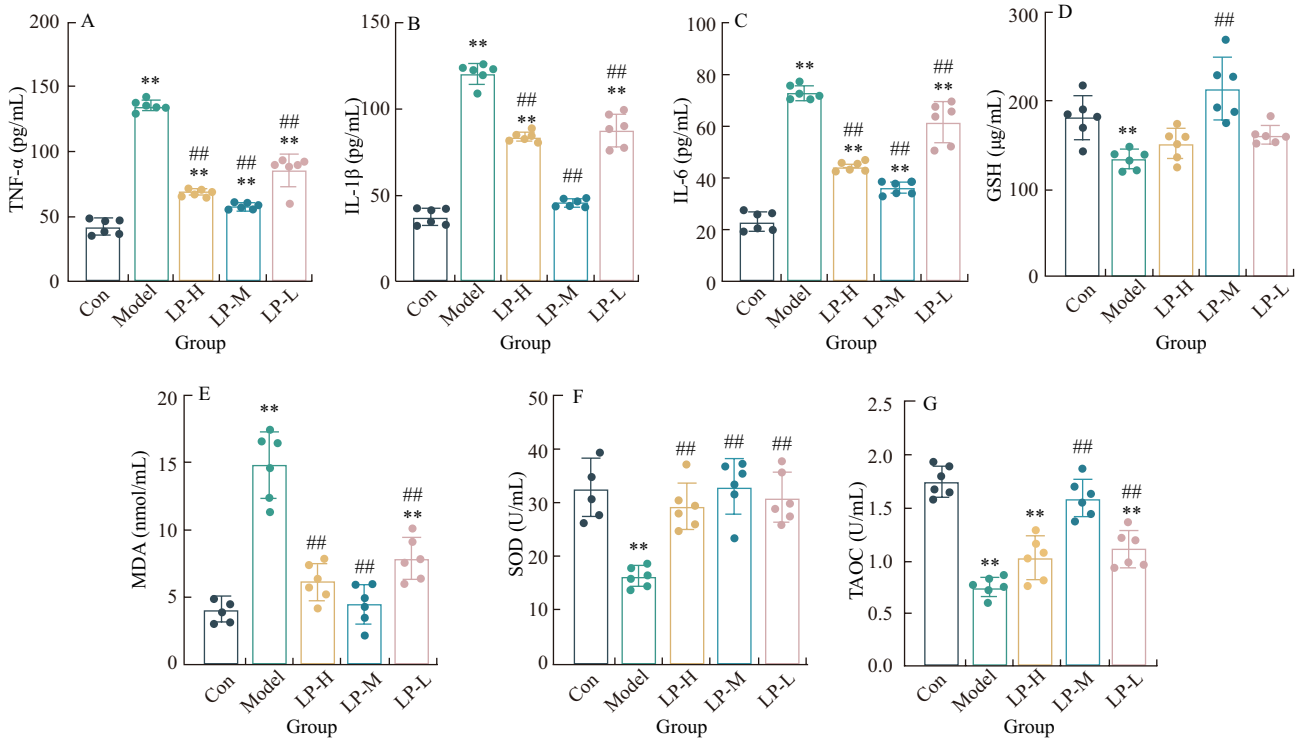

Fig. 3 *L. purpureus* suppresses inflammation and inflammation-induced oxidation in diarrhea rats. Serum levels of (A) TNF-α, (B) IL-1β, and (C) IL-6 in rats. Serum levels of (D) GSH, (E) MDA, (F) SOD, and (G) TAOC in rats. Con, control group; Model, diarrhea group; LP-H, *L. purpureus* high dose group; LP-M, *L. purpureus* middle dose group; LP-L, *L. purpureus* low dose group. $^{**}P < 0.01$ compared to the control group; $^{\#\#}P < 0.01$ compared to the model group.

3.4 Administration of L. purpureus decreased intestinal injury and bolstered intestinal barrier functionality

The intestinal mucosal barrier plays a crucial role in safeguarding against infiltration by harmful bacteria and foreign elements. It compromise serves as the foundation for the development of diarrhea. AB/PAS staining was utilized to quantify the abundance of goblet cells. The findings demonstrated a significant decrease in the number of goblet cells in the diarrhea group compared to the control group ($P < 0.01$). Notably, *L. purpureus* treatment effectively alleviated the depletion of goblet cells induced by diarrhea, with the *L. purpureus*-M intervention showing the most prominent effect (Fig. 4A). Intestinal barrier function is linked to gastrointestinal permeability, assessed using the FD4 test. When the intestinal barrier is compromised, FD4 can pass through the "leaky" gut and enter the bloodstream, detectable in the plasma. The diarrhea group showed significantly higher levels of plasma FD4 compared to the control group, whereas the *L. purpureus* group exhibited relatively lower levels of FD4 compared to the diarrhea group ($P < 0.01$) (Fig. 4B). TEM was used to further examine the intestinal ultrastructure and assess the protective effects of *L. purpureus* on the gut. In the diarrhea group, widespread damage

to microvilli, disruption of TJs, and cell death were observed in the intestinal samples. Nevertheless, pre-administration of *L. purpureus* effectively alleviated these injuries, preserving intact TJ structures (Fig. 4C).

3.5 Administration of *L. purpureus* increased the expression of TJ markers

Additionally, critical proteins essential for maintaining TJs, including Occludin, and Claudin 1, which signify intestinal barrier integrity and performance, exhibited markedly reduced expression in the intestines of diarrhea rats. Intriguingly, the *L. purpureus* group showed a substantial increase in the expression of these TJ markers ($P < 0.01$) (Figs. 5A and B). These findings were confirmed by qPCR results (Figs. 5C-E). Collectively, these findings indicate that *L. purpureus* treatment ameliorated intestinal injury and enhanced gut barrier function subsequent to rhubarb-induced damage.

3.6 Evaluating potential effects of *L. purpureus* treatment on gut microbiota

To explore the impact of *L. purpureus* administration on microbial composition, we conducted high-throughput gene sequencing of fecal bacterial DNA from diarrhea rats. Analysis of Chao1, ACE, Simpson,

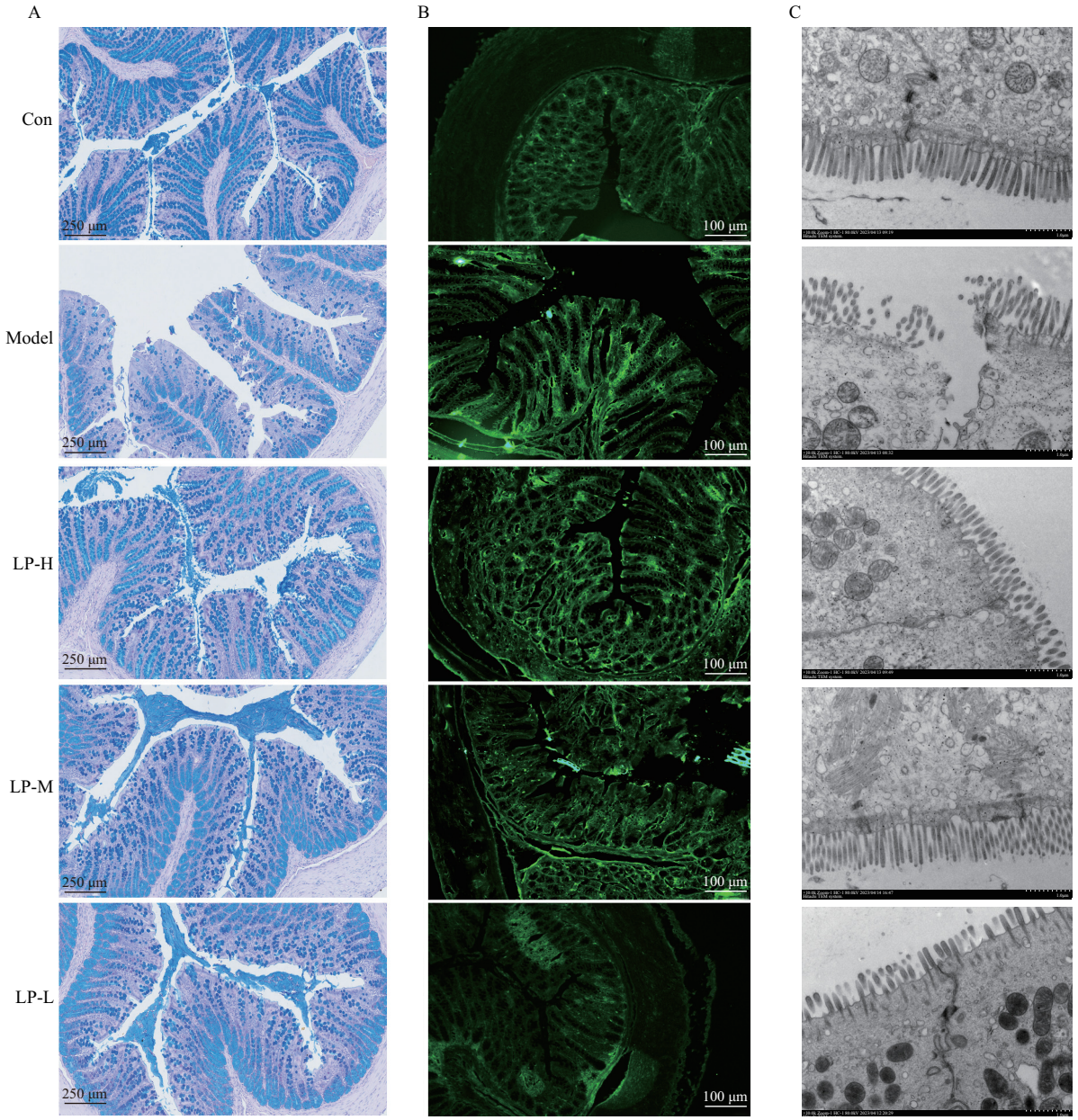

Fig. 4 *L. purpureus* administration mitigated intestinal damage and strengthened intestinal barrier integrity. (A) AB-PAS staining. (B) Effect of *L. purpureus* on the permeability of FITC-dextran. (C) Representative ultrastructural transmission electron photomicrographs of colon samples. (D) Integrated optical density per area based on AB-PAS staining. (E) FITC-dextran fluorescence intensity in intestinal tissues. Con, control group; Model, diarrhea group; LP-H, *L. purpureus* high dose group; LP-M, *L. purpureus* middle dose group; LP-L, *L. purpureus* low dose group. $^{*}P < 0.05$, $^{**}P < 0.01$ compared to the control group; $^{\#\#}P < 0.01$ compared to the model group.

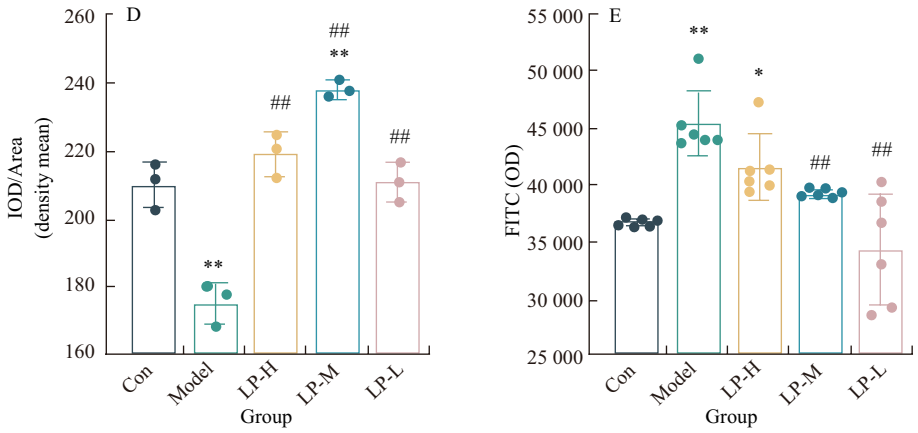

Fig. 4 (*Continued*)

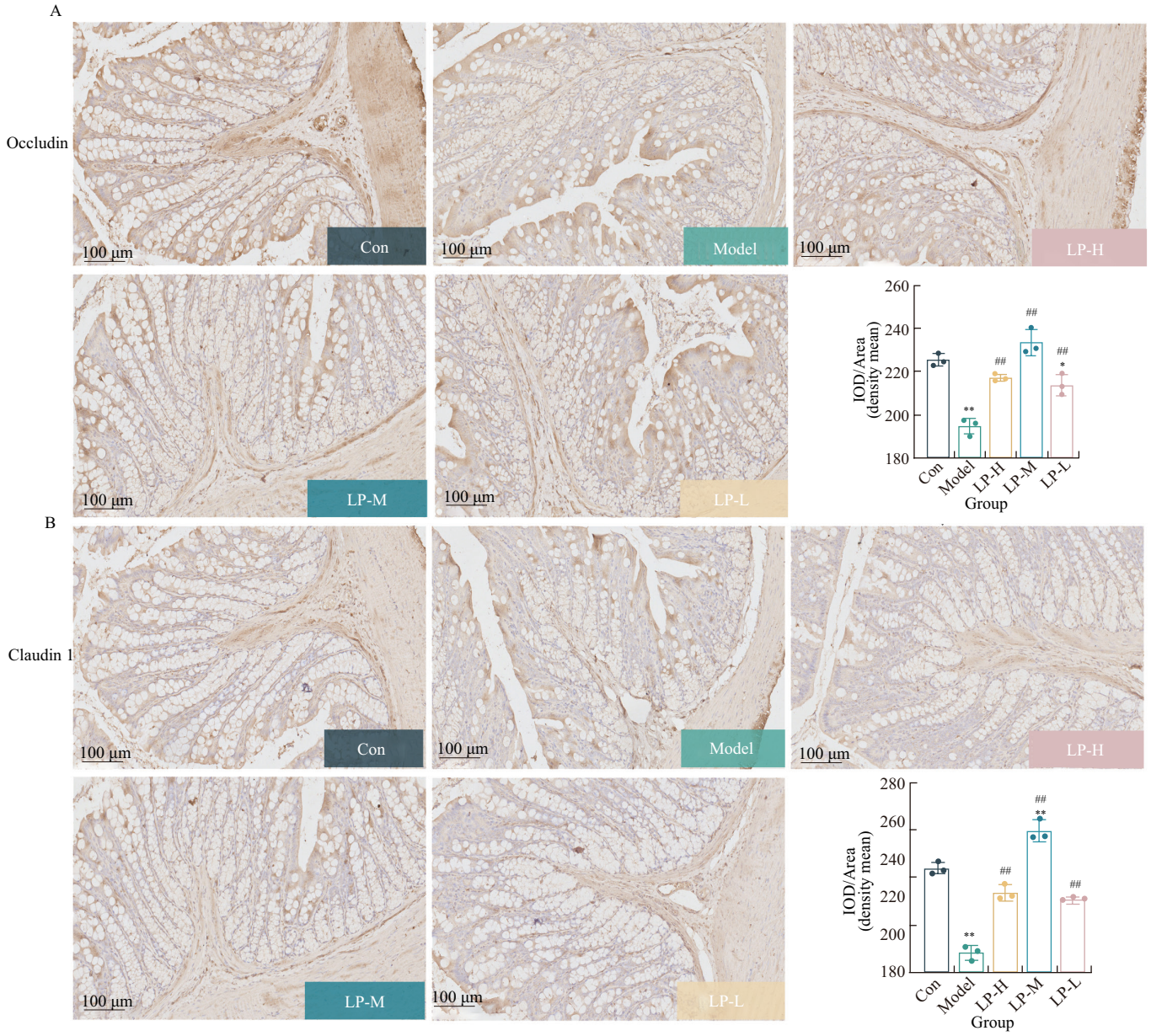

Fig. 5 (A) Representative images of immunohistochemical of Occludin in colon. (B) Representative images of immunohistochemical of Claudin-1 in colon. Relative mRNA levels of genes encoding the tight junction protein (C) *Ocln*, (D) *Claudin-1,* and (E) *ZO-1*. Con, control group; Model, diarrhea group; LP-H, *L. purpureus* high dose group; LP-M, *L. purpureus* middle dose group; LP-L, *L. purpureus* low dose group. $^{**}P < 0.01$ compared to the control group; $^{\#\#}P < 0.01$ compared to the model group.

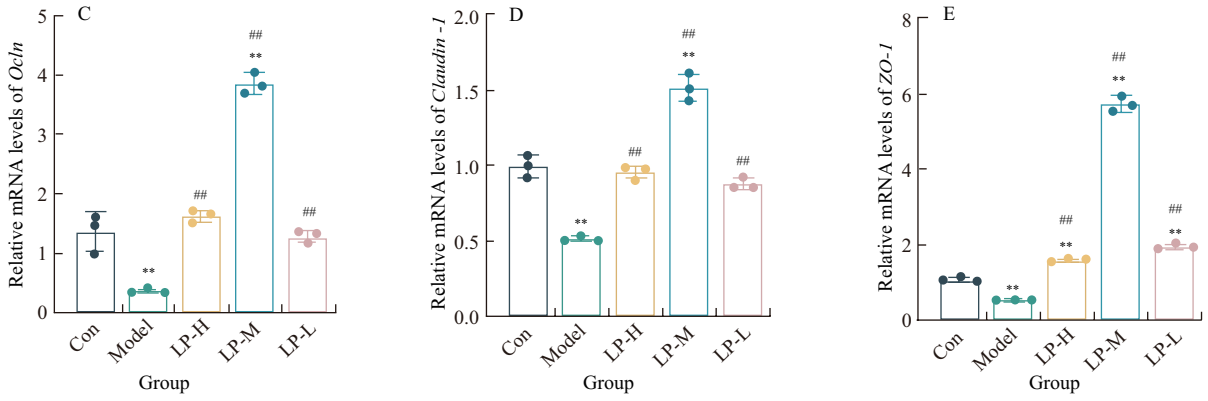

Fig. 5 (*Continued*)

and Shannon indices among the groups revealed no significant differences ($P > 0.05$) (Fig. S3). NMDS analysis highlighted distinct fecal flora community compositions in the Control, diarrhea, and *L. purpureus* groups, with *L. purpureus* treatment normalizing the bacterial community after diarrhea to approximate the control group (Fig. 6A). Figs. 6B and C display the relative abundance of intestinal flora at the phylum and genus levels in rats. In contrast to the control group, the diarrhea group displayed an elevated relative abundance of *Lactobacillus* and a reduced abundance of *Prevotella* and *Alloprevotella* ($P < 0.05$). Remarkably, *L. purpureus*-M treatment reinstated these bacterial genera to their normal proportions in diarrhea rats (Fig. 6D). Through LEfSe analysis, we detected significant variations in bacterial community dominance across the groups. Notably, *Alloprevotella* and *Prevotella* were more enriched in the control group (Fig. 6E). Investigating the influence of altered bacterial communities on microbial SCFA metabolism, we assessed fecal SCFA concentrations using targeted metabonomics. Compared with the control group, diarrhea rats exhibited notably decreased levels of propionic acid, butyric acid, isobutyric acid, valeric acid, isovaleric acid, and caproic acid ($P < 0.05$). *L. purpureus* treatment substantially elevated concentrations of propionic acid ($P < 0.05$) as well as acetic acid, butyric acid, isobutyric acid, valeric acid, isovaleric acid, and caproic acid ($P < 0.01$) in rat feces (Fig. 6F).

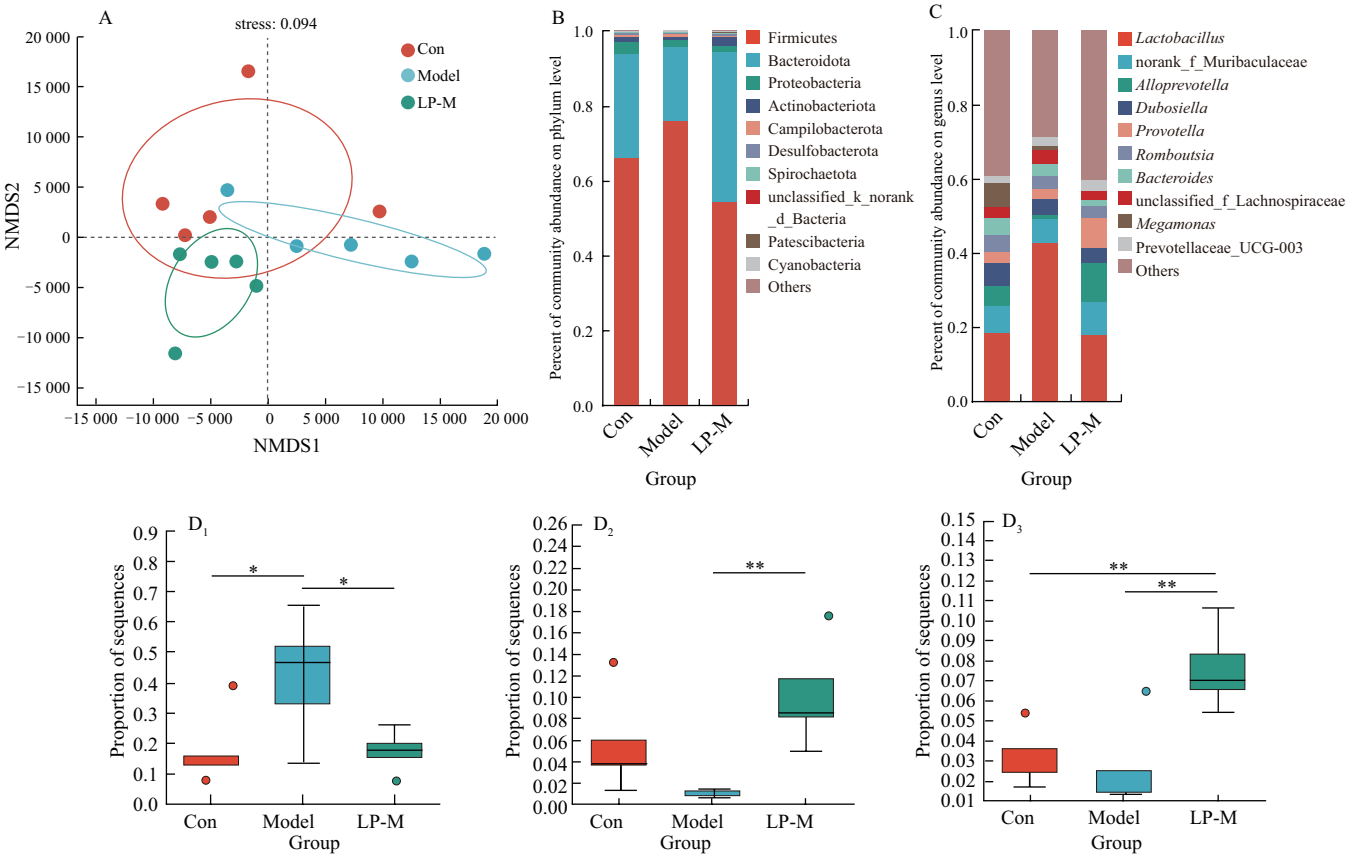

Fig. 6 The effect of *L. purpureus* on gut microbiota composition. (A) NMDS score plot of the gut microbe. (B) Relative abundance of phylum. (C) Relative abundance of genus. Proportion of (D_1) *Lactobacillus,* (D_2) *Alloprevotella,* (D_3) *Prevotella* (E) LEfSe analysis. (F) Fecal SCFAs contents in diarrhea rats. Con, control group; Model, diarrhea group; LP-H, *L. purpureus* high dose group; LP-M, *L. purpureus* middle dose group; LP-L, *L. purpureus* low dose group. $^{*}P < 0.05$, $^{**}P < 0.01$ compared to the control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared to the model group.

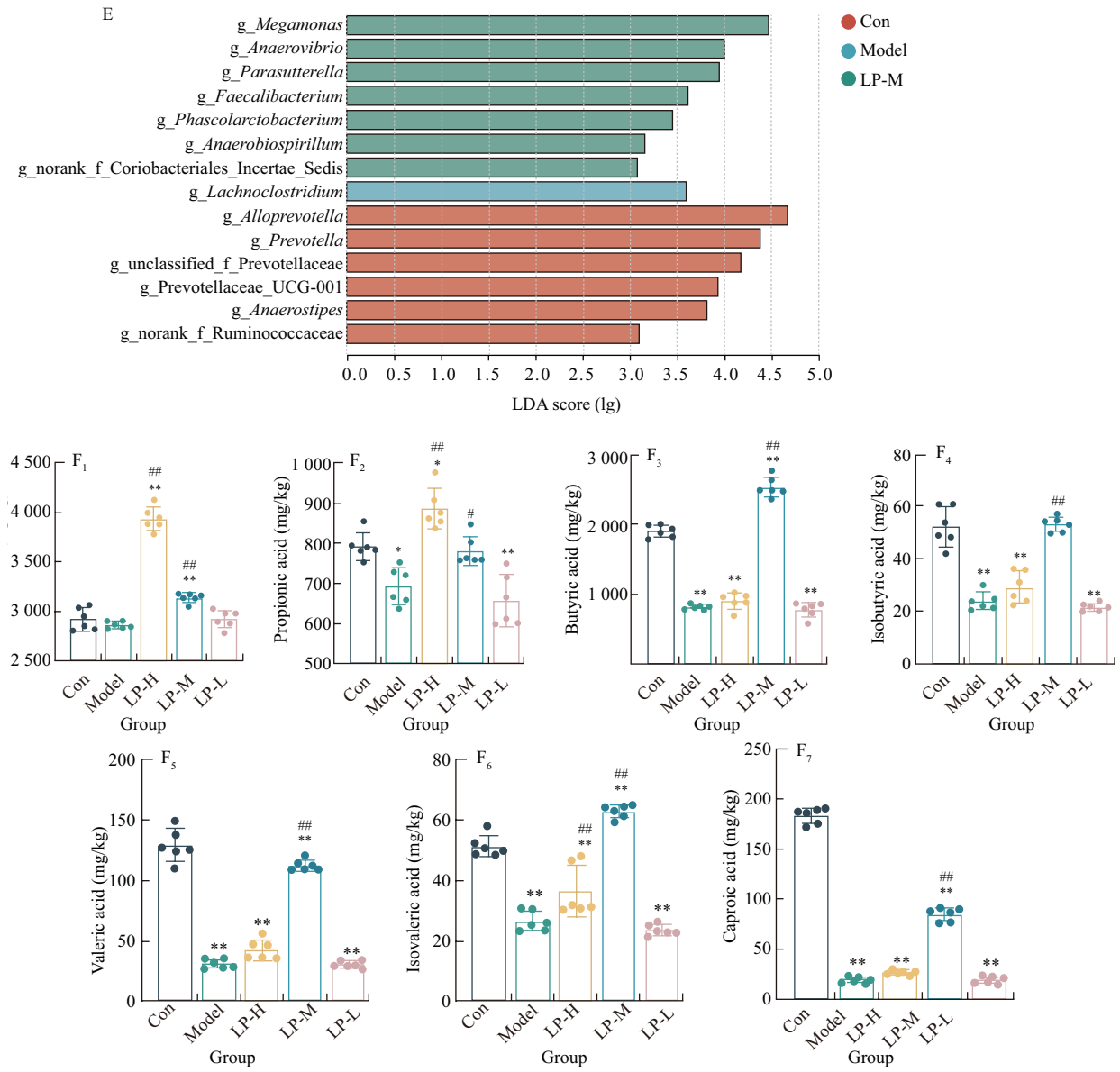

Fig. 6 (*Continued*)

3.7 L. purpureus relieved diarrhea in a gut-dependent manner

Significant changes in gut microbiota were observed following *L. purpureus* treatment. To elucidate the role of gut microbiota, a microbiota elimination experiment was conducted. Thirty rats were divided into 5 groups: Con, ABX, model, *L. purpureus*, and ABX-LP. Seven days prior to the study, ABX and ABX-LP groups received a quadruple antibiotic regimen to eliminate gut microbiota (Fig. 7A). No adverse reactions were observed in the antibiotic-treated groups. The protective effects of *L. purpureus* were diminished following gut flora depletion in rats. Fecal moisture (Fig. 7B) and colonic histological changes (Fig. 7C) showed no significant differences between the diarrhea and ABX-*L. purpureus* groups. In the ABX-*L. purpureus* group, serum levels of TNF-α, IL-1β, and IL-6 were significantly increased, whereas they were reduced in the *L. purpureus* group ($P < 0.01$) (Fig. 7D). Serum levels of TAOC, MDA, GSH-Px, and SOD were not significantly different between the diarrhea and ABX-*L. purpureus* groups (Fig. S4). Notably, *L. purpureus* treatment mitigated goblet cell depletion induced by diarrhea (Fig. 7E). The expression levels of *Ocln, Claudin-1* and *ZO-1*, markers of intestinal barrier function, were significantly lower in the diarrhea and ABX-*L. purpureus* groups compared to the *L. purpureus* group, which showed markedly higher expression ($P < 0.01$) (Fig. 7F). These findings indicate that the protective effect of *L. purpureus* against diarrhea is dependent on the presence of intestinal flora.

3.8 FMT confirmed that L. purpureus alleviated the diarrhea by regulating gut microflora

The therapeutic effect of *L. purpureus* is diminished by the depletion of gut flora. To investigate whether the gut microflora treated by *L. purpureus* has an ameliorative effect on diarrhea, we conducted FMT from control, diarrhea, or *L. purpureus*-M groups into gut microbiota-depleted rats for 10 days (Fig. 8A). The groups were designated as follows: FMT-Con (receiving control

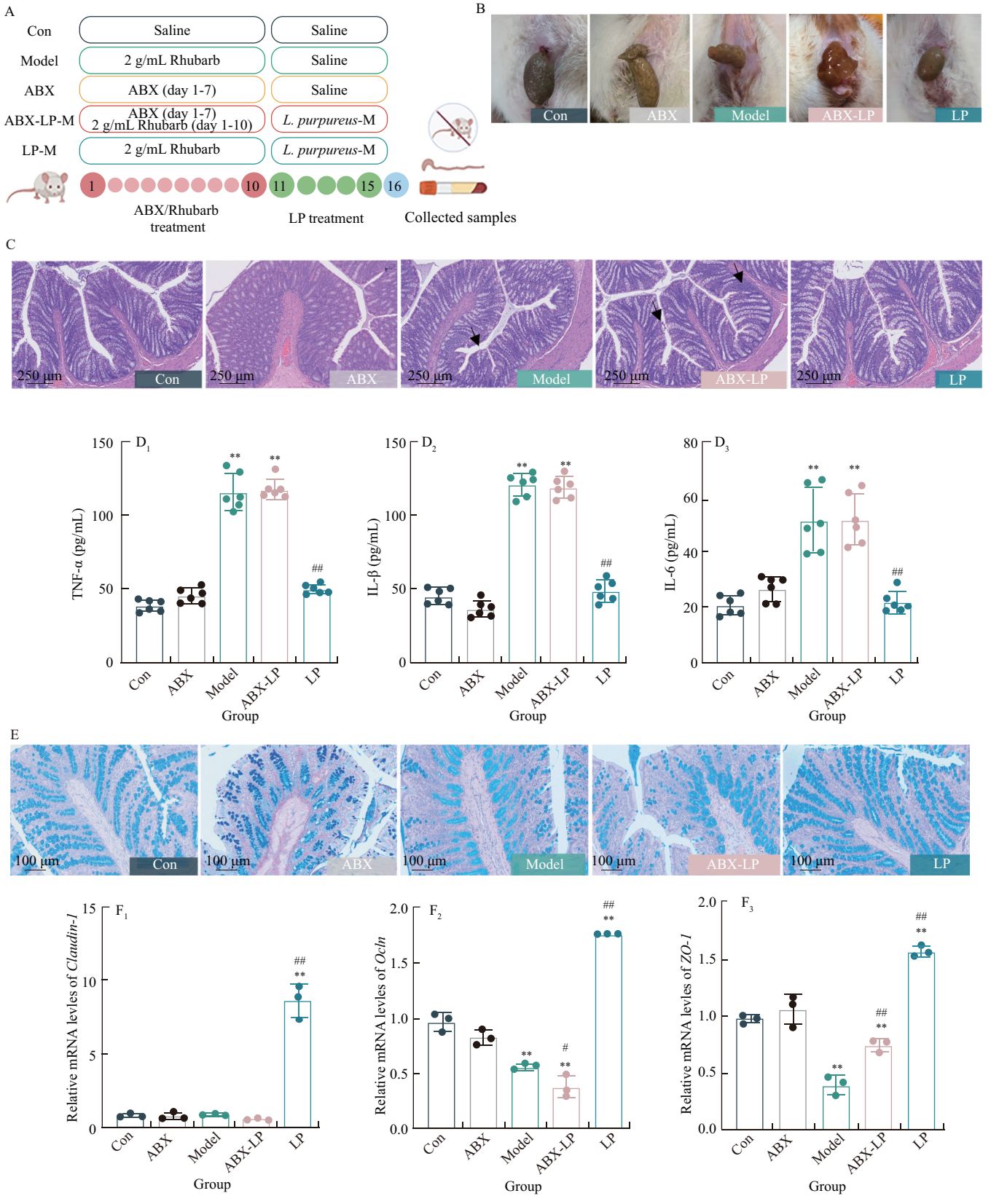

Fig. 7 *L. purpureus* relieved diarrhea in a gut-dependent manner. (A) The flow chart of animal treatments. (B) The incidence of diarrhea. (C) H&E staining.Black arrows point to inflammatory cells and disorganized cells. (D) The levels of TNF-α, IL-1β, and, IL-6 in the serum of rats. (E) AB-PAS staining. (F) Relative mRNA levels of *Ocln*, *Claudin-1* and *ZO-1*. Con, control group; ABX, antibiotic cocktails, Model, diarrhea group; ABX-LP, Antibiotic cocktails prior to *L. purpureus*-M treatment, LP, *L. purpureus* middle dose group. The significance levels of the *P*-values are shown below: $^{**}P < 0.01$ compared to the control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared to the model group.

microbiota), FMT-Model (receiving diarrhea model microbiota), FMT-LP (receiving *L. purpureus*-M microbiota) and FMT-LP-SFF (receiving *L. purpureus*-M microbiota sterile fecal filtrate) For the SFF procedure, supernatants were collected and filtered through 70 and 0.22 μm filters. Results revealed that rats receiving feces from *L. purpureus*-treated rats exhibited significantly lowered fecal moisture ($P < 0.01$) (Fig. 8B), and improved colonic histological changes (Fig. 8C) compared to those receiving feces from diarrhea rats. Rats administered LP-M-SFF displayed attenuated lesions compared to the diarrheal, presumably attributed to the SCFAs present in the fecal supernatants. FMT with "*L. purpureus* microbiota" effectively mitigated goblet cell depletion induced by diarrhea (Fig. 8D) and reduced anti-inflammatory factors significantly ($P < 0.01$) (Fig. 8E). Serum levels of TAOC, MDA, GSH-Px, and SOD showed no notable differences between FMT-Control and FMT-*L. purpureus* groups in diarrhea rats (Fig. S5). Notably, rats receiving feces from diarrhea rats exhibited decreased expression of TJ proteins (ZO-1, Occludin, and Claudin-1) while the FMT-*L. purpureus* group showed higher expression levels ($P < 0.05$) (Fig. 8F), suggesting *L. purpureus* in regulating diarrhea through gut microbiota modulation. Analysis of bacterial taxonomic composition using 16S rDNA gene sequencing revealed distinct microbial community compositions among recipient rats after FMT, as depicted by NMDS analysis (Fig. 8G). LEfSe analysis identified *Allobacterium* and *Bifidobacterium* as pivotal bacterial taxa within the FMT-*L. purpureus* group (Fig. 8H).

4. Discussion

In this study, rhubarb was used to create a model of diarrhea and rhubarb was chosen because it is inexpensive, easy to add, and clinically effective[17]. Our study found that rats in the rhubarb-induced group exhibited severe diarrhea symptoms, including loose stools, soiled perianal fur, and increased fecal water content. The laxative effect of Rhubarb involves complex physiological mechanisms, including the regulation of electrolyte balance, the metabolic actions of intestinal microbiota, and the comprehensive control of the neuromuscular system of the intestines. Research indicates that active components contained in Rhubarb can directly affect the gastrointestinal tract by reducing the activity of Na^+-K^+-ATPase and Ca^{2+}-ATPase within the interstitial cells of Cajal, leading to damage to the cell membrane and imbalance in energy metabolism, thereby intervening in the normal rhythm of colonic motility[18]. Furthermore, the conjugated anthraquinone compounds in Rhubarb are transformed into metabolites with higher biological activity, such as rhein anthrone, under the action of intestinal microbiota, significantly activating the nerve plexus in the submucosa and the muscular layer of the large intestine, thereby further promoting intestinal motility[31].

This study aimed to investigate the *in vivo* effects of *L. purpureus* on diarrhea and its underlying mechanisms. Our results provide compelling evidence that *L. purpureus* effectively protects against diarrhea induced by rhubarb. This protective effect is evident through various beneficial outcomes, including reduced weight loss, diarrhea rate, colon length shortening, and improved colonic histology exerting antioxidative and anti-inflammatory effects, as well as by reducing colonic permeability. Significantly, we employed gut bacteria depletion and FMT experiments to establish the gut-dependent nature of *L. purpureus*'s ability to alleviate diarrhea. Administration of *L. purpureus* resulted in enhanced gut microbial diversity and a favorable microbial composition, leading to increased production of SCFAs and improved integrity of the colonic mucosal barrier. These findings strongly support the potential of *L. purpureus* as a natural

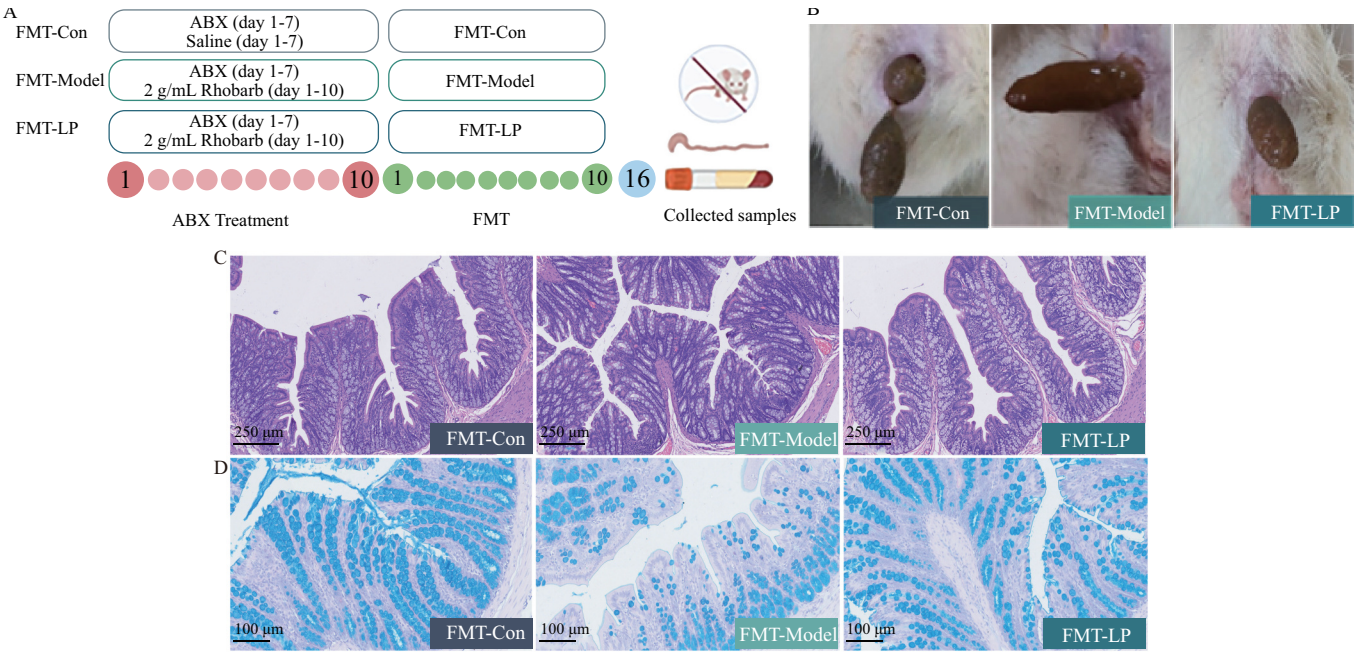

Fig. 8 FMT verification affirmed the role of *L. purpureus* in safeguarding colonic mucosal integrity through gut microflora modulation. (A) Flow chart of animal treatments. (B) The incidence of diarrhea. (C) Representative histopathological changes in the colon tissues of rats. (D) AB-PAS staining. (E) The levels of TNF-α, IL-1β, and, IL-6 in the serum of rats. (F) Relative mRNA levels of *Ocln*, *Claudin-1* and *ZO-1*. (G) NMDS score plot. (H) LEfSe. FMT-Con receiving control microbiota FMT rats; FMT-Model receiving model microbiota FMT rats FMT-LP receiving *L. purpureus*-M microbiota FMT rats; FMT- LP-SFF receiving *L. purpureus*-M microbiota sterile fecal filtrate.$^{**}P < 0.01$ compared to the control group; $^{\#\#}P < 0.01$ compared to the model group.

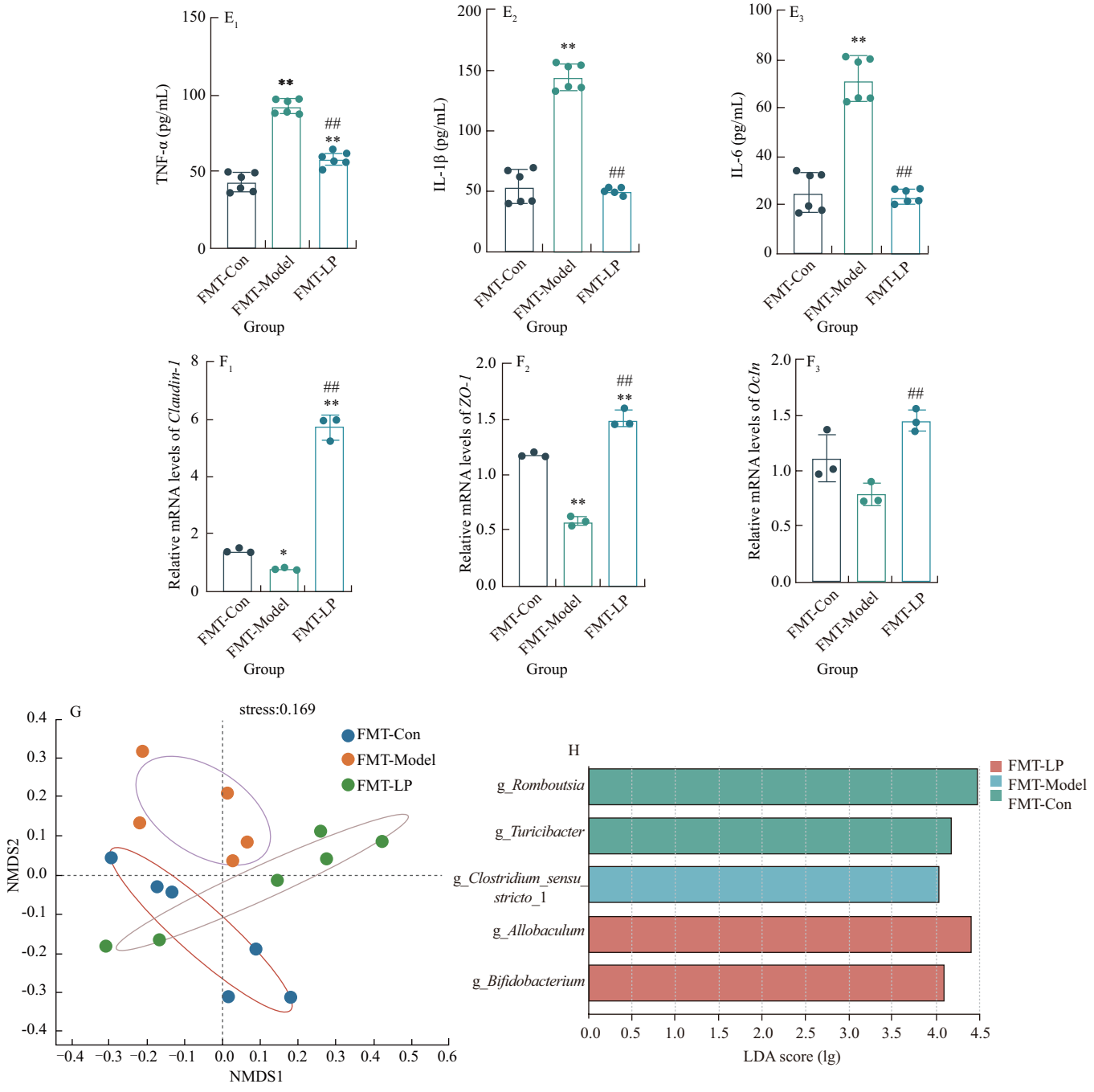

Fig. 8 (*Continued*)

therapeutic intervention for the prevention and treatment of diarrhea by regulating the gut microbial.

Oxidative stress gives rise to disruptions in the gastrointestinal mucosal layer and imbalances in microbial equilibrium, both characteristic indicators of inflammatory bowel disease[32]. The gastrointestinal environment is highly susceptible to ROS, which predisposes to intestinal inflammation, epithelial barrier disruption, diarrhea, and dysbiosis. These factors collectively lead to compromised nutrient absorption and reduced weight gain. As a result, countering systemic OS becomes a crucial therapeutic goal in managing diarrhea. UHPLC-MS/MS analysis highlighted trigonelline, pipecolic acid, *L*-(–)-malic acid as the pivotal constituents contributing to *L. purpureus*'s observed benefits. Trigonelline, an alkaloid found in various plants including *Trigonella foenum graecum*, peas, corn, and coffee, is linked to multiple medicinal properties such as exerting anti-diabetic effects[33], and displaying antioxidative activity. Pipecolic acid, derived from *L. purpureus*, exhibits therapeutic effects in treating diarrhea. *L*-(–)-malic acid possesses antioxidant prowess, antibacterial properties, and involvement in lipid metabolism[34]. In summation, *L. purpureus* potentially exerts its therapeutic impact on rhubarb-induced diarrhea through the antioxidative actions of these aforementioned components.

Intestinal barrier impairment is a hallmark in various intestinal conditions, including diarrhea and inflammatory bowel disease[35]. The intestinal mucosal barrier plays a crucial role in safeguarding the colon against pathogens and external substances, thereby

maintaining bodily equilibrium. Factors like increased intestinal permeability, heightened oxidative stress, and the migration of intestinal bacteria can compromise this barrier. Our research findings on anti-inflammatory and antioxidant markers, including MDA, SOD, GSH, TAOC, TNF-α, IL-6, and IL-1β, align with prior studies[28]. TJs are complex structures composed of transmembrane barrier proteins (e.g., Claudins, junctional adhesion molecules, Occludin, and tricellulin) and cytoplasmic scaffolding proteins (e.g., the ZO family, cingulin, and afadin)[36]. These proteins directly engage with intracellular cytoskeletal and regulatory proteins. In cases of intestinal injury, the intestinal barrier is compromised, evidenced by augmented permeability, reduced TJ protein expression, and disordered TJ structures. Our research observed similar alterations in diarrhea rats, characterized by reduced expression of TJ proteins (ZO-1, Occludin, and Claudin-1) and disrupted TJ structures, enterocytes, and microvilli as seen in TEM images. However, *L. purpureus* administration effectively mitigated these effects, thereby maintaining the integrity of the intestinal barrier. In conclusion, our results underscore the potential of *L. purpureus* administration to alleviate rhubarb-induced intestinal injury, thereby safeguarding intestinal function and integrity.

Considering the intricate interplay between inflammation and the adjustment of intestinal microbes and their metabolites, it becomes apparent that maintaining a harmonious equilibrium within the intestinal microecology assumes critical significance in preserving the host's overall well-being[37]. Our prior investigation also illuminated a reduction in the relative abundance of Prevotellaceae in diarrhea rats[24]. Correspondingly, in the current study, diarrhea rats exhibited diminished levels of *Prevotella*, juxtaposed with escalated levels of *Lactobacillus*. Prevotellaceae play a crucial role in preserving the health of the intestine. They inhibit the M1-type polarization of colonic macrophages and the synthesis of pro-inflammatory molecules like IL-1β and TNF-α[38]. The heightened level of *Lactobacillus* might be linked to the rhubarb administration[39]. Prior investigations have indicated potential correlations between *Lactobacillales* and escalated intestinal permeability along with systemic inflammation[40]. The gut microbiota's impact on host health is mediated through metabolite production[41]. As essential metabolites of gut microbiota, SCFAs primarily result from the anaerobic breakdown of dietary fibers within the intestine, with acetic, propionic, and butyric acids being the predominant forms[42]. Acetic acid's production has been attributed to *Bifidobacterium*, *Bacteroides*, and *Prevotella*[43], propionic acid is associated with *Akkermansia* and *Prevotella*[44], while *Roseburia* contributes to butyric acid production[45]. Our findings illustrated that *L. purpureus* significantly elevated the concentrations of propionic acid, acetic acid, and butyric acid in rat fecal samples compared to diarrhea rats.

To explore the impact of gut microbes on colon impairment triggered by diarrhea, we employed an ABX and FMT model for further investigation. In the ABX model, rats underwent antibiotic treatment to deplete the majority of their intestinal microbiota, creating a conducive environment for subsequent colonization by fecal bacteria. Fecal bacteria from rats in the Con, diarrhea, and *L. purpureus*-M groups were then transplanted into recipient rats. Following the depletion of intestinal microbiota, notable differences between the diarrhea and *L. purpureus* groups were not observed. Nevertheless, through FMT, it was revealed that recipient rats colonized with *L. purpureus* microbiota demonstrated significant improvements, encompassing enhanced body weight, colon length, reduced fecal moisture, and ameliorated colonic histological changes, in contrast to rats colonized with diarrhea microbiota. Additionally, the analysis of intestinal bacteria using 16S rDNA amplification and sequencing unveiled variations in the richness of intestinal bacterial communities among the treatment groups. The LEfSe analysis highlighted *Allobacterium* and *Bifidobacterium* as the pivotal bacterial types influencing the intestinal microecology in the FMT-*L. purpureus* group. Furthermore, *Romboutsia*, *Roseburia*, and *Alloprevotella* exhibited increased presence in the FMT-*L. purpureus* group, consistent with the *L. purpureus*-M group (Fig. S6). *Allobaculum*, regarded as a probiotic, synthesizes short-chain fatty acids and certain long-chain fatty acids, exerting anti-inflammatory effects[46]. *Bifidobacterium* is often associated with promoting intestinal motility, thereby maintaining gut equilibrium and host health. Notably, the anti-inflammatory potential of *Bifidobacterium* has garnered support in studies employing an animal model of drug/stress-induced intestinal inflammation[47]. The abundance of *Romboutsia*, as per previous research, displayed an inverse correlation with systemic inflammation and displayed a preference for chemoprevention in an animal model of colorectal cancer[48]. Given these results, it can be inferred that *L. purpureus* might play a role in maintaining the equilibrium of the host's intestines by influencing diverse physiological mechanisms through interactions with the gut microbiota.

5. Conclusion

In summary, our results establish that *L. purpureus* proficiently mitigates diarrhea through the modulation of gut microbiota. This safeguarding impact is accomplished through diverse mechanisms, encompassing bolstering antioxidant capability, reinforcing intestinal TJs, restraining the manifestation of pro-inflammatory agents within the intestine, elevating SCFAs production, and enhancing intestinal permeability. These findings deliver crucial theoretical underpinning for the prospective clinical deployment of *L. purpureus*.

Conflict of interest

The authors report there are no competing interests to declare.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://doi.org/10.26599/FSHW.2024.9250348.

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