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A unique polysaccharide from *Hericium erinaceus* mycelium ameliorates acetic acid-induced ulcerative colitis rats by modulating the composition of the gut microbiota, short chain fatty acids levels and GPR41/43 respectors

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ABSTRACT

Ulcerative colitis (UC) is an idiopathic, chronic inflammatory disorder of the colonic mucosa. Risk of colorectal cancer in ulcerative colitis is increased in patients with long-standing disease compared with the general population. Hericium erinaceus (HE) has been used in traditional folk medicine and medicinal cuisine in China, Korea and Japan with anti-gastritis and anti-ulcerative colitis activities. EP-1, a purified unique polysaccharide isolated from HE mycelium, has recently been identified as the active component responsible for anti- ulcerative colitis activity by using a cell model for identification. In this study, our data shows that EP-1 was effective in relieving the symptoms of acetic acid induced UC rats. Based on the Illumina MiSeq platform, 16S rRNA sequencing of the rat colonic contents indicated that the intestinal flora structure remarkably changed in the model rats and the tendency was alleviated to a certain degree by EP-1. The further results showed that in the acetic acid induced UC rats EP-1 modulated the gut microbiota community and increased short chain fatty acids (SCFAs). And immunoblot analyses showed that after treated by EP-1, GPR41 and GPR43 were significantly suppressed expression in colonic tissues of the UC rats. In the meanwhile, EP-1 also showed its antioxidant, anti-inflammatory and enhancing immune activities. Thus, the polysaccharide purified from HE showed potential for anti-UC activity and the complementary and alternative medicine (CAM) herb therapy.

1. Introduction

Ulcerative colitis and Crohn's disease are the two main forms of inflammatory bowel disease (IBD) [1]. Ulcerative colitis is an idiopathic, chronic inflammatory disorder of the colonic mucosa, which starts in the rectum and generally extends proximally in a continuous manner through part of, or the entire, colon [2]. The pathophysiology factors of UC including epithelial barrier, commensal microflora, dysregulation of immunological responses and etc. The risk of colorectal cancer (CRC) in UC increases with the duration of the disease [3]. The basis of ulcerative colitis treatment is medical, but about 20–30% of patients eventually need surgery [4]. Moreover, the higher cancelation rate and the recurrence after surgery bring great suffering to the patients of UC. Thus, more efforts should be made to enrich the treatment prevention methods of UC. Natural herbs and its products used by UC patients as an alternative treatment method for either induction or maintenance treatment were more and more popular worldwide [5]. Furthermore, herbal therapies in UC seemed to be effective in some proportion of patients with active UC [6,7]. It's very interesting comparing with the chemical medicine, herbal therapies exert their

Abbreviations: HE, *Hericium erinaceus*; UC, ulcerative colitis; AC-UC, acetic acid-induced ulcerative colitis; SCFAs, short chain fatty acids; CAM, complementary alternative medicine; IBD, inflammatory bowel disease; CRC, colorectal cancer; GC, gas chromatography; FID, flame ionization detector; SD, standard deviation; SNK, Student–Newman–Keuls; GC–MS, gas chromatography–mass spectrometry; HPLC, high-performance liquid chromatography; SOD, superoxide dismutase; MDA, malondialdehyde; TNF-α, tumor necrosis factor-α; OTUs, operational taxonomic units; PICRUSt, Reconstruction of Unobserved States

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therapeutic in UC benefit by different mechanisms including immune regulation [8], antioxidant activity [9] and anti-inflammatory [10] etc. However, in-depth studies on the exact mechanism and active ingredients of natural herbs were absent, which limited the widely recognized by both doctors and the patients.

Hericium erinaceus (HE) is well known in Asia as a health-promoting food and important medicinal fungus. The anti-gastric/colitis ulcer activity has been well documented, as it has been widely used as a traditional Chinese medicine and home remedy for many years, particularly in Japan and China [11]. "Houtoujun Tiquwu Keli" is one example of a marked drug in China (Chinese drug approval number H14023099), made from the mycelium extract of HE, which is used for gastritis, peptic gastric and duodenal ulcers, colitis, and dyspepsia. However, the mycelium extract of HE has not yet been analyzed for its active component that exhibits the effects.

In a recent study, we isolated and chemically characterized a unique polysaccharide fraction from HE mycelium, designated EP-1, Which has a molecular weight of approximately 3100 Da and is composed of glucose, mannose and galactose, and has a backbone of α-d-Glc(1 → 3) and β-d-Glc(1 → 3). The polysaccharide exhibits anti-ulcer and anti-gastritis activity [12,13]. Our study has been reported the polysaccharide purified from HE mycelium showed good biological activities on inflammation and ulcer in digestive mucosa [13]. We next demonstrated that EP-1 possessed anti-UC activity using an in vitro hydrogen peroxide (H_2O_2)-abused Caco-2 cell model [14]. EP-1 can increase SOD enzyme activity as well as decrease ROS content and oxidative damage both in vivo and in vitro [15]. However, anti-UC activity of EP-1 has not has not been described in detail. In the study, we used acetic acid induced ulcerative colitis on rats to demonstrate that EP-1 possessed anti-inflammatory, anti-antioxidant, and immunomodulatory activities in vivo and could alleviate inflammation. Furthermore, EP-1 is a polysaccharide with the molecular weight of 3100 Da, which are generally neither absorbable nor digestible by the human body after oral administration. Because gut microbiota are instrumental in metabolizing EP-1 and other polysaccharides, polysaccharide content can have varying impacts on bacterial populations and metabolites within the intestine [16]. Intestinal microbiota degrade polysaccharides into SCFAs (i.e. acetate, propionate, and butyrate) as final metabolic products [17], which can change the gut microbiota ecology and play important roles in maintaining epithelial barrier function, regulating epithelial proliferation [18], modulating immune responses [19], and preventing colorectal cancer [20]. SCFAs are ligands of two orphan G protein-coupled receptors, GPR41 and GPR43, which modulate cell proliferation and induce apoptosis. However, it is unclear if SCFAs enhance the effects of chemotherapy in a GPR41- or GPR43-dependent manner [21].

The aim of the present study was to clarify the anti-UC activity of EP-1 and evaluate its possible mechanism(s) of action at both the organismal level and at the tissue level using an acetic acid-induced UC (AC-UC) rat model. The results discussed here shed light on EP-1 mechanisms of action on gut microbiota after comparison of intestinal microflora community structure and fecal metabolite content among AC-UC rats treated with EP-1, untreated AC-UC rats, and healthy controls. Ultimately, after intragastric administration of EP-1, significant changes in fecal metabolite content and compositional ratio of SCFAs were observed. SCFAs can perform their functions via intermediary metabolism or via specific SCFA receptors, GPR41 and GPR43. These results revealed that the anti-UC activity of EP-1 holds promise for the development of new medicinal foods and complementary alternative medicine (CAM) herbal therapies for UC.

2. Materials and methods

2.1. Reagents

IL-1b, IL-6, TNF-a, MDA, SOD, C3, C4, IgG, and IgM were measured using commercial ELISA kits (Enzyme-linked Biotechnology Co., Ltd., Shanghai, China). Highly purified SCFAs were used to prepare standard solutions for GC-TOFMS (Leco, MA) determination. Acetic acid propanoic acid, 2-methylopropanoic acid, butanoic acid, 3-methylbutanoic acid, and pentanoic acid were obtained from Sigma (St. Louis, MO, USA All other reagents used were of analytical grade and were purchased from Shanghai Chemicals and Reagents Co. (Shanghai, China). Methyl chloroformate (MCF), chloroform, pyridine, sodium hydroxide, sodium bicarbonate, ammonium acetate, and anhydrous sodium sulfate were of analytical grade and obtained from Sigma-Aldrich (St. Louis, MO, USA). Ultrapure water was produced by a Milli-Q system. All the chemicals of other microbial metabolites were commercially purchased from Sigma-Aldrich (St. Louis, MO, USA), Santa Cruz (Dallas, TX, USA), and Nu-Chek Prep (Elysian, MN, USA).

2.2. Extraction and purification of EP-1

HE mycelium was extracted with five volumes of water at 70 °C with gentle stirring for 12 h. The residue was re-extracted twice as described above and all supernatants were combined, concentrated and precipitated with ethanol (final concentration 80%) to obtain an ethanol-soluble fraction and ethanol-precipitated fraction. The ethanol-precipitated fraction was further separated into two fractions by passing it through hollow fibre ultrafiltration cartridges (3 K). The resulting fractions included a concentrated solution fraction and a permeate fraction. The permeate fraction was further separated using a hollow fibre ultrafiltration column (0.2 K) to obtain concentrated solution and a permeate fraction. Next, the concentrated solution was then applied to a DEAE-Sephadex column and was eluted stepwise with distilled water followed by 0.2 M sodium chloride and finally 2.0 M sodium chloride. The fraction eluted with distilled water was concentrated and freeze-dried to obtain EP-1, which was subsequently subjected to structural characterization and determination of biological activity.

2.3. Animals model assessing the anti-colitis effect of EP-1

Forty 8-week-old Sprague-Dawley (SD) rats (200–250 g each, 5 males and 5 females per group) were obtained from Jilin University College of Pharmacy and were accompanied by a health and safety certificate of conformity administered by the Chinese government (ShengChanXuKe number SCXK2016-0001). Animal experiments were performed in accordance with the Regulations of Experimental Animal Administration. Rats were housed in a specific-pathogen-free (SPF) animal center for drug safety evaluation and research at Jilin University with air-conditioned animal quarters, 12 h light/12 h dark cycle, with temperature maintained within 23 ± 3 °C and relative humidity within 50% ± 10%. All rats were acclimatized for 7 days before they were used in any experiments and were fed standard chow and water ad libitum. All rats were randomly divided into the following four groups: Blank group (no UC), Model group (untreated UC), EP-1(L) (low-dose treated UC), and EP-1(H) (high-dose treated UC). Each group was composed of 10 rats.

The animal model of colitis was based on a protocol reported previously [22]. Briefly, including the exception of the blank, other groups of rats fasted and received only water for 24 h. After anesthesia with pentobarbital sodium (30 mg/kg IP), we inserted a silica gel enema tube with diameter of 3 mm slowly through the anus of each rat to a depth of about 8 cm. Next, a syringe was used to inject 1.5 ml of 4% acetic acid (untreated group and drug-treated group) or pure water (Blank group). After accurate timing of 15 s, rats were flushed with 4 ml saline. Rats were then housed and fed normally after awakening. For 10 d starting at day 2 post-UC induction, the Blank group and the Model group (untreated UC group) were administered by gavage equal volumes of distilled water, while high or low doses of EP-1 were administered to the corresponding UC rat treatment groups. On the 11th day, rats fasted and received no drinking water for 12 h then were weighed. After blood

was taken from the ocular orbit of each rat, animals were put to death by neck strangulation. After being weighed, the animals were killed. The distal 10 cm of the colon, measured by insertion of a ballpoint syringe, was removed and opened by making a longitudinal incision. The occurrence of diarrhea was noted. The colon segment was briefly washed in saline and scored for gross changes in morphology: 0 = no damage, 1 = hyperemia, 2 = ulcers < 25% of the total area, 3 = ulcers 25–50% of the total area, and 4 = ulcers > 50% of the total area. The segment was weighed to obtain the wet weight, and pieces were removed for histologic examination [23]. After blood was centrifuged, sera were collected. Tissue samples were cryopreserved and some samples were used to prepare paraffin-embedded sections.

2.4. Tissue sample preparation and serum cytokine ELISA analysis

The colonic tissues of 5 mice were randomly selected in each group. The histopathological analysis was done in the colon specimen fixed in 10% formalin in PBS and embedded in paraffin. About 4 mm thick sections of colon were prepared, stained with Eosin and Hematoxylin and observed under light microscope. All sections were analyzed and interpreted by a certified histopathologist. The other colonic tissue was homogenized in a commercial Pro-Prep Protein Extraction Solution (Shanghai Beyotime, China). Intestine tissue samples from 38 rats were evaluated. The relative level of IL-1b, IL-6, TNF-a, MDA, SOD, C3, C4, IgG, and IgM were determined using Elisa kits. According to the detecting step instructions, the concentration of cytokines in serum was calculated by the standard curve.

2.5. DNA extraction, PCR, and MiSeq sequencing

DNA was extracted from colon contents using an Omega Mag-Bind Soil DNA Kit (200) (Omega Bio-Tek, USA). Purified PCR products were prepared using Q5® High-Fidelity DNA Polymerase (NEB, USA) and products were quantified, then each PCR sample was diluted 5 times to 20 ng/μL. PCR Amplification System: PCR mixed product sample (2 μL), 5× reaction buffer (5 μL), 5× GC buffer (5 μL), dNTP (2.5 mM 2 μL), Forward primer (10 μM, 1 μL), Reverse primer (10 μM, 1 μL), Q5 DNA Polymerase (0.25 μL), DNA template (2 μL), ddH_2O 8.75 μL. PCR amplification of the 16S rRNA genes V3–V4 region was performed using the forward primer 338F 5′-ACTCCTACGGGAGGCAGCA-3′ and reverse primer 806R 5'-GGACTACHVGGGTWTCTAAT-3′. Sample-specific 7-bp barcodes were incorporated into the primers for multiplex sequencing. The PCR components contained 5 μl of Q5 reaction buffer (5×), 5 μl of Q5.High-Fidelity GC buffer (5×), 0.25 μl of Q5 High-Fidelity DNA Polymerase (5 U/μl), 2 μl (2.5 mM) of dNTPs, 1 μl (10 μM) of each Forward and Reverse primer, 2 μl of DNA Template, and 8.75 μl of ddH_2O. Thermal cycling consisted of initial denaturation at 98 °C for 2 min, followed by 25 cycles consisting of denaturation at 98 °C for 15 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s, with a final extension of 5 min at 72 °C. The amplicon library was then used for paired-end sequenced (2 × 250 bp) on an Illumina MiSeq platform (Illumina, San Diego, USA) according to standard protocols.

2.6. Determination of SCFAs in colonic portion by GC–MS

2.6.1. Chemicals and reagents

The stock solutions of ethanoic acid, propanoic acid, 2-methylopropanoic acid, butanoic acid, 3-methylbutanoic acid and pentanoic acid representative reference chemicals of microbial metabolites were prepared in methanol, ultrapure water, or sodium hydroxide solution at a concentration of either 5 mg/mL or 1 mg/mL. The three groups of mixed stock solutions were mixed to create stock calibration solutions based on their solubility and chemical properties. Further serial dilutions of the stock calibrations were made to obtain the eight calibrators at a wide concentration range covering from 0.008 to 250 μg/mL. Internal standards were added to monitor the data quality and compensate for matrix effects.

2.6.2. Sample preparation

The sample preparation and derivatization protocols with MCF were based on the method using our previously published procedures with modifications [1,2]. Samples were thawed on ice-bath to diminish sample degradation. Approximately 50 mg of the study materials was homogenized with 300 μL of NaOH (1 M) solution using a homogenizer (BB24, Next Advance, Inc., Averill Park, NY, USA) and centrifuged at 13,500 rpm and 4 °C for 20 min (Microfuge 20R, Beckman Coulter, Inc., Indianapolis, IN, USA). Each 200 μL of supernatant was transferred into an autosampler vial (Agilent Technologies, Foster City, CA, USA), and the residue was further exacted with 200 μL of cold methanol. After the second step of homogenization and centrifugation, each 167 μL of supernatant was combined with the first supernatant in the autosampler vial. The extracts in the autosampler vial was capped and submitted for automated sample derivatization with a robotic multipurpose sample MPS2 with dual heads (Gerstel, Muehlheim, Germany). Briefly, each 20 μL of MCF was added to the mixture and the sample was vortexed vigorously for exact 30 s. Another 20 μL of MCF was added for the second time derivatization. Four hundred microliter of chloroform followed by four hundred microliter of sodium bicarbonate solution (50 mM) was added to achieve the separation. The prepared samples were centrifuged at 4 °C and 4000*g* for 20 min, and the bottom chloroform layer was carefully transferred by the robotic preparation station to a capped empty autosampler vial preloaded with approximately 25 mg of anhydrous sodium sulfate. The sample pretreated with sodium sulfate was shaken on a laboratory shaker at 1,500 rpm and 4 °C for 20 min and further transferred to a capped empty autosampler vial for injection.

2.6.3. Instrumentation

A gas chromatography coupled to time-of-flight mass spectrometry (GC-TOFMS) system (Pegasus HT, Leco Corp., St. Joseph, MO, USA) operated in electron ionization (EI) mode was used to quantitate the microbial metabolite in this project. The optimized instrument settings are briefly described below. Instrument optimization was performed every 24 h.

GC-TOFMS instrument settings.

GC	
Column	Rxi-5MS (crossbond ® 5% diphenyl/95% dimethyl polysiloxane) 30 m (length) × 250 μm I.D., 0.25-μm film thickness
Oven programmed temp. (°C)	45 (1 min), 45–260 (20 °C/min), 260–320 (40 °C/min), 320 (2 min)
Inlet temp. (°C)	270
Injection vol. (μl)	1.0
Carrier gas	Helium (99.9999%)
Transfer interface temp. (°C)	270
Flow rate (mL/min)	1.0

Mass spectrometer	
Ionization mode	electron impact
Electron energy (EV)	−70
Detector voltage (V)	−1450
Source temp (°C)	220
Acquisition rate	20 spectra/s.
Mass range (Da)	38–550

2.7. Western blot analysis

For WB analysis, the colonic tissue was homogenized in a commercial Pro-Prep Protein Extraction Solution (Beyotime Institute of

Biotechnology, Shanghai, China). Protein concentrations in the extracts were quantified using the BCA protein Assay reagent (Beyotime Institute of Biotechnology, Shanghai, China) according to the manufacturer's instructions. Samples were loaded onto a 12% SDS-PAGE gels (30 μg for each sample) and then transferred to polyvinylidene difluoride (PVDF) member (Millipore Corp., Billerica, USA). The members were blocked with 5% BSA, and then, were incubated overnight with corresponding primary antibodies at the following dilutions: Anti-GPR41, 1:2000; anti-GPR41, 1:2000 and anti-GAPDH, 1:5000 (Santa Cruz Biotechnology, Inc). The protein bands were visualized with ECL detection reagents (Thermo Fisher Scientific, Rockford, IL, USA) after incubation with appropriate secondary antibodies for 1 h at room temperature. Results were expressed as the ratio of intensity of the target protein and that of the GAPDH loading reference band in the same lane.

2.8. Statistical analysis

All experiments were done in triplicate. Results were expressed as means ± standard deviation (SD). Data were evaluated by one-way analysis of variance using SPSS 17.0 software. Differences between groups were evaluated using the Student–Newman–Keuls (SNK) test. The level of significance was set at $p < 0.05$.

3. Results

3.1. Isolation and determination of EP-1

Mycelia of *Hericium erinaceus* were extracted with hot water and the extract was successively subjected to ethanol precipitation, hollow fibre ultra-filtration, and ion–exchange chromatography to obtain the purified anti-gastritis polysaccharide, designated EP-1. The purification procedure is shown in Fig. 1. The carbohydrate content of EP-1 was 95.7% and its molecular weight was approximately 3.1 KDa, as determined by high-performance liquid chromatography (Fig. 2). The results of methylation analyses of EP-1 obtained using gas chromatography–mass spectrometry (GC–MS) revealed that the glucosyl residues are mainly composed of (1 → 3)-linked Glu*p* units with approximately 10% each of (1→)-Man*p* units and (1 → 3,4)-Glu*p* units and 1.5% of (1 → 3,4)-Gal*p* units, as described previously [13].

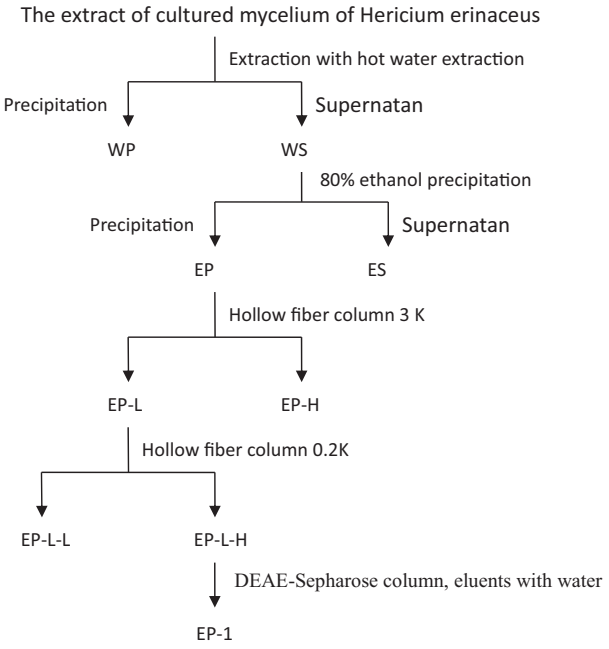

Fig. 1. Summarized extraction and isolation procedure of EP-1.

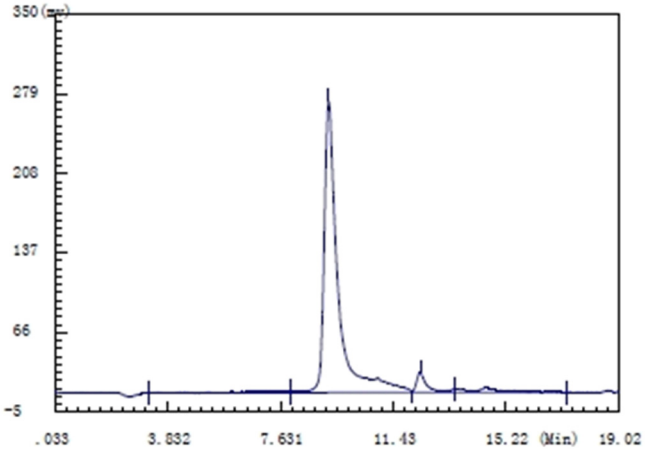

Fig. 2. GPC analysis of EP-1.

3.2. EP-1 relieves the symptoms of acetic acid induced UC rats

In this study, a UC rat model induced by acetic acid (AC-UC) was used to evaluate the anti-UC activity of EP-1. Compared to the Blank group, the Model group of rats with untreated AC-UC lost weight significantly during the trial period and some exhibited serious diarrhea or obvious rectal bleeding symptoms after two days post-UC induction. However, groups receiving different intragastric doses of EP-1 (0.6 and 1.2 g/kg) exhibited a trend of reduced weight loss, with remarkable increase in body weight observed for the group receiving high-dose (1.2 g/kg) EP-1 (Fig. 3a). Gross examination (with the unaided eye) of the colonic segment comprised of the proximal 10 cm-section of the colon adjacent to the anus demonstrated that non-diseased rat colon (Blank group) had a smooth surface and clear folds; however, in the untreated UC rats (Model group), the colonic mucosa were congested, with hyperemia and obvious mucosal epithelial erosion. In the low-dose EP-1 group, designated EP-1(L), epithelial mucosal hyperemia and erosion of the colonic epithelium were significantly reduced compared with the untreated UC group. In the high-dose treatment group, designated EP-1(H), the colonic mucosa exhibited only a small amount of hyperemia and the epithelial fold was basically restored to a non-diseased state (Fig. 3b). The gross morphologic scores in different groups were shown in Table 1. Upon evaluation of histopathological characteristics of colonic tissue by H&E staining, in the untreated AC-UC group histopathological observations included mucosal degeneration and necrosis, cryptitis and cystic dilatation of the crypts, and mucosal and submucosal infiltration of neutrophils and mononuclear inflammatory cells as compared to healthy controls. However, in all rats of the EP-1(L) group, infiltration and aggregation of mononuclear inflammatory cells were observed in the lamina propria of the colon, with improvement observed in epithelial degeneration and necrosis. In rats of the EP-1(H) group, hyperemia and linear healing of ulcers were observed in the colon; histopathologic examination revealed mucosal epithelial regeneration with hyperemia and infiltration of only a few neutrophils and mononuclear inflammatory cells (Fig. 3c).

3.3. EP-1 improved the structure of gut microbiota of acetic acid induced UC rats

3.3.1. Classification of colonic bacterial communities into operational taxonomic units

In this study, 19 colonic content samples from groups of healthy rats designated Blank (no disease), Model (untreated UC), EP-1(L) (treated UC), and EP (H) (treated UC) were studied to evaluate EP-1 amelioration of AC-UC (n = 5, 5, 4, 5 rats in each group, respectively). High throughput sequencing using the Illumina MiSeq platform was performed based on analysis of 16S rDNA (the V3-V4 region) and the

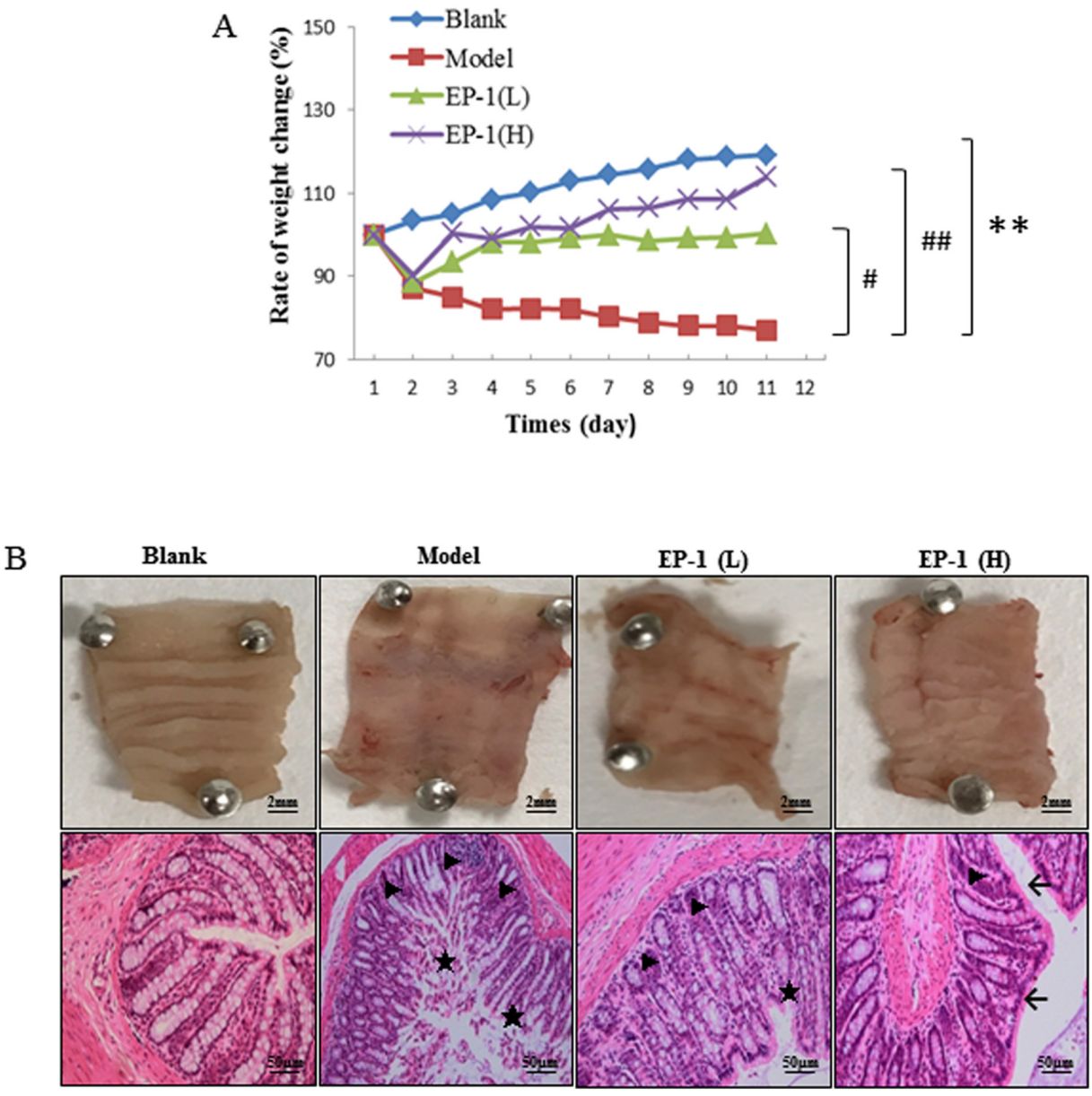

Fig. 3. EP-1 relieves the symptoms of acetic acid induced UC rats. (a) Change of body weight during the trail process. (b) Gross changes of colons in rats by naked eye. (c) Histopathological changes of colons. "▶" indicates the infiltration of neutrophils and mononuclear inflammatory cells in the mucosa or submucosa. "★"indicates the degeneration, necrosis of mucosal epithelium. "←"indicates linear healing of ulcers. Original magnification ×400. The results were presented as mean ± SD; $n = 10$ for each group; $^{**}P < 0.01$, versus normal control group; $\#P < 0.05$, $\#\#P < 0.01$ versus model group.

Table 1
Colonic mucosa score in each group.

Group	n	Scores (Mean ± SD)
Blank	10	0.2 ± 0.4
Group	10	2.8 ± 0.8**
EP-1(L)	10	1.5 ± 0.5#
EP-1(H)	10	1.2 ± 0.4##

** P < 0.01, versus normal control group.
# $P < 0.05$.
$P < 0.01$ versus model group.

results showed a total of 766,558 raw reads, which passed all quality filters set to a cutoff of at or above 97% identity to generate a total of 960 species for classification into operational taxonomic units (OTUs). OTUs for Blank, Model, EP-1(L), and EP-1(H) groups were determined from colonic content samples and were used to label the nodes within the bipartite network. OTUs analyses showed that the number of OTUs from the Model group was significantly lower than the number of OTUs from the Blank group ($P < 0.05$). However, the numbers of OTUs from EP-1(L) and EP-1(H) groups were both higher than for the Model group, with a significant difference between the EP-1(H) group and Model group (P < 0.05). The Venn diagram illustrating overlap of OTUs and the statistical maps of OTU divisions and classification results and OTU numerical results are shown in Fig. 4A and Fig. 4B. The Chao1 estimate, observed species, and Shannon rarefaction curves of samples were close to the saturation plateau (see Supplementary Fig. 1). These results suggest that the number of species of intestinal flora decreased significantly in the Model (untreated AC-UC) group compared with the Blank group and that EP-1 exerted a significant recovery effect on the decrease of the flora species.

3.3.2. Changes in intestinal bacterial communities

Community composition data at each classification level were

clustered according to relative abundance of taxa and were compared among experimental groups. The results showed that there were significant differences in the relative abundance of bacteria among the different groups (Fig. 5A). Then, we use QIIME software to obtain the composition and abundance of bacteria at each classification level for each sample. Compared with normal, the levels of Verrucomicrobia, Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria phyla were significantly altered in AC-UC rats. After administration of EP-1 by gavage, the most obvious recoveries were observed for Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria (Fig. 5B). Next, GraPhlAn was used to construct a hierarchical tree based on sample composition at all levels of classification using different colors to reflect different taxonomic groups, with the sizes of nodes reflecting their relative abundances. Subsequently, we found that dominant flora included members of the Ruminococcaceae family within the phylum Firmicutes, as well as members of Proteobacteria and Bacteroidetes phyla. Members of Allobaculum and Desulfovibrionales genera and S24-7 family members were also dominant microbial constituents (Fig. 6). Next, the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) method was applied to predict metabolic function by comparing existing 16S rDNA gene sequencing data to the microbial reference genome database of known metabolic functions. Using this method, carbohydrate metabolism was the most abundant metabolic function observed (see Supplementary Fig. 2). This is interesting because carbohydrate metabolism is relevant to the formation of short chain fatty acids such as acetic acid and butyric acid, which play an important role in intestinal mucosal structure and function, thus aligning with the intestinal short chain fatty acid changes observed in this study [24].

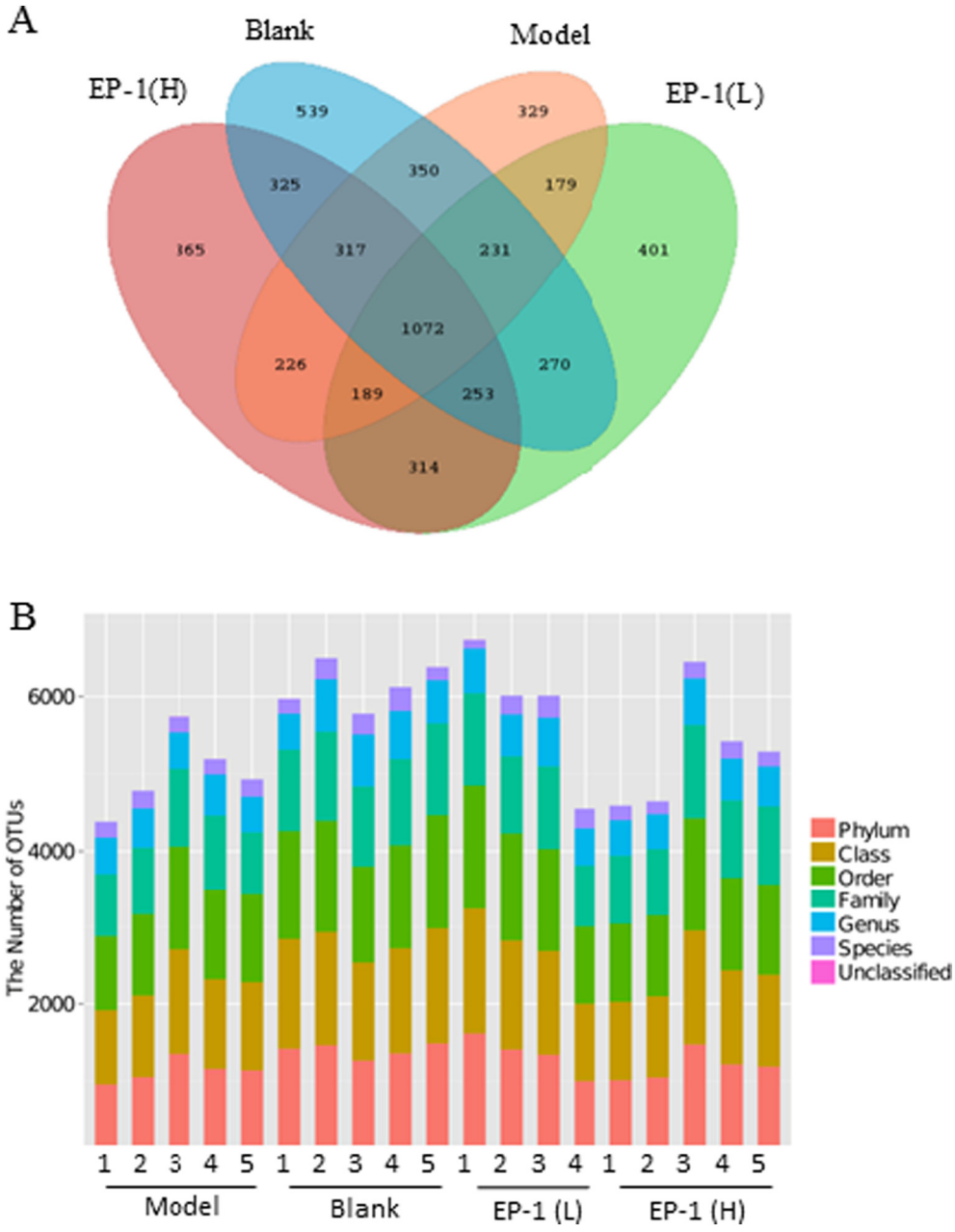

Fig. 4. EP-1 alters diversity and richness of intestinal flora in AC-UC rats (A) Venn diagram illustrating overlap of OTUs. (B) The statistical maps of OTU division and the classification of status identification results.

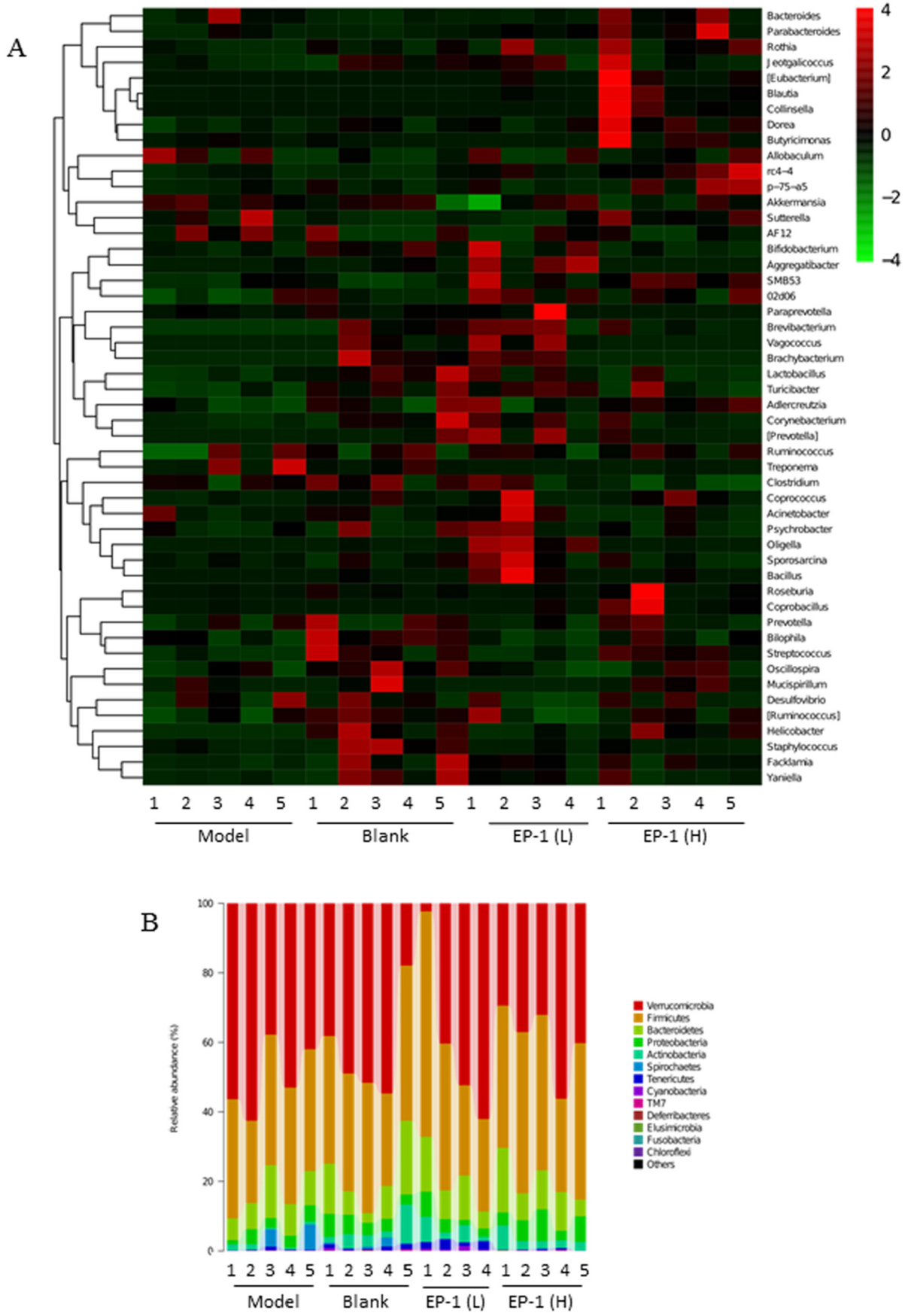

Fig. 5. EP-1 alters microbiota composition in UC rats. (A) Bacterial taxonomic profiling in the genus level of intestinal bacterial from different groups. (B) Heatmap correlation analysis of the genes level in the different groups.

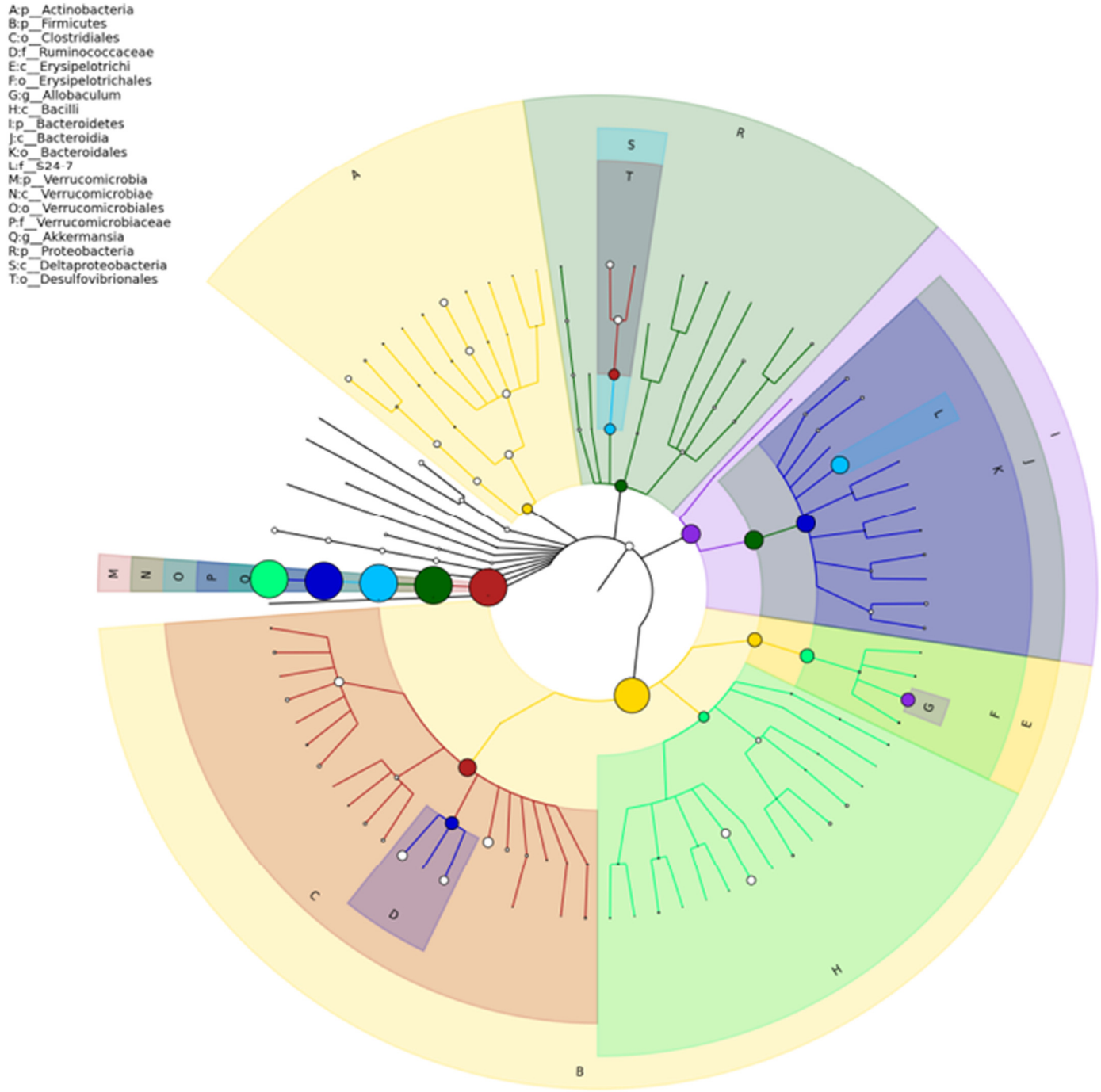

Fig. 6. Classification tree of sample population based on GraPhlAn.

3.4. EP-1 modulates short chain fatty acids (SCFAs) in the feces of UC rats

In this study, using GC–MS to test colonic contents samples of the four groups, we commonly detected C2 (Acetic acid), C3 (Propanoic acid), C4 (Isobutyric acid and Butanoic acid), and C5 (Isovaleric acid and Valeric acid) using main SCFAs as standards. The results showed that the SCFAs detected in feces among the different groups were mainly ethanoic acid, propionic acid, butanoic acid, and pentanoic acid, with ethanoic acid present in the highest proportion followed by butanoic acid then propionic acid, with pentanoic acid present in lowest proportion. In the Blank group, the relative molar ratio of SCFAs showed stable proportions as compared to the Model (untreated UC) group, wherein the quantity medmin of SCFAs was altered and relative proportions of ethanoic acid and butanoic acid were reduced significantly (Table 2). In AC-UC rats treated with different doses of EP-1, relative proportions of ethanoic acid and butanoic acid increased as compared to their proportions in the Model (untreated UC) group and showed an EC-1 dose-dependent effect; indeed, the relative molar ratio of SCFAs in the high-dose treatment group EP-1(H) resembled the Blank group most closely. Thus, the results demonstrated that EP-1 increased the relative proportions of ethanoic acid and butanoic acid SCFAs in feces of rats with AC-UC.

3.5. EP-1 activated GPR41 and GPR43 in acetic acid induced UC rats

The results showed that GRP41 and GRR43 proteins were

Table 2
Analytical parameters for GC–MS method of SCFAs in colonic contents.

Analyte Id	Coefficients R	Lineary range (ng/mg)	Blank (ng/mg)	Model (ng/mg)	EP-1(L) (ng/mg)	EP-1(H) (ng/mg)
Acetic acid	0.9950	40–5000	1303.65	1227.64	1310.95	1493.67#
Propionic acid	0.9937	10–2500	157.19	152.12	150.16	159.05
Butyric acid	0.9948	0.5–1000	200.7	39.31**	41.76#	67.74##
Valeric acid	0.9953	0.1–200	34.03	16.29*	26.27#	21.57##
Isobutyric acid	0.9980	0.5–1000	15.79	11.93*	18.43#	17.15#
Isovaleric acid	0.9984	0.25–500	9.66	5.41*	11.36##	9.79#
Total SCFAs			1721.02	1452.7*	1558.93#	1768.97##

Each value is the median (n = 8).
* P < 0.01.
** P < 0.01 versus normal control group.
# P < 0.05.
P < 0.01 versus model group.

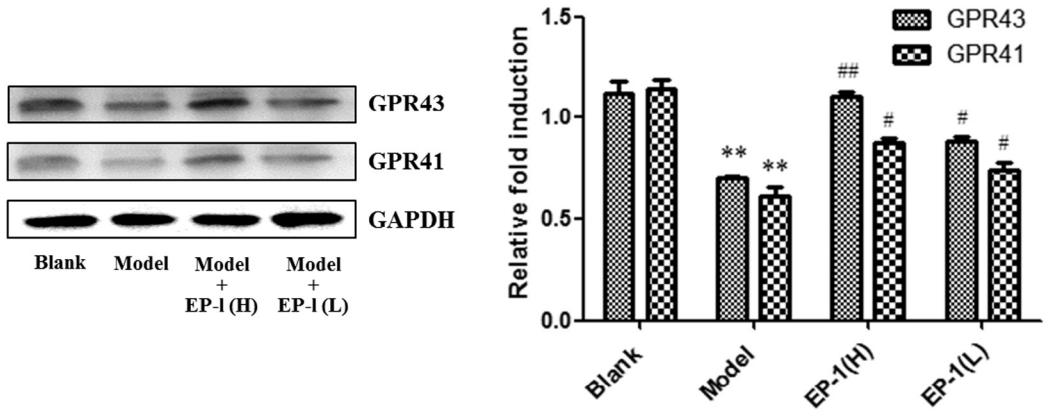

Fig. 8. Western blotting analyses of GPR41 and GPR43. (A) Representative western blotting of GPR41 and GPR43 with GAPDH as the internal control. (B) Expression ration of GPR41 and GPR43. Values represent mean ± SD of three independent experiments, *P < 0.05, **P < 0.01 versus normal control group; #P < 0.05, ##P < 0.01 versus H_2O_2 group.

significantly decreased in the Colon tissue of model rats, the decreased levels of both were inhibited by EP-1. The EP-1 also showed a dose dependent manner (Fig. 8). GPR41 and GPR43 are receptors of SCFAs which showed diverse functions in inflammation, metabolism, and allergic diseases [25]. Next, we examined whether EP-1enhanced antioxidant, anti-inflammatory and immune activities via GPR41 and GPR43.

3.6. Serum cytokine, immunoglobulin, and complement profiles in rats

In previous studies in humans, IL-1 and IL-6 have been observed to be the main pro-inflammatory factors closely related to UC in children [26]. In this study, sera of rats in the untreated AC-UC group also exhibited increased IL-1 and IL-6 levels. However, AC-UC rats that received intragastric administration of EP-1 exhibited cytokine production that was significantly reduced in an EP-1 dose-dependent manner (Fig. 9a). Meanwhile, oxidative stress has also been linked to UC [27]. Therefore, we examined the antioxidant effect of EP-1 on serum SOD and MDA levels among the different experimental groups and demonstrated that serum levels of MDA increased and SOD levels decreased in the untreated (Model) UC group. However, the effect of UC treatment on serum SOD and MDA levels depended on EP-1 dose, whereby one EP-1 dose caused increased SOD levels and reduced MDA levels (Fig. 9b, c). In addition, serum immunoglobulin and complement were also examined to evaluate the effects of EP-1 on immunologic function. Compared with the Blank group (without UC), the Model group exhibited significantly increased C3 levels and decreased IgM levels (P < 0.05). However, in the EP-1(H) group, significantly lower levels of both C3 and IgM were observed relative to corresponding levels of the Model group (Fig. 2e, g). By contrast, C4 and IgG levels observed in sera of either EP-1(H) or EP-1(L) groups did not exhibit any statistically significant differences from levels observed in the Blank group (Fig. 9f, h).

4. Discussion

UC is a type of chronic idiopathic IBD. The use of conventional UC treatment drugs, such as corticosteroids, immunosuppressants, and antibiotics, etc., is accompanied by the certain risk of side effects, some of which are quite severe [28]. Consequently, use of complementary or alternative medicines by 50% of IBD patients for at least a short period of time has been reported. Of these, 58% of patients have reported using herbal medicines to treat either active or quiescent disease [29]. *Hericium erinaceus* (HE) is a traditional Chinese medicine and medicinal food used in treating UC. However, its wider application is limited by its ambiguous pharmacodynamic composition, incomplete experimental verification of efficacy and its unclear mechanism of action. In our previous study, we purified a polysaccharide from HE, designated EP-1, and characterized its chemical structure [13]. Furthermore, we verified EP-1 as the major component responsible for anti-UC activity by cell model [14]. In this study, we used an acetic acid-induced ulcerative colitis rat model to confirm the anti-ulcerative activity of EP-1. Through observations of the polysaccharide's morphological and histological effects, the results preliminarily demonstrated EP-1's anti-inflammatory, anti-oxidative and immunoregulatory activities. These results laid a solid foundation upon which to build further studies to reveal the mechanism of action of EP-1.

In recent years, the association between ulcerative colitis and

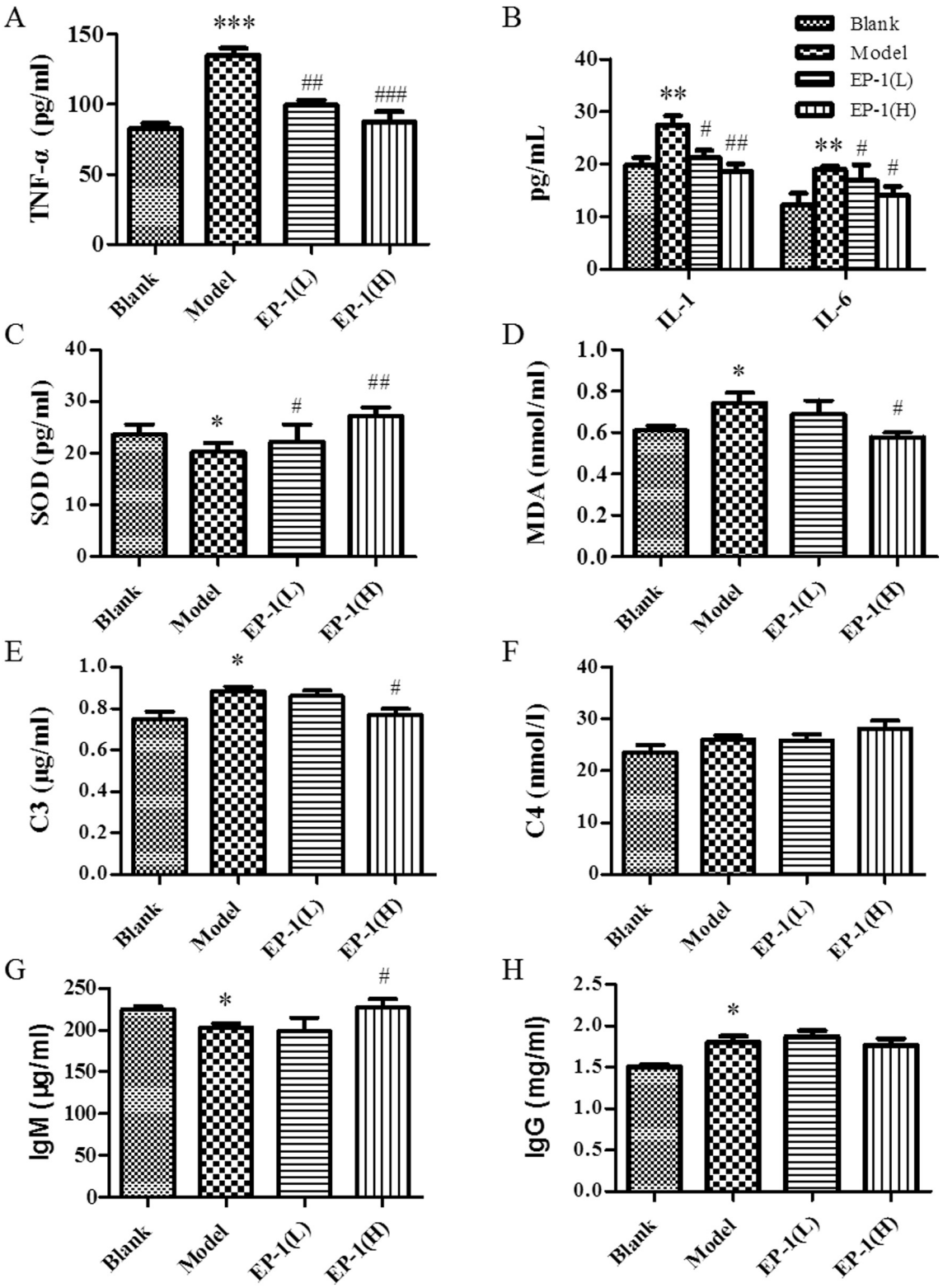

Fig. 9. EP-1 showed different activities on antioxidant, anti-inflammatory and immunoregulation. Following the animal treatment as Fig. 2, the level of IL-1, IL-6 (a), SOD (b), MDA (c), C3 (d), C4 (e), IgM and IgA (f) in the rats' serum were determined respectively by Elisa method. The results were presented as mean ± SD; $n = 10$ for each group; $^{**}P < 0.01$, versus normal control group; #P < 0.05, ##P < 0.01 versus model group.

intestinal flora imbalance has been confirmed [30]. Indeed, certain intestinal bacteria and their metabolites can activate the mucosal immune system, leading to intestinal mucosal dysfunction [31]. Moreover, studies elucidating the pathological condition of ulcerative colitis have shown that the diversity of intestinal flora in patients with ulcerative colitis is significantly reduced [32]. Interestingly, metabolic breakdown products of some herbal medicines have been shown to alter the composition of the intestinal microbial community. It has been reported that such medicines may alleviate ulcerative colitis by regulating the proportions of certain intestinal bacteria to achieve anti-inflammatory

effects and immunomodulation [33–35]. Our gut macrobiotic study showed that EP-1 can significantly increase the diversity and richness of intestinal flora in AC-UC rats. Compared with the healthy control group, the proportion of *Verrucomicrobia, Firmicutes, Bacteroidetes* and *Proteobacteria* in the AC-UC group changed significantly, but the polysaccharide-treated group resisted such changes, exhibiting a community profile similar to that of the normal group. Short-chain fatty acids (SCFAs) are the most relevant endogenously produced substances within the UC realm of metabolomics [36]. SCFAs mainly include acetic acid, propionic acid, butyric acid etc. Butyric acid is the principal source of energy for colonic epithelial cells, while acetic acid is involved in fat production and gluconeogenesis. Notably, acetic acid and butyric acid can activate GPR41 and GPR43 and inhibit histone deacetylase to play an anti-inflammatory role [37]. Because butyric acid, 2-methylopropanoic acid and acetic acid are decreased significantly in stools of UC patients [38], regulating the intestinal content of SCFAs has also become a UC treatment target. Notably, our results demonstrated that proportions of acetic acid and butyric acid in intestinal contents of the disease model group were significantly lower than in the normal group. However, these proportions increased significantly in UC rat intestinal contents after EP-1 administration, ultimately assuming normal SCFAs ratios, principally butyrate which provides the colonocyte with about 70% of its energy. In conclusion, the polysaccharide EP-1 improved both microbial diversity and richness of microbial communities in AC-UC rats while stimulating production of SCFAs. This study opens the way for the development of complementary alternative herbal medicine treatments by justifying a new UC treatment strategy based on herbal medicines that modulate intestinal flora. While our study only incorporated 16S rDNA sequencing to study gut microbiota, the future application of shotgun meta-omics sequencing technology will further clarify bacterial species present [39]. In addition, our sole analysis of specific metabolites, SCFAs, will be augmented in the future by metabonomics, which will provide more detailed analysis of metabolic pathways to understand mechanisms of action in greater detail [40]. Ultimately, we foresee that a combination of integrative omics studies will further demonstrate the efficacy and mechanisms of action of polysaccharides derived from traditional Chinese medicines to guide their development into effective treatments for UC.

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Conflict of interest

The authors declare no conflict of interest.

Authors' contribution

MW designed the experiment and contributed to the writing. MW and SS carried out the majority of the biochemical analysis and revised the manuscript. DW, JD and MW contributed to the supervision and drafting of the manuscript. XL, QZ contributed with technical support, scientific advice.

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